Quality, Safety and Efficacy of a Novel Nanoparticulate Imiquimod Formulation

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Mainz, 2023

1. Berichterstatter:

2. Berichterstatter:

Tag der mündlichen Prüfung:

„Time is life"

Dr. Albert Bourla, Chairman and CEO of Pfizer

during the "Project Lightspeed" leading to BioNTech-Pfizer's mRNA COMIRNATY™ vaccine

Für meine Eltern, meine Schwester, meine Onkel und meine Großeltern

Acknowledgements

I hereby declare that I wrote the dissertation submitted without any unauthorized external assistance and used only sources acknowledged in the work. All textual passages which are appropriated verbatim or paraphrased from published and unpublished texts as well as all information obtained from oral sources are duly indicated and listed in accordance with bibliographical rules. In carrying out this research, I complied with the rules of standard scientific practice as formulated in the statutes of Johannes Gutenberg University Mainz to insure standard scientific practice.

Chapters 1, 2 and 3 use text, tables and figures of the following publications:

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Abstract

Imiquimod (IMQ) is a well-established treatment for actinic keratosis (AK), basal cell carcinoma (BCC) and genital warts and is commercially available as the approved product Aldara™, an oil-in-water cream formulation containing IMQ solubilized in isostearic acid. While this treatment has demonstrated safety and efficacy in humans, treatment of AK with Aldara™ is associated with severe side effects due to the poorly tolerated excipient isostearic acid resulting in considerable adverse events e.g. severe erythema.

To overcome this issue, a novel nanoparticulate IMQ formulation "IMI-Gel" has previously been developed aiming at a decreased dermal drug permeation based on suspended drug nanoparticles with decreased permeation kinetics and follicular drug delivery. This thesis aims at applying the concept of translational medicine, supplying IMI-Gel to patients in a phase I clinical trial for treatment of AK. Thereby, the quality of the product was optimized by applying the concept of Quality by Design (QbD). In this context, the critical process parameters of milling time and rotational speed (identified in a risk based approach) were evaluated and optimized with identified optimal process conditions at 650 rpm for 135 min delivering IMQ nanocrystals at the targeted particle size of 300 - 400 nm. Critical process parameters (CPPs) and critical material attributes (CMAs) were linked to the critical quality attributes (CQAs) of IMI-Gel enabling installation of in-process controls (IPCs) and quality control tests (QC) to ensure each manufactured batch complies to the targeted quality. Trend analysis of IPC and QC data from batches manufactured according to Good Manufacturing Practice (GMP) reveal minimal batch-to-batch variability and consistent quality.

Moreover, the process of complaint management of a quality complaint filed by a participating subject in the clinical trial, is presented. The complaint was filed by a patient participating in the respective phase I clinical trial and concerned a quality defect of IMI-Gel. Root-cause analysis included a pH Test, evaluation of the primary packaging, studies on the physical stability inside and outside of the primary packaging and rheological analyses. Evaluation of the formulation pH as an indicator of the Carbopol gel stability indicated no change from the range defined in the acceptance criteria. Evaluation of the primary packaging revealed no damaging to the primary packaging. Studies on the physical stability of the formulation stored at 40 °C and 80 °C inside of their primary revealed no change related to the physical stability. Storage of formulations at the latter conditions inside of their primary packaging with an open screw cap and outside of their primary packaging on a petri dish however, revealed quick phase separation. Studies on the physical stability using rotational and oscillatory rheological tests revealed structural stability of the formulations stored at 25 °C/65 % rH over 12 months and at 40 °C/75 rH stored over 6 months as long as the formulation is stored inside of a tightly closed primary packaging. Based on the results it was concluded that the most probable root-cause is a handling error by the patient.

Evaluation of safety and efficacy from the patient related data generated during the clinical trial revealed comparable efficacy for both tested formulations Aldara™ and IMI-Gel. This was found by no significant difference in the response rates between the groups (number of patients whose lesions were completely cleared) and in the reduction of the AK skin lesion area. For IMI-Gel a superior tolerability was found with a statistically significant difference in the skin quality outcome. Moreover, a lower relative frequency of ulcerations and exudates was observed for patients receiving IMI-Gel, although not statistically significant.

Overall, IMI-Gel demonstrated its quality, safety and efficacy with superior properties related to its tolerability in humans.

Zusammenfassung

Imiquimod (IMQ) ist für die Behandlung der aktinischen Keratose (AK), des Basalzellkarzinoms (BCC) und von Genitalwarzen zugelassen. Kommerziell ist IMQ als Aldara™, eine Öl-in-Wasser-Creme-mit IMQ gelöst in Isotearinsäure, erhältlich. Obwohl die Anwendung von Aldara™ bei Menschen Sicherheit und Wirksamkeit gezeigt hat, kommt es während der Behandlung von AK aufgrund des schlecht verträglichen Hilfsstoffes Isostearinsäure und der hohen Hautpermeation zu Nebenwirkungen in der Form von schwerwiegenden Erythemen.

Um die Verträglichkeit von IMQ zu verbessern, wurde eine neuartige IMQ-Formulierung "IMI-Gel" ohne Isostearinsäure entwickelt, die auf suspendierten Wirkstoffnanopartikeln basiert und IMQ mittels follikulären Arzneistofftransport in die Haut abgibt. Ziel dieser Arbeit ist es, das Konzept der translationalen Medizin auf die IMI-Gel Formulierung anzuwenden, um diese für eine Phase-I-klinische Studie zur Behandlung von Patienten mit AK, bereitzustellen. In diesem Zusammenhang wurde die Qualität des Produkts mithilfe von Quality by Design (QbD) optimiert. Es konnte gezeigt werden, dass die optimalen Prozessbedingungen der als kritisch identifizierten Prozessparameter von Mahlzeit und Rotationsgeschwindigkeit bei 650 U/min für 135 Minuten liegen, um IMQ-Nanokristalle mit der Zielgröße von 300-400 nm herzustellen. Weiterhin wurden die kritische Prozessparameter (CPPs) und kritischen Materialattribute (CMAs) mit den kritischen Qualitätsattributen (CQAs) von IMI-Gel verknüpft, welche mithilfe von installierten In-Prozess-Kontrollen (IPCs) und Qualitätskontrolltests (QC) während und nach der Herstellung kontrolliert wurden. Dies hatte das Ziel sicherzustellen, dass jede hergestellte Charge den angestrebten Qualitätsstandards entspricht. Die Daten von IPC- und QC-Kontrollen der hergestellten Chargen (nach Good Manufacturing Practice (GMP) belegen eine hohe Qualität der hergestellten IMI-Gel Chargen mit kleiner Chargenvariabilität).

Trotz dieser Maßnahmen, wurde die Qualität einer Tube des Produkts IMI-Gel von Seiten eines Patienten der Phase I Studie beanstandet. Die Beanstandung betraf die physikalische Stabilität des Produktes. Im Rahmen einer Ursachenanalyse wurden der pH-Wert, die Primärverpackung, Untersuchungen zur physikalischen Stabilität der Formulierung innerhalb und außerhalb der Primärverpackung und rheologische Analysen durchgeführt. Dabei wies der pH-Wert, als Indikator für die Carbopol-Gel-Stabilität, keine Abweichung vom Akzeptanzkriterium auf. Eine Untersuchung der Primärverpackung ergab keine sichtbaren Beschädigung. Untersuchungen zur physikalischen Stabilität der Formulierung bei 40 °C und 80 °C innerhalb ihrer Primärverpackung zeigten keine Veränderungen. Eine Lagerung der Formulierung innerhalb der Primärverpackung mit geöffneter Verschlusskappe, sowie außerhalb der Verschlusskappe führte zu einer raschen Separation der dispersen Jojoba wax Phase. In einem Langzeitstabilitätstest für Formulierungen, die bei 25 °C/65 % rH über 12 Monate und bei 40 °C/75% rH über 6 Monate gelagert wurden, konnte mit Hilfe von rheologischen Rotations- und Oszillationanalysen gezeigt werden, dass die Formulierung physikalisch stabil ist und nur minimale strukturelle Veränderungen aufweist, sofern diese dicht verschlossen in ihrem Primärpackmittel gelagert wird. Als wahrscheinlichste Ursache für den Qualitätsmangel wurde ein fehlerhafter Umgang von Seiten des Patienten mit der Prüfmedikation ausgemacht.

Weiterhin wurde die Wirksamkeit und Sicherheit der neuen Formulierung IMI-Gel basierend auf den Patientendaten der Phase I Studie ausgewertet. Es konnte gezeigt werden, dass die Formulierung IMI-Gel die gleiche Wirksamkeit wie das Vergleichspräparat Aldara™ zeigt. Dies beruht auf einer vergleichbaren Eliminationsrate der AK Läsionen (keine unterschiedliche Signifikanz), sowie einer nicht signifikant unterschiedlichen Reduktion der AK Läsionsflächen für beide Behandlungsgruppen. Bei Analyse der Verträglichkeit zeigte sich eine signifikant bessere Verträglichkeit für das Produkt IMI-Gel, basierend auf der Analyse des „Cosmetic outcome“, wenn gleich, die Effektgröße als gering bestimmt wurde. Darüber hinaus zeigten Patienten, die mit IMI-Gel behandelt wurden eine geringere Rate an Ulzerationen und Exudaten, wenn auch nicht signifikant unterschiedlich zu Patienten aus der Aldara Behandlungsgruppe.

Zusammenfassend lässt sich festhalten, dass die Sicherheit, Wirksamkeit und Verträglichkeit von IMI-Gel gezeigt werden konnte, wobei IMI-Gel dem Vergleichspräparat hinsichtlich der Verträglichkeit überlegen ist.

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1. Introduction

1.1. Translational medicine

Medicinal products for human use are required to set high standards with respect to their quality, safety and efficacy with the goal to protect the public health and generate a positive benefit-risk balance for the treatment of patients. Throughout the development process of medicinal products, developing institutions such as pharmaceutical industry or academia are required to demonstrate the safety in preclinical studies, the quality of the product in the framework of Good Manufacturing Practice (GMP) and the safety and efficacy in humans in phase I, phase II and phase III clinical trials in order to obtain a marketing authorization for their product(s).

The process of driving medicinal products from development to administration in patients (from "bench-to-beside") is commonly referred to as translational medicine. More specifically, it is a process in which the ideas, insights, and discoveries generated through basic scientific inquiry are applied to the treatment and prevention of human disease – thus playing a critical role between basic research and clinical research [1]. Translational medicine therefore embodies many loosely integrated distinct activities distributed across the academic, pharmaceutical or biotech industry, and governmental and other private sectors [1].

In translational research, research is conducted in different therapeutic areas where oncology marks up for the largest number of products in the research and development pipeline as indicated by Figure 1.

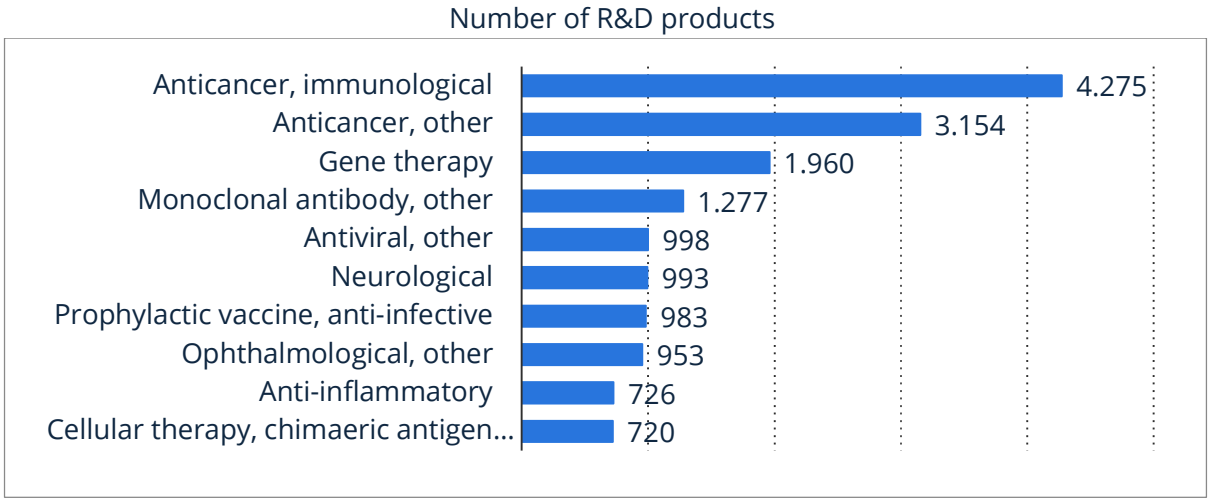

Figure 1: Leading 10 therapeutic categories worldwide by number of R&D products as of 2022. Adapted from [2]

This is underlined by Figure 2 revealing the number of FDA approved drugs by therapeutic area. There, anticancer drugs dominate the 5-year average and 2022 approval rate. Interestingly, the second largest approval rate is observed for the field of dermatology for the year 2022.

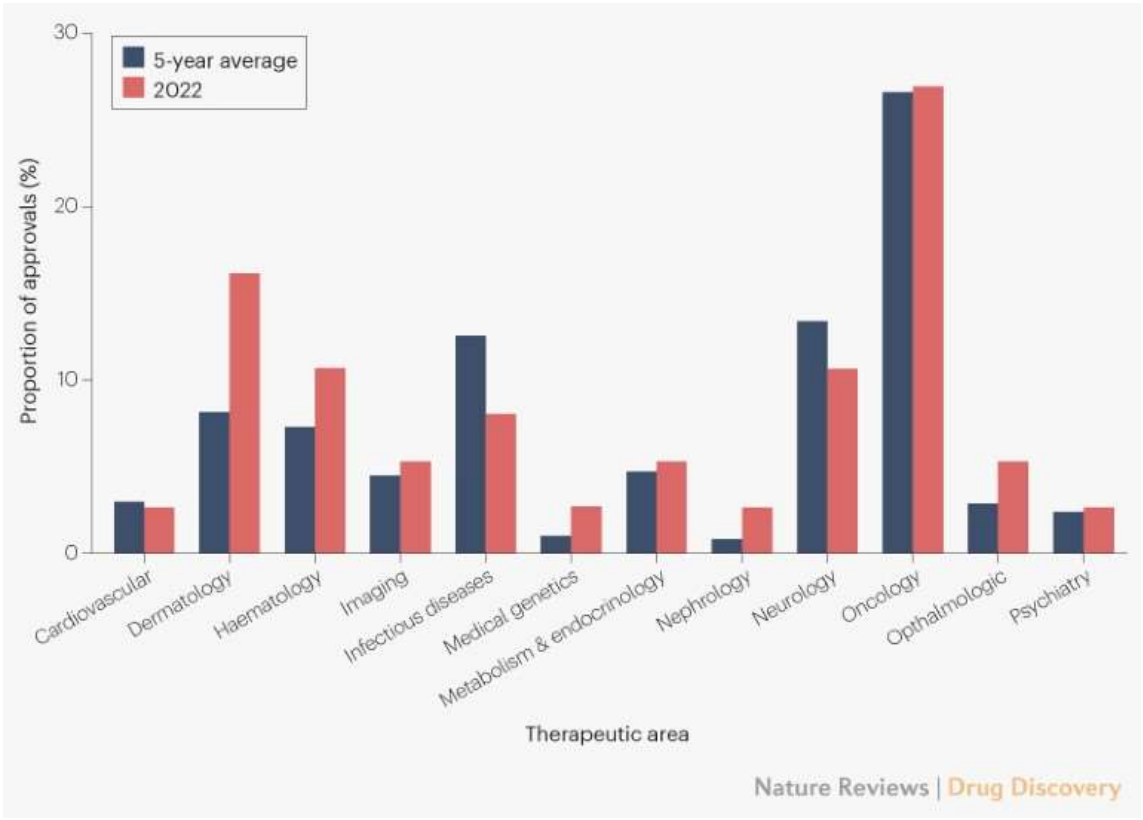

Figure 2: FDA's Center for Drug Evaluation and Research (CDER) approvals by therapeutic area, adapted from [3]

Despite enormous research efforts in different therapeutic areas, often drug development fails to reach application in patients with an overall reported drug development failure rate of 96 % [4] including a 90% failure rate during clinical trials [5–10]. Many of the basic research findings fail to get into the therapeutic development process but get stuck in the "valley of death" [11,12], the ever-widening gap between basic research and clinical research. There are many reasons why the translational process fails at the early, basic research stage or throughout clinical research including lack of reproducibility of the findings, lack of clinical relevance, lack of a fundamental understanding of the health and disease state, lack funding to cover for the high drug development costs e.g. ranging from 944 million $ to 4.54 billion $ for anticancer drugs [13], lack of incentives and technical expertise to advance any further [1], or a lack in efficacy or safety [14] observed during clinical trials that were not predicted in preclinical and animal studies [9,15]. To increase the efficacy and success rate of the translational process, it has been suggested that the scientific community, academic and research institutes, industry representatives, policy makers and the public in general must be open to the idea of integrating more inter-institutional, multidisciplinary collaborations, continue to invest in the next generation of researchers who may not fit into the traditional academic profile and continue to build partnerships between academia and industry to utilize the strengths and expertise of all parties [16].

This work aims at overcoming the "valley of death", translating a novel nanoparticulate imiquimod (IMQ) formulation from preclinical development to investigating in humans for its safety and efficacy against in the dermatological field for treatment of the skin disease actinic keratosis (AK).

1.1. Actinic Keratosis (AK)

Actinic keratoses (AKs) are common dysplastic cutaneous lesions that arise from chronic solar UV exposure of the skin. Together with basal cell carcinomas and squamous cell carcinomas, they constitute a major public health problem in the general (pale-skinned) population with reported prevalence rates ranging from 11 to 25% in various northern hemisphere populations and up to 60% amongst Australian adults [17]. Untreated, AK may progress to other non-melanoma skin cancers e.g. squamous cell carcinoma (SCC). Hence, there is a clear medical need to effectively treat AK which is further supported by the fact that AK has been recognized as an occupational disease in Germany (SG Aachen, Urt. v. 16.3.2012 – S 6 U 63/10). Established AK treatment options include photodynamic therapy (PDT), cryotherapy, topical imiquimod (IMQ), ingenol mebutate (IMB), 5-fluorouracil (5-FU), trichloroacetic acid (TCA), diclofenac sodium, ablative fractional laser and combinations of the latter therapies [18]. In a recent review, non-inferiority in terms of the overall AK clearance between the different treatment groups was observed, except for diclofenac showing the lowest overall clearance rate [18].

1.2. Imiquimod (IMQ)

In this work, IMQ was used as the active pharmaceutical ingredient (API) for treatment of AK. IMQ, a nucleoside analogue of the imidazoquinoline family, is a synthetic small molecule with a molecular weight of 240.30 Da. IMQ is a toll-like receptor (TLR) agonist, mainly activating TLR 7 but also TLR-8 [19]. Toll-like receptors belong to the group of pattern-recognition receptors (PRRs) who recognize structures conserved among microorganisms termed pathogen-associated molecular patterns (PAMPs) [20]. These receptors are expressed in cells of the innate immune system such as dendritic cells (DCs) and macrophages and also in various nonprofessional immune cells such as fibroblasts and epithelial cells. Depending on their cellular localization, the TLR receptor family is categorized into two sub families. Cell surface TLRs include TLR 1, 2, 4, 5, 6 and 10 who recognize mainly microbial membrane components such as lipids, lipoproteins and proteins [21]. Intracellular TLRs are localized in the endosome and include TLR 3, 7, 8, 9, 11, 12, 13 who recognize nucleic acids derived from bacteria and also recognize self-nucleic acids in disease conditions such as autoimmunity [22]. The stimulation of TLR receptors by PAMPs or synthetic molecules such as by IMQ (activating TLR 7 who occurs mainly in the endosome of plasmacytoid DCs), leads to upregulation of genes involved in inflammatory responses, orchestrated by the release of proinflammatory cytokines [20].

In AK, the precise mechanism of action for IMQ is unknown, however, it can be assumed that IMQ stimulates the innate and acquired immune response through the TLR 7 and 8 receptors, followed by an inflammatory cell infiltration at the field of drug administration, ultimately, leading to apoptosis of the diseased tissue [23]. AK patients administrate IMQ on the AK lesions which typically results in an induction of a local inflammatory response in a dose dependent manner, also, leading to adverse reactions such as erythema and burning/itching that adversely affects patient compliance and overall efficacy [24].

Commercially, IMQ was introduced with the product Aldara™ at a dosage strength of 5% (w/w) IMQ. Aldara™ received marketing authorization in the USA in 1997 and in the European Union in 1998 [25]. Since 2012, other strengths of IMQ have seen market authorization in the form of the product Zyclara® at dosage strength of 3.75 % (w/w) and 2.5. % (w/w). They are indicated for short-term AK cycle therapy and expanded treatment areas [26].

A recent review outlines the efficacy of topical IMQ in AK patients reporting an overall percent reduction rate of AK plaques of 68.0 ± 1.6 % at 6-12 months after treatment. Despites the demonstrated efficacy of topical IMQ for treatment of AK, also significant side effects occur over the treatment period with the main reported side effects in that same review being erythema (53.3 %), stinging/itching (41.6 %), and crusting (33.5 %) taken from 26 conducted clinical trials [18]. While the side effects can be attributed to the API itself, they may

also be due to the used excipients in the commercial product(s). For example, Aldara™ contains isostearic acid, an excipient known to contribute to adverse skin reactions [27]. Hence, it would be beneficial for patients to have an IMQ containing formulation at similar efficacy with a superior tolerability. To achieve this, IMQ has to be reformulated and successfully transported into the skin. In addition, a newly developed product must be of high quality and safety for patients. As a basis, the anatomy of the skin to identify a suitable formulation concept for dermal or intra/transdermal drug delivery, has to be considered.

1.3. Anatomy of the skin

The description of the immunological network in this chapter was previously published as:

Pielenhofer J, Sohl J, Windbergs M, Langguth P and Radsak MP (2020) Current Progress in Particle-Based Systems for Transdermal Vaccine Delivery. Front. Immunol. 11:266. doi: 10.3389/fimmu.2020.00266.

I contributed in that work by performing a literature review related to relevant information on delivery technologies used for transcutaneous vaccination and writing of the sections "particle based systems for transcutaneous vaccination" and "conclusion" of the manuscript.

The skin is the largest organ of the body executing a multitude of essential functions including maintenance of fluid levels, governance of the body temperature, sensing of pain and protection against exogenous noxious agents or (micro)organisms. This barrier function is allocated to (i) the unique structural arrangement of the skin serving as a physical barrier and (ii) to its associated immunological network.

1.3.1. Structural arrangement of the skin

Human skin is composed of three major layers: The epidermis, dermis and subcutis. The superficial layer of the epidermis, the stratum corneum, is the outermost skin layer between the environment and the body, forming the main penetration barrier for pathogen entry but also for pharmaceutically active compound delivery into or across the skin. It is arranged as a 10-20 µm thick skin layer of dead corneocytes forming a "brick and mortar" structure where the corneocytes form the "bricks" and interlamellar lipids (free fatty acids and ceramides), form the "mortar" [28,29]. Located beneath the stratum corneum is the viable epidermis (50-100 µm thick), consisting of basal, spinous and granular cells, which are responsible for generating the stratum corneum [28]. The generation of the stratum corneum is a complex differentiation process starting at the basal epidermal layer, during which the keratinocytes undergo various changes in their structure and composition ultimately leading to a transition into chemically and physically resistant cornified squames of the stratum corneum, called corneocytes before being sloughed by abrasion [28,29].

The dermis is located adjacent to the epidermis, hosting various cells including fibroblasts and myofibroblasts whose function it is to support the structural integrity of the skin. In addition, various immune cells including macrophages, lymphocytes and mast cells are resident in the dermis [30].

Underlying the dermis is the subcutis. The subcutis serves as an energy reservoir by storing fatty acids, serves as an endocrine hormone by contributing to glucose homeostasis and lipid metabolism, produces a variety of mediators such as growth factors, adipokines, and cytokines, contains multiple immune cells and serves as an insulating layer for the body, as fat is a poor conductor of heat [30]. Figure 3 illustrates the anatomical arrangement of the skin.

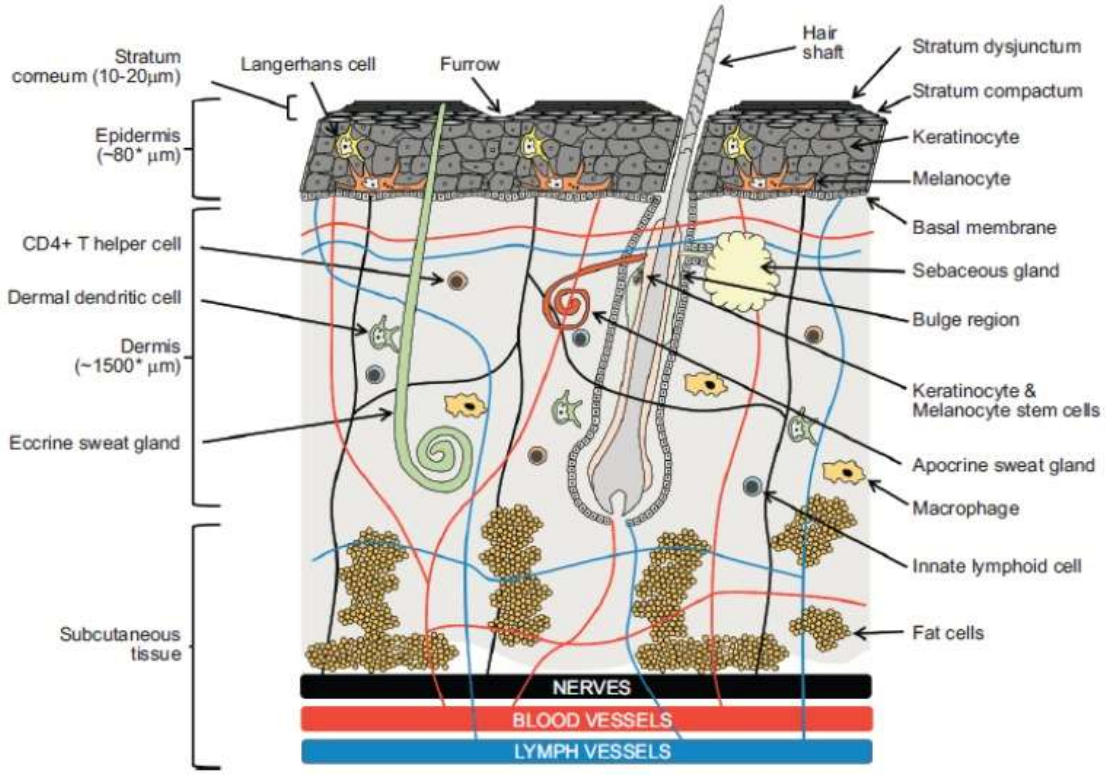

Figure 3: Diagrammatic illustration of human skin, taken from [31]

1.3.2. Immunological network of the skin

The immunological cell network of the skin acts as a sentinel of the surrounding tissue, promoting immunological tolerance or induction of robust protective immune response in case of antigen or pathogen entry. The basal part of the epidermis is populated by a specialized subtype of dendritic cells, named Langerhans cells (LCs). Langerhans cells are uniquely located in the epidermal layer and build up the first line of antigen presenting cells (APCs) that encounter skin-invading antigens. A multitude of scientific reports indicate a crucial role for LCs in the induction of CD8+ T cell responses, likely due to their ability to cross-present antigens to naïve or memory CD8+ T cells [32].

Dermal DCs represent a highly mixed subset with functional heterogeneity and have been identified as key players in the induction of immune responses both in cutaneous infection and in skin vaccination [33]. Based on their developmental origin, surface markers, and function, dDCs can be broadly subdivided in steady-state conditions. The dermis is inhabited by two conventional subtypes of dDCs, both originating from a common bone-marrow-derived Lin− cKitint M-CSFR+ Flt3+ precursor. The XCR1+ cDC1 subtype is functionally specialized in antigen cross-presentation, polarization of T helper cells into the TH1 subset, and secretion of IFNγ, which emphasizes its crucial role in acting against intracellular pathogens [34]. The CD4+CD11b+ subset represents a separate DC lineage ("cDC2") specialized in the presentation of antigen to CD4+ T cells and with the unique ability to favor polarization toward TH2 or TH17 responses, which emphasizes their importance during immune responses to extracellular pathogens. The development of the cDC2 lineage is highly dependent on the transcription factor IRF4 [35]. Moreover, it has been shown that the cDC2 lineage is also able to prime CD8+ T cells independently [36]. Additional and more detailed information on the diversity of the cutaneous APC network facilitating a profound understanding of immunological processes in the skin can be found in some recently published reviews [37,38].

1.4. Dermal and Transdermal Drug Delivery

Dermal and transdermal drug delivery offers an easy, accessible and convenient route for drug administration leading to good patient compliance, avoidance of first past metabolism among other advantages. This has led to the development and authorization of various products for dermal and transdermal drug delivery of therapeutic drug molecules. In addition, the density of the skin's immunological network has attracted researchers to work on a novel vaccination concept termed transcutaneous vaccination in which vaccine compounds such as antigenic peptides, proteins or mRNA are formulated alone or together with adjuvant compounds in such a way that they are delivered towards LCs or dermal DCs to induce a protective immune response against infections and cancer [39,40].

1.4.1. Percutaneous Absorption and Drug Release

The main challenge in drug delivery through the skin lies in the stratum corneum, as it represents the major entrance barrier for hydrophilic molecules but also for lipophilic molecules, into the skin. Usually, drugs with molecular weights >500 Da are prohibited from entering the skin [41]. Several conventional dosage forms have been developed for application and are established pharmaceutical products in the market such as ointments, creams, gels and patches. The passage of drug molecules from these vehicles into and through the skin (percutaneous penetration) is a passive process that can be described by diffusion. The fundamental relationships governing the diffusion process occurring in pharmaceutical systems is expressed in Fick's first law of diffusion:

$$J = -D\frac{dC}{dx} \tag{1}$$

Where J is the flux of the solute, D is the diffusion coefficient of a penetrant, C is its concentration in g/cm^3 and x is the distance in centimeters of movement perpendicular to the surface of the barrier. As expressed above, often the skin represents the major barrier for dermal and transdermal drug delivery, hence serving as the rate limiting step for percutaneous absorption. When including the skin as a barrier, the following approximate relationship between the steady-state rate of penetration ($\frac{dq}{dt}$) and various properties of a fairly water-soluble drug as postulated by Higuchi can be written, under the assumption, that the vehicle containing the drug does not appreciably affect the skin [42].

$$\frac{dq}{dt} = (P.C.)\frac{(Conc.of\ Drug)DA}{L} \tag{2}$$

Where *(P.C.)* is the effective distribution coefficient of the penetration agent between the vehicle and the barrier of the skin, *(Conc. of Drug),* the concentration of the agent in the vehicle, D, the effective average diffusivity of the agent in the barrier phase, A, the effective cross-sectional area and L, the effective thickness of the barrier phase.

Under consideration of the thermodynamic activity of the drug in its vehicle, the equation can be written as:

$$\frac{dq}{dt} = \frac{a}{\gamma}\frac{DA}{L} \tag{3}$$

Where a is the thermodynamic activity of the drug in its vehicle and γ is the effective activity coefficient of the compound in the skin barrier phase.

For substances having an extremely low affinity towards the lower aqueous layers of the skin, the rate limiting step for percutaneous absorption is the transfer from the barrier phase towards the underlying tissue. In such a scenario, the resistant barrier is considered as two dissimilar layers, one lipoidal layer and one hydrous skin layer. Higuchi expressed this relationship mathematically as [42]:

$$\frac{dq}{dt} = \frac{aA}{\frac{h_1}{P_1} + \frac{h_2}{P_2} + \cdots \frac{h_n}{P_n}} \tag{4}$$

Where h is the thickness of the respective skin layers and $P = D/\gamma$ for the n layer system.

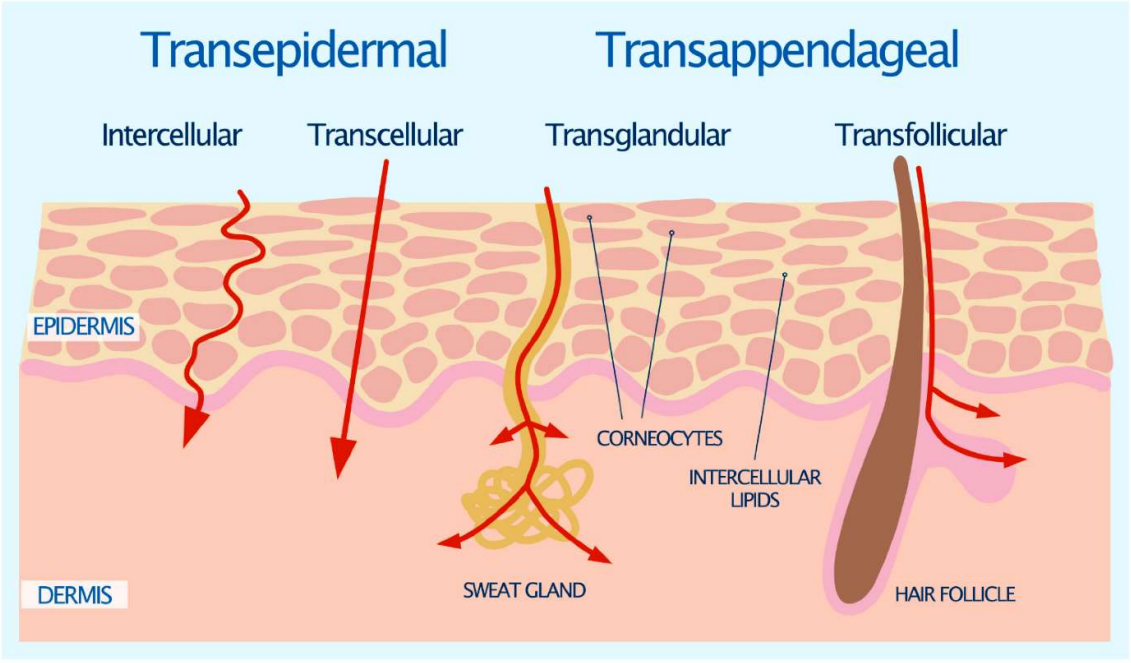

Figure 4: Diagrammatic illustration of the absorption pathways for drug molecules, taken from [43]

In the process of percutaneous absorption, drug molecules can enter the intact skin through different pathways either transepidermal or transappendageal i.e. through the associated tissues of the sweat glands and hair follicles. Figure 4 shows the percutaneous absorption pathways for drug molecules. In the transepidermal pathway, molecules may diffuse in between the corneocytes taking the intercellular pathway or diffuse transcellularly i.e. through the corneocytes. In general none of both pathways seems to be dominating, however it can be assumed that the transcellular pathways predominates once steady state conditions have been reached.

In recent years, the transfollicular route has received increasing attention. The group of Lademann and Co-workers have published a number of articles outlining the important role of drug delivery via the hair follicle when using nanoparticles. In these articles, it is outlined, that nanoparticles are able to migrate and accumulate inside of the hair follicular in a size dependent manner with the deepest follicular penetration achieved at a size of around 600 nm [44,45]. The altered barrier capacities of the hair follicle and its ability to store nanoparticles for up to ten days [46] opens new opportunities for extended dermal drug delivery of not only small and lipophilic but also hydrophilic molecules into the skin.

Drugs in pharmaceutical dosage forms for dermal drug delivery may be present in a suspended or dissolved state. Depending on their physical state, the release of drugs may involve both factors, dissolution of the drug in its vehicle and diffusion towards the upper skin layer and into the skin. Multiple variables contribute to the drug release of the compounds from its vehicle including the physicochemical properties of the API and its vehicle (including used excipients) as well as physiological aspects of the biologic system such as patient population, administration site, age, ethnicity etc. The physicochemical properties for the API include drug concentration, molecular size, partitioning coefficient, crystal form, pKa, aqueous solubility among other factors. For new API compounds, these factors have to be determined and understood to the extent to select and develop a suitable drug delivery system that enables delivery towards the targeted tissue at the targeted release characteristics.

In a suspended condition, the release of suspended drug particles from ointments is given by Higuchi [47]:

$$Q = \sqrt{2ADC_s t} \tag{5}$$

With Q = the amount of drug absorbed at time t per unit area of exposure, A = the concentration of drugs expressed in units/cm^3, D = diffusion constant of the drug molecule in the external phase, C_s = the solubility of the drug as units/cm^3 in the external phase. Equation (5) is valid for the common case where $A >> C_s$. When the conditions of A, D, C_s are kept constant, the amount of drug released is directly proportional to the square route of time.

For solubilized compounds, the Higuchi model can be described by the following equation if the percent released, R, is ≤ 30 % of the drug and the drug is present in a molecularly dispersed state (solubilized) [48].

$$Q = 2C_0\left(\frac{Dt}{\pi}\right)^{1/2} \tag{6}$$

With Q = amount of drug released per unit area of application, C_0 = initial concentration of drug in ointment, D = diffusion coefficient of the drug in the ointment, t = time after application. This simple equation reveals that the amount of drug released is directly proportional to the initial drug concentration in the ointment vehicle. Furthermore, a major contributing factor is the diffusion coefficient which is a function of the formulations viscosity, the temperature and the molecular size.

For heterogeneous systems as present in creams, the following equation can be used:

$$D_e = \frac{D_1}{V_1 + kV_2}\left[1 + 3V_2\frac{kD_2 - D_1}{kD_2 + 2D_1}\right] \tag{7}$$

With D_e = effective diffusion constant of the system, D_1 = diffusion constant of the drug in the outer phase, D_2 = diffusion constant of the drug in the inner phase, V_1 = volume fraction of the outer phase, V_2 = volume fraction of the inner phase and k = partitioning coefficient. The complexity of heterogeneous systems allows

only for a limited applicability of equation (7) where it is assumed that the drug substance is solubilized and distributed in both phases, that the disperse phase consists of small spheres and makes up for a small fraction. However equation (7) reveals that the effective diffusion constant of the system requires knowledge of the individual diffusion constants of the phases as well as of the partitioning coefficient. When the diffusion constants for both phases are almost similar, the partitioning coefficient is the major factor influencing the drug release rate. Favorable drug release occurs when the partitioning coefficient determines presence of the drug predominantly in the outer phase. If the drug is predominantly present in the inner phase, less favorable drug release may be observed.

The design and development of a suitable formulation for a targeted drug delivery towards the targeted tissue requires consideration of the drug release kinetics for drugs present in suspended or dissolved state, consideration of the targeted skin tissue, the percutaneous absorption pathways and consideration of the disease intended to become treated. To achieve this, multiple formulation concepts have been introduced including more conventional semi-solid products outlined in the European Pharmacopeia (Ph.Eur.) and novel drug delivery systems (see below).

1.4.2. Delivery systems

Delivery systems for dermal and transdermal drug delivery can be broadly classified into active delivery approaches where the skin is disrupted to facilitate skin entrance of the applied APIs and passive delivery systems where the API in its vehicle is applied on the intact skin.
Active delivery systems facilitate skin entrance by physical disruption of the main skin penetration barrier, the stratum corneum. Such approaches include iontophoresis [49], sonophoresis [50], electroporation [51], ultra sound, skin radiofrequency/thermal and laser ablation [52] and tape stripping [53]. Moreover, some efforts have been made in developing drug delivery devices that actively disrupt the stratum corneum for example epidermal power injectors [54], jet injectors [55] and microneedle drug delivery systems [56]. Power and jet injectors have found application in the context of transcutaneous vaccination. While the disruption of the stratum corneum by the above listed approaches enhances skin entrance of topically applied compounds, these approaches are associated with discomfort and pain in patients.

Passive delivery approaches, such as dermal and transdermal delivery, rely on the passive diffusion of topically applied drug compounds from their vehicle into the skin. For suspended compounds, the APIs need to dissolve in their vehicle before they can diffuse into the skin, whereas solubilized compounds don't require this step. Traditional formulation approaches for passive delivery include hydrophobic and hydrophilic ointments, water-emulsifying ointments, lipophilic and hydrophilic creams, hydrophilic and lipophilic gels, pastes, and transdermal patches. While these systems have limitations in terms of the amount and type of APIs delivered, penetration enhancers can be added to increase the transport of incorporated compounds. Examples of penetration enhancers include e.g. poly-alcohols, alcohols, amines, pyrrolidones, amides, sulphoxides, fatty acids, alkanes, esters, surfactants, terpenes, and phospholipids, and much more [57].

In addition to the transepidermal route, there is increasing interest in delivering drugs through hair follicles. Nanocarrier systems have gained significant attention as a promising approach for delivering drugs topically into the skin via the hair follicles. Nanocarriers are colloidal structures with particle or droplet sizes less than 500 nm [58]. The nanocarrier systems used for dermal drug delivery include polymeric nanoparticles, lipid-based nanoparticles (such as liposomes, niosomes, exosomes, lipid-based vesicles, solid lipid nanoparticles, and nanostructured lipid carriers), as well as micro- and nanoemulsions and nanocrystals. The selection of the type and size of the carrier systems depend on various factors, including the physicochemical properties of the drug substance, such as its solubility in the carrier matrix, the required patient dosage, and the targeted percutaneous absorption pathways.

For IMQ, most of the nanocarrier concepts appears to be unsuitable owing to IMQs poor solubility in water, commonly used lipid(s), matrices or organic solvents. The commercial IMQ product Aldara™ is an oil-in-

water cream, containing IMQ solubilized in the disperse isostearic acid phase. However, reports indicate that isostearic acid induces adverse skin reactions in patients, negatively affecting the tolerability of the product, making it rather unsuitable as a carrier component for IMQ delivery in novel formulations that aim to increase its tolerability. Moreover, previous studies have shown also shown that IMQ has a high skin permeation rate over murine skin when formulated in Aldara™ [59] potentially causing additional levels of side effects due to systemic exposure. To address these findings, nanosuspensions were identified as a suitable formulation concept, leading to decreased IMQ absorption through migration into hair follicles and slow but constant passive diffusion.

1.4.3. Nanosuspensions

Nanocrystals are a type of drug nanoparticle formulation composed of 100% active drug substance, stabilized by a surfactant and/or polymeric layer [60]. These drug nanoparticles are commonly utilized to enhance the bioavailability of poorly water-soluble drugs [61], as their increased surface area leads to a higher dissolution rate, as described by the Noyes-Whitney equation [62]. This ultimately results in improved bioavailability [63].

Compared to other nanocarriers with polymeric or lipid matrices, nanocrystals exhibit a much higher loading capacity since they are made entirely of the active substance. This characteristic is particularly advantageous for dermal and transdermal drug delivery, as a high drug loading reduces the amount of formulation needed to reach the desired therapeutic concentration. Moreover, nanocrystals offer sustained release and absorption of the administered compounds into the skin, further augmenting their effectiveness.

The decelerated percutaneous absorption of nanosuspended IMQ is based on two mechanisms: (i) the solubilized fraction of IMQ can penetrate the skin via passive diffusion, and (ii) the IMQ nanocrystals can migrate and accumulate into the hair follicles.

In passive diffusion, the concentration gradient between the vehicle and the skin is the main driving force in percutaneous absorption. Reducing the size of IMQ drug particles to the nanoscale increases their particulate surface area, in turn, leading to a higher dissolution velocity of IMQ in its vehicle. As one IMQ molecule diffuses into the skin, it is immediately replaced by the next molecule. This continuous replacement process creates a constant concentration gradient between the solubilized IMQ in the vehicle and the skin, resulting in zero-order absorption kinetics. The low water solubility of IMQ ensures, that only a small fraction of IMQ is absorbed from its vehicle, decreasing the absorption kinetics of IMQ. However, this assumption is based on the rate-limiting step in percutaneous absorption being the entrance of the molecules into the skin rather than the diffusion of the compounds from the stratum corneum to deeper tissues, which could change the concentration gradient between the formulation and the skin, altering the drug flux.

In addition, follicular penetration appears as a promising concept for extended drug release. Recent research has shown that hair follicles can serve as a long-term reservoir for nanoparticles including nanocrystals making them an attractive target for drug delivery.

Preparation of nanocrystals may be realized by using bottom-up or top-down methods. The most frequently used methods for preparation of nanocrystals are the top-down methods of wet media milling, including wet stirred media milling, planetary milling and ball milling [64]. In wet media milling, drug nanoparticles are prepared by dispersing drug particles in aqueous polymeric/surfactant stabilizer solution, together with grinding beads [65]. During milling, the coarse micron-size particles are repeatedly stressed when captured between the colliding beads, over time leading to a breakdown of the drug particles to the nanoscale, yielding a nanosuspension [64,66]. Throughout processing and storage, stability issues such as nanoparticle aggregation, formation of large clusters; surface amorphization, which would lead to the immediate dissolution of the amorphous part and the recrystallization onto the present drug crystals; or Ostwald ripening, leading to

a loss of the benefits of the large surface area and an inability to exhibit the targeted product performance, may occur [64,67]. The manufacturing of physically stable drug nanoparticles via wet media milling at the targeted size entails (i) the selection and optimization of effective equipment process parameters, (ii) the consideration of the physicochemical and mechanical properties of the drug substance and (iii) the selection of a suitable polymeric/surfactant stabilizer [67].

Alternatively, nanocrystals can also be prepared using high-pressure homogenization (HPH). With e.g. a piston-gap homogenizer, drug particles dispersed in an aqueous surfactant solution are passed through a small gap of e.g. 10 µm in height at high velocity (e.g. 500 m/s) where the coarse drug particles are broken up to the nanoscale by cavitation forces. Thereby due to the narrowness of the gap, the streaming velocity of the suspension increases meaning that the dynamic pressure of the fluid increases [68]. In parallel, the static pressure of the fluid decreases below the boiling point of water at room temperature which in consequence leads to boiling at water at room temperature, formation and implosion of gas bubbles (cavitation) and hence forces strong enough to break the drug microparticles to drug nanoparticles [68]. This technology was developed by Müller et al. [69] and leads to nanosuspensions termed DissoCubes ®.

Overall, nanocrystals appear to be a promising formulation concept for IMQ. They provide targeted decelerated drug permeation through passive diffusion of the solubilized part and via the hair follicles. This could potentially improve drug delivery and increase the effectiveness of treatments using IMQ. For preparation of nanosuspensions, in general top down methods appear more attractive than bottom-up techniques due to the lack of organic solvents used in these processes. With respect to the manufacturing processes used in the literatire, most studies for dermal nanosuspensions use the top down processes of wet media milling. For example various drugs such as diclofenac, azithromycin, miconazole, luliconazole and flurbiprofen were formulated as nanosuspensions using wet media milling processes [70–74]. The technology for preparation of nanosuspensions type DissoCubes ® was mostly applied for poorly water-soluble drugs for peroral administration to improve oral bioavailability for example for spironolactone [75].

For IMQ, previous formulation efforts resulted in an oil-in-water emulsion gel formulation containing nano-dispersed IMQ crystals used in the context of transcutaneous immunization experiments prepared using a combination of wet media ball milling and high-pressure homogenization [59].

1.5. Aim of the thesis

The objective of this thesis was to evaluate the effectiveness of a new nanocrystalline formulation of IMQ as an investigational medicinal product (IMP) for the treatment of AK in a phase I clinical trial. Ensuring the quality of the IMP was crucial to minimizing risk for the trial participants. To achieve this, the target product quality characteristics of IMI-Gel, the nanocrystalline IMQ formulation, developed by Gogoll [76], and continued by Denny [77], were defined before submitting relevant documents for regulatory approval of the clinical trial.

Quality by Design (QbD) was employed as the approach for this thesis, involving the development of a quality target product profile (QTPP) to identify the critical quality attributes (CQAs) of the IMP. Given that the new IMQ formulation utilizes nanocrystals for follicular drug delivery, the process conditions were optimized to achieve a mean particle size of 300-400 nm using the statistical modeling approach of Design of Experiments (DoE).

Implementation of adequate controls for incoming materials, intermediate products as In-Process-Controls (IPCs), and Quality Control (QC) tests were also essential components of the control strategy for the IMP. Good Manufacturing Practice (GMP) rules were followed in the manufacturing and quality control testing process to provide the appropriate quantity of IMP for enrolling patients in the clinical trial. Continuous quality monitoring was conducted to identify out-of-specification (OOS), out-of-trend (OOT), or out-of-expectation (OOE) results and minimize bias in the clinical trial resulting from large variability in the product quality.

Finally, the safety and efficacy of the novel nanocrystalline IMQ formulation against the reference product Aldara™ were evaluated in patients with a clinical diagnosis of AK.

2. Quality by Design

Note:

The content of this chapter uses text, tables and figures of the following publication:

Pielenhofer, J.; Meiser, S.L.; Gogoll, K.; Ciciliani, A.-M.; Denny, M.; Klak, M.; Lang, B.M.; Staubach, P.; Grabbe, S.; Schild, H.; et al. Quality by Design (QbD) Approach for a Nanoparticulate Imiquimod Formulation as an Investigational Medicinal Product. Pharmaceutics 2023, 15, 514. https://doi.org/10.3390/pharmaceutics15020514

The introduction of this part was adapted from the above mentioned publication in order to make the text fit to the content of the thesis. The first paragraph contains exact text and wording as published. The section "This thesis […]" until "[..] pharmaceutical product suitable for AK patients" was modified. Afterwards the text, tables and figures were used as published with the exceptions of an added explanation of alpha in chapter 2.2.4. "(an alpha of 1 means that the star points are on the faces of the cube)", *removal of the Figure legend in Figure 5 within the figure and modification of the headers in the supplemental material section of the S1. HPLC Assay 1, S2. HPLC Assay 2, S3. HPLC Assay 3 to match them to the headers in section 2.2.7.*

I contributed in that work by designing the QTPP, designing and performing the DoE study, performing laboratory investigations and writing of the publication. The (Co)-authors contributed to this work as follows:

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- Methodology: *J.P., S.L.M., Karsten Gogoll (K.G.), Berenice M. Lang (B.M.L.), Petra Staubach (P.S.), Stephan Grabbe (S.G.), M.P.R., H.S.-L. and P.L.;*
- Software: *J.P. and S.L.M.;*
- Validation*: J.P., S.L.M. and K.G.;*
- Formal analysis*, S.L.M., K.G., Anna-Maria Ciciliani (A.-M.C.), Mark Denny (M.D.), Michael Klak (M.K.), B.M.L., P.S., S.G., Hansjörg Schild (H.S.), M.P.R., H.S.-L. and P.L.;*
- Investigation*: J.P., S.L.M., K.G., A.-M.C., M.D., M.K., M.P.R., H.S.-L. and P.L.;*
- Resources*: M.P.R., H.S.-L. and P.L.;*
- Data curation*: J.P., S.L.M., K.G., M.P.R., H.S.-L. and P.L.*
- Writing—original draft preparation: *J.P. and S.L.M.;*
- Writing—review and editing: *K.G., A.-M.C., M.K., B.M.L., P.S., M.P.R., H.S.-L. and P.L.;*
- Visualization: *J.P., S.L.M. and H.S.-L.;*
- Supervision: *B.M.L., P.S., S.G., M.P.R., H.S.-L. and P.L.;*
- Project administration*: B.M.L., P.S., S.G., M.P.R., H.S.-L. and P.L.;*
- Funding acquisition*: M.P.R. and P.L.*

2.1. Introduction

Quality by design (QbD) is a modern tool aiming at a systematic development of medicines by employing statistical, analytical and risk management methodology in the design, development and manufacturing of drug products [78]. Particularly for investigational medicinal products (IMPs), quality is of high concern thanks to yet-incomplete knowledge of the final toxicity and potency of the API and the safety of the product. With QbD, the quality of the product is achieved in a multistage process [79], beginning with the definition of the target product quality characteristics in a quality target product profile (QTPP), followed by the identification of critical quality attributes (CQAs) taking into account the efficacy and safety of the drug product [80,81]. In the next steps, the product design and understanding phase is initiated, enabling the identification of the critical material attributes (CMAs) of the input materials, including the drug substance and the excipients. Thereafter, the process design and understanding phase begins by identifying critical process parameters (CPPs) as sources for critical variability in the product quality being managed by establishing defined limits for the CPPs (and CMAs) within which the desired product quality is assured [81]. Afterward, a planned set of controls derived from product and process understanding is installed as a part of the control strategy. The intention is to consistently monitor process performance and the product quality by quality control testing (for more details on the quality by design approach, the reader is referred to the excellent review by Yu et al. [81] as well as to related published guidelines ICH Q8 (R2) (pharmaceutical development) [80], ICH Q9 (quality risk management) [82] and ICH Q10 (pharmaceutical quality system) [83]).

This thesis utilizes the QbD approach to optimize the quality of the nanocrystalline IMQ formulation, IMI-Gel. To achieve effective follicular drug delivery, it is necessary to break down the initial micron-sized particles (d10 4.96 µm, d50 13.02 µm, d90 26.39 µm) to the nanoscale. In the preparation of IMQ nanocrystals, the selection and evaluation of appropriate process parameters and stabilizing excipients are crucial to produce physically stable drug nanoparticles at the desired size. This is essential for the development of a high-quality pharmaceutical product suitable for AK patients [67,84]. To achieve this objective, the critical process parameters (CPPs) of the milling time and the rotational speed, identified in an Ishikawa diagram as a risk-based approach, are evaluated and optimized by applying the concept of the design of experiments (DoE) in a face-centered central composite design (CCD). The target particle size and polydispersity are identified on the basis of a designed quality target product profile (QTPP) outlining the biopharmaceutically critical quality attributes (CQAs) that are most relevant for the IMP used for the treatment of AK patients in the respective phase-I/II clinical trial. Moreover, the critical factors from the input materials affecting the target product quality referred, (critical material attributes (CMAs)) to in previously conducted studies are discussed. In addition, the installation of appropriate controls during manufacturing and for batch release testing are put in place. Additionally, quality metric data from manufactured and released batches are presented.

2.2. Materials and Methods

2.2.1. Materials

IMQ drug substance of GMP grade was purchased from Teva Pharmaceuticals Industries Ltd.—Teva API Division (Debrecen, Hungary). Aponorm® 7 mL tubes, Aqua conservata DAC, Carbopol 974P, jojoba wax, polysorbate 80 and sodium hydroxide were purchased from Caesar & Loretz GmbH (Hillscheid, Germany). Zirconium oxide milling spheres of 1 mm diameter were purchased from Fritsch GmbH (Idar-Oberstein, Germany). Acetonitrile ≥ 99.9%, HiPerSolv CHROMANORM gradient grade and methanol ≥ 99.8%, HiPerSolv CHROMANORM gradient grade, WTW technical buffers TEP 2, STP-7 and STP-10 trace were purchased from VWR International GmbH (Darmstadt, Germany). Phosphoric acid LiChropur > 85%, sulfuric acid ≥ 98% LiChropur, Millex nylon syringe filters of 0.45 µm pore size and a diameter of 25 mm, triethylamine ≥ 99.5% LiChropur and IMQ USP Reference Standard were purchased from Sigma Aldrich Chemie GmbH (Taufkirchen, Germany). Ethanol Rotisolv HPLC gradient grade, heptane-1-sulphonic acid sodium salt, sodium lauryl sulfate ≥ 99% and Omnifix disposable 50 mL syringes with Luer-Lock fitting were purchased from Carl Roth GmbH (Karlsruhe, Germany). Inertsil ODS3-5 µm 250 mm × 4.6 mm and Zorbax

RX-C8 150 mm × 4.6 mm 5 µm HPLC columns were purchased from MZ Analysentechnik GmbH (Mainz, Germany).

2.2.2. Design of the QTPP and Identification of CQAs

As a basis for the QTPP, the product characteristics of the comparator product Aldara™ were compared against the ICH Q8 R(2) guideline on pharmaceutical development [80], and a recent review [85] was used. The target dosage form, the route of administration and the dosage strength were defined on the basis of the disease intended to be treated and the properties of the comparator product Aldara™. The target stability was defined on the basis of the required time period from manufacturing, quality control testing, batch releasing, delivery to the study site and treatment period with optional extension and additional stability testing, if required. The drug product quality attributes were defined to meet the applicable standards in terms of physical attributes, including the IMQ drug particle size, rheological properties, disperse jojoba wax droplet size and pH of the formulation, as well as for the identification, the assay, the homogeneity of the formulation and tube uniformity of filled units, the limits for the impurities, the degradation products, the content for the preservatives and the microbiological limits. The container closure system and the integrity thereof were included, aiming at ensuring appropriateness for the dosage form to ensure the target shelf life.
The CQAs were identified on the basis of the projected severity of harm to the patient in case the attribute were to fall outside of the targeted range.

2.2.3. Product Design

The product is designed to meet the patients' needs in terms of a safe and easily administrable formulation. For the treatment of AK, it is required to transport IMQ toward the immunocompetent cells in the epidermis and dermis to activate the innate and acquired immune system, ultimately leading to the apoptosis of the atypic keratinocytes that cause the clinical presentation of AK [23].

2.2.4. Process Design, Identification and Evaluation of CMAs and CPPs

Preparation of the aqueous nanosuspension: to create an IMQ nanosuspension, the top-down method of a wet media ball-milling process was selected. CMAs and CPPs were identified by using an Ishikawa diagram and estimated for their criticality in a risk-estimation matrix. The CMAs have been previously studied by Gogoll [59,76] and Denny [77]. In this study, the identified CPPs of the wet media ball-milling process's milling time and rotational speed were evaluated by using DoE in a face-centered CCD and were set up by using Minitab Statistical Software (version 21.1). The CPPs were studied at two levels: low (−1) at 250 rpm for 60 min and high (+1) at 650 rpm for 240 min in 2 replicates with an alpha of 1 indicating the distance of the star points from the central point in the CCD (an alpha of 1 means that the star points are on the faces of the cube). Milling time was segmented into 20 min cycles, each cycle with a 10 min pause interval in between each cycle, using a Fritsch Pulverisette 6 planetary mill. For the experimental runs with 150 min of milling time, a 10 min cycle was added after the seventh milling cycle. All experimental runs were randomized. The model contained 8 corner points, 10 center points and 8 star points, totaling 26 conducted experimental runs. For each experimental run, the z-Average and PdI were measured in triplicates. The ratio of milling balls to drug substance mass, the media volume and the milling ball size were kept as used previously(see [59,76]). For the study and manufacturing of IMPs, a total mass of 25 g of 1 mm diameter zirconium oxide milling balls, a volume of 17 mL of a 9 % (w/w) polysorbate 80 solution and an IMQ mass of 3 g were added into a 45 mL zirconium oxide grinding vessel [77]. Based on the identified optimal process conditions, milling for IMP manufacturing was performed at 650 rpm for 7 20 min repetitive cycles, each with a 10 min pause interval in between each milling cycle.

Homogenization of the o/w emulsion: for the dispersion of jojoba wax in the aqueous IMQ suspension, a high-pressure homogenization (HPH) process was selected. The number of cycles and pressure necessary to disperse the jojoba wax were identified as a critical process parameter and empirically determined until the emulsion appeared homogeneously. For the manufacturing of IMPs, the emulsion was homogenized for 5 cycles at 500 bar and 10 cycles at 1000 bar after the addition of stochiometric amounts of jojoba wax, Aqua conservata DAC and polysorbate 80 using an Avestin Emulsiflex C3.

Adjustment of the cream consistency: to create a cream formulation, a neutralized 3% (w/w) Carbomer 974P gel was prepared (pH 6.5–7.0). After gelling, a stochiometric amount of the neutralized carbomer gel was added to the homogenized o/w emulsion after the HPH step. The emulsion was incorporated into the gel matrix in a bowl with a pestle and mixed until the gel had transitioned from a sol to the gel state, and the formulation appeared as a cream. The resulting formulation was packaged in a 7 mL Aponorm® aluminum tube with 3 g ± 15% of the formulation and labeled according to regulatory requirements on the labeling of IMPs.

2.2.5. Dynamic Scanning Calorimetry (DSC) Measurements of Milled Suspension

The melting point of the as-received IMQ crystals and that of dried IMQ nanosuspension (milled for 240 min at 650 rpm without stabilizer) were determined by using a Mettler Toledo DSC 1 (Mettler Toledo AG, Analytical, Schwerzenbach, Switzerland). A sample of 2–3 mg of powder was placed in a sealed perforated aluminum pan. The samples were heated under nitrogen flow at between 200 °C and 335 °C at a rate of 5 °C/min.

2.2.6. Manufacturing Process of the IMP IMI-Gel, Based on the Process Design and CPPs

Figure 5 reveals the manufacturing process of the IMP IMI-Gel as described in Section 2.4.

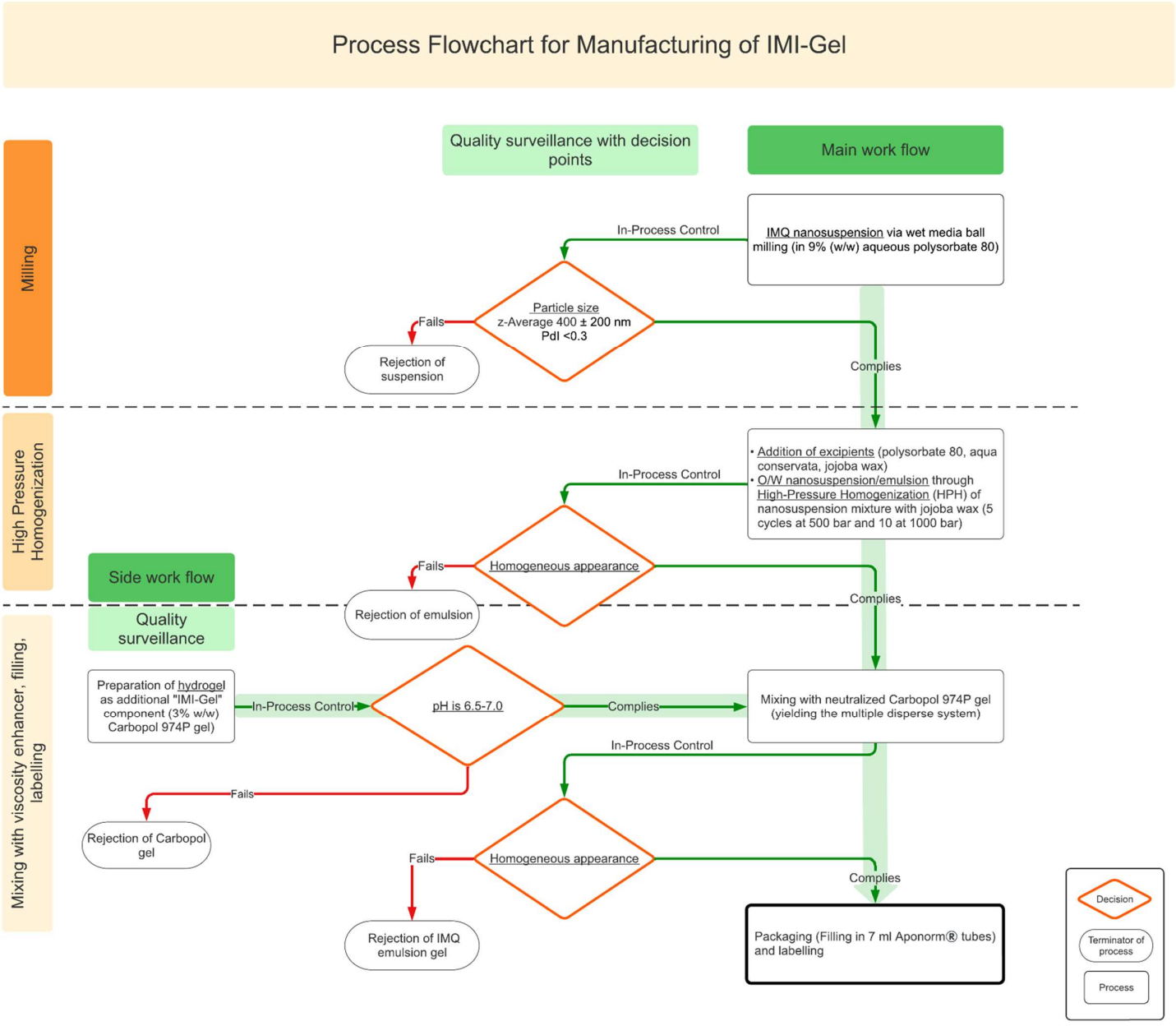


Figure 5: Process flowchart for manufacturing of IMI-Gel. IMQ nanocrystals are manufactured in a wet media ball-milling process, followed by an In-Process-Control of the resulting mean particle size and the polydispersity index. If both parameters comply with the acceptance criteria of a z-Average of 400 ± 200 nm and a PdI < 0.3, the jojoba wax phase (together with the remaining stochiometric amounts of Aqua conservata DAC and polysorbate 80) are added and homogenized to fine-disperse the nanoparticle emulsion. If the formulation appears homogeneous, a stochiometric amount of a precisely neutralized Carbopol 974P gel (pH 6.5–7.0) is added and mixed with the emulsion. If the IPC of the homogeneous appearance of the formulation complies, the formulation is transferred into 7 mL Aponorm® tubes.

2.2.7. Control Strategy

2.2.7.1. Particle-Size Distribution Analysis

The particle-size distribution of the IMQ nanocrystals was measured via dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS (Malvern Panalytical Ltd., Malvern, UK) as an IPC after the manufacturing process. Three samples of 2.8 µL of the IMQ suspension were withdrawn from the grinding vessel, diluted with 4.99 mL of MilliQ water at 25 °C and measured in three consecutive runs so that 18 data points were obtained per batch. Acceptance criterion: z-Average 400 ± 200 nm, PdI < 0.3.

2.2.7.2. Homogeneity of the Formulation

Homogeneity of the formulation was visually assessed by inspecting the emulsion after HPH for indicators of inhomogeneity, e.g., phase separation. Acceptance criterion: homogeneous appearance.

2.2.7.3. Weighing of Filled Tubes

All filled units of each manufactured batch were weighed by placing each unit without a label onto an analytical balance Precisa XR205SM-DR (Dietikon, Switzerland). Acceptance criterion: 5 g ± 15%.

2.2.7.4. HPLC Assay 1: Therapeutically Active Ingredient IMQ

The content of the drug substance was analyzed per high performance liquid chromatography (HPLC)-UV using a Jasco HPLC equipped with a LC-Net II/ADC interface box, an Autosampler AS-950, a PU-980 pump, an UV/VIS UV-975 detector, a DG980-50 degasser, a LG-980-50 mixer and a Jet Stream ATP-CHY 501 column oven. ChromNav CFR 2 software was used to integrate and analyze the generated peaks of the chromatograms. As the analytical method, the method from the USP Monograph Imiquimod Cream Assay was used [86] (for details, see S1). Acceptance criterion 90% ≤ × ≤ 110% of labeled amount.

2.2.7.5. HPLC Assay 2: Content of Preservatives (Methyl- and Propylparaben)

For the analysis of the content of preservatives, a previously published method was used, with some slight modifications [87] (for details, see S2). For analysis, the same system as under Section 2.6.4. was used. Acceptance criterion: total preservative content of 0.04–0.06% (w/w).

2.2.7.6. HPLC Assay 3: Impurities and Degradants of IMQ

As the assay for the impurities, the method as described in the Pending USP Mono-graph IMQ Cream under the section “Impurities” was used [86]. For analysis, the same system as under Section 2.6.4. was used (see S3 for details). Acceptance criteria: impurity levels for the 5 major impurities not more than 0.2%; for unknown impurities, not more than 0.1%; and for the total amount of impurities, not more than 0.5%.

2.2.7.7. Jojoba Wax Droplet Size

The jojoba wax droplet size was assessed by using a Leica DM IL (Wetzlar, Germany) inverted microscope at 400× magnification using a Hund Wetzlar 40/0.60-LD objective. One filled tube per batch was separated into three 750 mg samples. From these samples, one sample, per 750 mg sample, of 250 mg weight was transferred into a 50 mL volumetric flask and diluted with MilliQ water up to the mark. The samples were diluted at a 1 to 10 ratio. Here, 50 µL of each sample was placed on microscopic slides, from which 3 photos per sample were recorded. Acceptance criterion: oil droplet diameter of <10 µm for all droplets.

2.2.7.8. The pH Measurement of the Final Formulation

The pH of the formulation was measured on the basis of the method described in USP 791, stating "where approximate pH values suffice, indicators and test papers may be suitable" [88]. The pH indicator papers were considered suitable for pH analysis and approved upon regulatory submission from the federal authority. The pH of the formulations was assessed by using nonbleeding pH indicator strips from pH 4.0–7.0 (4.0–4.4–4.7–5.0–5.3–5.5–5.8–6.1–6.5–7.0). Three samples per batch were measured. Acceptance criterion: pH of final formulation = 4.0–6.0.

2.2.7.9. Microbiological Testing according to Ph.Eur. 2.6.12. with Acceptance Criteria according to Ph.Eur. 5.1.4.

Microbiological testing was externally performed at BioChem GmbH (Karlsruhe, Germany). Samples for microbiological quality testing were transported under controlled conditions to Karlsruhe and handed over to BioChem in person. As the test method, the pour plate method as described under Ph.Eur. 2.6.12. with acceptance criteria from Ph.Eur. 5.1.4. was used. Acceptance criteria: limits as defined in the Ph.Eur. 5.1.4. monograph.

2.2.8. Statistical Data Analysis and Generation of Graphs

The design of experiments (DoE) experimental plan was created and analyzed by using Minitab Statistical Software (version 21.1). The response-surface plots and DSC graphs were plotted using OriginPro 2023. The Ishikawa fishbone diagram and process flowchart were plotted using Lucidchart 2023. The HPLC chromatograms and peaks were integrated using ChromNav2 CFR software (version 2.02.08). The peaks were analyzed using Microsoft Excel 2019. The QC graphs were plotted using GraphPad Prism 9. Descriptive statistics for the observed QC data were calculated using GraphPad Prism 9.

2.3. Results

2.3.1. Definition of QTPP and Identification of CQAs

Table 1 revels the quality targeted product profile (QTPP) with the identification of the CQAs of a nanoparticulate emulsion gel formulation for the product IMI-Gel. The particle size of IMQ is identified as a crucial CQA thanks to a size-dependent follicular migration of nanoparticles [44,89].

Table 1. Quality target product profile (QTPP) for the investigational medicinal product (IMP) IMI-Gel, where identification of CQAs are the stability, the particle size of IMQ, the rheological properties, the pH of the formulation, the content of the API, the homogeneity, the limits for impurities, the in vitro release profile and the microbiological limits

QTPP Elements	Target	Criticality	Justification
Dosage form	Cream	----	Suitable dosage form for treatment of skin disease AK
Route of administration	topical	----	AK is a skin disease
Dosage strength	5% *w*/*w*	----	Similar strength to comparator Aldara™ product
Dosage design	Oil-in-water emulsion with dispersed IMQ nanocrystals and dispersed jojoba wax	----	---------
Stability	ICH Q1A: At least 6 months at room temperature	Yes	Quality requirement: maintain quality of product over shelf life
Drug product quality attributes	Physical attributes:		To meet applicable quality standards
	• Mean particle size of IMQ nanocrystals in the range of 300–400 nm, PdI ≤ 0.3	• Yes	• Target particle size to deliver IMQ into the hair follicles for slow and sustained absorption [44,45]
	• Rheological properties	• Yes	• Relevant for residence time of drug at administration site and stability [89]
	• Oil droplet size < 10 µm	• No	• Ensure dispersity of the oil phase but droplet size not relevant for mode of action
	• pH 4.0–6.0	• Yes	• Relevant for efficacy of preservatives, prevention of pH-dependent solubilization of IMQ within the formulation (<1% solubilized at pH 4 [90] and gel formation of the polyacrylate gel
	Identification	----	---------
	Assay: 90 ≤ × ≤ 110% of labeled amount based on USP 42 NF 37 IMQ Cream Monograph	Yes	Relevant for dosing uniformity, ensuring desired mode of action and limited adverse events owing to overdosing
	Homogeneity	Yes	Relevant for stability over treatment period
	Degradation products/ impurities based on USP 42 NF 37 IMQ Cream Monograph	Yes	Should be maintained below limits owing to relevance for efficacy and safety
	In vitro release profile	Yes	Relevant for slower permeation rate across the skin in comparison to reference AldaraTM product
	Content of preservatives: consult Ph.Eur. 5.1.3.	-----	-----
	Microbiological limits: consult Ph.Eur. 2.6.12. and Ph.Eur 5.1.4.	Yes	Must be maintained below specified limits in the pharmacopeia
Container closure system	Suitable for dosage form	----	Influences product stability, handling and acceptability/acceptance by the patient
Packaging integrity	Suitable over shelf life	----	Needed for stability, clinical effectiveness and safety

Additional CQAs are identified on the basis of their relevance for the safety and efficacy of the formulation. For example, the rheological properties are known to be relevant for the residence time of the formulation on the skin (for ensuring suitable drug release, as indicated by the in vitro release profile relevant for the sustained absorption of IMQ in the skin). For maintaining the stability over the shelf life, the pH of the formulation is crucial for the prevention of the pH-dependent solubilization of IMQ [90]. The pH is also relevant for ensuring the efficacy of the preservatives, which are the prerequisites for microbiological quality, as the outer continuous phase of the formulation is water. Furthermore, the pH is maintaining the gel structure of the gelling agent. Also critical for efficacy/tolerability are the content of IMQ and the impurities/degradants.

2.3.2. Product Design

2.3.2.1. IMQ Drug Substance: Considerations Regarding the Physicochemical Properties of IMQ and Nanocrystals

IMQ is a small molecule with a molecular weight of 240.30 Da and a pKa of 7.3. Its physicochemical properties make IMQ a suitable candidate for being formulated as a nanosuspension because IMQ neither dissolves in water nor changes its polymorphic form upon processing, which might alter the dissolution rate and lead to instabilities in nanosuspensions (see DSC graphs in Figure Appendix Figure S1).

2.3.2.2. Stabilizing Excipients for IMQ Nanocrystals and Disperse Oil Phase

For IMI-Gel, different stabilizers to prevent Ostwald ripening were considered as suitable, including the surfactant polysorbate 80 and crystal growth inhibitors PVP K30 and PVP K90. It was found that the addition of polysorbate 80 leads to the appropriate stabilization of the IMQ nanocrystals, whereas the crystal growth inhibitors PVP K30 and PVP K90 do not positively influence the stability of IMQ nanocrystals but instead lead to the fast sedimentation of IMQ particles when PVP K90 is used [76]. Given the fine dispersion of jojoba wax, polysorbate 80 is able to stabilize the dispersed jojoba wax droplets, leading to a fine emulsion.

2.3.2.3. Selection of the Oil Component

As potential oil components, different pharmaceutically acceptable oils (including middle chain triglycerides (MCT), jojoba wax, squalene and oleic acid) were considered. A major decision criterion for selection was their influence on IMQ permeation behavior across murine skin. Among the tested oils, jojoba wax led to the strongest deceleration of IMQ permeation and was hence selected as the oil component [76].

2.3.2.4. Gelling Agent and Neutralizing Agent

As the thickening agent, Carbopol was selected thanks to its ability to form optically pleasant hydrogels at low concentrations of 0.5–1% (w/w) upon neutralization with inorganic or organic bases. As a suitable gelling agent, it provides structural stability over a broad pH range of 4–11, where the viscosity is largely independent of the pH [91].

2.3.2.5. 3.1.2.5. Preservatives and Antioxidants

Preservatives, which have their optimal efficacy in the strong acidic pH ranges, are not suitable for IMI-Gel because this might lead to the solubilization of the nanocrystals and collapse of the carbomer gel. Furthermore, ionic preservatives affecting the Carbopol stability are excluded. Parabens (methyl- and propylparaben) show efficacy over the pH range in which Carbopol forms and maintains its structure and IMQ nanocrystals are not solubilized (from 4–8). Hence, they were selected as the preservatives.

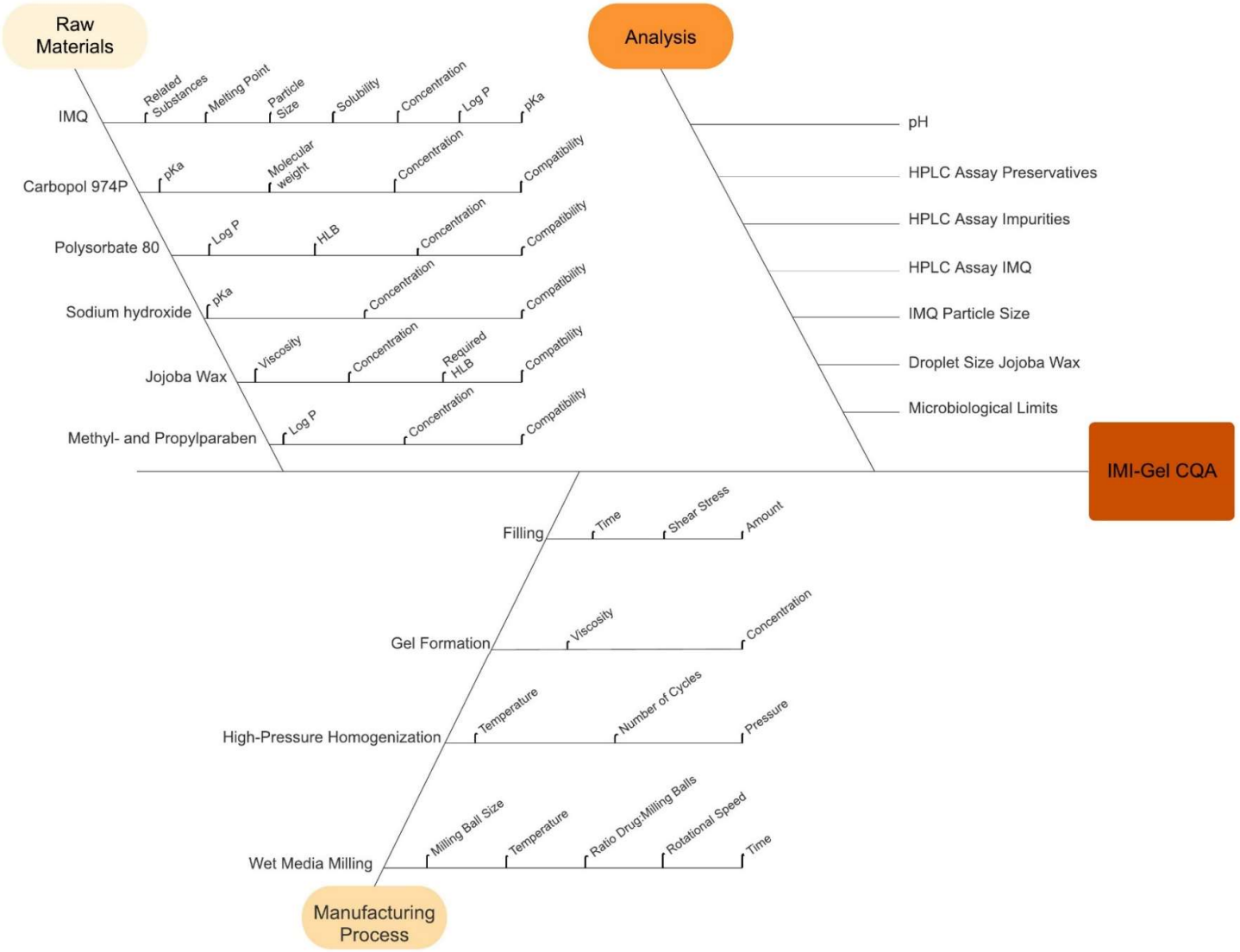
2.3.3. Process Design and Identification of CMAs and CPPs

2.3.3.1. Process Flowchart

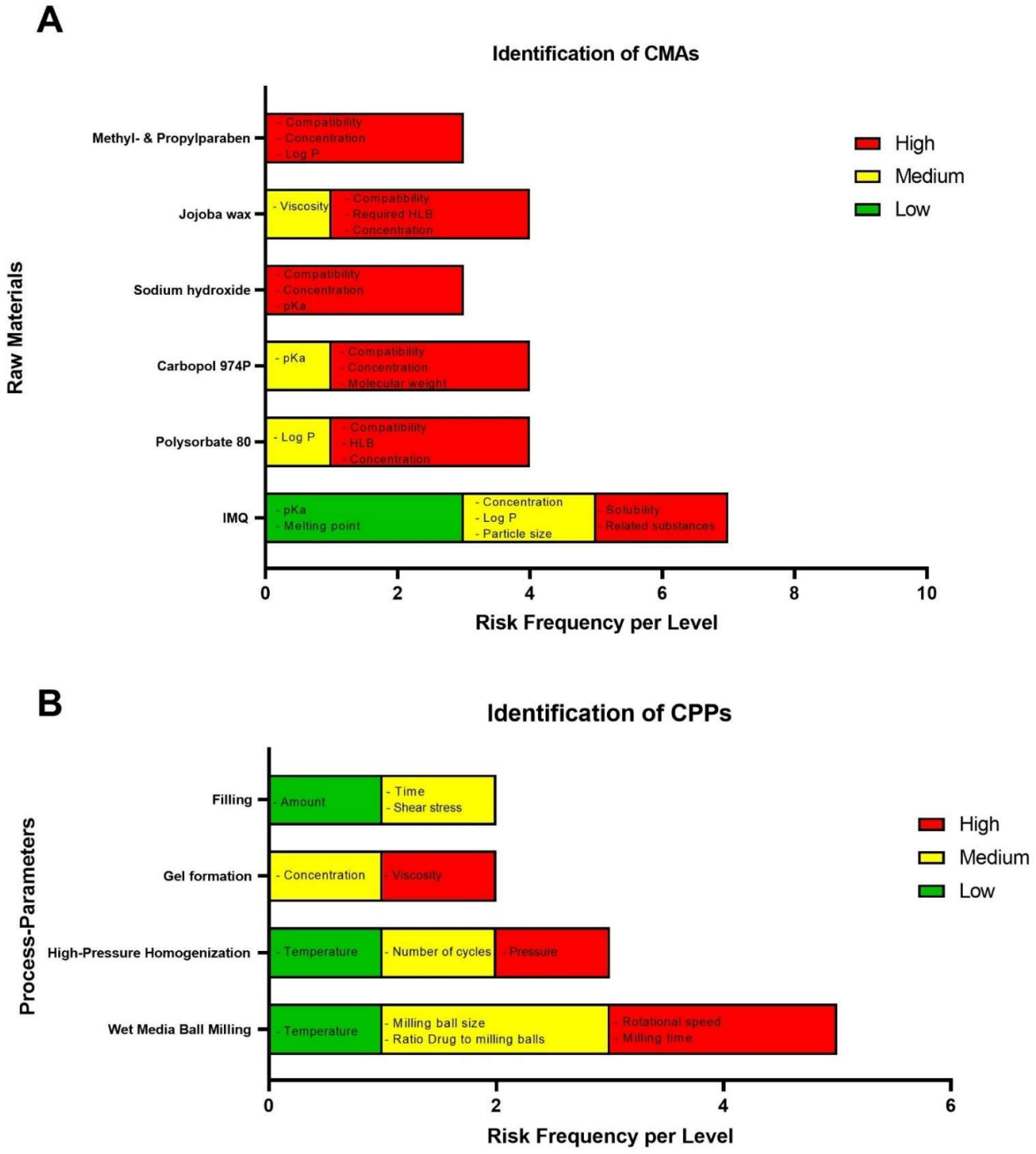
Figure 5 shows the process design in form of a process flowchart for the manufacturing of IMI-Gel. For IMI-Gel, a top-down wet media ball-milling process is selected, followed by a HPH unit operation to finely disperse the oil phase. A semisolid formulation is then created by the addition of a precisely neutralized carbomer gel.

2.3.3.2. Ishikawa Diagram and Risk-Estimation Matrix (REM) for IMI-Gel

In risk assessment, factors affecting the CQAs for IMI-Gel from the input materials and during manufacturing are identified by using an Ishikawa diagram and evaluated for their criticality in a risk-estimation matrix. Figure 6a depicts the Ishikawa diagram for IMI-Gel. Figure 6b depicts the major results out of the respective risk-estimation matrix for IMI-Gel (for the original matrix, see Supplement Table S1). Table 2 lists the identified CMAs.



(a)



(**b**)

Figure 6. (a) Ishikawa diagram for the IMP IMI-Gel showing the critical parameters affecting the quality of the IMP related to the raw materials, the manufacturing process and the analytical characterization. Additional factors not depicted here are "Environment" and "People". (b) Frequency histogram for identification of critical material attributes (CMAs) (A) and critical process parameters (CPPs) (B) for the IMP IMI-Gel showing the critical parameters affecting the quality of the IMP related to the raw materials and for manufacturing. CMAs and CPPs are categorized as low (in green), medium (yellow) and high (red).

Table 2: Summary of identified potential CMAs with effects on the CQAs' stability (including nanocrystal stability, cream stability, emulsion stability, microbiological stability and chemical stability), permeation rate, rheological properties, pH, content uniformity and particle-size distribution.

Formulation Components CMAs	CQAs
Drug substance IMQ	
Solubility	Stability of nanocrystals, permeation rate, pH, particle-size distribution
Related substances	Stability (degradants/impurities)
Surfactant polysorbate 80	
Compatibility	Content uniformity, stability (nanocrystals, emulsion), particle-size distribution
HLB	Stability (of nanocrystals and emulsion)
Concentration	Stability (of nanocrystals and emulsion), particle-size distribution
Gelling agent Carbopol 974P	
Compatibility	pH, cream stability, rheological properties
Concentration	Rheological properties, cream stability
Molecular weight	Rheological properties, cream stability
Neutralizing agent sodium hydroxide	
Compatibility	pH, rheological properties
Concentration	pH, rheological properties, stability (of nanocrystals, cream)
pKa	pH, rheological properties, stability (of nanocrystals, cream)
Oil component jojoba wax	
Compatibility	Permeation rate, stability (of emulsion)
Required HLB	Permeation rate, stability (of emulsion)
Concentration	Rheological properties, permeation rate, stability (of emulsion)
Preservatives methyl- and propylparaben	
Compatibility	Stability (microbiological limits)
Concentration	Stability (microbiological limits)
Log P	Stability (microbiological limits)

2.3.4. Considerations and Evaluation of Selected Potential CMAs

2.3.4.1. IMQ Drug Substance

For the IMQ drug substance, critical material attributes identified on the basis of the REM (Figure 2b and Table S1) and Ishikawa diagram (Figure 6a) are the IMQ solubility and the occurrence of related substances. Previously conducted chemical stability investigations into the criticality on the occurrence of related substances revealed no detectable levels of related substances over 6 months of storage [77].

2.3.4.2. Stabilizing Excipients for IMQ Drug Substance

The identified CMAs for the surfactant are the compatibility between the surfactant and the drug substance, the concentration of the surfactant and the hydrophilic–lipophilic balance (HLB).

During formulation development, it was found that polysorbate 80 at a concentration of 0.9% (w/w) enabled the sufficient wettability of IMQ for the preparation of nanosuspensions [76]. Stable nanosuspensions manufactured with 0.9 % (w/w) polysorbate 80 show the following characteristics: (a) a negative zeta potential of −33.1 ± 0.93 mV; (b) a size scale range of 100–500 nm, measured per scanning electron microscopy using Martins' diameter; and (c) a mean hydrodynamic size of 285 nm, measured per DLS. The stability testing of the nanosuspension over 9 months revealed no change in the mean particle size (z-Average) of the IMQ nanocrystals at the tested 0.9 % (w/w) concentration [76].

2.3.4.3. Oil Component

As CMAs for the oil component, three parameters are identified: (a) the compatibility of the formulation, (b) the concentration and (c) the required HLB (which should be met by the selected surfactant in order to achieve the ideal dispersion of the oil phase). For the emulsification of 40 % (w/w) jojoba wax as the disperse phase, the required HLB is 12.5 [92] at a required final surfactant concentration of ≥4 % (w/w) in the system [92]. Given the appropriate stabilization of the IMQ nanoparticles, polysorbate 80 was evaluated as the only surfactant for the emulsification of jojoba wax. For the highest impact of the deceleration of IMQ, a high concentration of jojoba wax (45%) was selected. The concentration of polysorbate 80 was selected to be 5 % (w/w) on the basis of the reported ≥ 4 % polysorbate 80 required for the emulsification of 40 % (w/w) [92] plus the tested 0.9 % (w/w) of polysorbate 80 for the stabilization of the IMQ nanocrystals. The evaluation of the compatibility revealed compatibility between the surfactant oil and IMQ nanocrystals at the given polysorbate 80 concentration (at HLB 15) and at a jojoba wax concentration of 45 % (w/w) [76,77].

2.3.4.4. Gelling Agent and Neutralizing Agent

As depicted in the REM, the CMAs of the gelling agent are identified as its concentration, its molecular weight, the target pH of the formulation thanks to the pH-dependent gel formation of Carbopol hydrogels (after neutralization) and its compatibility with IMQ. From the available Carbopol types, Carbopol 974P (medium range molar mass of 35,000 Da) is selected thanks to its ability to form hydrogels with a plastic flow behavior of a high viscosity of ~30,000 cP at pH 7.5 and at a concentration of 0.5% (w/w) [93].
For neutralization, sodium hydroxide is selected. As a CMA, its concentration is identified because it influences the final pH of the formulation (which is controlled through the addition of stochiometric NaOH and by monitoring the final pH).

2.3.4.5. Preservatives and Antioxidants

Methyl- and propylparaben are selected as the preservatives thanks to their optimal activity between pH 4 and pH 8, which matches the targeted pH range of the formulation [94]. The effect of different methyl- and propyl-4-hydroxybenzoate concentrations on the inhibition of microbiological growth was evaluated in a conservation load test, according to Ph.Eur. 5.1.3. There, no difference at the tested concentrations ranging from 0.04 % (w/w) to 1.5 % (w/w) was observed [77]. All tested concentrations inhibited the microbiological growth of the inoculated microorganisms with a partial reduction in the total number of inoculated microorganisms. The required log reduction number of viable microorganisms defined in the Ph.Eur.5.1.3 was not met. The evaluation of the microbiological examination of nonsterile products specified in Ph.Eur. 2.6.12. for formulations stored over 6 months under intermediate storage conditions at 30 °C /65% RH, according to ICH Q 1 A (R2) [95] revealed that the Ph.Eur. criteria for all tested concentrations were met.
The addition of antioxidants is considered unnecessary because jojoba wax as a material contains natural antioxidants [94], hence showing high oxidative stability.

2.3.5. Evaluation of Potential CPPs

2.3.5.1. Design of Experiments

Table 3 lists the identified most critical process parameters (CPPs) affecting the IMQ particle-size distribution during the wet media ball milling of IMQ (depicted in red in the REM and frequency histogram) and the homogeneity of the formulation after the addition of the jojoba wax phase.

Table 3. Identification of CPPs (depicted in red in the REM and frequency histogram) affecting the CQAs of the particle-size distribution and the stability of the formulation, by factoring in the milling and the homogenization process steps.

Risk Assessment of Critical Process Parameters	CQAs
Wet media ball milling	
Milling time	Particle-size distribution
Rotational speed	Particle-size distribution
High-pressure homogenization	
Pressure	Stability (homogeneity)

In wet media milling, the frequently studied process parameters significantly affecting the breakage rate of the drug particles are milling time, milling medium (most importantly bead size and the amount of milling medium, e.g., number of beads), milling speed, drug amount and milling design [67,96–98]. In the current study, the effect of factors such as (a) the rotational speed (X_1) and (b) the milling time (X_2) on the following CQAs are studied during wet media ball milling: (I) the particle-size distribution measured as the responses of the intensity-weighted mean hydrodynamic particle size (z-Average [d.nm]) and (II) the polydispersity index. A face-centered CCD is applied to determine the optimal process parameters for a mean particle size in the range of 300–400 nm and with a minimal PdI. Other parameters, including drug amount, type and amount of milling medium, bead size and milling design, are taken as previously described [76,77]. The response data for all 26 conducted experimental runs are presented in Table S2.

The statistical significance of the linear and quadratic impacts of the studied factors and their interactions with the responses as calculated by analysis of variance (ANOVA) are presented in Table 4.

Table 4. Analysis of variance (ANOVA) table revealing a statistically significant influence of the linear terms of the rotational speed (X_1) and the milling time (X_2), of the quadratic term of the rotational speed $(X_1)^2$ and for the term of 2FI (X_1X_2) on the response factor of the z-Average and statistical significance for the linear term for the milling time X_2 on the response factor PdI.

Source	Mean particle size (z-Average)				Polydispersity Index (PdI)			
	Degrees of freedom (DF)	Sequential Sum of Squares (Seq SS)	F value	*p* value	Degrees of freedom (DF)	Sequential Sum of Squares (Seq SS)	F value	*p* value
Model	5	97143	60.15	0.000	5	0.037890	9.66	0.000
X_1	1	33507	103.74	0.000	1	0.000161	0.21	0.655
X_2	1	53373	165.25	0.000	1	0.037074	47.28	0.000
$(X_1)^2$	1	6664	13.88	0.003	1	0.000194	0.04	0.835
$(X_2)^2$	1	1310	11.78	0.058	1	0.000333	0.42	0.522
$X_1 \cdot X_2$	1	2288	4.06	0.015	1	0.000128	0.16	0.690
Lack of fit test	3	1529	1.76	0.193	3	0.000188	0.07	0.976
Pure error	17	4930	----	-----	17	0.015495	----	----
Total	25	103603	----	-----	25	0.053574	----	----

The ANOVA table for the z-Average reveals that linear, quadratic and two-factor interactions (2FI), except for the squared milling time, are statistically significant for the model parameters, with p-values of < 0.05. An analysis of the model fit reveals an acceptable correlation between the predicted and observed values, as expressed by the regression coefficient (with the corrected regression coefficient of 0.9221 and the predicted regression coefficient of 0.8732 and an insignificant lack of fit value with a p of 0.193 (see Table 6)).

The analysis of the model yields the following regression equation:

$$Particle\ size = 837.4 - 0.996 \cdot X_1 - 1.734 \cdot X_2 + 0.000656 \cdot (X_1)^2 + 0.001901 \cdot (X_2)^2 + 0.000904 \cdot X_1 \cdot X_2 \quad (8)$$

where X_1 = rotational speed and X_2 = milling time.

When for the PdI model, a Box-Cox transformation at a λ of 1 is conducted, the ANOVA table for the linear, quadratic and 2FI reveals statistical significance for the linear factor of the milling time (X_2) with a $p < 0.05$ (see Table 4). Statistical insignificance is observed for the linear term for rotational speed (X_1) with a p of 0.655; the quadratic terms for the rotational speed $(X_1)^2$ with a p of 0.835; the milling time $(X_2)^2$ with a p of 0.522; and the 2FI (X_1X_2) with a p of 0.690. The reduction in the model by the removal of the insignificant quadratic terms of the rotational speed $(X_1)^2$, the milling time $(X_2)^2$ and the 2FI (X_1X_2) (see Table 5) to improve the accuracy of the predicted values reveals an improved fit of the model, indicated by a reduced difference between the R-squared adjusted and R-squared predicted values (see Table 6). The linear term for the rotational speed (X_1) is not removed from the model, because its removal would not increase the prediction accuracy to a significant extent.

Table 5. Analysis of variance (ANOVA) table after a reduction in the model revealing a statistically significant influence of the linear term the milling time (X_2) on the PdI with an improved fit, indicated by the *p*-value of 0.986 for the lack of fit test.

Source	**Polydispersity Index (PdI)**			
	Degrees of Freedom (DF)	**Sequential Sum of Squares (Seq SS)**	**F-Value**	***p*-Value**
Model	2	0.037235	26.21	0.000
X_1	1	0.000161	0.23	0.638
X_2	1	0.037074	52.19	0.000
Lack of fit test	6	0.000843	0.15	0.986
Pure error	17	0.015495	----	----
Total	25	0.053574	----	----

The analysis of the model yields the following regression equation:

$$PdI = 0.3003 - 0.000018 \cdot X_1 - 0.000618 \cdot X_2 \quad (9)$$

where X_1 = rotational speed and X_2 = milling time.

Table 6. Summary of the model fit for the studied response variables of the mean particle size (z-Average [d.nm]) of the IMQ

Responses	**Standard Deviation S**	**R-Squared**	**R-Squared Adjusted**	**R-Squared Predicted**
z-Average [d.nm]	17.9720	0.9376	0.9221	0.8732
PdI (full factors)	0.0280	0.7073	0.6341	0.3522
PdI (reduced factors)	0.0267	0.6950	0.6685	0.5679

nanocrystals and for the polydispersity index (PdI) with the complete and the reduced model.

Figure 7 visualizes the effect of the milling time and rotational speed on the mean particle size (z-Average [d.nm]) and the polydispersity index. The response-surface plot for the reduction in the mean particle size in Figure 7A reveals a curvature, indicating a *nonlinear* relationship between particle-size reduction and the studied factors of the milling time and rotational speed approaching a lower limit of ~300 nm. The lowest mean hydrodynamic particle size is observed at the highest rotational speed and the longest milling time, with a z-Average of 292.8 nm (run 11) and 305.2 nm (run 17), as shown in Table S2 (see Appendix).

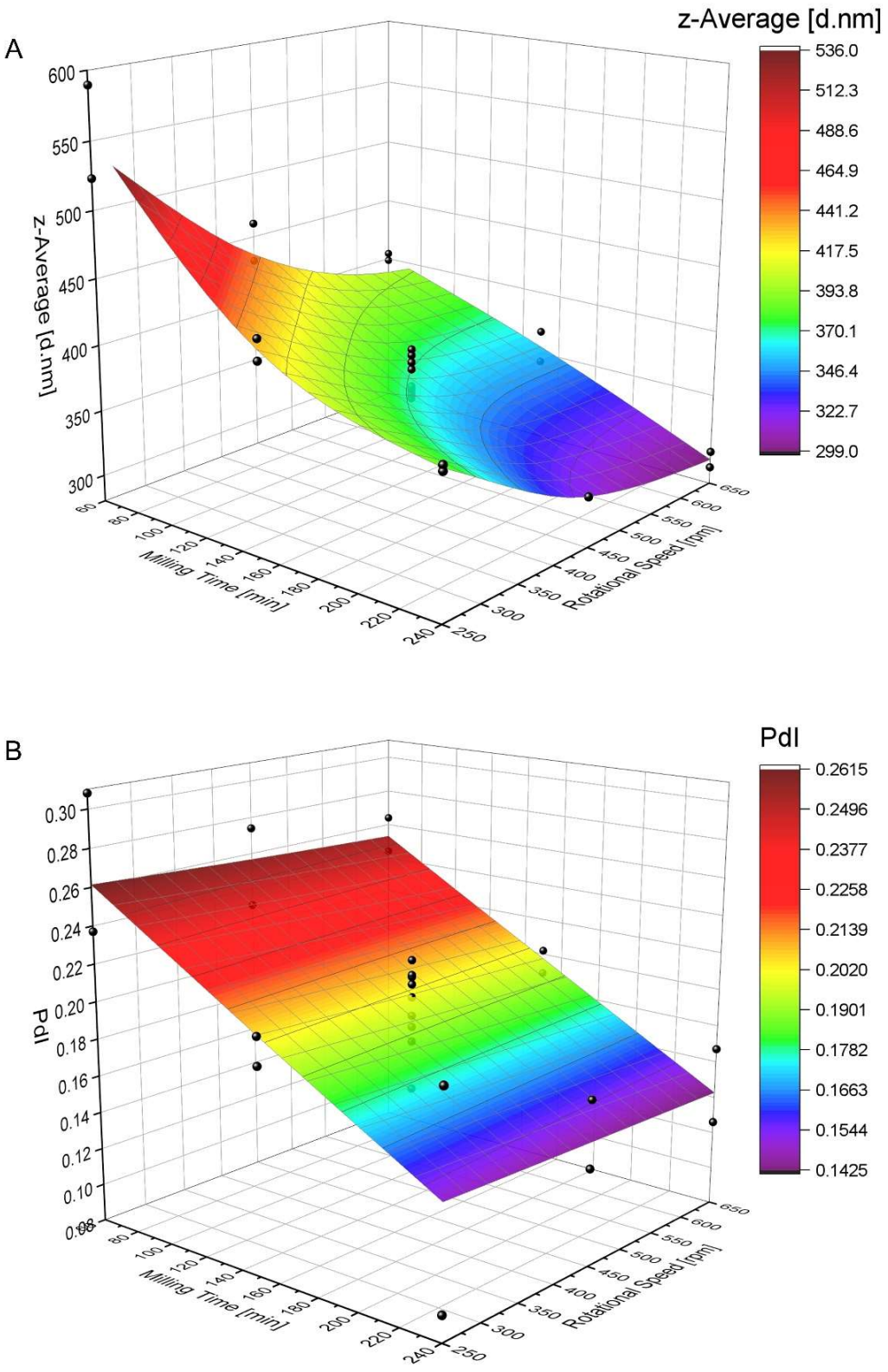


Figure 7. Response-surface plots with experimental points in black, illustrating the effect of the milling time and rotational speed on the z-Average (**A**) and the PdI (**B**) (for optimized parameters, see Table 7).

Figure 7B reveals that a reduction in the PdI follows a *linear* model, where the minimal achievable PdI is a function of time.

2.3.5.2. Validation of the Model

The purpose of validation is to evaluate the accuracy of the predictability of the model and to find optimal process conditions to achieve a mean particle size in the range of 300–400 nm with a minimal PdI (at least smaller than 0.3) at a reasonable milling time for manufacturing under GMP conditions. To evaluate these conditions, a milling time range of 80–140 min, with a target particle size of 350 nm and a minimal PdI with starting conditions of 650 rpm and 140 min, is chosen. The predicted solution is a rotational speed at 650 rpm for 135 min, resulting in a mean particle size (z-Average) of 349.99 nm and a PdI of 0.205. The optimal conditions with the observed and predicted values are given in Table 7. Because the milling process was segmented into 20 min cycles, the factors were chosen to be 650 rpm and 140 min of milling time.

Table 7. Result from QbD approach: Optimized parameters with predicted and observed values of responses.

Factors		**Predicted Values**		**Observed Values**	
Rotational Speed [rpm]	**Milling Time [min]**	**z-Average [d.nm]**	**PdI**	**z-Average [d.nm]**	**PdI**
650	135	349.99	0.205	378.8 ± 11.22	0.195 ± 0.03

An analysis of the particle size reveals that with the conditions of 650 rpm and 140 min, a slightly larger mean hydrodynamic particle size is observed, with a difference of 8.23% ± 3.22% and a difference for the PdI of 4.88 % ± 0.034 %.

2.3.5.3. High-Pressure Homogenization

The number of cycles to achieve a homogeneous emulsion was empirically deter-mined until the emulsion appeared homogeneous because the droplet size was not identified as a CQA. During evaluation, it was found that with 5 cycles at 500 bar and 10 cycles at 1000 bar led to a homogeneous formulation.

2.3.6. Formulation Performance Testing

For long-term performance testing, formulations containing 45 % (w/w) jojoba wax, 5 % (w/w) polysorbate 80 and 5 % (w/w) IMQ nanocrystals preserved with 0.045 % methyl- and propylparaben were used. Formulations were stored over 6 months under intermediate storage conditions, according to ICH Q 1 A (R2), and evaluated for their chemical stability. There, it was observed that the mean IMQ content of the formulation varies from 99.85 % to 100.08 % with no detectable impurities, revealing chemical stability [77].
The evaluation of the microbiological quality, according to Ph.Eur. 2.6.12., in an unopened primary packaging over 6 months as well as in an in-use stability test over 2 and 4 weeks simulating potential microbiological contamination revealed no microbiological contamination, fulfilling the acceptance criteria of the Ph.Eur.

The evaluation of the formulation pH over the storage period revealed no change in pH from the targeted pH range of 4–6 over the storage period [77].

The rheological characterization of the formulation with 0.5 % (w/w) Carbopol 974P revealed plastic flow behavior in the formulations and an approximately 20-fold increase in shear stress at similar shear rates for the Carbopol-based formulation when compared to the reference product Aldara™ (thin, fluid consistency) [76]. An assessment of the structural stability for IMI-Gel assessed in a three-interval step test for formulations stored for 12 months at ICH Q1 A (R2) under long-term conditions (25 °C/65 % rH) and over 6 months under

accelerated storage conditions (40 °C/75 % rH) revealed structural stability for formulations precisely adjusted to a pH range of 4–6 (see [99,100] for the study).

2.3.7. Control Strategy

Data of In-Process Control and Quality Control Tests from Manufactured Batches
Figure 8 shows the results obtained from the IPC and QC analyses. Given the trend analysis for the particle size (Figure 8A), the mean particle size and PdI of the IMQ nanoparticles (analyzed as IPCs during the manufacturing of IMP batches) is found to be slightly larger than predicted in the model but within the defined limits and with low batch-to-batch variability. The measured values of the mean particle-size range from 358.58 nm up to 451.4 nm, with a median of 384.4 nm and a 95 % confidence interval of the median of 372.2 nm to 399.5 nm (see Table S3 for the full data). For the PdI, slightly larger values are observed in comparison to those in the model. The measured values range from 0.185 to 0.260, with a 95% confidence interval of 0.215–0.241 (see Table S4 for the full data).

The data from the content analysis of IMQ reveal minor fluctuations for the mean content between batches (ranging from 94.38 % ± 1.02 % to 104.93 % ± 3.57 %) with no out-of-specification (OOS) or out-of-trend (OOT) observations (see Figure 8B). All tested batches are within the defined acceptance criteria (mean content of 90 % ≤ × ≤ 110 % of the targeted dose of 5 % (w/w) (see Table S5 for the full data)).

For the level of impurities, the data reveal that detected levels of impurities are observed for 6 out of the 13 manufactured batches. The detected levels are all significantly below the acceptance criteria (see Table S6 for the full data).

The data for the level of the preservatives reveal minor fluctuations in the mean preservative content, all within the acceptance criteria, where the latest manufactured batches reveal levels closer to the lower acceptance limit (see Table S7 for the full data).

Given the weight of the filled tubes, the data reveal that the box plot for the batch IMI-Gel-170219 exceeds the upper specification limit. An investigation of this OOS result revealed that 6 out of the 28 manufactured tubes are OOS. A root-cause analysis of this observation revealed that the mean weight of the primary packaging batch used for manufacturing exceeded the weight of previously used batches, while the filled mass of the formulation was within the targeted range of 3 g ± 15 %. As a consequence, the affected primary packaging batch was withdrawn. The weight of the filled tubes for the remaining batches revealed no OOS or OOT results (see Table S8 for the full data).

The data for the measured pH of the formulation reveal a consistently measured pH for the tested batches. The measurements reveal a pH toward the upper specification limit (pH 6).

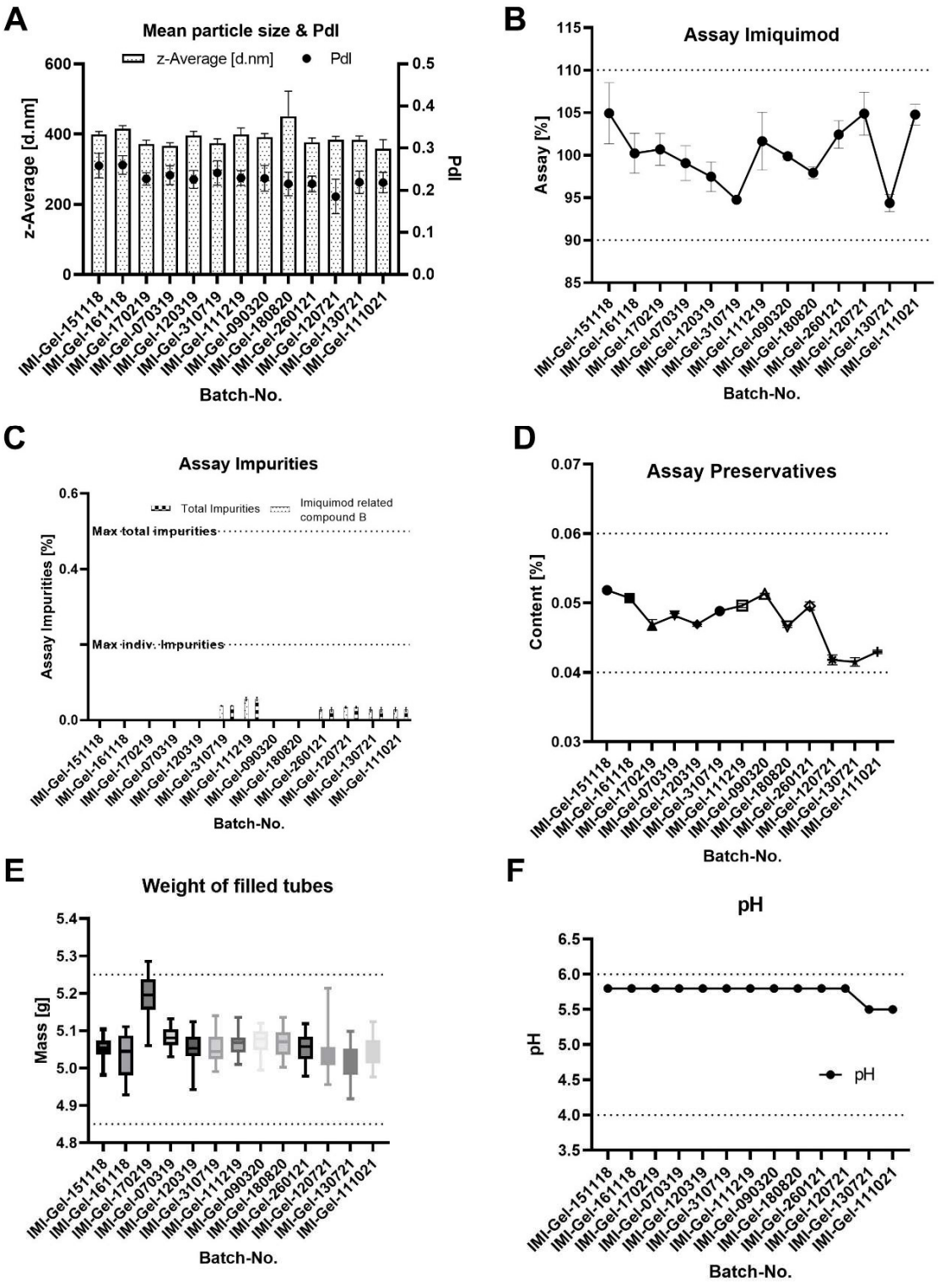


Figure 8. Batch-to-batch variability in the In-Process Control data for the mean particle size of the IMQ nanocrystals (A) and from QC data of the mean IMQ content (B), the level of impurities and degradants (C), the content of the preservatives methyl- and propylparaben shown as the total content (D), the weight of the filled tube units (E) and the pH (F). The data reveal minimal batch-to-batch variability in the manufactured batches. The grid lines represent the respective lower and upper specification limits.

2.4. Discussion

2.4.1. Product Design

In general, a variety of formulation concepts for transportation of IMQ across the stratum corneum toward the target tissue are available, e.g., microemulsions (ME), microneedles (MN) and nanocrystals. The poor solubility of IMQ in most pharmaceutical oils (except for isostearic acid) and the ME concept to increase drug permeation across the skin outline the unsuitability of ME for the delivery of IMQ to the targeted upper skin layers. Additionally, MNs appear as unsuitable formulation concepts owing to their limited drug loading capacity (the required IMQ dose is 50 mg/g). Nanocrystals appear as suitable concepts matching the targeted decelerated IMQ skin permeation. Together with a suitable oil component, yielding an oil-in-water (O/W) emulsion with nanocrystalline IMQ particles, the further deceleration of IMQ skin permeation is achieved.
The thermodynamic instability in nanosuspensions and (nano)emulsions owing to the large surface free energy (ΔG) of the nanosized disperse particles and oil droplets [76,77] requires the addition of stabilizing excipients to the product. The reduction in the large surface area in these systems appears (i) through the dissolution of incipient crystalline nuclei, causing particulate precipitation; (ii) through the agglomeration of small particles to reduce the surface free energy [76]; or (iii) by its leading to conventional phase separation phenomena in (nano)emulsions such as creaming, sedimentation, coalescence and flocculation [101] Conventionally, stabilizing excipients such as surfactants (reducing the interfacial tension between the solid–liquid phase or liquid–liquid phase), polymers (to sterically stabilize the particles and droplets) and crystal growth inhibitors (to prevent Ostwald ripening) are added. The choice of the stabilizer is mostly empirical, but it must take into account interaction forces (hydrophilic, hydrophobic, hydrogen bonding or ionic forces) and a chain length long enough to create a steric barrier and the required HLB of the oil type. For IMI-Gel, the selected surfactant polysorbate 80 and polymer Carbopol were found to be suitable candidates to create and maintain nanocrystals while stabilizing the (nano)dispersed oil phase.

With respect to the type of the oil component, the strongest decrease in release of IMQ was identified as jojoba wax and selected for the product IMI-Gel. This observed strongest IMQ decelerated permeation is in line with previously reported decelerated diclofenac permeation across the skin for formulations containing jojoba wax [102]. This is supposed to be associated with the three-dimensional structure of jojoba wax.

The viscosity-enhancing agent is required for (i) the creation of a cream formulation of increased viscosity (~93 cP at a shear rate of 10 s−1 at 32 °C) for the prolonged residence time of the formulation on the skin while in parallel (ii) stabilizing the disperse IMQ particles and the oil phase. Given the available types of other macromolecular gelling agents, the synthetic gelling agent Carbopol is selected thanks to its good skin tolerability and maintenance of the gel structure over a broad pH range [103]. Other gelling agents, e.g., cellulose-based excipients, were previously identified as unsuitable for suspended IMQ [90]. As the neutralizing agent, sodium hydroxide as an inorganic base is selected because common reports suggest that nitrosamines (classified as probable human carcinogens) form when using neutralizing agents, such as triethanolamine.

2.4.2. Process Design and Identification of CMAs and CPPs

The designed process to manufacture IMI-Gel contains two major unit operations. (i) Wet media ball milling is selected for the creation of IMQ nanosuspensions as it is a widely used process to create nanosuspensions offering a robust, reproducible, scalable and solvent-free process. During milling, the suspension undergoes high-energy input where particles are broken down to the nanoscale by bead–bead collisions. Only a small fraction of the energy input is used to deform the particles [104] while most of the energy is converted into heat through dissipative mechanisms, e.g., viscous losses, inelastic bead–bead collisions and bead–wall collisions [105]. This leads to a rise in the temperature of the suspension, facilitating Ostwald ripening, the growth of the nanoparticles or surface modification and amorphization. Therefore, it is important to identify

the material attributes of the drug substance that may be critical to a temperature rise, requiring temperature control by the adjustment of the process parameters of the milling process, as demonstrated by Guner et al. In their study, it was shown that the rate of the temperature increase in the suspension during a wet stirred media milling process depends on the selected process parameters of the stirrer speed, bead loading and bead size [106]. When selecting process conditions with lower energetic levels (the lowest stirrer speed, lowest bead loading and smallest beads), an overall temperature rise of 6 °C over 60 min was observed for the suspension [106]. In contrast, high energetic conditions (the fastest stirrer speed, largest bead loading and largest beads) caused a temperature jump from 18 °C to 45 °C in 2 min [106]. A helpful tool from a temperature rise perspective, designed in this study, is the thermal desirability score (TDS). The TDS rates a milling process on the basis of the temperature increase and the number of cycles—as excellent (corresponding to almost isothermal conditions), good, acceptable or poor [106]. Because the material attributes of IMQ, which has a high melting point (297–299 °C), features practically insolubility in water (also at elevated temperatures) and lacks known polymorphic forms with no change in the crystallinity of the drug before and after milling (see Figure S1), are uncritical for physicochemical instabilities, thanks to a temperature rise during milling, temperature control is not required.

(ii) HPH is chosen for the dispersion of jojoba wax because it offers a versatile and scalable process disrupting oils under high pressure to fine (nano)emulsions. During homogenization, high operational temperatures are generated, which are on the one hand advantageous as they decrease the viscosity of the formulation, enabling the passage of the formulation through the narrow gap of the homogenizer. On the other hand, high temperatures may lead to similar instabilities in nanosuspensions, as outlined above, and for oils sensitive to oxidation. For IMI-Gel, IMQ and jojoba wax are insensitive to high temperatures and oxidation. Therefore, a temperature rise during the process is uncritical.

2.4.3. Considerations and Evaluation of Selected CMAs

2.4.3.1. IMQ Drug Substance

The criticality of the CMA of the solubility for IMQ is a function of the resulting formulation pH because IMQ is a weak base. The originator of the product Aldara™ found that increased aqueous solubilization for IMQ occurs when the pH is lowered, but with an in-capability to solubilize the required 5 % (w/w) IMQ dose even at an extremely acidic pH [90]. For the disperse systems present in IMI-Gel, the partial dissolution of the particles is undesirable as this may cause physical instabilities, e.g., Ostwald ripening in nanosuspensions. By defining and maintaining the lowest acceptable pH, of 4, solubility issues causing such instabilities can be avoided.

The CMA for the occurrence of related substances for IMQ is a function of the chemical stability of the IMQ drug substance. IMQ is reported to be a relatively stable drug (the originator of the Aldara™ formulation applied for a retest interval of 2 years after manufacturing [107]). In suspensions, degradation kinetics appear to be zero-order processes, where the large reservoir of solid drug particles leads to a constant concentration of the drug in the solution and, hence, a constant degradation rate for the drug molecules in the solution [108]. Thanks to the good chemical stability and suspended state of the particles, it is expected that the occurrence of related substances is uncritical. The results of the chemical stability investigations for IMQ confirmed these expectations [77].

2.4.3.2. Stabilizing Excipients for IMQ Nanocrystals and Disperse Oil Phase

For polysorbate 80 as the stabilizing excipient for IMQ nanocrystals and disperse jojoba wax, the material attributes of compatibility, HLB and concentration reveal their criticality as they determine the stability of IMQ nanocrystals and the jojoba wax phase. Compatibility between IMQ and polysorbate 80 results when the surfactant ameliorates the increased wettability of the particles for the creation of nanoparticles, decreases interfacial tension and leads to sterical stabilization in the disperse solid nanocrystals, which is in turn a

function of the used concentration. For the preparation of physically stable (nano)suspensions, it is recommended to select concentrations below the critical micellar concentration (CMC) to prevent instabilities such as aggregation or Ostwald ripening due to the solubilization of the particles. [109,110]. For IMQ, the used concentration of 0.9 % (w/w) revealed the good compatibility and stabilization of the particles while exceeding the CMC (the CMC for polysorbate 80 has been reported to be from 13.4 ± 0.6 mg/L to 24.7 ± 1.4 mg/L [111]). However, even if small IMQ quantities may be solubilized at this concentration above the CMC, it was shown that the used concentration did not negatively affect the stability of the nanosuspension over 9 months of storage [76]. In contrast, the usage of concentrations above the CMC might be beneficial during processing, creating an excess of available surfactant for the rewetting of the newly created hydrophobic surface upon particle breakage [112]. Otherwise, particles might dewet or position themselves at an existing liquid–gas interface, leading to the formation of an undesirable Pickering foam during processing [112]. For the optimization of the surfactant concentration (preventing foam formation), a mathematical approach may be used to calculate the required surfactant/stabilizer concentration by calculating the specific surface area of the drug from the targeted particle-size distribution, followed by the theoretical surfactant quantity, estimated from the head area of the surfactant considered for drug stabilization [112]. Afterwards, the actual quantity of surfactant can be calculated from the required volume of the suspension to achieve the required available extent of surfactant [112].

With regard to the required HLB for jojoba wax (~12.5), it is found that an HLB of 15, as used with polysorbate 80 in IMI-Gel, adequately stabilizes jojoba wax in the aqueous phase, even at a high concentration, of 45%. Because concentration optimization studies for polysorbate 80 for jojoba wax stabilization were not conducted but instead taken from the literature [92], it is obvious that the optimization of surfactant concentration or the usage of surfactant combinations will lead to the better/increased dispersity of jojoba wax.

2.4.3.3. Oil Component

For the oil component of jojoba wax, its material attributes of compatibility, required HLB and concentration affect the permeation rate, stability and rheological properties of IMI-Gel. These attributes are significant contributors to the formulation performance and are therefore identified as CMAs. As outlined above, the optimal compatibility between the surfactant and the jojoba wax phase is a function of the required HLB. The used concentration influences the permeation rate. Therefore, the addition of high concentrations is required to maximize the deceleration of IMQ permeation.

2.4.3.4. Gelling and Neutralizing Agent

The identified critical material attributes of the gelling and neutralizing agent of compatibility, concentration, molecular weight and pKa contribute to the resulting consistency of IMI-Gel, affecting the CQAs of the pH, cream stability and rheological properties and are therefore categorized as critical. Compatibility is given as long as the pH of the formulation is adjusted to a weak acidic or neutral pH to ensure the stability of IMQ nanocrystals and the sol-gel transitioning of Carbopol 974. The consistency of IMI-Gel is a function of the used concentration of the gelling agent and its molecular weight. The selection of the medium molecular weight Carbopol 974P as a gelling agent is a compromise between obtaining a formulation with a high viscosity (~30,000 cP at pH 7.5 at a concentration of 0.5% (w/w) [93]) to contribute to the stabilization of the disperse particulate and of the oil phase and to the prolonged residence time at the administration site while in parallel achieve the good spreadability of the formulation. Given the CMAs for the neutralizing agent, the attributes go hand in hand with the viscosity and pH of the formulation and therefore with the stability of the nanocrystals requiring stochiometric addition and pH control.

2.4.3.5. Preservatives

The CMAs of the preservative's compatibility, concentration and log P are relevant in that they determine the efficacy of the used preservatives and determine the microbiological stability of the formulation. While concentration is observed as uncritical, compatibility and log P revealed criticality, demonstrated in the incapability to fulfill the acceptance criteria of a conservation load test from Ph.Eur. 5.1.3. Parabens are known to show partitioning between hydrophilic and lipophilic phases and may be included in surfactant micelles, leading to a loss in efficacy [113]. Related to IMI-Gel, partitioning in the jojoba wax phase or inclusion in polysorbate 80 micelles might be the cause for the observed limited efficacy (for additional discussion, see Section 4.5), revealing that log P and compatibility are CMAs. As reported in the literature, parabens interact to a considerable extent with the polyoxyethylene macromolecules used in IMI-Gel (polysorbate 80) [113].

2.4.4. Evaluation of Potential CPPs

The milling process is a critical unit operation as it creates the IMQ nanocrystals, a fundamental component of the product. Consequently, the optimization of the process parameters, determining the resulting breakage kinetics and particle size, is required. This study focused on the evaluation and optimization of the critical process parameters of milling speed and milling time (as identified in the REM) as they determine the energy input and therefore the breakage rate of the drug particles, among other important process parameters [67]. In wet media milling, it is suggested that the breakup kinetics follow a first-order exponential decay, where the mean/median particle size approaches a limiting minimal particle-size value as a function of time [66,114–116]. The approximation of a lower particle-size plateau in prolonged milling can be explained by two major factors: (i) the formation of a dynamic equilibrium between particle breakage and the aggregation of the generated finer particles [70,109] and (ii) a decrease in the particle breakage rate thanks to the high strength of the nanoparticles, making them harder to break [105,117].
In this study, the evolution of the IMQ particle size during the wet media milling process follows an exponential decay (as outlined above), approaching a lower value of around 300 nm (although the grinding limit was not determined). In the generated DoE model, this is indicated by the fact that the linear and quadratic terms for the studied process parameters also have statistically significant impacts on the z-Average, leading to a curved shape for the response-surface plot. Other studies in the literature also observed similar results with statistical significance for the milling time and the rotational speed on the particle size [118,119]. However, the application of a first-order kinetic model is known to have limitations, particularly that the early milling requires the elimination of early-milling kinetic data [105,116]. In the generated model, this is observed in the regression equation for the particle size, predicting an apparent initial mean IMQ particle size of 837.4 nm when the rotational speed and the milling time approach zero, although the initial particle size is much larger (d10 4.96 µm, d50 13.02 µm, d90 26.39 µm).

The observed narrowing of the particle-size distribution over time (with the statistical significance for the linear term of the milling time) shows that an increased number of bead–bead collisions and drug particle compression is required in order to increase the extent of particle breakage and reduce the fraction of unmilled particles. When approaching the submicron (colloidal) size domain during milling, coarser particles continue to break (albeit slowly). In contrast, finer particles below a certain size limit will not break at all, or will break much slower, because they are harder to capture by beads and because the intensity/number of stressing events by the colliding beads may not be sufficiently high to further break them up [105,120]. Hence, this over time leads to a tightening of the particle-size distribution, as observed in this study.

Among the conducted pharmaceutical wet media milling studies in the literature, statistical approaches, including the response-surface method, as used in this study, are reported to be the most widely used [61]. Such empirical approaches allow a good understanding of the interaction effects and the selection of optimal experimental conditions, as observed in this study. The identified optimal process conditions lead to IMQ nanocrystal manufacturing at the targeted particle-size region with an acceptable accuracy between the

predicted and observed values of the z-Average and the PdI. Conducting additional experimental runs and studying other important process parameters, e.g., the number of beads and the bead size, will improve the predictability of the model between the process parameters, particle size and PdI.

A major limitation of statistical approaches, however, is their lack of including the underlying physics governing the milling process. One method to overcome this drawback is to use mechanistic modeling. In this approach, the complex physics occurring during wet media milling are modeled at different length scales (fluid mechanics, contact mechanics, bead collisions, particle capture, particle breakage, etc.), providing mechanistic-phenomenological modeling on a spectrum [61]. With, e.g., computational fluid dynamics (CFD), the discrete element method (DEM), population balance modeling (PBM) and microhydrodynamic modeling (MHD), the modeling of the wet media milling process may provide an improved quantitative fundamental understanding of the process, allowing for better process understanding and optimization. For example, Bilgili's group adapted and refined a comprehensive MHD model first developed by Eskin [104] for the wet stirred media milling of pharmaceutical drug substances.

Despite the capability of the wet media milling process to produce nanosuspension for a broad variety of poorly water-soluble drugs, the process is associated with several drawbacks and knowledge gaps, particularly its processing-operational aspects: (i) the process is costly and energy intensive [121]; (ii) the process is time-consuming, with processing times ranging from hours to a day(s); (iii) the understanding of the stabilizer impact during milling beyond its stabilizing role is inadequate [61,105]; (iv) bead wear during processing leads to product contamination [67,120,122]; and (v) the scale-up of the process is mostly empirical [105,116]. When transferring between different equipment types for the manufacturing of nanosuspensions, challenges often occur because of differences in the geometry, mode of operation and power density of the mills, differently affecting the breakage kinetics and milling efficiency [67]. The milling behavior of a specific mill is characterized by the stress event intensity and the stress energy connected to each of the stress events [123]. Hence, the outcome of the comminution process is determined by the stress event frequency and intensity. While the optimized process showed an acceptable milling efficiency, an increase in milling efficiency may be required when moving to a larger device with a similar or different operating principle. Planetary ball mills, as used here, are more subject to early-phase development when the drug substance availability is limited, delivering small-scale suspension volumes, whereas wet stirred media mills are frequently used for pilot- and large-scale manufacturing, delivering larger suspension batch sizes at comparable or faster milling times [124]. The milling efficiency of the presented process may be increased by increasing the bead loading, as shown by Colombo et al. In their study, an increased milling efficiency for higher numbers of beads during the preparation of dexamethasone nanocrystals was observed [125]. Alternatively, the potential usage of various hydrodynamic parameters, e.g., the proposed milling intensity factor F by Afolabi et al. [66], may be useful for scale-up.

2.4.5. Formulation Performance Testing

For IMI-Gel, the essential evaluated attributes are the chemical stability ensuring the pharmacodynamic activity; the physical stability influencing the decelerated IMQ skin permeation as a function of the mean particle size; the stability of the emulsion and cream; and the microbiological stability (quality). As observed in chemical stability testing, the chemical stability of IMQ is not a critical attribute, indicated by the complete lack of loss in the drug product content and the complete lack of detectable levels of related substances, revealing that the pharmacodynamic activity is ensured over the shelf life of the product.
Physical stability for IMI-Gel refers to the physical stability of the IMQ nanocrystals in that they determine the decelerated IMQ permeation into the skin. As discussed in Section 4.3.2., the used surfactant polysorbate 80 leads to the good stabilization of the IMQ nanocrystals, revealing the physical stability of the nanosuspension over the shelf life and ensuring the targeted performance. In addition, physical stability refers to the structural stability of the product in that this determines the residence time at the site of action and the

release of IMQ. Structural stability investigations by rheological analysis reveal good structural stability and no sensitivity toward shear or thermal stress (see [99,100]).

In terms of the microbiological stability, the stability tests reveal acceptable performance in terms of the inhibition of microbiological growth, but with the inability of the used surfactants to fulfil the test criteria of the monograph Ph.Eur. 5.1.3. on the efficacy of antimicrobial preservation. Thanks to the ability of the preservatives to inhibit microbiological growth, the regular authority agreed on a short in-use stability over 2 weeks after opening the primary packaging upon consultation. For further proceeding in drug product development, an evaluation of alternative preservatives, e.g., propylene glycol, isopropanol or ethanol, may yield favorable results fulfilling the test criteria of a conservation load test in Ph.Eur. 5.1.3. However, the compatibility with the IMQ nanocrystals and the oil phase needs to be demonstrated.

2.4.6. Control Strategy

The implementation of IPC and QC tests on the intermediate and final product(s) is intended to meet the targeted CQAs of the product, as defined in the acceptance criteria. While for all identified CQAs, the appropriate IPC or QC tests are installed, rheological quality control testing is excluded. The rationale for this decision is based on the fact that individual viscosity values or yield points do not represent material constants [126] and are therefore not considered as predictors of formulation performance. Instead, it is necessary to evaluate the structural stability of the formulation over its shelf life with the target attribute to maintain the structural stability over the shelf life of the formulation. This is indicated by no significant change in the flow properties and the ability to achieve structural recovery after the application of shear stress. The results from an organoleptic evaluation and, second, a rheological investigation using oscillatory three-interval-step tests have shown this attribute for IMI-Gel (see [99,100]).
Given the IPC and QC data, the application of the QbD concept enabled the development and manufacturing of high-quality IMPs with small batch-to-batch variability. For the mean particle size of the product, its observed small batch-to-batch variability outlines a robust and reproducible manufacturing process for the creation of nanocrystals close to the targeted range of ~400 nm, achieving the targeted product quality in terms of the particle size CQA. While for the batch IMI-Gel-180820, a higher mean particle size with a higher standard deviation than for the other batches is observed, the observation is not considered critical, because the resulting mean particle size is well within the acceptance criterion. In addition, the observed range of the particle size falls within the size range in which the particles migrate into the hair follicles.

For the content of IMQ, fluctuations are observed with a range of 10.15 % (difference between the batch with lowest mean content and with the highest mean content). While this difference may appear significant, it is uncritical as values do not approach lower or upper specification limits, being well within the defined acceptance criterion of the product. In general, observing drug substance concentrations at the lower acceptance limit might be critical for substances with limited chemical stability (meaning that the concertation of the drug reaches a content below 90% during or at the end of the shelf life). For IMQ, however, achieving lower concentrations within the defined limits is not critical, thanks to the good chemical stability and limited degradation. Achieving higher dosing than that targeted may lead to overdosing and increased side effects, which are not observed with the manufactured batches.

As for the detected level of impurities, negligibly low measured levels underline the good chemical stability of IMQ.

Given the level of preservatives, the observed trend toward lower levels for the latest batches is considered uncritical because it was shown that the efficacy of the preservative is not concentration dependent. Manufactured batches should have at least a minimal content of 0.04 % (that was found to inhibit microbiological growth), whereas larger concentrations do not positively affect microbiological growth

The QC test of the weight of the filled tubes is based on the requirement of minimum fill USP<755> included in the USP monograph imiquimod cream. The test is intended to test whether the patient has been provided with sufficient formulation. In terms of the weight of the filled tubes, it is important to provide the patient with sufficient formulation for the treatment of their AK lesions. Therefore, trends toward upper specification limits are not critical. During further proceeding, it is suitable to change the test by taking the mean weight of 10 packaging units and defining the total weight ± 15 % from the detected mean weight of the packaging unit batch.

In terms of the pH, minor variabilities may be neglected because the material attributes potentially affected by the pH (such as the gelling agent Carbopol and the paraben preservatives) are compatible with a broad pH range. The trend of the QC outlines pH values toward the upper specification limit. This achieved pH is not considered critical, because pH values toward the neutral range are reported to enable the maintenance of a stable gel structure for Carbopol-based gels, prevent IMQ crystals from solubilization and are well within the pH range where the paraben preservatives are able to inhibit microbiological growth.

In terms of the microbiological evaluation of IMI-Gel, no trend is observed, in that all tested batches fulfill the test criteria of the chapter Ph.Eur. 2.6.12.

2.5. Conclusions

QbD is a systematic process for the development of high-quality medicines with a prospective design of the product in a QTPP, the identification of CQAs and the collection, evaluation and optimization of material attributes and process parameters to meet the predefined quality objectives, linking CMAs and CPPs to the CQAs. In this part of the thesis, the concept of QbD was successfully employed for the development of a high-quality nanoparticulate IMQ emulsion gel formulation used as an IMP. The optimization of the product included the design of a QTPP with the identification of CQAs and a discussion on the selected CMAs that allowed for the selection of the excipients for the product. In addition, the optimization of the CPPs of milling time and milling speed by employing the statistical tool of DoE enabled the optimization of the process conditions for the manufacturing of IMQ nanocrystals in the target range of 300–400 nm at a minimal PdI. The results of the QbD process are depicted in the IPC and QC of the manufactured IMP batches, revealing small batch-to-batch variability and a product of high quality. Further studies on, e.g., material attributes, including other or additional surfactants at different concentrations or other process parameters in the milling process, e.g., number of beads and bead size or the optimization of the HPH process, may lead to a product of even higher quality.

3. Complaint Management

The content of this chapter uses text, tables and figures of the following publications:

Pielenhofer, J.; Meiser, S.L.; Gogoll, K.; Ciciliani, A. M.;Klak, M.; Lang, B.M.; Staubach, P.; Grabbe, S.; Schild, H.; Spahn-Langguth, H.; Langguth, P.; Complaint management of a quality defect in a nanoparticulate Imiquimod formulation in an investigator initiated academic phase I/II clinical trial Part 1 PharmInd, 2023 85(2), 182-186

And

Pielenhofer, J.; Meiser, S.L.; Gogoll, K.; Ciciliani, A. M.;Klak, M.; Lang, B.M.; Staubach, P.; Grabbe, S.; Schild, H.; Spahn-Langguth, H.; Langguth, P.; Complaint management of a quality defect in a nanoparticulate Imiquimod formulation in an investigator initiated academic phase I/II clinical trial Part 2 PharmInd, 2023 85(3), 294-299

The introduction of this part was modified from the above mentioned publications in order to make the text fit to the content of the thesis. In the first paragraph, exact text and wording was used in the parts from "In pharmaceutical industry[…]" to "[…] Good Manufacturing Practice (GMP) and Quality Risk Management (QRM)". In the second paragraph, exact wording and text was used from "[…] according to internally valid Standard Operating Procedures (SOPs)" to "[…] concerning the quality of the IMP". From there onwards, exact wording, text and figures of the published articles were used with the following exceptions:

- *As similar materials and manufacturing procedure were used as described in chapters 2.2.1., 2.2.4 and 2.2.6. the content was excluded in this section.*
- *Figures 16 and 17 were published as one figure in the journal articles whereas they were separated into two figures in this dissertation.*
- *In Figure 12, it was indicated which cream was stored at which temperature by adding "(left)" behind "40°C" and "(right)" behind "80°C"*
- *The content of table 8 was used as published, only the table format was adapted.*

I contributed in that work by designing the investigation, designing and performing the root-cause analysis, performing laboratory investigations and writing of the publication. The Co-authors contributed as follows:

- Conceptualization*: J.P., S.L.M., M.P.R, H.S.-L. and P.L.;*
- Methodology*, J.P., S.L.M., K.G., B.M.L., P.S., S.G., M.P.R., H.S.-L. and P.L.;*
- Software*, J.P. and S.L.M.;*
- Screening & inclusion of patients: *B.M.L., P.S., S.G.*
- Handing over IMPs: *B.M.L., P.S.; S.G*.
- Formal analysis: *S.L.M., K.G., A.-M.C., M.K., B.M.L., P.S., S.G., H.S., M.P.R., H.S.-L. and P.L.*
- Investigation*, J.P., S.L.M., K.G., A.-M.C., M.K., M.P.R., H.S.-L. and P.L.;*
- Resources*, M.P.R., H.S.-L. and P.L.;*
- Data curation*, J.P., S.L.M., K.G., M.P.R., H.S.-L. and P.L.*
- Writing—original draft preparation*, J.P. and S.L.M.;*
- Writing—review and editing*, K.G., A.-M.C., M.K., B.M.L., P.S., M.P.R., H.S.-L. and P.L.;*
- Visualization*, J.P., S.L.M. and H.S.-L.;*
- Supervision*, B.M.L., P.S., S.G., M.P.R., H.S.-L. and P.L.;*
- Project administration*, B.M.L., P.S., S.G., M.P.R., H.S.-L. and P.L.;*
- Funding acquisition*, M.P.R. and P.L.*

3.1. Introduction

Pharmaceutical manufacturers are obligated by law to establish and maintain procedures for recording, assessing, investigating and reviewing of occurring complaints including potential quality defects. The complaint management process is an integral part of the Pharmaceutical Quality Management system of the manufacturing organization. If necessary, this system may initiate an efficient recall of medicinal products for both human and veterinary use, as well as for IMPs, from the distribution network in case of inadequate product quality that poses a threat to public health [127]. The legal basis for these procedures is provided by the EudraLex Volume 4 Part 1 Chapter 8 "Complaints, Quality Defects and Product Recalls" and Annex 13.
In pharmaceutical industry, entire departments may be responsible for the management of complaints, which are related to quality defects concerning marketed medicinal products (according to the previously outlined regulatory framework). In an academic setting, however, manpower may be significantly smaller, but nevertheless, the system needs to adhere to the concepts of Quality Management (QM), Good Manufacturing Practice (GMP) and Quality Risk Management (QRM).

Although the previously disclosed QC data indicated that all batches of the IMP IMI-Gel tested in the respective clinical trial, were of high quality, a patient included in the clinical study filed a complaint regarding physical instability of the semisolid product when opening one of the tubes from one batch. This quality defect was reported while the clinical study was still ongoing. It is worth noting that a total of 12 batches were manufactured and sent to the Clinical Research Center (CRC) at the Department of Dermatology of the University Medical Center Mainz. Each batch typically produces 28 tubes, each containing 3 g of formulation, as well as 3 tubes filled with 5 g of formulation for microbiological analysis. The process of complaint management was conducted according to internally valid Standard Operating Procedures (SOPs). This includes first measures such as a site visit to the study center to assess the number of affected tubes or batches, informing of the regulatory agency and other related parties, initiation of a root cause analysis incl. structural stability testing by rheological analysis at storage and elevated temperatures and decision making regarding the necessity for Corrective and Preventive Actions (CAPAs) (see Figure 9) concerning the quality of the IMP.

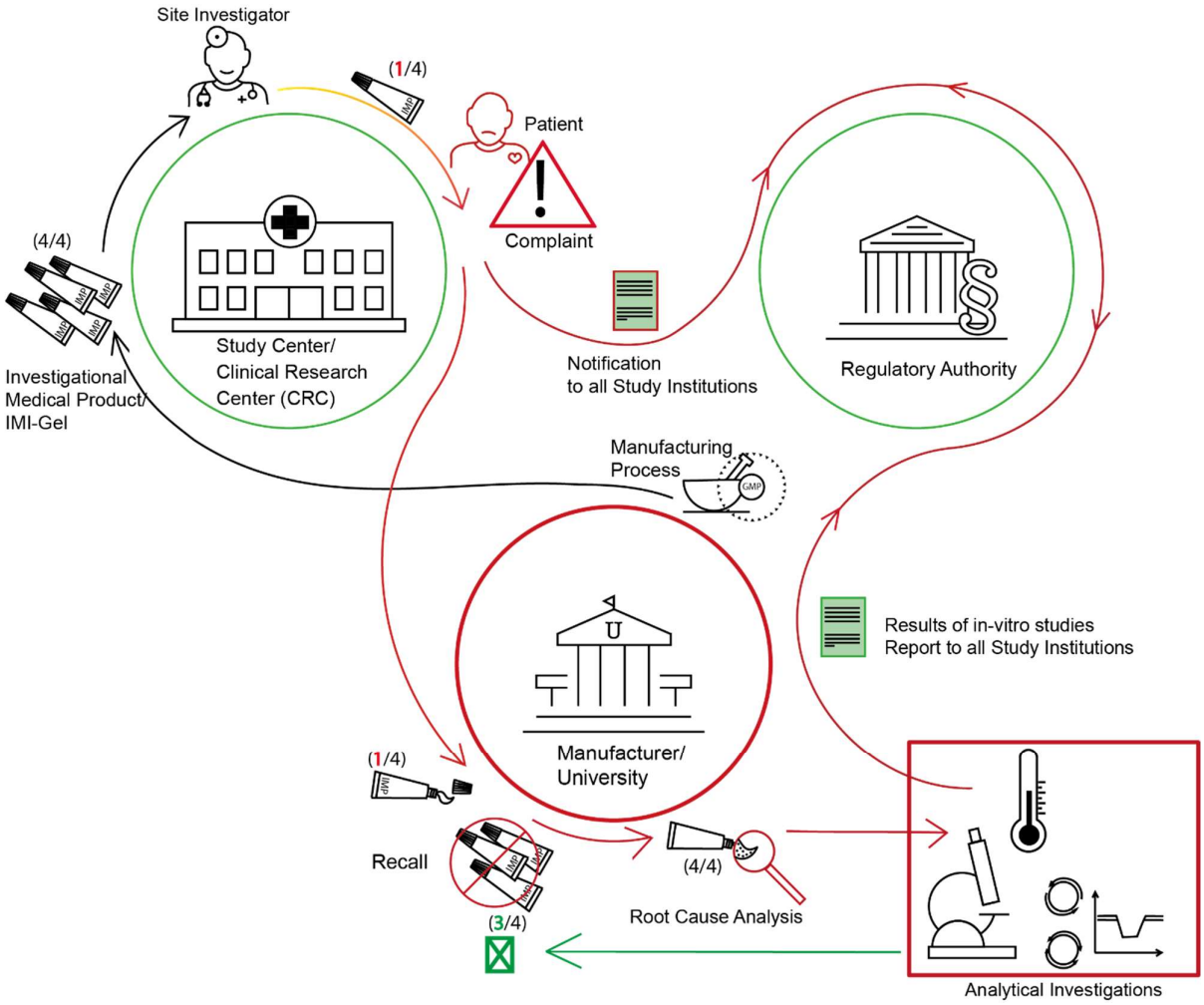


Figure 9. Schematic procedure for complaint management related to IMI-Gel. Manufactured and released batches are delivered to the study center where each patient in the IMP treatment arm receives 4 tubes of the IMP IMI-Gel. .Patient 56 filed a complaint related to the physical instability of the IMP the day after he received the first out of 4 tubes that were planned for his treatment. The responsible people for manufacturing of the IMP incl. the head of the Quality Assurance, the head of the Quality Control and the Qualified Person were informed about the filed complaint by the Site Investigator. The affected tube and the 3 remaining packaging units were replaced with 4 new IMP units of a different batch. Afterwards, the sponsor and the competent authority were informed by the Qualified Person regarding the quality defect. The quality defect was investigated by laboratory analytical investigations for root cause analysis and captured in a report.

3.2. Materials

For materials see 2.2.1.

3.3. Manufacturing of IMI-Gel

For the manufacturing process see 2.2.4. and 2.2.6.

3.4. The Complaining Patient within the Clinical Study Setup, Complaint Report and Complaint Event Management Strategy

3.4.1. Clinical Study Setup

Patients who fulfilled inclusion/exclusion criteria and for whom informed consent was obtained were randomly assigned to either the treatment arm of the reference product Aldara™ or the IMP treatment arm IMI-Gel according to the randomization schedule (1:1). AK lesions were treated in either 1 or 2 treatment cycles 3 times per week either with the reference product Aldara™ or the IMP IMI-Gel depending on the responsiveness to the treatment (complete responders after the first cycle are transferred to the follow-up period without the necessity to receive a second treatment cycle). In total, patients were treated either 4 or 8 weeks with the reference product Aldara™ or IMI-Gel. Patients in the treatment arm of IMI-Gel received a total of 4 tubes of the same batch of the IMP IMI-Gel covering the whole treatment period of 2 possible treatment cycles. During the treatment cycle, each tube was used over the period of 2 weeks. Following this first treatment phase the used tube was replaced during the next study site visit so that the patient possessed only 1 of the 4 tubes at a time. Patients were instructed on the appropriate handling and storage of the IMPs prior to receiving the first tube of the IMP from the clinical site staff/personnel (see also fig. 9).

3.4.2. Complaint Event

On the respective Day 1 of an open clinical phase-I/II study in 82 subjects (having been performed during a total period of 30 months at that time), Patient 56 reported a quality defect to the study center, stating that the cream of the first tube he had received (from the 4 tubes planned for his treatment) was not a cream and not homogenous, but liquid was dropping out of the tube opening and the remaining yellowish-white material could not be easily applied on the skin. This represented the first and only complaint filed regarding the quality of the IMP from this study, for which a total of 12 batches had been manufactured and released. The tube the complaint referred to was from the batch IMI-Gel 260121 and at the time 7 months old, i.e., well within the 12 months shelf life of the formulation.
After having received the complaint, the following steps were taken immediately:

1. The study center informed the manufacturer regarding the quality defect for one tube of the IMP IMI-Gel.
2. The patient was assigned to 4 new packaging units out of an alternative batch from the Clinical Research Center. One of the four tubes from the alternative batch was handed to the patient, while he returned the complained tube he had received the day before. The 3 remaining tubes of the complained batch (which were assigned to this patient and still located at the study center) were transported back to the manufacturing and quality control site. Moreover, the patient was asked to report how he handled the IMP he complained about.
3. The sponsor of the clinical trial, the site investigator at the study center and the competent authority were informed by the Qualified Person regarding the quality defect and the withdrawal and replacement of the packaging units the patient had received.

4. The action plan included: All 4 tubes (1 of them opened and returned, 3 unopened and withdrawn from the study center) were transferred back to the manufacturer for evaluation and cause analysis. It was considered, which component of the emulsion represents the liquid phase that was leaking out. Known causes for polyacrylate gel (continuous phase) instabilities might be deviations in pH, the presence of bi- to multivalent cations and temperature stress.
5. The action plan included: (A) Identification of the liquid component released from the emulsion, (B) a pH test with non-bleeding pH indicator strips pH 4.0–7.0 and (C) an evaluation of quality defects in the primary packaging leading to a potential presence of aluminum ions and (D) studies on temperature dependance of physical stability inside the tube (open vs. closed) and outside of the tube.

3.5. Temperature Stress Tests under Different Conditions and Evaluation of the Structural Stability

3.5.1. Visual Organoleptic Observation of Formulations kept at 40 °C and 80 °C

The temperature stress test under different conditions was performed to evaluate the effect of elevated temperatures on the physical stability of the IMP. The day the tube was collected by the patient was a warm summer day in the mid of August with temperatures reaching up to 29 °C. Storage of the IMP inside the patient's vehicle might have led to temperatures beyond the defined storage conditions from 15–25 °C in a scenario of direct sun exposure or even at warm summer temperatures. To experimentally simulate such a potential handling incorrectness, 3 samples of 3 different batches of IMI-Gel were stored:

i. inside of their original primary packaging for 24 hours (screw caps kept closed),
ii. inside of their primary packaging with a removed screw cap and
iii. outside of their primary packaging on a petri dish placed inside of 2 Ecocell 55 drying chambers set to 40 °C or 80 °C, respectively, for 3 hrs. Photos were taken for documentation after 1, 2, and 3 hours of storage at the respective temperature.

The appearance of the formulations was ranked based on the organoleptic evaluation of the surface structure and regarding the extent of phase separation with the following characteristics (see also fig. 10):

- 0 = initial appearance maintained, no oil ring detectable at the tube outlet (no change in quality),
- + = slight change in quality, a shiny appearance of the surface, however, with no significant phase separation (oil ring ≤ 5 % of the total surface at the tube outlet)
- ++ = start of significant phase separation, uneven surface structure (oil ring >5–10 % of the surface at the tube outlet)
- +++ = clear phase separation with rough, frizzy, and irregularly rugged surface structure (oil ring >10–20 % of the surface at the tube outlet).

The temperatures for the stress test were chosen to simulate extreme short-term conditions. If exposed to direct sun, the temperature inside a vehicle would have risen up to 40 °C at an external temperature of 24 °C after 30 min of standing time/parking [128] or to even 70 °C [129] to 80 °C [130] after 60 min of standing time/parking at higher outside temperatures.

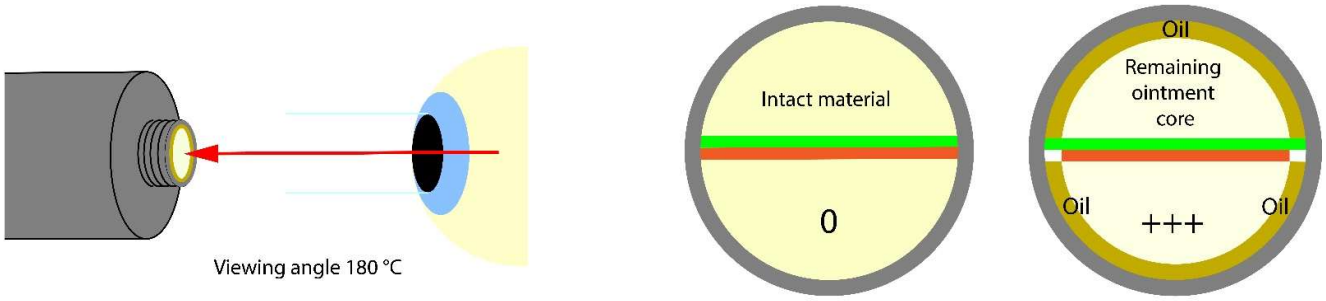


Figure 10. Scheme for 3.5.1. (ii) – Semi-quantitative estimation of extent of oil leakage out of the drug formulation: The grey circles represent the outlet of the aluminum tube when the cap is removed from the screw thread. After having exposed the unscrewed (opened) tubes to elevated temperatures, the content was inspected at the outlet. For this purpose, the outlet was viewed at a surface angle of 90°, i.e., the tube was oriented in direct line in front of the eyes as depicted on the right side of the scheme. The surface was monitored and the percent reduction of diameter of the ointment core was estimated (see scheme on the right). In the current example, a 14.6 % reduction of the size of the ointment core was detected/is depicted schematically, the distance to inner surface of the tube outlet being filled with oil. (Green line: inner diameter, red line: diameter of the remaining semisolid part of the preparation, white line (behind red line): liquid (oil); the extent of oil leaking, which is schematically depicted on the right side of this scheme (reference in the middle), would be ranked +++.)

3.5.2. Temperature Sweep Tests

For the temperature sweep test, 8 oscillating temperature ramps with 4 heating and 4 cooling intervals were performed. Each heating interval consisted of heating of the formulations from 20–80 °C, over the period of 600 s, followed by a cooling interval where formulations were cooled from 80–20 °C also over 600 s. During the test, behavior nearly at rest was recorded by oscillating the formulations within the linear viscoelastic (LVE) region at a controlled stress of τ_0 = 2.5 Pa at a frequency of 1 Hz. The test was performed using a Thermo Fisher Haake Rheostress 1 Rheometer equipped with a titanium cone with a shaft length of 77 mm, model P 35 Ti L.

3.5.3. Structural Stability Analysis by Rheological Tests

Structural stability of the IMP IMI-Gel was assessed for 3 batches stored at 25 ± 2 °C/60 ± 5 % relative humidity (RH) over 12 months and at 40 ± 2 °C/75 ± 5 % RH over 6 months according to long-term and accelerated storage conditions as outlined in the ICH Q1 A (R2) guideline [10]. Rheological tests were performed using a Thermo Fisher Haake Rheostress 1 Rheometer equipped with a titanium cone with a shaft length of 77 mm, model P 35 Ti L. Formulations were rheology characterized at different time points during storage for the respective batches being initially after the manufacturing process and after 1, 3,6, and 12 months with the 12 months' time point being recorded for the long-term storage conditions (25 ± 2 °C/60 ± 5 % RH). The flow behavior of the batches was assessed by applying a rotational test using a rotational ramp (step) at a controlled rate (CR, $\dot{\gamma}$) from $\dot{\gamma}$ 0.000–100.0 1/s over a period (t) of 30 s at a defined temperature (T) of 25 °C. Prior to designing the step test to evaluate the structural regeneration of formulations stored under the two ICH conditions over the shelf life of the product, the LVE region of the product was assessed by performing an oscillatory amplitude sweep test. The amplitude sweep was performed at controlled stress (CS, τ_0) from τ_0 0.000–100.0 Pa at a frequency (f) of 1 000 Hz. The LVE was identified of being within 1–10 Pa. The structural regeneration was assessed by performing a step test with 3 test intervals Oscillation/Rotation/Oscillation (ORO) with the oscillatory intervals being performed at CS of τ_0 2.000 Pa and f 1.000 Hz for t 120.00 s at T 20.00 °C inside of the LVE region followed by a rotational test at $\dot{\gamma}$ 100 1/s for t 60 s at T 20.00 °C followed by an oscillatory interval with similar conditions as in interval I.

3.6. Data Analysis

3.6.1. Measures

Extent of oil leakage was detected via observation and estimated semi-quantitatively through measurement of the diameters inside the unscrewed tubes. Rheological data were recorded using a Haake Rheo Win Job Manager Version 4.30.0001. For the respective measurements the following parameters are of relevance: G' (which measures the elastic component) and G'' (which measures the plastic component). The shear viscosity (η) is another important output of the rheological tests.

3.6.2. Graphs

Rheological graphs were plotted using Haake RheoWin Data Manager Version 4.88.001. The temperature sweep test was plotted as a logarithmic plot for G' and G'' and the temperature as the second y-axis against the time. The flow curves were plotted as overlayed curves from the data obtained from the different measurement time points as double logarithmic plots with the viscosity against the shear rate $\dot{\gamma}$. For the Oscillatory step test or interval test, G' and G'' were plotted as a logarithmic plot in the first and third interval against the time. In the second interval, the logarithm of the viscosity was plotted against the time. The scheme for the semi-quantitative estimation of the extent of oil leakage and the procedure of complaint management was designed using Adobe Illustrator 2022 Software.

3.7. Results

3.7.1. Evaluation of the General Relevance and Related Steps

Figure 9 demonstrates the procedural steps taken concerning the complaint for the IMP IMI-Gel. Following that procedure captured in an SOP, within the existing QM system, the first action after being noticed of the complaint was a site visit at the study center to investigate which tube(s) or batch(es) were affected. There, it was observed that during the whole study and from all the batches manufactured and used only 1 single tube of 1 single batch was affected, yet revealing a clear quality defect related to the physical (in)stability of the IMP IMI-Gel as shown in fig. 11. A clear phase separation between the disperse oil phase and the continuous phase of the formulation was visible. Other remaining tubes from the same batch, which were stored in the CRC, were also investigated for quality defects, however, no quality defect was observed in any other case (see fig. 11). Internally for safety reasons, it was decided to recall all remaining tubes of the affected batch from the study site (the only remaining tubes of this batch were the 4 tubes of the complaining patient). The patient who filed the complaint received 4 new tubes from a different batch (each patient in the treatment arm of the IMP receives 4 tubes each containing 3 g of the IMI-Gel formulation for the treatment). Secondly, the complaint related to the quality defect was documented in a report in collaboration with the Qualified Person, the Head of the Quality Control Unit and the Head of the Quality Assurance and reported to all relevant parties being

i. the regional regulatory authority „Landesamt für Jugend, Soziales und Versorgung“ (LSJV)
ii. the sponsor being the University Medical Center Mainz and
iii. the site investigator at the CRC study site

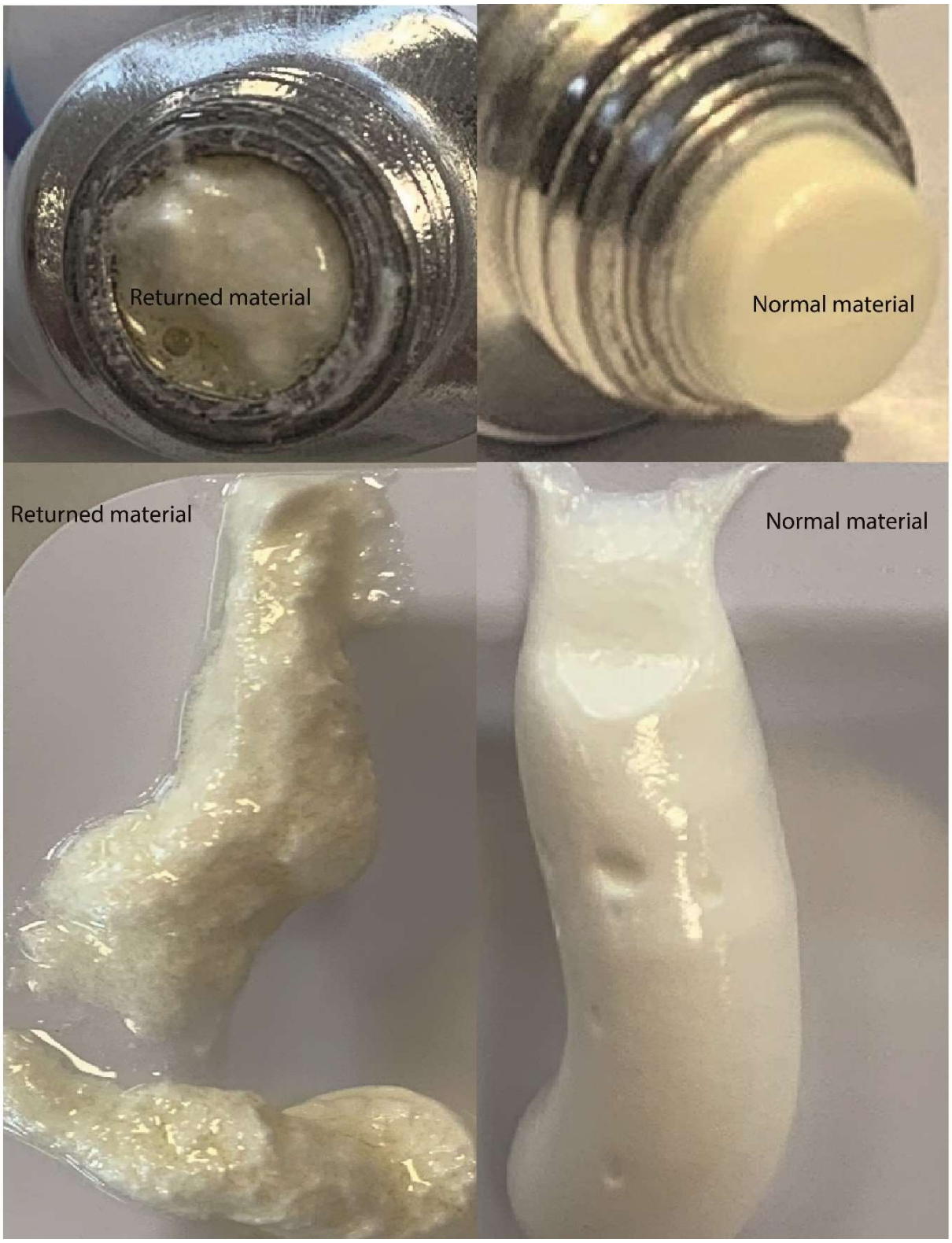


Figure 11. Comparison of the appearance of the returned material (left) and "normal" material (right). Opened tubes (top) and content (bottom). The returned material on the left is showing leakage of liquid from the formulation, where the remaining semisolid core exhibits a rough, frizzy, irregularly rugged structure (zoom factor similar for all images).

3.7.2. pH Test and Evaluation of Primary Packaging for Potential Defect

The pH of the affected tube and the remaining tubes was measured with non-bleeding pH test strips since known causes for a polyacrylate gel (continuous phase) instabilities might be deviations in pH. Measurement of the pH did not indicate any pH shift with the measured pH being 5.8, which is not different from the measured pH of 5.8 measured during quality control testing. In addition, the retention samples were investigated for changes in their quality regarding the pH. There as well no change in the pH was observed. Visual inspection of the affected tube for damage leading to transitioning of multivalent cations from the primary aluminum packaging into the formulation or slow evaporation of water out of the formulation did not reveal damaging on the interior or exterior part of the primary packaging. Consultation of the manufacturer of the primary packaging for known complaints regarding the used batches resulted in no reported quality defects.

3.7.3. Studies on the Structural Stability

As a route-cause analysis, further structural stability testing was initiated of the IMI-Gel formulation. The primary hypothesis was that the disputed tube was stored at elevated temperatures potentially affecting the physical stability of the IMP outside of the declared storage conditions of 15–25 °C. This hypothesis was based on the fact that on the day the tube was collected by the respective patient, outside-temperatures were reaching up to 29 °C. In cases where the IMP would have been left inside of the patient's vehicle, the overall temperature inside of the vehicle might have risen up to 56 °C during a vehicle standing time of 1 h [128]. Temperatures may be even higher in parts directly exposed to sun (possibly reaching 70 –80 °C at the dashboard [129,130].

3.7.3.1. Physical Stability Inside the Closed Tube

Following up on that hypothesis, a first test was performed where the 3 remaining tubes of the respective batch were stored inside of their primary packaging in a temperature stress test at 40 °C and 80 °C for 24 hrs. There, no phase separation was observed as depicted in Figure 12

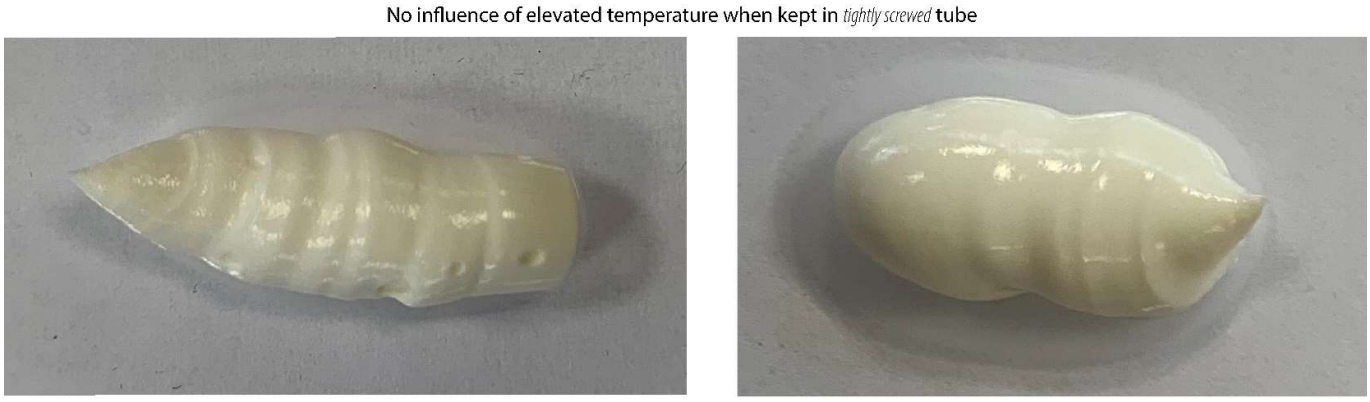


Figure 12: Visual appearance of IMI-Gel formulations after 24 hrs of storage at 40°C (left) and 80°C (right) inside the tightly screwed primary packaging: Inspection of the physical stability of the emulsion did not indicate apparent instabilities. (Here, the shadow zones (surrounding liquid at the border of the formulation) represent water (condensed from the heated formulations which were poured onto cooler petri dishes after the storage period).)

3.7.3.2. Physical Stability upon Elevated Temperature when Outside the Tube

When in the second test formulations were stressed by storing them outside of their primary packaging at 40 °C and 80 °C, it was observed that phase separation occurred quickly after a storage period of as short as 1 h at

80 °C (see Figure 11 "++"). For the samples stored at 40 °C it was observed that the formulation already showed a shiny surface structure, shinier in comparison to the initial appearance when stored for 1 h outside of its primary packaging, which is indicating the start of the phase separation process and leaking of jojoba wax. Clear phase separation was observed after 3 hours of storage at 40 °C or higher with the oil phase being separated out from the formulation (see Figure 13 and 14 as well as table 8). The phase separation of the formulation is explained based on the evaporation of water when the formulations are stored outside of their primary packaging leading to a collapse of the gel structure due to insufficient hydration of the polyacrylate gelling agent and coalescence of the disperse oil phase

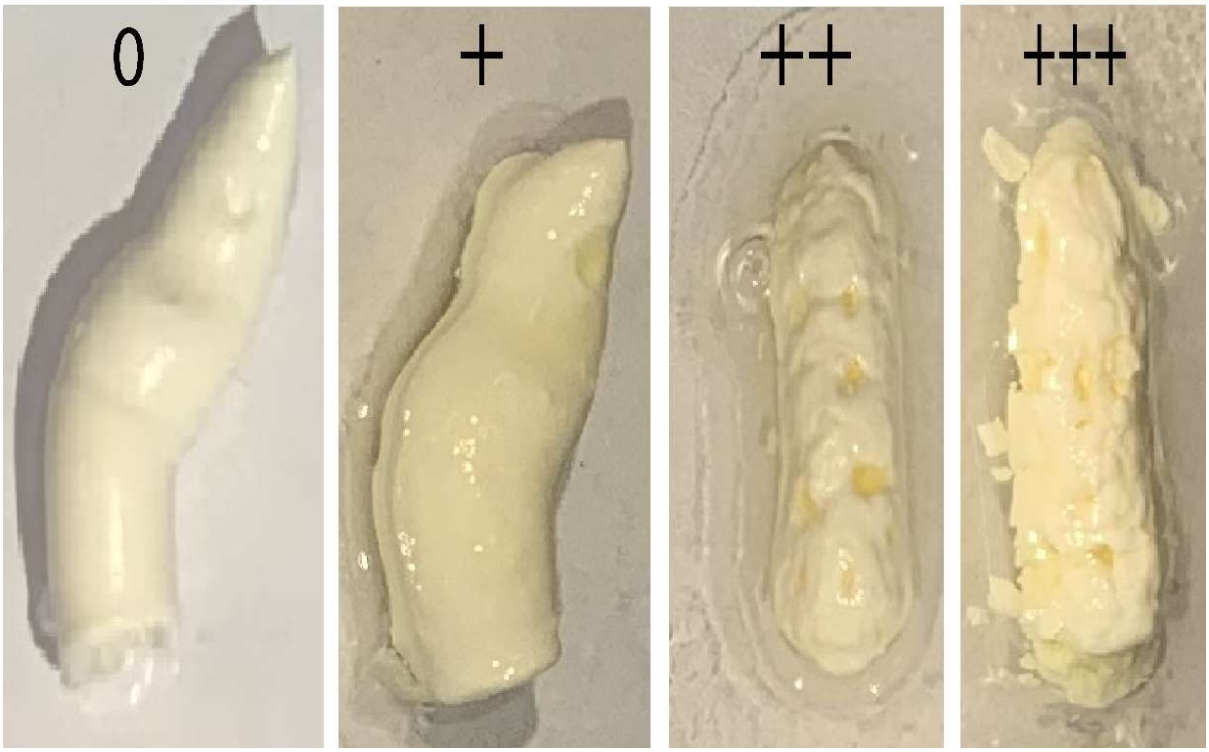


Figure 13:: Physical stability A – visual appearance of IMI-Gel formulations when kept at elevated temperature after being taken from the tube (see also Figure 11, table 8 and Figure and scheme for 3.5.1 for ranking and respective categories): 0 = start conditions or no change upon treatment, + = slight oil leakage, ++ = phase separation apparent (reduced strand diameter), +++ = significant phase separation with loss of regular strand shape (Here, the liquid surrounding the emulsion core is jojoba wax.), +++ was found, e.g., after 3 hours at 80 °C (cream strand being kept at elevated temperature after being taken out of the tube).

Table 8. Semiquantitative (visual) analyses of the formulation's physical stability when incubating *unscrewed* tubes (= opened primary packaging) under different defined temperature conditions [quality ranking according to the definitions given in 3.5.1].

Measure	Initial condition	After 1 hour	After 2 hours	After 3 hours
Surface condition/ 40 °C (*)	0	+	+	+
Phase separation / 40 °C (*)	0	0	+	++
Surface condition / 80 °C	0	++	+++	+++
Phase separation / 80 °C	0	++	+++	+++

(* When stored at 40 °C with an opened cap, only the first third of the formulation directly exposed to elevated temperature and to other environment factors showed a commencing phase separation and slightly rigid surface structure, while the other fraction in the tube remained unchanged.)

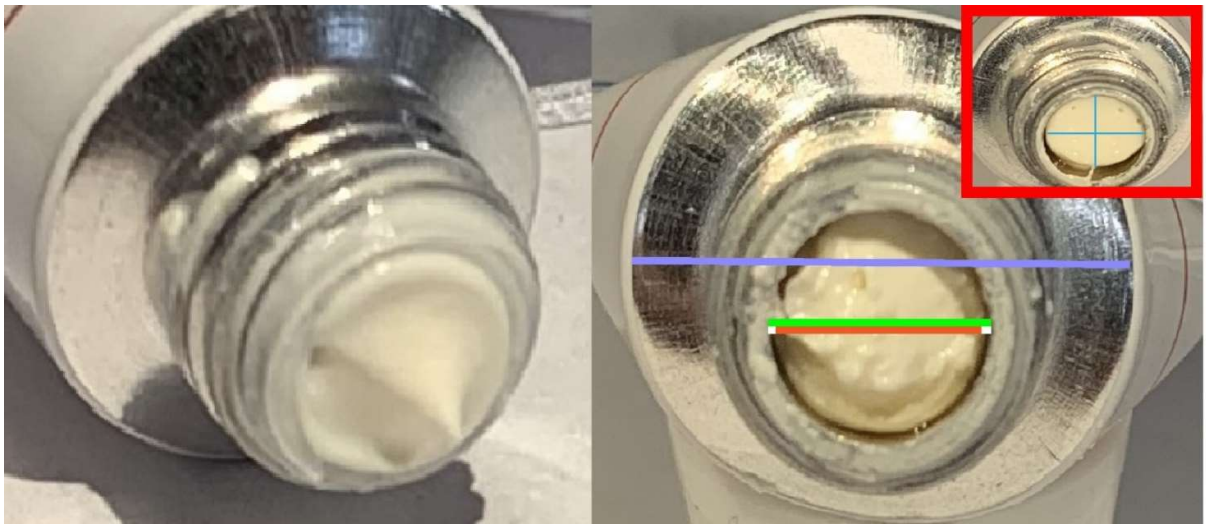


Figure 14:: Physical stability B – Visual appearance of IMI-Gel formulations inside the tubes with unscrewed caps. Tube examples as well as the mode of estimation/approximation of the extent of oil leaking are depicted (see also 2.4.1 (ii), scheme). Left: Unaffected content, right: open tube incubated at 80 °C for 2 hours. The blue line indicates the 13.5-mm diameter of the Aponorm tube. (The window in the right upper corner shows an ellipsoid instead of a circle. In such a case a comparable diameter was estimated through area calculation.)

3.7.3.3. Rheological Analyses

In a third step, rheological analyses were performed by an oscillatory temperature cycle test where the elastic module G' and the loss module G'' were recorded during heating and cooling of the formulation (see Figure 15). With this test, structural instability or changes in the microstructure of the formulations may be observed when the curves for the elastic modulus G' and the loss modulus G'' are approaching or when G'' is exceeding G' giving a hint of structural loss and incapability of the formulation to recover its structure. Considering the results for the IMP, the recorded graphs show an oscillating behavior of the elastic module G' and the loss module G''. Approximation of the curves or exceeding of G'' > G' was not observed revealing structural stability of the tested batches with insensitivity towards temperature changes.

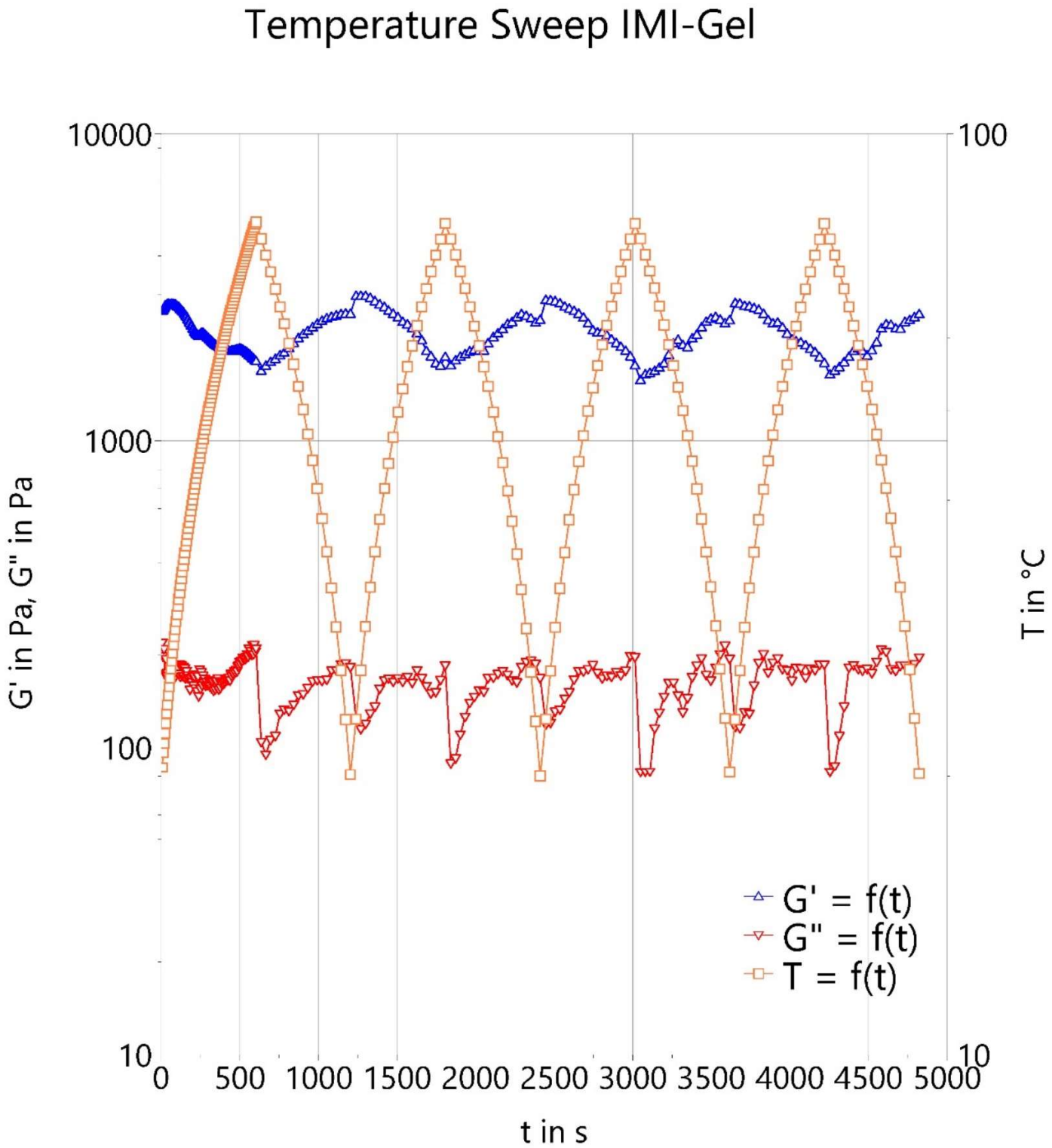


Figure 15:*:* Results of the temperature sweep test. The orange graph represents the 4 heating and cooling cycles where formulations were heated from 20–80 °C over the period of 600 s followed by cooling from 80–20 °C over the period of 600 s. The diagram shows the graphs for the storage modulus G' in blue and the loss modulus G'' in red revealing a periodic pattern for the G' and G'' graphs indicating structural stability of the formulation and temperature insensitivity.

To investigate whether changes in the microstructure occur over the shelf life of the formulation the flow behavior and the structural regeneration of the formulations stored at (i) 25 ± 2 °C/60 ± 5 % RH over 12 months and (ii) at 40 °C ± 2 °C/75 ± 5 % RH over 6 months were analyzed. Figure 16 reveals the obtained flow curves for the IMP stored under the respective conditions with graph A showing the flow curves for formulations stored at regular conditions according to ICH Q1A (R2) at 25 °C ± 2 °C/60 ± 5 % RH and graph B showing the flow curves for formulations stored at accelerated conditions according to ICH Q1A (R2) at 40 °C ± 2 °C/75 ± 5 % RH. The obtained flow curves reveal consistent plastic flow over the storage period with minor variabilities in the apparent shear viscosities for the tested samples. Figure 17 A and B reveal the results of the performed ORO step test simulating

(i) the behavior nearly at rest,
(ii) the structural breakdown behavior and
(iii) the structural recovery nearly at rest for formulations stored under accelerated conditions (17A) and long-term storage conditions (17B).

This is achieved by (i) oscillation within the linear viscoelastic region (LVE) within the first interval (evaluated to be between 1–10 Pa and 1–10 Hz) followed by (ii) application of shear stress through rotation followed by (iii) oscillation within the LVE region. The results of the data are displayed as the time-dependent functions of the storage modulus G', the loss modulus G'' and shear viscosity η. The diagrams for both storage conditions show a solid structure in the first test interval with G' > G'', a short liquid structure in the third interval with a quick recovery of the solid structure shown by a quick appearance of the crossover point G' = G''.

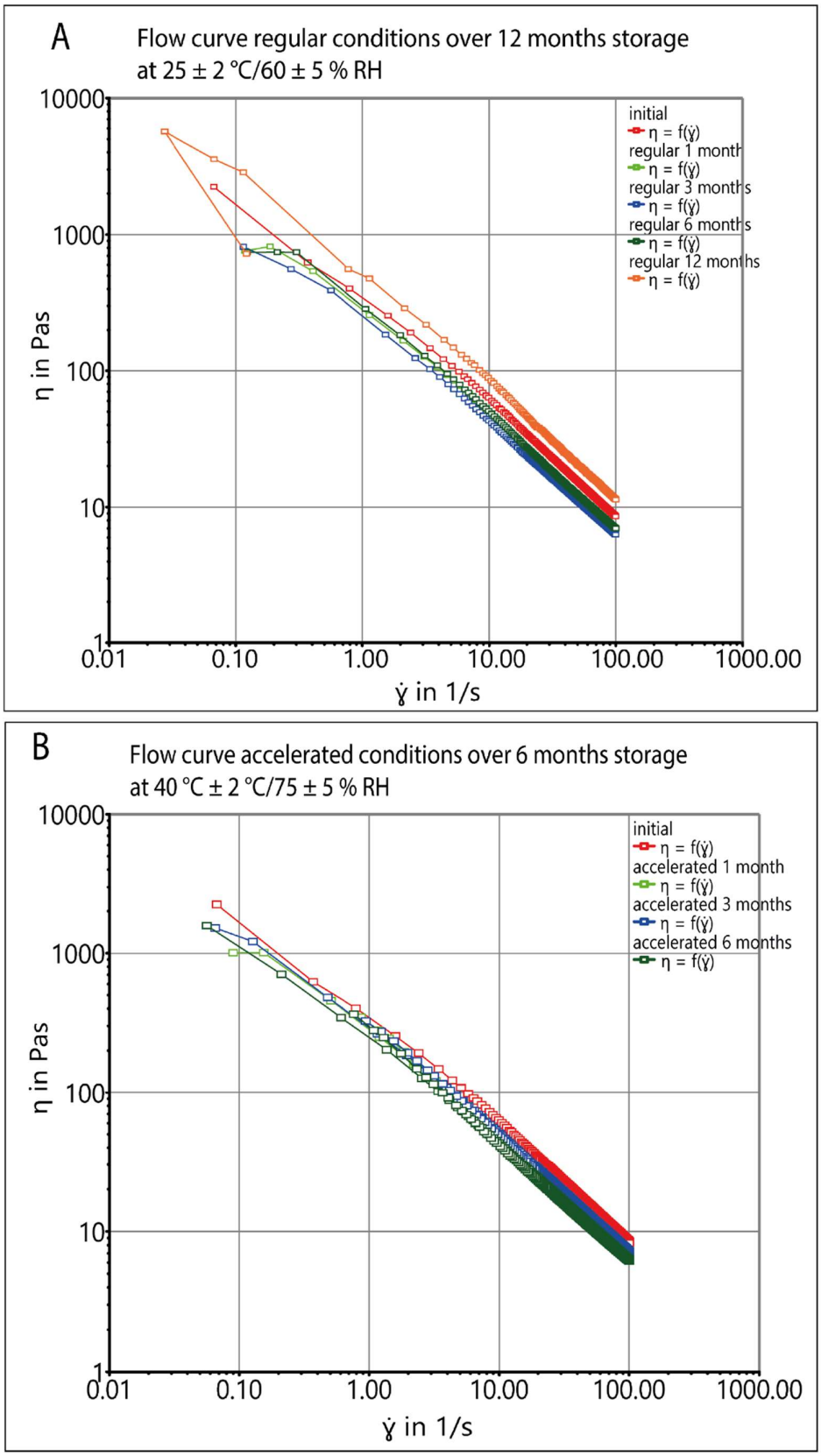


Figure 16: Flow curves for the formulations stored over 12 months under ICH regular storage conditions A and over 6 months under ICH accelerated storage conditions B. The storage period of the tested formulations is shown in red for initial analysis after manufacturing, in blue for 1 months, in magenta for 3 months, in brown for 6 months and in turquoise for 12 months of storage. The curves reveal no significant change in the flow behavior over the respective storage period, with the formulation revealing constant plastic flow behavior.

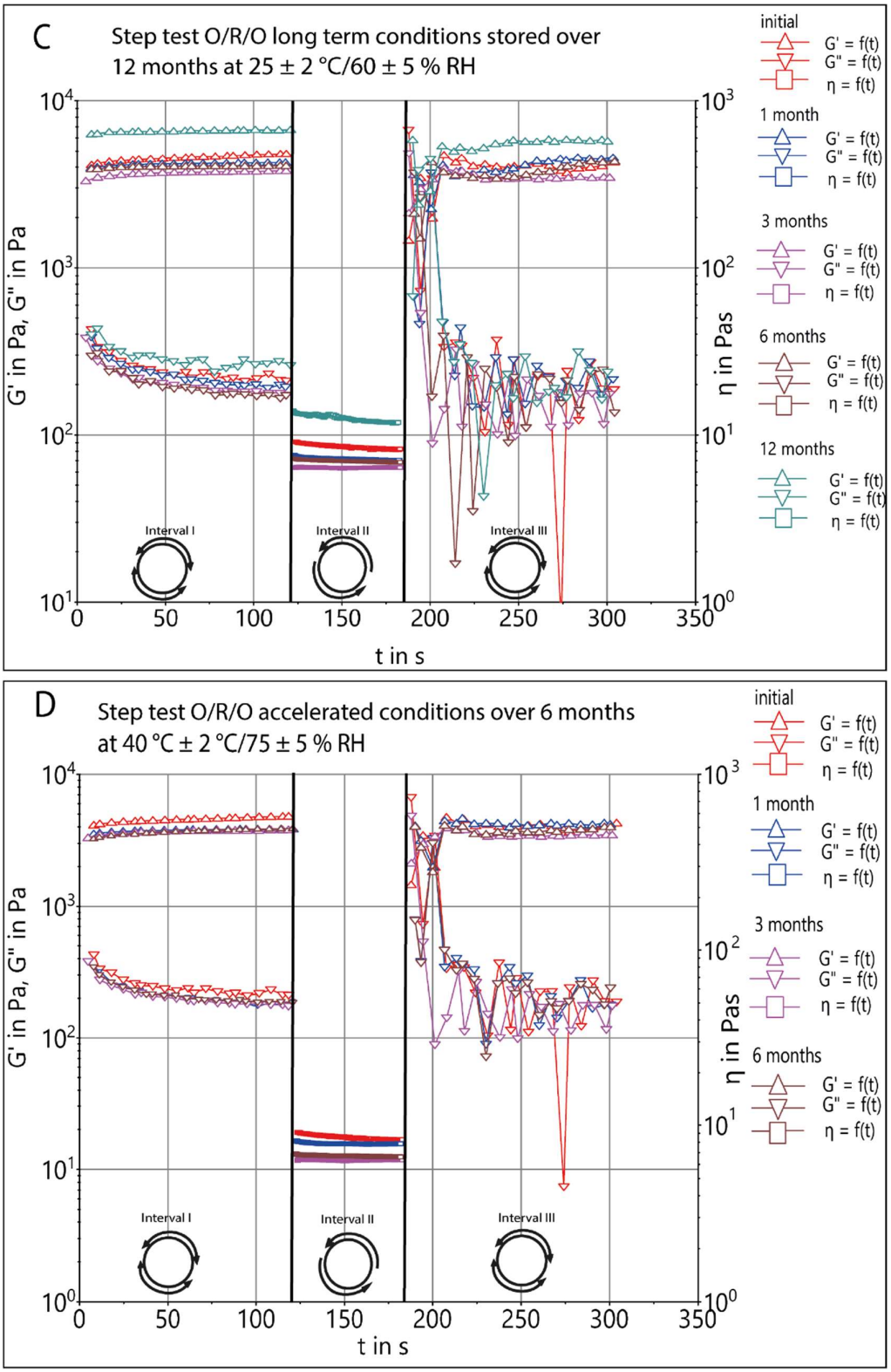


Figure 17: Three-interval step test: (i) oscillation within the LVE region from 0–120 sec showing the behavior of the formulation nearly at rest, (ii) application of shear stress by rotation at 100 s–1 from 120–180 s and (iii) regeneration of the structure measured by oscillation within the LVE region from 180–300 s for formulations stored under regular (C) and accelerated (D) conditions. The storage period of the tested formulations is shown in red for initial analysis after manufacturing, in blue for 1 months, in magenta for 3 months, in brown for 6 months and in turquoise for 12 months of storage. The curves reveal structural stability of the tested formulations over the storage period indicated by G' exceeding G" as well as the capability of the formulations to recover its structure after application of shear stress independently of temperature or applied shear stress.

3.8. Discussion

3.8.1. Considerations Regarding the Cause of the Physical Instability in 1 out of 384 Tubes Manufactured at that Time

The long-term physical stability of nano-disperse systems as present in IMI-Gel is a consequence of the small size of the disperse jojoba wax droplets and the IMQ nanocrystals impairing conventional destabilization phenomena like creaming, flotation, Ostwald ripening, sedimentation or coalescence [131]. Stabilization of the disperse jojoba wax droplets and the IMQ nanoparticles in the IMI-Gel formulation is achieved by addition of stabilizing excipients such as the surfactant polysorbate 80 (reducing the interfacial tension between the disperse oil phase, the IMQ nanocrystals and the continuous aqueous phase) and by addition of the hydrogel forming agent polyacrylic acid (increasing the viscosity of the continuous aqueous phase and reducing the diffusivity of the disperse phase within the formulation). Possible factors leading to the presented quality defect (the phase separation) include changes in the physicochemical properties of the formulation leading to failure of the stabilizing function for one of the contained excipients or induced stress in form of thermal or shear stress. For polyacrylate hydrogels, it is well known that pH [132] (alkaline and acidic conditions) as well as the presence of multivalent cations – even it traces – may lead to polyacrylate gel bleeding or destruction of the gel network, facilitating coalescence and phase separation of the disperse oil phase. A change in pH leads to the reduction of the total number of charged acidic groups and reduced repulsion of the acidic moieties destabilizing the gel network. However, pH testing of the affected tube as well as the other 3 tubes from the affected batch did not reveal changes in pH. An instability through pH alteration was therefore excluded as root cause. Presence of aluminum ions would be a consequence of damage to the interior epoxy phenol resin coating of the primary packaging causing transition of aluminum from the primary packaging into the formulation, which would then be leading to destruction of the gel network. Moreover, damaging of the primary packaging facilitating evaporation of water and insufficient hydration of the gelling agent is another consideration, since such damage would cause collapse of the gel structure. However, visual inspection of the inner and outer part of the tube did not reveal any apparent defect. In addition, consultation of the supplier in terms of reported quality defects revealed no reported quality defect of the interior coating resulting. This leads to the exclusion of primary packaging as a root cause. Therefore, thermal and shear stress were evaluated.

3.8.2. Extended Stability Studies under Stress Conditions

The investigations concerning the physical stability of IMI-Gel under stress conditions reveal that only minor changes in the microstructure occur over the shelf life of IMI-Gel, when it is stored at ICH long term or under accelerated conditions. In addition, it was shown that temperature stress for formulations being adequately stored inside of their primary packaging do not affect physical stability, whereas exposure to elevated temperatures for IMI-Gel outside of its primary packaging leads to quick phase separation. While the root cause of the filed complaint related to the physical stability of IMI-Gel was not clearly identified, it was shown that the quality of IMI-Gel when stored and handled correctly is not impacted. EudraLex Volume 4 Part 1 Chapter 8 8.16 outlines this scenario as follows: “In cases where the true root cause(s) of the quality defect cannot be determined, consideration should be given to identifying the most likely root cause(s) and to addressing those” [127]. Considering the presented quality deficiency, the most likely root cause is an inconsistency (product handling not according to general instructions), e.g., the patient may have opened the tube and exposed it to stability affecting conditions, such as sun or generally elevated temperatures. The investigations regarding the quality of the IMP have shown that in the clinical trial risks for participating subjects receiving treatment with the IMP IMI-Gel, which may be related to defects in quality of the formulation itself, are to be excluded with a high probability. Whether product handling or a rare defect of the primary packaging may play a role, cannot be finally concluded. However, based on the investigations performed and possible identified root-causes, it was decided that initiation of appropriate CAPAs is not

required since this was the first and only quality complaint filed for all of the manufactured tubes. In cases of additional quality defects, further investigation enabling the decision making of the relevance and implementation of appropriate CAPA measures will be performed.

4. Clinical Trial Results

Note:

The content of this chapter uses content and methodology of the clinical study protocol as submitted to the regulatory authority Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM) and to the ethics committee of the Medical Center of the Johannes Gutenberg-University Mainz written by Prof. Dr. Markus Radsak:

Radsak, M. Clinical Study Protocol Version 2.3. Open Label, Randomized Pilot Study to Evaluate Safety and Tolerability of a Novel Imiquimod Formulation in the Treatment of Actinic Keratosis (AK) 2018, 1–77.

I participated in the clinical study part as the clinical monitor by conducting monitoring visits prior to and during the clinical study in according to GCP principles, as well as collecting data from the case report files and performing the statistical analysis of the collected data. As previously outlined, I also performed the quality control analysis of the IMP manufactured for patients in this trial and coordinated the timely supply of the study medication IMI-Gel.

4.1. Introduction

The purpose of clinical trials is to evaluate the safety and efficacy of medical interventions, such as drugs, vaccines, and medical devices, in humans. Clinical trials aim to determine the optimal dosage and regimen of a given intervention, as well as to identify and mitigate potential risks or adverse effects associated with its use. By conducting clinical trials, researchers can collect robust data on the safety and effectiveness of interventions, which can inform regulatory decisions and ultimately improve patient outcomes. Clinical trials also play a critical role in advancing medical knowledge and driving innovation in healthcare.
In this monocentric, open-label, randomized control trial, the novel IMQ formulation IMI-Gel is investigated in humans for its safety and efficacy. In general, it is expected that patients benefit from the treatment with either IMQ formulation with a potential curative outcome since previous clinical trials with the approved product Aldara™ for the treatment of AK have demonstrated its efficacy in humans. Hence, no inferior efficacy or unexpected adverse events (AEs) resulting in a disadvantage for patients treated in the IMI-Gel group is expected.

Given that both formulations contain IMQ and have a similar mode of action, it is assumed that local AEs in both treatment groups will be comparable to those observed in previous clinical trials using Aldara™. These trials showed that approximately 96% of patients experienced local skin reactions at the application site, with symptoms typically of mild to moderate intensity (as assessed on a 4-point scale by investigators) and lasting for 1 to 4 days. In some cases, symptoms persisted for 1 to 2 weeks or longer, and in rare instances, adverse reactions necessitated treatment interruption or discontinuation. To minimize potential risks associated with the administration of Aldara™ or IMI-Gel and to ensure a uniform study population, specific inclusion and exclusion criteria were established for this study.

In total, 82 patients with at least 2 AK lesions were planned on being included in the study. Patients were randomized at a 1:1 ratio to either the Aldara™ group or the IMI-Gel group. Figure 18 and Figure 19 show the designed visit schedule according to the clinical study protocol.

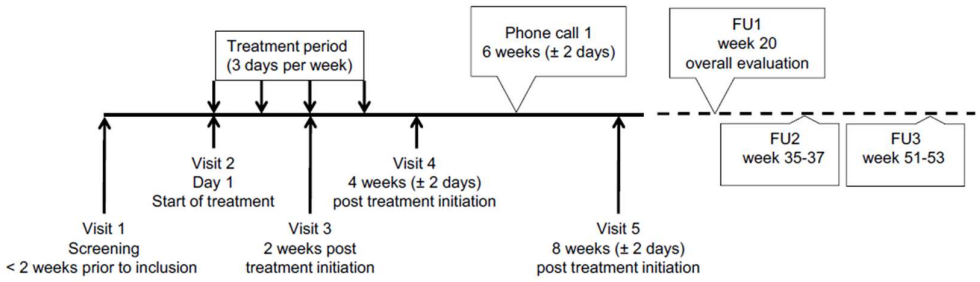


Figure 18: Summary of schedule for complete responding patients, taken from [133] After positive screening and patient inclusion, patients were either treated with Aldara™ or IMI-Gel depending on the randomization schedule over the course of 4-weeks followed by a 4-week off-treatment period. The outcome of the treatment was assessed at visit 5. Patients who showed complete clearance of the treated AK lesions (complete responders) were then directly transferred to the follow up period where during the follow-up visits (FU1, FU2 and FU3) reoccurrence of AK lesions and the cosmetic outcome were assessed.

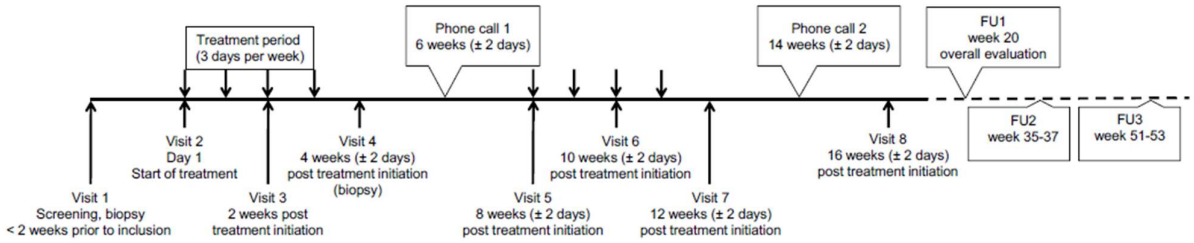


Figure 19: Summary of schedule for partial and non-responding patients, taken from [133]. After positive screening and patient inclusion, patients were either treated with Aldara™ or IMI-Gel depending on the randomization schedule over the course of 4-weeks followed by a 4-week off-treatment period. The outcome of the treatment was assessed at visit 5. Patients who showed no or partial reduction of the treated AK lesions (partial and non-responders) were then treated in a second treatment cycle over the course of 4-weeks followed by 4-week off treatment period with either Aldara™ or IMI-Gel. The outcome for patients of this group was assessed at week 8. Afterwards, patients were transferred to the follow up period where during the follow-up visits (FU1, FU2 and FU3) reoccurrence of AK lesions and the cosmetic outcome were assessed.

Patients were treated with either Aldara™ or IMI-Gel in 1 or 2 treatment cycles. Each cycle consisted of 4-week treatment in which patients administered the respective IMQ formulation 3x per week onto the AK lesions followed by a 4-week off-treatment period. Depending on the outcome after the first treatment cycle assessed at visit 5 (8weeks post treatment initiation), patients were directly transferred to the follow-up of the study in case all AK lesions were cleared (Figure 18).or had to start a second treatment cycle (Figure 19). During the follow up visits FU1, FU2 and FU3 reoccurrence of AK lesions and the cosmetic outcome were assessed.

To follow up on the hypothesis that IMI-Gel and Aldara™ are of comparable efficacy with superior tolerability for IMI-Gel, local skin reactions (LSRs) as measures for the tolerability were monitored and rated on a scale from 0-3 during the trial. These LSRs included erythema as the main LSR criterion and other skin reactions including edema, vesicles, erosions, ulcerations, scaling/flaking, scabbing/crusting and weeping/exudates. In addition, a comparative analysis of the skin quality after the treatment was conducted. For analysis of the efficacy, the reduction of the total lesion area and the patient response rates were measured and analyzed here.

4.2. Methods

4.2.1. Study Population

Study subjects were required to be ≥ 18 years of age with a clinical diagnosis being AK that is (1) not on the eyelid, lip vermilion, auditory canal or nostril and (2) not hyperkeratotic and at least two AK lesions (one may be used for biopsy) with an Eastern Cooperative Oncology Group (ECOG) score of 0-1 (score for physical activity with 0 meaning normal, unlimited activity and 1 meaning limited activity only under physical exertion but with the option to walk). Women of childbearing potential were permitted to participate in this study only if they had a negative serum pregnancy test at screening and a willingness to use a highly effective method of contraception. Subjects were required to be willing and able to sign the informed consent form.
Subjects were excluded from the study if they had used topical therapy with IMQ, diclofenac, ingenolmebutat, 5-FU, salicylic vaseline, steroid, retinoids; photodynamic therapy; UVA/UVB/PUVA therapy; laser abrasion or dermabrasion; chemical peel within the past two months or/and conducted topical treatment with moisturizers (creams, lotions, oils and other formulations) at the time point of screening and for the whole treatment period in the treatment region. Moreover, subjects were excluded from the study if they had surgery, therapy refractory AKs, or basal cell carcinoma, Morbus Bowen or squamous cell carcinoma (SCC) in the treatment region. Subjects were also required to not have used systemic steroids (inhal.+nasal allowed up to 1200µg/d Beclomethasone), immunosuppressive drugs (steroids, interferon, AZA, CsA) or retinoids in the past 4 weeks and no other study medication within the past three months. Other exclusion criteria included known hypersensitivity to imiquimod or any of the excipients in use in IMI-Gel or Aldara™, ECOG score >1, known HIV, HBV or HCV infection, any malignant tumors (other than the above mentioned), severe neurologic or mental disorders interfering with the ability to give informed consent, clinical severe auto-immune disease planned high UV exposition within treating phase (e.g. vacation, occupational exposure), organ transplant recipients, pregnant or lactating patients.

4.2.2. Primary Objective

4.2.2.1. Erythema Rating

The primary objective was a comparative analysis with regard to the overall patient local erythema rate in the Aldara™ and IMI-Gel treatment groups, assessed 4 weeks after the last treatment (at Visit 5 or 8 depending on the number of treatment cycles patients had to undergo). The primary null hypothesis (H_0, one-sided) was that the local erythema rate is equal upon treatment with Aldara™ and IMI-Gel. Based on the publication by Gebauer et al. [24] a local erythema rate (Rating ≥2) of 35 % for Aldara™, with IMI-Gel < 10 %. ($\Delta = 25$ %) was expected.

H_0: Local Skin reaction rate IMI-Gel = Local Skin reaction rate Aldara™

The primary alternative hypothesis (H_1, one-sided) is that IMI-Gel was associated with a lower erythema rate compared to Aldara™.

H_1: Local Skin reaction rate IMI-Gel < Local Skin reaction rate Aldara™

The analysis of the difference in Erythema rating was conducted using Fisher's exact test.

4.2.3. Secondary Objectives

4.2.3.1. Reduction of Total Lesion Area

According to the clinical study protocol, the size of each AK lesion was determined by measuring the 2 largest perpendicular diameters of the AK lesions at each study visit. The total AK lesion size is a result from the sum of all single lesion areas. The reduction of AK lesion area per patient was assessed by comparing the total lesion area (the size of all treated lesions added up) pre-treatment at baseline with the total lesion area at all visits. To calculate the lesion areas, an ellipsoid form of the lesions was assumed. The individual and total lesion area(s) were calculated as:

$$Lesion\ 1\ area\ \ [cm^2] = (radius\ 1\ Lesion\ 1\ x\ radius\ 2\ Lesion\ 1)x\ \pi \quad (10)$$

$$\text{Lesion 2 area } [cm^2] = \ (radius\ 1 \text{ Lesion 2 } x\ radius\ 2 \text{ Lesion 2})x\ \pi \quad (11)$$

$$Total\ lesion\ area\ [cm^2] = \ Lesion\ 1\ area + Lesion\ 2\ area \quad (12)$$

For the analysis, the difference of the AK lesions area at week 20 (equals Follow up 1 visit) from baseline was determined by conducting an analysis of covariance (ANCOVA) with the treatment groups treated as fixed factors, the difference in the mean skin area (skin area baseline – skin area at week 20) treated as the dependent variables and the baseline skin area of the respective lesions treated as the covariates. Prior to conducting an ANCOVA, the homogeneity of the covariate of the baseline skin area between the groups was calculated by conducting an ANOVA. There, it was confirmed that no significant difference between the baseline skin areas of the groups exists (p-value = 0.142 > 0.05). In addition, the homogeneity of the regression slopes of the baseline skin area and the treatment group was confirmed in an ANCOVA with a p-value of 0.158 revealing no significant difference between the regression slopes.

4.2.3.2. Patient Complete Response

Patient complete response rates at week 20 were evaluated by means of Pearson's chi-square test with Yates' continuity correction and assessed by means of the difference between response rates. A complete responder is defined as a patient in whom all AK lesions are cleared (no AK lesions were remaining).

4.2.3.3. Skin Quality Assessment and Overall Cosmetic Outcome

According to the clinical study protocol, the study personnel assessed and recorded the following characteristics on a scale from 0 = none, to 3 = severe, to describe the skin quality at the treatment area at baseline and at the end of the study:

- Skin surface (roughness, dryness, scaliness).
- Hyperpigmentation (independent of texture change or hypopigmentation).
- Hypopigmentation (independent of texture change or hyperpigmentation).
- Mottled or irregular pigmentation (both hyper- and hypopigmentation).
- Degree of scarring (independent of pigmentary changes).
- Atrophy.

Based on the skin quality assessment, the cosmetic outcome was calculated as described in table 9:

Table 9. Rating of the skin quality outcome for AK lesions based on the change of the skin quality characteristics

Rating	Basis
0 (very good)	The cosmetic outcome is rated as very good if the sum score of the previously mentioned ratings (all ratings for each sign added up) at a given visit has improved by at least 2 points as compared to baseline. If at least 1 sign has worsened by 1 point, the sum score must have improved by at least 3 points.
1 (good)	The cosmetic outcome is rated as good if the sum score at a given visit has improved by at least 1 point as compared to baseline.
2 (satisfactory)	The cosmetic outcome is rated as satisfactory if the sum score at a given visit is identical to the one at baseline.
3 (unsatisfactory):	The cosmetic outcome is rated as unsatisfactory if the sum score at a given visit has worsened by 1 point compared to baseline.
4 (impaired):	The cosmetic outcome is rated as impaired if the sum score at a given visit has worsened by at least 2 points compared to baseline.

The differences in cosmetic outcome between Aldara™ and IMI-Gel were analyzed at week 20 (Follow up 1), at follow-up 2 (FU2) and follow up 3 (FU3) visits by means of the Wilcoxon-Mann-Whitney test. Differences between the treatment groups were analyzed by means of the relative effect size.

4.3. Safety Analysis

To assess the safety of both formulations, the frequency of serious adverse events (SAEs) which occurred in the study in each treatment group was analyzed. In addition, the frequency of local skin reactions at the treatment area with the respective CIs for each treatment group was analyzed.

4.3.1. Data Analysis

The statistical analyses were conducted using GraphPad Prism 9.5.1. and SPSS 27.

4.4. Results

4.4.1. Study Population Analysis

At the time of analysis, 77 patients (93.9%) from the planned 82 patients were randomized. The first patient in was on 18.02.2019. From the 77 randomized patients, 51 patients (66.23 %) completed the study according to the clinical study protocol from whom 26 (50.98 %) had received Aldara™ and 25 (49.02 %) IMI-Gel, respectively. 11 patients (14.29 %) were in the follow up treatment. 14 patients (18.18 %) terminated the study early, 7 (50.00 %) from the Aldara™ group and 7 (50.00 %) from the IMI-Gel group. Table 10 reveals the reasons for discontinuation per treatment group:

Table 10. Reasons for patient discontinuation on the clinical trial per treatment group

Aldara™	**IMI-Gel**
• Withdrawal of informed consent: Participation is to complicated/burdensome (2 patients) • Discontinuation due to corona pandemic, risk of participation to high as patient has COPD disease • Discontinuation due to corona pandemic • Termination due to SAE (patient died on Covid-19, unrelated) • Termination due to Adverse event (fever, purulent plaque, facial swelling) • Patient signed ICF, but not randomized	• Withdrawal of informed consent: Participation is to complicated/burdensome • Termination due to incompliance of the patient • Withdrawal of informed consent prior to follow up 3 • Termination due to SAE (stroke, unrelated) • Patient does not appear to visits and cannot be reached • Patient does not appear to follow up 3 visit due to knee surgery, wanted to send pictures • Patient terminated treatment with IMP due to AE (strong itching on scalp)

The study population were predominantly male (79.22 %) with a mean ± SD age of 72 ± 7.8 years. Table 11 summarizes the population analysis data per treatment group.

Table 11. Summary of population analysis data from Aldara™ and IMI-Gel group

Variable	**Aldara™**	**IMI-Gel**
Age		
Mean	73.00	71.00
Standard Error Mean	1.22	1.28
Standard Deviation	7.55	7.99
Minimum	57.00	44.00
Q1	67.75	67.00
Median	75.50	72.00
Q3	78.25	87.00
Maximum	89.00	84.00
Gender		
Male	33 (42.86 %)	28 (36.36 %)
Female	5 (6.49 %)	11 (14.29 %)
Study completed according to protocol	26/38 (68.42 %)	25/39 (61.10 %)
Early Terminated	7/38 (18.42 %)	7/39 (17.95 %)
Subjects in follow-up	5/38 (13.15 %)	6/39 (15.38 %)
Subjects in treatment	0/38 (0.00 %)	1/39 (2.56 %)
Subjects to be included	3/41 (7.31 %)	2/41 (4.88 %)
Treatment cycles		
One cycle	13/38 (34.21 %)	10/39 (25.64 %)
Two cycles	25/38 (65.78 %)	29/39 (74.36 %)

4.4.2. Primary Objective

4.4.2.1. Erythema Rating

The analysis of the erythema rating is conducted for all patients in the Per Protocol (PP) population set. The PP-set includes all randomized patients treated at least once with the IMP without any major protocol violations. For both groups, this also includes patients which are currently in the follow up.
In the Aldara™ group, 1 event of an erythema rating ≥2 from 60 analyzed AK lesions is reported whereas 6 erythema ratings ≥2 from 69 AK lesions are observed in the IMI-Gel group. Analysis of the population differences in the erythema rating using Fisher's exact tests reveals no statistically significant difference between the group. Hence, the H_0 Hypothesis cannot be rejected. Table 12 reveals the summary of Fisher's exact test (for full data set see supplement table S9).

Table 12. Results of Fisher's exact test for analysis of the difference in the erythema rating per treatment group

Data analyzed	Aldara™	IMI-Gel
Erythema Rating ≥2	1	6
Erythema Rating <2	59	63
Total	60	69
P-value and statistical significance		
Test	Fisher's exact test	
P-value	0.0828	
P-value summary	ns	
One- or two sided	One-sided	
Statistically significant (p < 0.05)	No	
Percentage of row total		
Erythema Rating ≥2	14.29 %	85.71%
Erythema Rating <2	48.36 %	51.64 %
Percentage of column total		
Erythema Rating ≥2	1.67 %	8.70 %
Erythema Rating <2	98.33 %	91.30 %
Percentage of grand total		
Erythema Rating ≥2	0.78 %	4.65 %
Erythema Rating <2	45.74 %	48.84 %

4.4.3. Secondary Objectives

4.4.3.1. Reduction of Total Lesion Area

Table 13 reveals the results of the between subjects effects for the calculated ANCOVA revealing no significant difference in reduction of the total lesion area between the two treatment groups with a p-value of 0.142 (for full data see supplement tables S10 and S11).

Table 13. Results of the ANCOVA test to assess differences in reduction of the treated lesion area between the two treatment groups Aldara™ and IMI-Gel

Source	Sum of Squares	df	Mean square	F	Significance (p-value)	Partial eta squared
Corrected Model	232.711[a]	2	115.355	372.390	<0.001	0.924
Intercept	1.455	1	1.455	4.698	0.034	0.072
Baseline	230.283	1	230.232	743.399	<0.001	0.924
Treatment Group	0.609	1	0.609	1.966	0.166	0.031
Error	18.896	61	0.310			
Total	343.510	64				
Corrected Total	249.607	63				

a. R Squared = 0.924 (Adjusted R Squared = 0.922)

Table 14 reveals the differences in the marginal means (least square means) between the treatment groups.

Table 14. Results of the ANCOVA test to assess differences in reduction of the treated lesion area between the two treatment groups Aldara™ and IMI-Gel

Treatment group	Mean difference in the skin area from baseline to week 20 [cm^2]	Std. Error [cm^2]	95 % Confidence Interval	
			Lower Bound [cm^2]	Upper Bound [cm^2]
Aldara™	1.104[a]	0.103	0.897	1.311
IMI-Gel	1.300[a]	0.094	1.112	1.488

a. Covariates appearing in the model are evaluated at the following values: Skin area [cm^2] = 1.51495

Table 15 reveals the pairwise comparison of the two treatment groups

Table 15 Pairwise comparison of the mean difference in lesion reduction between the groups

Treatment group (I)	Treatment group (J)	Mean Difference (I-J)	Std. Error	Significance (p-value)	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
Aldara™	IMI-Gel	-0.196	0.140	0.166	-0.475	0.084
IMI-Gel	Aldara™	0.196	0.140	0.166	-0.084	0.475

Based on estimated marginal means

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments)

4.4.3.2. Patient Complete Response

Table 16 reveals the summary of Pearson's Chi-square test with Yates' continuity correction to assess the difference in the complete response rates per treatment group. The data reveal no significant difference in the response rates between the two treatment groups with a p-value of 0.8880.

Table 16 Pearson's Chi-square test with Yates continuity correction, to assess the difference in complete response rates per treatment group at week 20

Data analyzed	Aldara™	IMI-Gel
Number of complete responders	14	17
Number of partial responders	14	18
Total	28	35
P-value and statistical significance		
Test	Chi-square with Yates' correction	
Chi-square, df	0.01985, 1	
Z	0.1409	
P-value	0.8880	
P-value summary	ns	
One- or two sided	Two-sided	
Statistically significant ($p < 0.05$)	No	
Percentage of row total		
Complete response rate	45.16 %	54.84 %
Partial response rate	43.75 %	56.25 %
Percentage of column total		
Complete response rate	50.00 %	48.57 %
Partial response rate	50.00 %	51.43 %
Percentage of grand total		
Complete response rate	22.22 %	26.98 %
Partial response rate	22.22 %	28.57 %

4.4.3.3. Skin Quality Assessment and Overall Cosmetic Outcome

The difference in the ranked skin quality from each patient per treatment group was calculated using a Mann-Whitney U test. Tables 17 and 18 summarize the result for the Mann-Whitney U tests calculated to test both treatment groups for statistical differences with regards to the skin quality ratings of week 20, 36 and 52 (follow up 1,2,3). For all visits, the data reveal a statistically significant difference between the treatment groups

Table 17 Summary of the Mann-Whitney U test for differences in the skin quality assessment between the two treatment groups Aldara™ and IMI-Gel.

Treatment group	N	Mean Rank	Sum of ranks
Follow up 1			
Aldara™	60	74.22	4453.50
IMI-Gel	70	58.02	4061.50
Total	130		
Follow up 2			
Aldara™	56	68.52	3837.00
IMI-Gel	66	55.55	3666.00
Total	122		
Follow up 3			
Aldara™	48	59.93	2876.50
IMI-Gel	58	48.18	2794.50
Total	106		

Table 18 Test statistics of the Mann-Whitney U test for differences in the skin quality outcome between the two treatment groups Aldara™ and IMI-Gel revealing a statistically significant difference between the treatment groups at all visits p-value = 0.009 (Follow up 1), p-value = 0.031 (Follow up 2), p-value = 0.039 (Follow up 3)

Test Statistics	
Follow up 1	
Mann-Whitney U	1576.500
Wilcoxon W	4061.500
Z	-2.598
Asymp. Significance (2-tailed) (p-value)	0.009
Follow up 2	
Mann-Whitney U	1455.000
Wilcoxon W	3666.000
Z	-2.152
Asymp. Significance (2-tailed) (p-value)	0.031
Follow up 3	
Mann-Whitney U	1083.500
Wilcoxon W	2794.500
Z	-2.061
Asymp. Significance (2-tailed) (p-value)	0.039

The Pearson correlation coefficient *r* is calculated to determine the effect size using the following formula.

$$r = \left|\frac{z}{\sqrt{n}}\right| \tag{11}$$

Inserting the calculated Z-values and the number of pairs *n* yields the following effect size

$$r\ (follow\ up\ 1) = \left|\frac{-2.598}{\sqrt{130}}\right| = 0.23 \quad (12)$$

$$r\ (follow\ up\ 2) = \left|\frac{-2.152}{\sqrt{122}}\right| = 0.19 \quad (13)$$

$$r\ (follow\ up\ 3) = \left|\frac{-2.061}{\sqrt{122}}\right| = 0.20 \quad (14)$$

According to Cohen (1988) [134] the effect size is small if the correlation coefficient *r* is 0.1, medium when *r* is 0.3 and strong when *r* reaches 0.5. The analysis of the correlation coefficients reveal a small effect size with respect to a superior skin quality outcome for patients treated with IMI-Gel since all calculated values are <0.3.

4.4.4. Safety Analysis

Throughout the study, eight serious adverse events (SAEs) occurred in patients, with an SAE rate of 10.38%. None of these events were related to the treatment. Five patients in the Aldara™ group and three patients in the IMI-Gel group experienced SAEs.

In addition to tracking SAEs, the study recorded the frequency and intensity of local skin reactions (LSRs), including edema, vesicles, erosion, ulcerations, scaling/flaking, scabbing/crusting, and exudates. These reactions were assessed at every visit and rated on a scale from 0 (none) to 3 (severe) for each patient.

In both the Aldara™ and IMI-Gel groups, patients experienced various local skin reactions. In the Aldara™ group, 19 out of 30 patients (63.33%) experienced edema, while in the IMI-Gel group, 24 out of 36 patients (66.67%) experienced edema. Vesicles occurred in 5 out of 30 patients (16.67%) in the Aldara™ group and in 8 out of 36 patients (22.22%) in the IMI-Gel group. Erosions were seen in 83.33% of patients in both groups (25 out of 30 in the Aldara™ group and 30 out of 36 in the IMI-Gel group). Ulcerations occurred in 50.00% of patients in the Aldara™ group (15 out of 30) and 27.78% of patients in the IMI-Gel group (10 out of 36). Scaling/flaking was observed in 93.33% of patients in the Aldara™ group (28 out of 30) and 94.44% of patients in the IMI-Gel group (34 out of 36). Scabbing/crusting occurred in 90.00% of patients in the Aldara™ group (27 out of 30) and 88.89% of patients in the IMI-Gel group (32 out of 36). Exudates were reported in 43.33% of patients in the Aldara™ group (13 out of 30) and 22.22% of patients in the IMI-Gel group (8 out of 36). Figure 19 reveals the relative frequency of occurred LSRs per treatment group

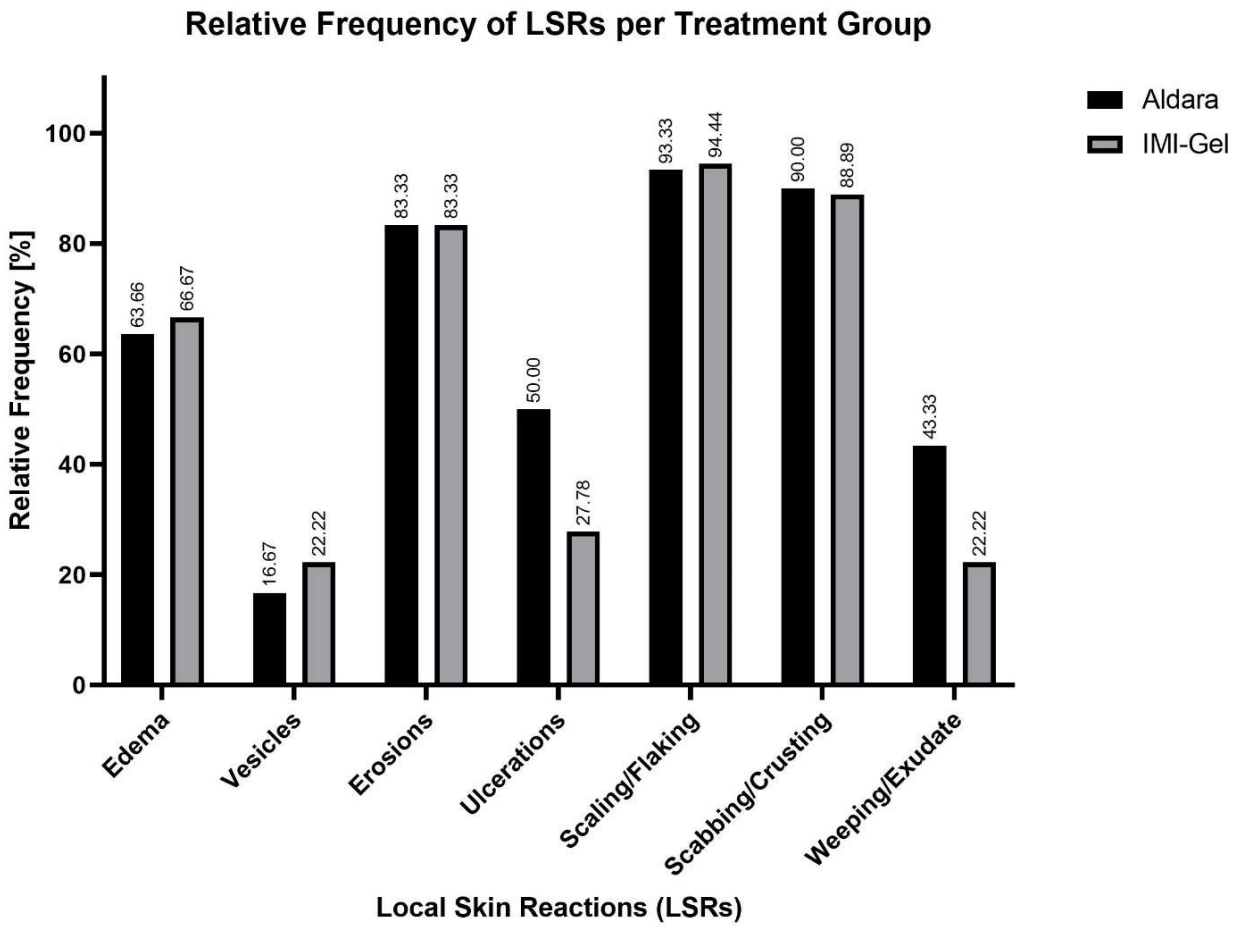


Figure 20: Relative frequency of LSRs per treatment group revealing a lower percentage of ulcerations and exudates in IMI-Gel patients, however not statistically significant (tested with Fischer's exact test, p-value (Ulcerations) = 0.0785, p-value (Weeping/Exudate) = 0.1144)

A considerable difference in relative local skin reaction frequencies between the treatment groups is observed for ulcerations and exudates where IMI-Gel patients experienced a lower frequency of both LSRs. An analysis for statistical significance however revealed no statistical significant difference in the number of patients experiencing ulcerations and exudates between the treatment groups, with a p-value of 0.0785 for ulcerations and a p-value of 0.1144 for exudates (analyzed using Fisher's exact test).

Table 19 reveals the absolute and relative frequencies of patients with the respective LSR (from 30 Aldara™ and 36 IMI-Gel patients).

Table 19 Absolute and relative frequencies of LSRs in the two treatment groups Aldara™ and IMI-Gel

LSR	Aldara™	IMI-Gel
Edema	19 (63.66 %)	24 (66.67 %)
Vesicles	5 (16.67 %)	8 (22.22 %)
Erosion	25 (83.33 %)	30 (83.33 %)
Ulcerations	15 (50.00 %)	10 (27.78 %)
Scaling/Flaking	28 (93.33 %)	34 (94.44 %)
Scabbing/Crusting	27 (90.00 %)	32 (88.89 %)
Weeping/Exudate	13 (43.33 %)	8 (22.22 %)

Figure 21 and Figure 22 reveal the relative event frequency of local skin reactions per treatment group calculated for all recorded events at baseline, visit 1,2,3,4,5,6,7,8 and follow ups 1,2,3.

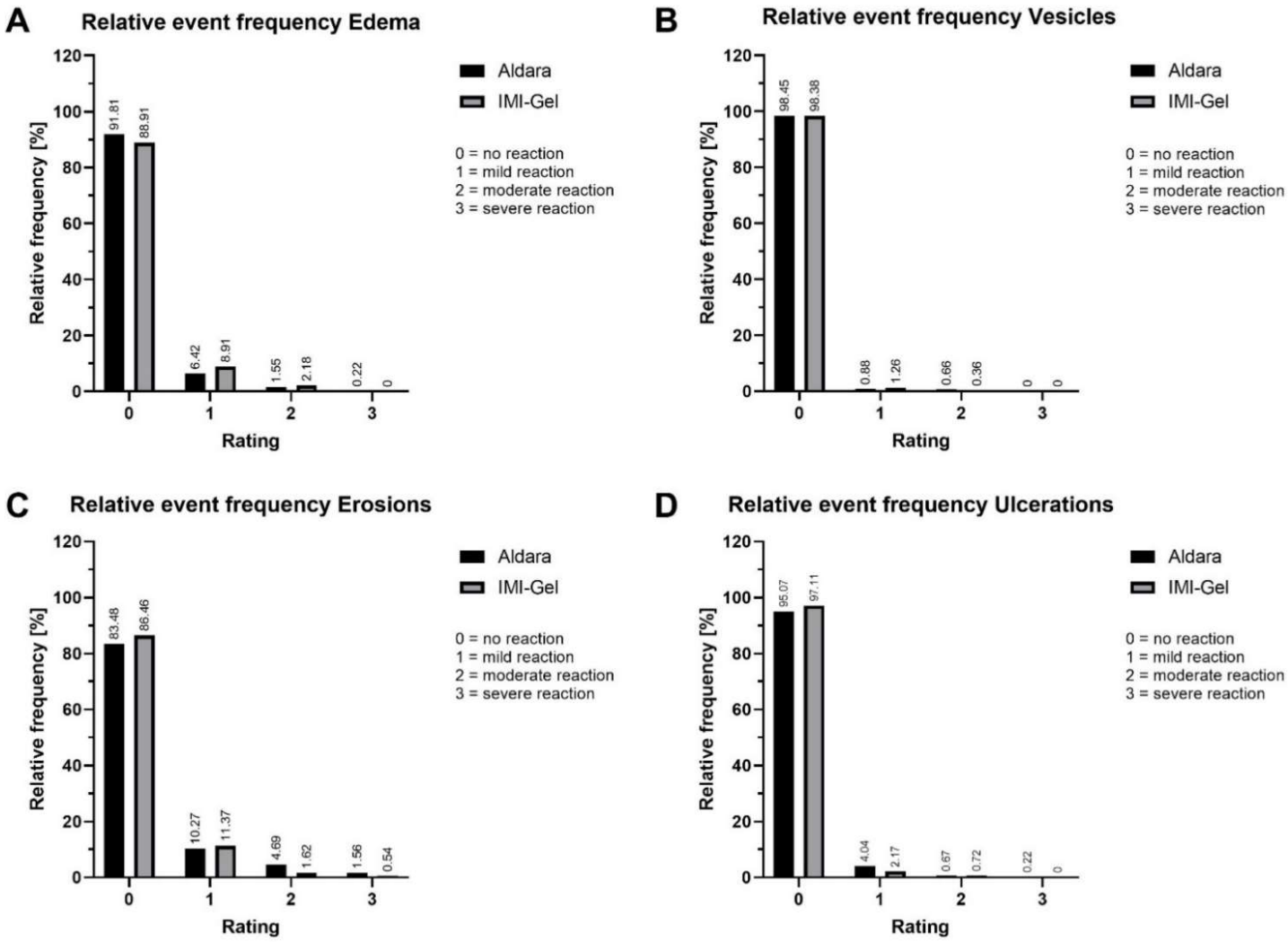


Figure 21: Relative event frequency of (A) edema, (B) vesicles, (C) erosions and (D) ulceration per rating and treatment group. The data reveal comparable relative event frequencies for both treatment groups.

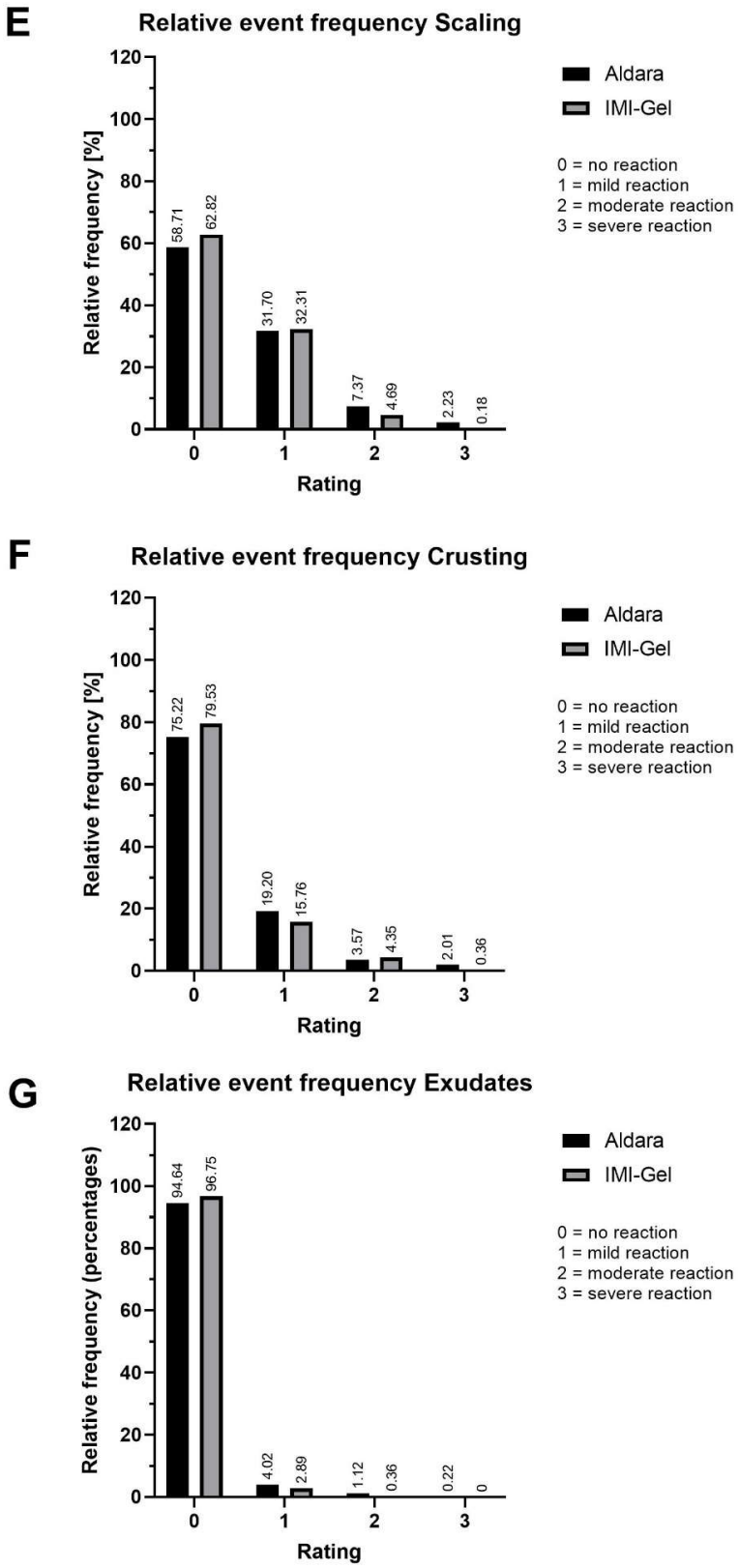


Figure 22: Relative event frequency of scaling, crusting and exudates peer rating and treatment group. The data reveal comparable frequencies for the presented LSRs, however with the tendency that severe scaling and crusting appears at a higher frequency in the Aldara™ group.

4.5. Discussion

The results of the clinical trial have demonstrated, that IMI-Gel is a safe and efficacious drug product. The hypothesis of an erythema rating of ≥2 in <10 % for patients treated with IMI-Gel could be confirmed. However, the primary alternative hypothesis of a lower erythema rating for patients treated with IMI-Gel in comparison to patients treated with Aldara™ and hence a rejection of the H_0 hypothesis was not revealed. In contrast, a surprisingly low erythema rating of 1.67% in Aldara™ patients was observed. The study of the publication by Gebauer et al. [24] reports a rate of 34.5 % of severe erythema rating observed in patients treated 3x week with 5% IMQ which is much higher than observed in this study. A difference caused by a difference in the study population can be excluded since in the study by Gebauer, study participants were of comparable mean age (71 ±10.2 years), also predominantly male (63 %) and all of white ethnicity. A further comparative analysis of the erythema events occurring at other visits might reveal a different overall erythema event frequency of ratings ≥2 with a recalculation of the required sample size based on the observed difference using a power analysis.

With regards to the overall efficacy, it can be concluded that both formulations show the same efficacy in humans indicated by no significant difference in the clearance rate of AK skin lesions as well in the reduction of the AK skin lesion area. These results are in line with the expected results of comparable efficacy for both treatments. Interestingly, patients receiving IMI-Gel experienced a significant improved cosmetic outcome after treatment even though the effect size is small. Most likely, this effect is attributed to the exclusion of isostearic acid in IMI-Gel and inclusion of jojoba wax.

Despite the non-significant differences between the treatment groups with respect to the LSRs, there is a noticeable difference in ulcerations and exudates with lower frequencies for the IMI-Gel group. Assuming that the sample size is not large enough to demonstrate a significant difference for both treatment groups, recalculating of the required sample size at a power of 0.8 with an estimated difference of 22.22 % for ulcerations and 21.11 % for exudates at an α of 0.05 results in a required sample size of 68 per group for the ulcerations and 75 per treatment group for exudates to demonstrate statistically significant differences. Hence, more patients would be needed if a statistically significant difference is aimed to be demonstrated. Regarding the observed SAEs, it is observed that both medical interventions are safe, where all SAEs are unrelated to the treatment.

Overall, it could be demonstrated that a high quality IMP batches of the formulation IMI-Gel were supplied to patients in the clinical trial resulting in an equally effective medicinal product in comparison to the reference product Aldara™ with superior tolerability for IMI-Gel. Completing the patient data and analysis of additional parameters e.g. treatment emergent adverse events, patient's quality of life by the dermatological life quality index (DLQI) questionnaire, Patient's and physician's assessment will allow a more precise analysis of the outcomes of the trial. However, the data presented in this thesis demonstrate additional benefit of the product IMI-Gel in comparison to the established IMQ formulation Aldara™. This might form a suitable basis for proceeding to phase II clinical trials. When pursuing this goal, some additional formulation characterization studies should be conducted including evaluation of the required quantity of jojoba wax, dispersity of the jojoba wax droplets, potentially evaluation of additional or other surfactants as well as optimization of the preservatives in the formulation in order to increase the product quality even further. Also, upscaling and process optimization to increase the manufacturing efficiency should be conducted (switching from a planetary ball mill to a wet stirred media mill and increasing the homogenization equipment).

Whether or not the observed benefits for the IMI-Gel product are sufficient for market authorization and price negotiations in Germany remains yet unknown.

Appendix

Supplementary Material

S1. HPLC Assay 1: Therapeutically Active Ingredient IMQ extracted from IMI-Gel with acetonitrile: water: phosphoric acid (250:750:10)

Briefly, 200 mg of the formulation equivalent to 10 mg of IMQ was transferred into a 50 mL volumetric flask. 20 mL of a diluent (acetonitrile:water:phosphoric acid 250:750:10, v/v) was added to the flask. The sample solution was heated to 70 °C in a water bath for 5 min. After the samples were cooled to room temperature, the volume was filled up to the mark with the diluent, mixed and filtered through a Millex Nylon Syringe filter with a pore size of 0.45 µm and a diameter of 25 mm. For the reference standard, 100 mg of USP IMQ Reference Standard was transferred into a 50 mL volumetric flask and dissolved in the similar diluent as used for the sample. For the mobile phase, 2.0 g of heptane-1-sulphonic acid sodium salt was dissolved in 750 mL of water followed by addition of 1.5 mL of triethylamine and 250 mL of acetonitrile. The pH of the solution was adjusted to 2.7 ± 0.05 with phosphoric acid ≥85% and the mobile phase was degassed for 15 min in an ultrasonic bath. As the stationary phase, a Zorbax RX-C8 column of 150 mm length with a diameter of 4.6 mm and a particle size of 5 µm was used. 20 µL of the sample solution was injected into the system at a flow rate of 1.5 mL/min with a column temperature of 30 °C. The IMQ Peak appeared at ~11 min. The content of IMQ was calculated as the ratio of peak response for the sample peak divided by the peak response of the IMQ standard multiplied with the concentration of the IMQ reference standard solution divided by through the nominal concentration of the sample solution multiplied with 100. For QC analysis three samples were prepared as described above. The reference standard and samples were measured in triplicates.

S2. HPLC Assay 2: Content of Preservatives (Methyl- and Propylparaben) extracted from IMI-Gel with ethanol:water:sulfuric acid (81:9:2)

All solvents used were of HPLC gradient grade. Briefly 1 g of IMI-Gel was transferred into a 20 mL volumetric flask followed by the addition of 1 mL of 2 M sulfuric acid solution. The flask was filled up to the mark with ethanol:water 90:10 diluent and heated to 60 °C for 5 minutes. Afterwards, the solution was quickly cooled to room temperature (RT) and kept at RT over night. For the analysis, the clear supernatant was used. For the HPLC analysis, the same system as de-scribed under 2.6.4. was used, equipped with the Zorbax RX-C8 of 150 mm length, 4.6 mm diameter and a 5 µm particle size. The mobile phase consisted of methanol:water (60:40) with the aqueous phase being adjusted to pH 2.2 ± 0.05 us-ing phosphoric acid ≥ 85%. For the analysis, one sample per batch was prepared and analyzed in triplicates. Per sam-ple, 20 µL of the sample solution was injected. As the calibration standards, 5 concentrations of 2.5 µg/mL, 5 µg/mL, 10 µg/mL, 20 µg/mL and 40 µg/mL of the reference standards were prepared from 1 mg/mL stock solutions of methyl- and propylparaben reference standard solutions and analyzed in triplicates. The retention times of the peaks methyl- and propylparaben were around 3 min and 5 min. The IMQ peak appeared immediately after the injection peak. The content of the preservatives was calculated by inserting the peak response of methyl- and propylparaben from the sample solutions into the regression equation obtained from the standards.

S3. HPLC Assay 3: Impurities and Degradants of IMQ extracted from IMI-Gel with acetonitrile: water: phosphoric acid (650:350:1)

Briefly, 400 mg of IMI-Gel was transferred into a 50 mL volumetric flask. 40 mL of a diluent of acetonitrile:water:phosphoric acid (650:350:1) was added to the sample followed by heating of the sample solution to 70 °C for 3 min with occasional stirring to suspend the formulation within the solution. Afterwards, the sample solution was cooled to room temperature, filled up to the mark with diluent and filtered through a Millex Nylon Syringe filter with a pore size of 0.45 µm and a diameter of 25 mm. For the IMQ reference standard solution, 50 mg of the USP IMQ reference standard was transferred accurately into a 50 mL volumetric flask and dissolved in the same diluent used for the sample solution. From this stock solution, a standard solution of 4 µg/mL was prepared by adding 20 µL of the stock solution to 4.98 mL of diluent. As the HPLC system, the same system as described under 2.6.4. and 2.6.5. was used. For the method, a gradient was used with three mobile phases. Mobile phase A was prepared by dissolving 1.0 g of heptane-1-sulphonic acid sodium salt, 0.8 g of sodium dodecyl sulfate and 1.0 g of dibasic potassium phosphate in 800 mL of water followed by addition of 200 mL of acetonitrile and mixing. Once at room temperature, the solution was adjusted with phosphoric acid ≥85 % to pH of 6.4 ± 0.05. Mobile phase B and C were prepared in analogous manner except for the ratio of water to acetonitrile being 400 ml to 600 mL for mobile phase B and 250 mL to 750 mL for mobile phase C. As the stationary phase, an Inertsil ODS-3 5 µm column with a length of 250 mm and an inner diameter of 4.6 mm was selected. For separation of IMQ and the related substances a gradient was used with an isocratic phase from 0-5 min composed of 80 % mobile phase A and 20 % mobile phase B, a gradient phase from 5-53 min from 80 % mobile phase A and 20 % mobile phase B to 40 % mobile phase A and 60 % mobile phase B, and isocratic phase of 100 % mobile phase C from 53-59 min and an equilibrium isocratic phase from 60-65 min of 80 % mobile phase A and 20 % mobile phase B. A flow rate of 1.2 mL/min at a column temperature of 30 °C was selected.

Dynamic Scanning Calorimetry (DSC) of IMQ before and after milling

For QC analysis, one sample per batch was prepared. The reference standard solution and the sample solution were analyzed in triplicates.

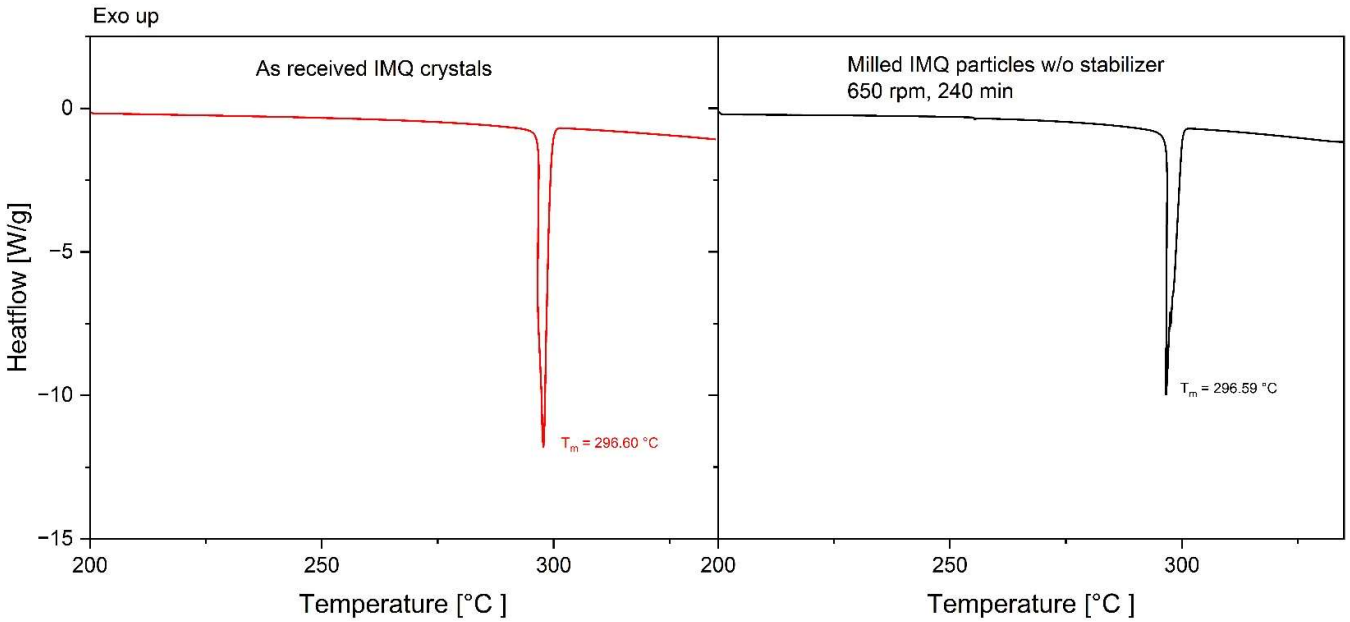


Figure S1. Dynamic Scanning Calorimetry (DSC) Thermograms of as received crystals in red (left) and after milling in black (right). The graphs show no change in the crystallinity of the drug after milling with a sharp melting point peak at a melting temperature Tm = 296.60 °C for the as received IMQ crystals and Tm = 296.50 °C for the milled IMQ particles

Risk Estimation Matrix (REM)

Supplement Table S1. Risk estimation matrix presenting initial risk assessment levels of individual material and process parameters: Low, low-risk parameter, medium, medium-risk parameter, High, high-risk parameter

		Attribute	Content Uniformity	Particle size distribution	pH	Rheological properties	Permeation rate	Stability*
Raw Materials	IMQ	pKa	Low	Low	Low	Low	Low	Low
		Log P	Low	Low	Low	Low	Low	Medium
		Concentration	Low	Medium	Medium	Medium	Medium	Medium
		Solubility	Medium	High	High	Low	High	High
		Particle Size	Low	Medium	Low	Low	Low	Low
		Melting point	Low	Low	Low	Low	Low	Low
		Related substances	Medium	Low	Low	Low	Low	High
	Surfactant	Compatibility	High	High	Low	Low	Low	High
		HLB	Medium	Medium	Low	Medium	Low	High
		Concentration	Medium	Medium	Low	Medium	Low	High
		Log P	Medium	Medium	Low	Low	Low	Medium
	Gelling agent	Compatibility	Low	Low	High	High	Low	High
		Concentration	Low	Low	Medium	High	Medium	High
		Molecular weight	Low	Low	Low	High	Medium	High
		pKa	Low	Low	Medium	Medium	Low	Medium
	Neutralizing agent	Compatibility	Low	Low	High	High	Low	Medium
		Concentration	Low	Low	High	High	Medium	High
		pKa	Low	Low	High	High	Medium	High
	Oil component	Compatibility	Low	Low	Low	Medium	High	High
		Required HLB	Low	Low	Low	Medium	High	High
		Concentration	Low	Low	Low	High	High	High
		Viscosity	Low	Low	Low	Medium	Medium	Medium
	Preservatives	Compatibility	Low	Low	Low	Low	Low	High
		Concentration	Low	Low	Low	Low	Low	High
		Log P	Low	Low	Low	Low	Low	High
Process Parameters	Wet Media ball milling	Milling ball size	Low	Medium	Low	Low	Low	Medium
		Temperature	Low	Low	Low	Low	Low	Low
		Ratio Drug to Milling balls	Low	Medium	Low	Low	Low	Medium
		Rotational speed	Low	High	Low	Low	Low	High
		Milling time	Low	High	Low	Low	Low	High
	High Pressure Homogenization	Temperature	Low	Low	Low	Low	Low	Low
		Number of Cycles	Medium	Medium	Low	Medium	Low	Medium
		Pressure	Medium	Medium	Low	Medium	Low	High
	Gel formation	Viscosity	Low	Low	Low	Medium	Low	High
		Concentration	Low	Low	Low	Medium	Low	Medium
	Filling	Time	Low	Low	Low	Medium	Low	Medium
		Shear stress	Low	Low	Low	Medium	Low	Medium
		Amount	Low	Low	Low	Low	Low	Low

*Stability includes the CQAs Homogeneity, Impurities/Degradants and microbiological limits

Design of Experiment (DoE) Data IMQ Particle size distribution

Supplement Table S2 z-Average and the PdI of the prepared IMQ nanosuspensions. The presented data from each condition ("run") are arithmetical means of triplicate measurements (n=3).

Run	Factors		Responses	
	Rotational speed [rpm]	Milling time [min]	Particle size (z-Average) [d.nm]	Polydispersity Index (PdI)
1	650	60	404.4	0.264
2	450	150	357.6	0.149
3	450	240	318.2	0.132
4	450	150	353.9	0.183
5	250	240	388.5	0.204
6	450	60	460.5	0.274
7	450	240	367.7	0.202
8	450	150	391.6	0.199
9	650	150	327.5	0.136
10	450	150	356.2	0.189
11	650	240	292.8	0.126
12	450	150	387.2	0.175
13	450	150	360.3	0.211
14	250	150	422.1	0.204
15	450	150	363.1	0.219
16	250	60	523.2	0.308
17	650	240	305.2	0.167
18	450	60	430.7	0.231
19	450	150	376.3	0.206
20	250	60	589.7	0.237
21	250	240	384.2	0.094
22	650	60	398.5	0.244
23	450	150	361.9	0.210
24	650	150	342.9	0.127
25	250	150	437.9	0.189
26	450	150	381.7	0.210

Quality Control Data IMI-Gel

Supplement Table S3. Mean particle sizes of manufactured IMI-Gel batches measured per DLS with the Standard Deviation, the Median, 95 % Confidence Interval for the Median and the Coefficient of Variation for all measured data

Batch-No.	Mean z-Average [d.nm]	Standard Deviation [d.nm]	N	Acceptance criterion
IMI-Gel-151118	399.22	8.74	18	z-Average 400 ± 200 nm
IMI-Gel-161118	415.37	8.75	18	
IMI-Gel-170219	372.24	10.77	18	
IMI-Gel-070319	366.85	8.82	18	
IMI-Gel-120319	396.31	11.84	18	
IMI-Gel-310719	374.04	12.85	18	
IMI-Gel-111219	399.51	18.29	18	
IMI-Gel-090320	391.76	9.82	18	
IMI-Gel-180820	451.41	70.93	18	
IMI-Gel-260121	377.39	11.64	18	
IMI-Gel-120721	384.43	9.46	18	
IMI-Gel-130721	383.54	11.17	18	
IMI-Gel-111021	358.58	25.71	18	
Median	384.4	--------	-----------	--------
Range	92.83	--------	-----------	--------
95 % Confidence interval for the Median	372.2 – 399.5	--------	-----------	--------
Coefficient of Variation [%]	6.17	--------	-----------	--------

Supplement Table S4. Mean PdI values of manufactured IMI-Gel batches measured per DLS with the Standard Deviation, the Median, 95 % Confidence Interval for the Median and the Coefficient of Variation for all measured data

Batch-No.	PdI	Standard Deviation	N	Acceptance criterion
IMI-Gel-151118	0.259	0.029	18	PdI < 0.3
IMI-Gel-161118	0.260	0.022	18	
IMI-Gel-170219	0.227	0.015	18	
IMI-Gel-070319	0.236	0.022	18	
IMI-Gel-120319	0.226	0.021	18	
IMI-Gel-310719	0.241	0.029	18	
IMI-Gel-111219	0.229	0.017	18	
IMI-Gel-090320	0.229	0.030	18	
IMI-Gel-180820	0.215	0.028	18	
IMI-Gel-260121	0.215	0.019	18	
IMI-Gel-120721	0.185	0.041	18	
IMI-Gel-130721	0.219	0.026	18	
IMI-Gel-111021	0.219	0.024	18	
Median	0.227	--------	--------	--------
Range	0.075	--------	--------	--------
95 % Confidence interval for the Median	0.215 - 0.241	--------	--------	--------
Coefficient of Variation [%]	6.17	--------	--------	

Supplement Table S5. Mean content for manufactured IMI-Gel batches with respective Standard Deviation and Confidence Interval

Batch-No.	Mean content [%]	Standard Deviation [%]	95 % Confidence Interval [%]	N	Acceptance criterion
IMI-Gel-151118	104.93	3.58	102.2-107.70	9	$90 \leq x \leq 110$ % of 5 % (w/w)
IMI-Gel-161118	100.23	2.33	98.28-102.20	8*	
IMI-Gel-170219	100.68	1.87	99.25-102.10	9	
IMI-Gel-070319	99.07	2.06	97.48-100.70	9	
IMI-Gel-120319	97.46	1.74	96.12-98.80	9	
IMI-Gel-310719	94.75	0.35	94.49-95.02	9	
IMI-Gel-111219	101.64	3.41	99.02-104.3	9	
IMI-Gel-090320	99.87	0.42	99.55-100.2	9	
IMI-Gel-180820	97.94	0.70	97.40-98.48	9	
IMI-Gel-260121	102.43	1.62	101.2-103.7	9	
IMI-Gel-120721	104.89	2.53	102.9-106.8	9	
IMI-Gel-130721	94.38	1.02	93.60-95.17	9	
IMI-Gel-111021	104.77	1.21	103.8-105.7	9	

* one was identified as a statistical significant outlier in an outlier test with a $p < 0.05$ using Grubb's outlier test and excluded from analysis

Supplement Table S6. Level of impurities for the manufactured IMI-Gel batches with type of impurity (related compound A, B, C, D, E, or unknown)

Batch-No.	Detected Impurity type	Level [%]	Total level of Impurities [%]	N	Acceptance criterion
IMI-Gel-151118	-----	< 0.1	< 0.1	3	Individual level of impurities ≤ 0.2 % Unknown impurities ≤ 0.1 % Total level of impurities ≤ 0.5 %
IMI-Gel-161118	-----	< 0.1	< 0.1	3	
IMI-Gel-170219	-----	< 0.1	< 0.1	3	
IMI-Gel-070319	-----	< 0.1	< 0.1	3	
IMI-Gel-120319	-----	< 0.1	< 0.1	3	
IMI-Gel-310719	B	0.038	0.038	3	
IMI-Gel-111219	B	0.056	0.056	3	
IMI-Gel-090320	-----	< 0.1	< 0.1	3	
IMI-Gel-180820	-----	< 0.1	< 0.1	3	
IMI-Gel-260121	B	0.028	0.028	3	
IMI-Gel-120721	B	0.034	0.034	3	
IMI-Gel-130721	B	0.028	0.028	3	
IMI-Gel-111021	B	0.028	0.028	3	

Supplement Table S7. Assay Preservatives for the manufactured IMI-Gel batches

Batch-No.	Assay Preservatives [%]	Standard Deviation [%]	N	Acceptance criterion
IMI-Gel-151118	0.052	0.0002	3	0.04 – 0.06 % (w/w)
IMI-Gel-161118	0.051	0.0001	3	
IMI-Gel-170219	0.047	0.0008	3	
IMI-Gel-070319	0.048	0.0003	3	
IMI-Gel-120319	0.047	0.0003	3	
IMI-Gel-310719	0.049	0.0002	3	
IMI-Gel-111219	0.050	0.0003	3	
IMI-Gel-090320	0.051	0.0001	3	
IMI-Gel-180820	0.047	0.0002	3	
IMI-Gel-260121	0.050	0.0006	3	
IMI-Gel-120721	0.042	0.0007	3	
IMI-Gel-130721	0.042	0.0006	3	
IMI-Gel-111021	0.043	0.0003	3	

Supplement Table S8. Minimum, 25% Percentile, Median, 75% Percentile and maximum weight of filled tubes from the manufactured IMI-Gel batches

Batch-No.	Minimum [g]	25% Percentile [g]	Median [g]	75% Percentile [g]	Maximum [g]	N	Acceptance criterion
IMI-Gel-151118	4.982	5.036	5.052	5.074	5.104	28	Weight of filled tubes: 5 g ± 15 %
IMI-Gel-161118	4.928	4.981	5.045	5.087	5.110	28	
IMI-Gel-170219	5.060	5.156	5.195	5.238	5.285	28	
IMI-Gel-070319	5.030	5.061	5.080	5.104	5.131	24	
IMI-Gel-120319	4.943	5.032	5.053	5.084	5.124	30	
IMI-Gel-310719	4.991	5.023	5.045	5.084	5.140	28	
IMI-Gel-111219	5.010	5.041	5.068	5.081	5.135	27	
IMI-Gel-090320	4.995	5.048	5.079	5.099	5.120	27	
IMI-Gel-180820	5.002	5.035	5.070	5.096	5.135	30	
IMI-Gel-260121	4.979	5.025	5.058	5.084	5.118	28	
IMI-Gel-120721	4.956	5.007	5.043	5.056	5.213	30	
IMI-Gel-130721	4.917	4.982	5.004	5.052	5.098	31	
IMI-Gel-111021	4.976	5.012	5.032	5.075	5.124	26	

Supplement Table S9. Data erythema ratings per patient 4 weeks after the last treatment, tbd = to be determined. Data in red indicate the last observation carried out forward method. a = patient only had 1 AK lesion which is an exclusion criteria according to the study protocol but showed good adherence, for completeness, these data are also included.

Erythema rating

Per Protocol Group Aldara™				Per Protocol Group IMI-Gel			
Patient Nr.	**Treatment endpoint**	**Erythema Rating Endpoint Lesion 1**	**Erythema Rating Endpoint Lesion 2**	**Patient Nr.**	**Treatment endpoint**	**Erythema Rating Endpoint Lesion 1**	**Erythema Rating Endpoint Lesion 2**
002 (102)	V8	2	0	003 (103)	V8	1	1
004 (104)	V5	0	0	007 (107)	V8	0	0
005 (105)	V5	1	1	008 (108)	V8	0	0
011 (112)	V8	0	0	009 (109)	V5	0	0
012 (110)	V8	1	1	010 (111)	V8	0	0
014 (114)	V8	0	1	013 (113)	V5	2	2
015 (115)	V5	0	0	016 (116)	V8	0	0
020 (120)	V8	1	1	017 (117)	V8	0	0
023 (123)	V8	0	0	018 (118)	V8	0	0
024 (124)	V8	0	0	021 (121)	V8	1	0
027 (127)	V8	1	1	022 (122)	V8	0	1
031 (131)	V5	1	1	026 (126)	V5	0	0
033 (133)	V8	1	0	028 (128)	V8	0	0
034 (134)	V8	0	0	029 (129)	V8	0	0
039 (140)	V5	0	0	032 (132)	V8	2	2
043 (144)	V8	0	1	035 (137)	V8	1	0
045 (146)	V5	0	0	036 (136	V8	1	1
047 (148)	V5	0	0	037 (138)	V5	0	0
050 (151)	V5	0	0	040 (141)	V8	0	1
052(153)	V8	0	0	041 (142)	V8	1	0
053 (154)	V8	1	0	042 (143)	V8	0	0
057 (158)	V8	1	1	046 (147) (1 Lesion)[a]	V5	0	----

Erythema rating

Per Protocol Group Aldara™				Per Protocol Group IMI-Gel			
Patient Nr.	**Treatment endpoint**	**Erythema Rating Endpoint Lesion 1**	**Erythema Rating Endpoint Lesion 2**	**Patient Nr.**	**Treatment endpoint**	**Erythema Rating Endpoint Lesion 1**	**Erythema Rating Endpoint Lesion 2**
058 (159)	V8	0	0	048 (149)	V5	0	0
064 (165)	V8	1	0	049 (150)	V8	0	0
065 (166)	V8	1	1	051 (152)	V5	0	0
066 (167)	V5	0	0	055 (156)	V5	1	1
069 (170)	V5	0	0	059 (160	V5	0	0
072 (173)	V5	1	1	061 (162)	V8	2	2
074 (175)	V8	1	0	062 (163)	V8	0	0
075 (176)	V5	0	0	067 (168)	V8	1	1
78	tbd	tbd	tbd	068 (169)	V5	1	1
80	tbd	tbd	tbd	070 (171)	V8	1	1
				071 (172)	V8	0	0
				073 (173)	V8	1	1
				076 (177)	V5	1	1
				077 (178)	tbd	tbd	tbd
				79	tbd	tbd	tbd

Skin lesion area

Supplement Table S10. Full data diameters skin lesions at baseline &follow up 1 for the AldaraTM group, in red are missing data replaced by the last observation carried out forward (LOCF) method

Aldara™: Skin lesions area

	Baseline							Follow Up 1 (FU1)							
Rando-mization Nr. (Patient Nr.)	Diameter 1 Lesion 1 (L1) [cm]	Diameter 2 Lesion 1 (L1) [cm]	Diameter 1 Lesion 2 (L1) [cm]	Diameter 2 Lesion 2 (L1) [cm]	Area Lesion 1 [cm^2]	Area Lesion 2 [cm^2]	Area sum [cm^2]	Diameter 1 Lesion 1 (L1) [cm]	Diameter 2 Lesion 1 (L1) [cm]	Diameter 1 Lesion 2 (L1) [cm]	Diameter 2 Lesion 2 (L1) [cm]	Area Lesion 1 [cm^2]	Area Lesion 2 [cm^2]	Area sum [cm^2]	Difference Baseline-FU1
002 (102)	1.20	0.60	0.50	0.50	0.57	0.20	0.762	0.50	0.50	0.30	0.30	0.20	0.07	0.267	0.495
004 (104)	1.00	1.00	0.50	0.70	0.79	0.27	1.060	0.00	0.00	0.00	0.00	0.00	0.00	0.000	1.060
005 (105)	0.70	0.50	0.60	0.50	0.27	0.24	0.511	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.511
011 (112)	2.00	2.00	1.00	2.00	3.14	1.57	4.712	0.50	0.40	0.00	0.00	0.16	0.00	0.157	4.555
012 (110)	2.00	1.00	1.50	1.00	1.57	1.18	2.749	1.00	1.00	0.20	0.20	0.79	0.03	0.817	1.932
014 (114)	1.00	1.00	1.00	1.00	0.79	0.79	1.571	0.00	0.00	0.30	0.50	0.00	0.12	0.118	1.453
015 (115)	0.50	0.50	1.00	0.50	0.20	0.39	0.589	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.589
020 (120)	1.50	1.00	1.00	1.00	1.18	0.79	1.963	1.30	1.00	1.00	1.00	1.02	0.79	1.806	0.157
023 (123)	0.50	1.00	0.50	1.00	0.39	0.39	0.785	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.785
027 (127)	2.00	1.00	1.50	1.00	1.57	1.18	2.749	1.50	1.50	1.00	1.00	1.77	0.79	2.553	0.196
031 (131)	1.50	1.00	0.50	0.80	1.18	0.31	1.492	0.00	0.00	0.00	0.00	0.00	0.00	0.000	1.492
033 (133)	0.80	0.50	0.50	0.50	0.31	0.20	0.511	0.50	0.30	0.50	0.20	0.12	0.08	0.196	0.314
034 (134)	1.00	0.70	0.50	0.50	0.55	0.20	0.746	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.746
039 (140)	0.50	0.50	1.00	0.80	0.20	0.63	0.825	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.825
043 (144)	1.50	1.00	1.00	0.50	1.18	0.39	1.571	0.00	0.00	0.00	0.00	0.00	0.00	0.000	1.571
045 (146)	2.50	1.50	0.50	0.40	2.95	0.16	3.102	1.50	1.00	0.50	0.50	1.18	0.20	1.374	1.728
047 (148)	1.00	1.00	1.00	0.50	0.79	0.39	1.178	0.00	0.00	0.00	0.00	0.00	0.00	0.000	1.178
050 (151)	0.50	0.50	0.50	0.50	0.20	0.20	0.393	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.393
052(153)	1.00	1.00	0.50	0.50	0.79	0.20	0.982	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.982
053 (154)	1.00	1.50	0.50	1.00	1.18	0.39	1.571	0.30	0.30	0.00	0.00	0.07	0.00	0.071	1.500
057 (158)	1.00	1.00	1.00	0.50	0.79	0.39	1.178	1.20	0.50	0.90	0.60	0.47	0.42	0.895	0.283
058 (159)	2.00	1.00	0.50	0.50	1.57	0.20	1.767	0.50	0.50	0.00	0.00	0.20	0.00	0.196	1.571
064 (165)	0.50	0.75	1.50	1.00	0.29	1.18	1.473	0.50	1.00	1.50	0.50	0.39	0.59	0.982	0.491
065 (166)	1.50	1.30	2.00	1.70	1.53	2.67	4.202	1.00	0.50	2.00	1.00	0.39	1.57	1.963	2.238
066 (167)	0.50	0.50	0.75	0.50	0.20	0.29	0.491	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.491
069 (170)	0.30	0.30	0.20	0.30	0.07	0.05	0.118	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.118
072 (173)	1.00	1.00	1.50	1.50	0.79	1.77	2.553	0.50	0.20	0.00	0.00	0.08	0.00	0.079	2.474
074 (175)	1.30	1.00	1.50	1.30	1.02	1.53	2.553	0.00	0.00	1.50	1.00	0.00	1.18	1.178	1.374
075 (176)	1.00	0.80	1.00	0.50	0.63	0.39	1.021	0.00	0.00	0.00	0.00	0.00	0.00	0.000	1.021

Supplement Table S11. Full data diameters skin lesions at baseline &follow up 1 for the IMI-Gel group, in red are missing data which were replaced by to the last observation carried out forward (LOCF) method

IMI-Gel: Skin lesions area

	Baseline							Follow Up 1 (FU1)							
Rando-mization Nr. (Patient Nr.)	Diameter 1 Lesion 1 (L1) [cm]	Diameter 2 Lesion 1 (L1) [cm]	Diameter 1 Lesion 2 (L1) [cm]	Diameter 2 Lesion 2 (L1) [cm]	Area Lesion 1 [cm^2]	Area Lesion 2 [cm^2]	Area sum [cm^2]	Diameter 1 Lesion 1 (L1) [cm]	Diameter 2 Lesion 1 (L1) [cm]	Diameter 1 Lesion 2 (L1) [cm]	Diameter 2 Lesion 2 (L1) [cm]	Area Lesion 1 [cm^2]	Area Lesion 2 [cm^2]	Area sum [cm^2]	Diff. Baseline-FU1
003 (103)	0.7	0.5	0.5	0.5	0,27	0.20	0.471	0.6	0.6	0	0	0.28	0.00	0.283	0.188
007 (107)	0.5	0.5	0.5	0.5	0,20	0.20	0.393	0.5	0.5	0.3	0.3	0.20	0.07	0.267	0.126
008 (108)	1	1	0.6	0.6	0,79	0.28	1.068	0	0	0	0	0.00	0.00	0.000	1.068
009 (109)	0.6	0.5	0.7	0.5	0,24	0.27	0.511	0	0	0	0	0.00	0.00	0.000	0.511
010 (111)	0.5	0.5	0.5	0.5	0,20	0.20	0.393	0.2	0.2	0.2	0.2	0.03	0.03	0.063	0.330
013 (113)	2	1	6	3	1,57	14.14	15.708	0.5	0.5	1	1	0.20	0.79	0.982	14.726
016 (116)	1	1	2	2	0,79	3.14	3.927	0.4	0.4	1.5	1	0.13	1.18	1.304	2.623
017 (117)	0.5	0.5	1	1	0,20	0.79	0.982	0	0	0	0	0.00	0.00	0.000	0.982
018 (118)	0.5	0.5	0.5	0.5	0,20	0.20	0.393	0	0	0	0	0.00	0.00	0.000	0.393
021 (121)	0.6	0.6	0.7	0.6	0,28	0.33	0.613	0	0	0	0	0.00	0.00	0.000	0.613
022 (122)	1	0.6	1.2	0.8	0,47	0.75	1.225	0.4	0.3	1	1	0.09	0.79	0.880	0.346
026 (126)	0.7	0.6	0.8	1	0,33	0.63	0.958	0.5	0.5	0.4	0.4	0.20	0.13	0.322	0.636
028 (128)	1	0.3	0.5	0.5	0,24	0.20	0.432	0	0	0	0	0.00	0.00	0.000	0.432
029 (129)	1.5	1	1.5	0.8	1,18	0.94	2.121	0	0	0	0	0.00	0.00	0.000	2.121
032 (132)	1	1	2.2	1.1	0,79	1.90	2.686	1	0.8	1	0.8	0.63	0.63	1.257	1.429
035 (137)	0.5	0.5	0.5	0.5	0,20	0.20	0.393	0.2	0.2	0.4	0.4	0.03	0.13	0.157	0.236
036 (136	1.3	0.6	0.5	0.4	0,61	0.16	0.770	1.5	1	0	0	1.18	0.00	1.178	-0.408
037 (138)	0.6	0.6	8	0.8	0,28	5.03	5.309	0	0	0	0	0.00	0.00	0.000	5.309
040 (141)	1	0.6	0.5	0.4	0,47	0.16	0.628	0	0	0.3	0.3	0.00	0.07	0.071	0.558
041 (142)	1	0.5	1	0.4	0,39	0.31	0.707	0.8	0.4	0	0	0.25	0.00	0.251	0.456
042 (143)	0.7	0.5	0.4	0.3	0,27	0.09	0.369	0	0	0	0	0.00	0.00	0.000	0.369
046 (147)	0.7	0.5	--	--	0,27	0.00	0.275	0	0	--	--	0.00	0.00	0.000	0.275
048 (149)	1.5	0.5	0.2	0.2	0,59	0.03	0.620	0	0	0	0	0.00	0.00	0.000	0.620
049 (150)	0.5	0.5	0.5	0.5	0,20	0.20	0.393	0	0	0	0	0.00	0.00	0.000	0.393
051 (152)	0.5	0.5	0.5	0.5	0,20	0.20	0.393	0	0	0	0	0.00	0.00	0.000	0.393
055 (156)	0.5	0.5	1	1	0,20	0.79	0.982	0	0	0	0	0.00	0.00	0.000	0.982
059 (160	0.5	0.5	0.5	0.5	0,20	0.20	0.393	0	0	0	0	0.00	0.00	0.000	0.393
061 (162)	1	0.5	2	1	0,39	1.57	1.963	0	0	1	0.5	0.00	0.39	0.393	1.571
062 (163)	1.5	0.8	1.5	1	0,94	1.18	2.121	0	0	0.3	1	0.00	0.24	0.236	1.885
067 (168)	1	0.5	0.75	0.75	0,39	0.44	0.834	0.3	0.2	0	0	0.05	0.00	0.047	0.787
068 (169)	0.5	1	0.75	0.75	0,39	0.44	0.834	0	0	1	1	0.00	0.79	0.785	0.049
070 (171)	1	0.7	0.5	0.3	0,55	0.12	0.668	1	1	0	0	0.79	0.00	0.785	-0.118
071 (172)	0.5	0.5	0.5	0.5	0,20	0.20	0.393	0.5	0.2	0	0	0.08	0.00	0.079	0.314
073 (173)	1.3	1.1	0.75	0.75	1,12	0.44	1.565	0	0	0	0	0.00	0.00	0.000	1.565
076 (177)	1	0.8	1.5	1	0,63	1.18	1.806	0	0	0	0	0.00	0.00	0.000	1.806
077 (178)	0.7	0.5	0.5	0.5	0,27	0.20	0.471	0.6	0.6	0	0	0.28	0.00	0.283	0.188

Skin quality outcome

Supplement Table S12 Skin quality assessment Aldara™ at Baseline, Follow up 1, Follow up 2, Follow up 3, rating from (0 = very good when a diff. of ≥2 to 4 = impaired with diff. ≥-2), tbd = to be determined, patients are in follow up. Data in red indicate missing data filled according to the last observation carried out forward (LOCF) method.

Skin quality assessment Aldara™																				
Patient Nr.	**Sum score Baseline**		**Sum Score FU1**		**Sum score FU2**		**Sum score FU3**		**Difference B-Fu1**		**Rating**		**Difference B-Fu2**		**Rating**		**Difference Baseline-Fu3**		**Rating**	
	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
002 (102)	2	0	5	0	5	0	1	0	-3	0	4	2	-3	0	4	2	1	0	1	2
004 (104)	3	0	3	0	5	0	0	0	0	0	2	2	-2	0	4	2	3	0	0	2
005 (105)	2	0	0	0	1	0	2	0	2	0	1	2	1	0	1	2	0	0	2	2
011 (112)	2	1	3	2	2	0	5	5	-1	-1	3	4	0	1	2	1	-3	-4	4	4
012 (110)	7	0	6	4	4	0	4	4	1	-4	1	4	3	0	0	2	3	-4	0	4
014 (114)	2	0	2	4	0	5	2	3	0	-4	2	4	2	-5	0	4	0	-3	2	4
015 (115)	3	0	4	3	4	5	0	0	-1	-3	3	4	-1	-5	3	4	3	0	0	2
020 (120)	5	5	3	2	6	5	3	2	2	3	1	0	-1	0	3	2	2	3	1	0
023 (123)	4	4	0	0	0	0	0	0	4	4	0	0	4	4	0	0	4	4	0	0
024 (124)	6	5	1	5	1	5	1	5	5	0	0	2	5	0	0	2	5	0	0	2
027 (127)	2	3	6	6	3	3	12	6	-4	-3	4	4	-1	0	3	2	-10	-3	4	4
031 (131)	1	4	2	2	0	0	0	0	-1	2	3	0	1	4	1	0	1	4	1	0
033 (133)	4	5	2	3	2	2	6	6	2	2	0	0	2	3	1	0	-2	-1	4	3
034 (134)	2	2	0	0	0	0	0	0	2	2	0	0	2	2	0	0	2	2	0	0
039 (140)	1	3	1	1	1	1	11	11	0	2	2	0	0	2	2	0	-10	-8	4	4
043 (144)	9	8	4	4	9	4	12	5	5	4	0	0	0	4	2	0	-3	3	4	0
045 (146)	6	2	9	6	6	6	7	3	-3	-4	4	4	0	-4	2	4	-1	-1	3	3
047 (148)	3	2	6	6	6	6	7	7	-3	-4	4	4	-3	-4	4	4	-4	-5	4	4
050 (151)	6	5	6	6	2	2	4	4	0	-1	2	3	4	3	0	0	2	1	1	1
052(153)	3	2	6	7	0	0	6	6	-3	-5	4	4	3	2	0	0	-3	-4	4	4
053 (154)	6	6	1	0	0	0	0	0	5	6	0	0	6	6	0	0	6	6	0	0
057 (158)	9	9	8	9	8	8	7	7	1	0	1	2	1	1	1	1	2	2	0	0
058 (159)	11	9	3	3	0	0	8	7	8	6	0	0	11	9	0	0	3	2	0	0
064 (165)	3	3	8	8	0	0	4	4	-5	-5	4	4	3	3	0	0	-1	-1	3	3
065 (166)	7	7	8	8	8	8	tbd	tbd	-1	-1	3	3	-1	-1	3	3	tbd	tbd	tbd	tbd
066 (167)	7	7	2	5	6	6	tbd	tbd	5	2	0	0	1	1	1	1	tbd	tbd	tbd	tbd
069 (170)	5	5	8	8	7	7	tbd	tbd	-3	-3	4	4	-2	-2	3	3	tbd	tbd	tbd	tbd
072 (173)	10	10	0	1	8	1	tbd	tbd	10	9	0	0	2	9	1	0	tbd	tbd	tbd	tbd
074 (175)	2	2	3	5	tbd	tbd	tbd	tbd	-1	-3	3	4	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd
075 (176)	9	9	1	0	tbd	tbd	tbd	tbd	8	9	0	0	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd

Supplement Table S13 Skin quality assessment IMI-Gel at Baseline, Follow up (FU) 1, FU 2, FU 3, rating from (0 = very good when a diff. of ≥2 to 4 = impaired with diff. ≥-2), red missing data filled according to LOFC, tbd = to be determined, patient in follow up

Skin quality assessment IMI-Gel

Patient Nr.	Sum score Baseline (B)		Sum Score FU 1		Sum score FU2		Sum score FU3		Difference B-FU1		Rating B-FU1		Difference. B-FU2		Rating B-FU2		Difference B-FU3		Rating B-Fu3	
	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
003 (103)	5	5	4	5	2	5	1	5	1	0	1	2	3	0	0	2	4	0	0	2
007 (107)	2	2	3	1	2	3	1	1	-1	1	3	1	0	-1	2	3	1	1	1	1
008 (108)	2	2	2	2	1	1	2	2	0	0	2	2	1	1	1	1	0	0	2	2
009 (109)	5	6	3	4	1	1	1	1	2	2	0	0	4	5	0	0	4	5	0	0
010 (111)	7	8	6	6	2	1	2	0	1	2	1	0	5	7	0	0	5	8	0	0
013 (113)	10	10	4	3	1	2	1	1	6	7	0	0	9	8	0	0	9	9	0	0
016 (116)	3	4	3	2	2	2	3	2	0	2	2	0	1	2	1	0	0	2	2	1
017 (117)	1	5	0	8	0	8	0	8	1	-3	1	4	1	-3	1	4	1	-3	1	4
018 (118)	4	4	2	2	4	4	2	2	2	2	1	1	0	0	2	2	2	2	0	1
021 (121)	7	3	0	0	1	0	1	0	7	3	0	0	6	3	0	0	6	3	0	0
022 (122)	10	10	0	6	2	2	0	0	10	4	0	0	8	8	0	0	10	10	0	0
026 (126)	5	5	1	2	1	2	1	2	4	3	0	0	4	3	0	0	4	3	0	0
028 (128)	3	4	0	0	0	1	0	0	3	4	0	0	3	3	0	0	3	4	0	0
029 (129)	5	4	4	4	4	4	4	4	1	0	1	2	1	0	1	2	1	0	1	2
032 (132)	3	3	6	6	3	4	10	10	-3	-3	4	4	0	-1	2	3	-7	-7	4	4
035 (137)	1	1	5	6	0	1	2	2	-4	-5	4	4	1	0	1	3	-1	-1	3	3
036 (136	4	4	1	0	1	1	1	3	3	4	0	0	3	3	0	0	3	1	0	1
037 (138)	1	1	7	7	7	7	7	7	-6	-6	4	4	-6	-6	4	4	-6	-6	4	4
040 (141)	2	3	2	0	3	1	0	0	0	3	2	0	-1	2	3	1	2	3	0	0
041 (142)	2	3	1	0	2	2	2	2	1	3	1	0	0	1	2	1	0	1	2	1
042 (143)	10	9	6	6	6	6	17	14	4	3	0	0	4	3	0	0	-7	-5	4	4
046 (147)	1	0	5	0	1	0	3	0	-4	0	4	2	0	0	2	2	-2	0	4	2
048 (149)	11	11	1	1	6	6	6	6	10	10	0	0	5	5	0	0	5	5	0	0
049 (150)	3	2	2	2	0	0	4	5	1	0	1	2	3	2	0	0	-1	-3	3	4
051 (152)	4	4	1	1	4	4	1	1	3	3	0	0	0	0	2	2	3	3	0	0
055 (156)	8	8	7	7	7	7	7	7	1	1	1	1	1	1	1	1	1	1	1	1
059 (160	7	7	2	1	1	1	0	1	5	6	0	0	6	6	0	0	7	6	0	0
061 (162)	8	8	0	6	6	6	1	1	8	2	0	0	2	2	0	0	7	7	0	0
062 (163)	7	12	0	6	0	4	1	1	7	6	0	0	7	8	0	0	6	11	0	0
067 (168)	7	7	2	0	0	2	tbd	tbd	5	7	0	0	7	5	0	0	tbd	tbd	tbd	tbd
068 (169)	6	6	0	1	5	5	tbd	tbd	6	5	0	0	1	1	1	1	tbd	tbd	tbd	tbd
070 (171)	2	2	8	8	tbd	tbd	tbd	tbd	-6	-6	4	4	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd
071 (172	6	6	1	0	1	0	tbd	tbd	5	6	0	0	5	6	0	0	tbd	tbd	tbd	tbd
073 (174)	3	3	6	6	0	0	tbd	tbd	-3	-3	4	4	3	3	0	0	tbd	tbd	tbd	tbd
076 (177)	7	7	0	0	tbd	tbd	tbd	tbd	7	7	0	0	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd

Local Skin Reactions

Supplement Table S14. Edema rating (0 = none, 1 = mild, 2 = moderate, 3 = severe) Aldara™ group, data in red missing and replaced according to LOFC, tbd = to be determined, patient in follow-up

Aldara™ Edema

Patient Nr.	Baseline/Visit 3		Visit 4		Visit 5		Visit 6		Visit 7		V8		FU1		FU2		Fu3	
	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)
002 (102)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
004 (104)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
005 (105)	2	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
011 (112)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
012 (110)	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
014 (114)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
015 (115)	0	0	1	1	0	0	-	-	-	-	-	-	0	0	0	0	0	0
020 (120)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
023 (123)	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
024 (124)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
027 (127)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	2	0
031 (131)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
033 (133)	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
034 (134)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
039 (140)	1	1	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
043 (144)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
045 (146)	1	1	1	0	0	0	-	-	-	-	-	-	1	0	0	0	0	0
047 (148)	1	1	1	1	0	0	-	-	-	-	-	-	0	0	0	0	0	0
050 (151)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
052(153)	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
053 (154)	0	0	1	2	0	0	-	-	-	-	-	-	0	0	1	0	0	0
057 (158)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
058 (159)	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
064 (165)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
065 (166)	2	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd
066 (167)	0	0	1	0	0	0	-	-	-	-	-	-	0	0	0	0	tbd	tbd
069 (170)	0	1	2	3	0	0	-	-	-	-	-	-	0	0	0	0	0	0
072 (173)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	tbd	tbd
074 (175)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd	tbd	tbd
075 (176)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	tbd	tbd	tbd	tbd

Supplement Table S15. Edema rating (0 = none, 1 = mild, 2 = moderate, 3 = severe) IMI-Gel group, data in red missing and replaced according to LOFC, tbd = to be determined, patient in follow-up

IMI-Gel Edema

	Baseline/Visit 3		Visit 4		Visit 5		Visit 6		Visit 7		Visit 8		Follow up 1		Follow up 2		Follow up 3	
Patient Nr.	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)
003 (103)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
007 (107)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
008 (108)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
009 (109)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
010 (111)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
013 (113)	1	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
016 (116)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
017 (117)	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
018 (118)	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
021 (121)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
022 (122)	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
026 (126)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
028 (128)	1	0	2	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0
029 (129)	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
032 (132)	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
035 (137)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
036 (136	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
037 (138)	1	1	1	1	0	0	-	-	-	-	-	-	0	0	0	0	0	0
040 (141)	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
041 (142)	2	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
042 (143)	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
046 (147)	1	-	0	-	0	-	-	-	-	-	-	-	0	-	1	-	0	-
048 (149)	1	1	2	2	0	0	-	-	-	-	-	-	0	0	0	0	0	0
049 (150)	1	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
051 (152)	0	0	1	1	0	0	-	-	-	-	-	-	0	0	0	0	0	0
055 (156)	0	0	1	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
059 (160)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
061 (162)	0	0	1	0	0	0	-	-	-	-	-	-	0	0	0	0	1	1
062 (163)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
067 (168)	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	tbd	tbd
068 (169)	1	1	1	1	0	0	-	-	-	-	-	-	0	0	0	0	tbd	tbd
070 (171)	0	1	0	1	0	0	0	0	0	0	0	0	0	0	tbd	tbd	tbd	tbd
071 (172)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd
073 (173)	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd
076 (177)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	tbd	tbd	tbd	tbd
077 (178)	0	0	0	0	0	0	0	0	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd

Supplement Table S16. Vesicle rating (0 = none, 1 = mild, 2 = moderate, 3 = severe), Aldara™ group, data in red missing and replaced according to LOFC, tbd = to be determined, patient in follow-up

Aldara™ Vesicles																		
	Baseline/Visit 3		Visit 4		Visit 5		Visit 6		Visit 7		Visit 8		Follow up 1		Follow up 2		Follow up 3	
Patient Nr.	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)
002 (102)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
004 (104)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
005 (105)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
011 (112)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
012 (110)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
014 (114)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
015 (115)	0	0	2	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
020 (120)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
023 (123)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
024 (124)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
027 (127)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
031 (131)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
033 (133)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
034 (134)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
039 (140)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
043 (144)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
045 (146)	1	0	0	0	0	0	-	-	-	-	-	-	1	0	0	0	0	0
047 (148)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
050 (151)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
052(153)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
053 (154)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
057 (158)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
058 (159)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
064 (165)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
065 (166)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd
066 (167)	0	0	2	0	0	0	-	-	-	-	-	-	0	0	0	0	tbd	tbd
069 (170)	0	0	2	1	0	0	-	-	-	-	-	-	0	0	0	0	0	0
072 (173)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	tbd	tbd
074 (175)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd	tbd	tbd
075 (176)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	tbd	tbd	tbd	tbd

Supplement Table S17. Vesicle rating (0 = none, 1 = mild, 2 = moderate, 3 = severe) IMI-Gel group, data in red missing and replaced according to LOFC, tbd = to be determined, patient in follow-up

IMI-Gel Vesicles

Patient Nr.	Baseline/Visit 3		Visit 4		Visit 5		Visit 6		Visit 7		Visit 8		Follow up 1		Follow up 2		Follow up 3	
	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)
003 (103)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
007 (107)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
008 (108)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
009 (109)	0	0	0	1	0	0	-	-	-	-	-	-	0	0	0	0	0	0
010 (111)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
013 (113)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
016 (116)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
017 (117)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
018 (118)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
021 (121)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
022 (122)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
026 (126)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
028 (128)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
029 (129)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
032 (132)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
035 (137)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
036 (136	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
037 (138)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
040 (141)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
041 (142)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
042 (143)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
046 (147)	1	-	0	-	0	-	-	-	-	-	-	-	0	-	0	-	0	-
048 (149)	1	0	1	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
049 (150)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
051 (152)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
055 (156)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
059 (160)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
061 (162)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
062 (163)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
067 (168)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd
068 (169)	0	0	2	2	0	0							0	0	0	0	tbd	tbd
070 (171)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd	tbd	tbd
071 (172)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd
073 (173)	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd
076 (177)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	tbd	tbd	tbd	tbd
077 (178)	0	0	0	0	0	0	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd

Supplement Table S18. Erosion rating (0 = none, 1 = mild, 2 = moderate, 3 = severe), Aldara™ group, data in red missing and replaced according to LOFC, tbd = to be determined, patient in follow-up

Aldara™ Erosions

	Baseline/Visit 3		Visit 4		Visit 5		Visit 6		Visit 7		Visit 8		Follow up 1		Follow up 2		Follow up 3	
Patient Nr.	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)
002 (102)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
004 (104)	1	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
005 (105)	2	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
011 (112)	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
012 (110)	0	1	1	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0
014 (114)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
015 (115)	2	1	1	2	0	0	-	-	-	-	-	-	0	0	0	0	0	1
020 (120)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
023 (123)	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
024 (124)	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
027 (127)	1	0	0	0	0	0	1	2	0	0	2	0	1	2	2	1	2	0
031 (131)	1	1	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
033 (133)	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
034 (134)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
039 (140)	1	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
043 (144)	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
045 (146)	0	1	2	1	0	0	-	-	-	-	-	-	1	1	0	0	0	0
047 (148)	1	1	3	3	0	0	-	-	-	-	-	-	0	0	0	0	0	0
050 (151)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
052(153)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
053 (154)	0	0	2	2	0	0	-	-	-	-	-	-	0	0	0	0	0	0
057 (158)	1	1	2	3	0	0	1	1	1	1	0	0	0	0	0	0	0	0
058 (159)	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0
064 (165)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
065 (166)	1	2	3	3	0	0	0	0	0	0	0	0	0	0	1	0	tbd	tbd
066 (167)	1	0	2	0	0	0	-	-	-	-	-	-	0	0	0	0	tbd	tbd
069 (170)	1	3	2	3	0	0	-	-	-	-	-	-	0	0	0	0	0	0
072 (173)	1	0	0	0	0	0	-	-	-	-	-	-	0	0	2	0	tbd	tbd
074 (175)	1	0	1	0	0	0	0	2	0	0	0	0	0	0	tbd	tbd	tbd	tbd
075 (176)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	tbd	tbd	tbd	tbd

Supplement Table S19. Erosion rating (0 = none, 1 = mild, 2 = moderate, 3 = severe), IMI-Gel group, data in red missing and replaced according to LOFC, tbd = to be determined, patient in follow-up

IMI-Gel Erosion

Patient Nr.	Baseline/Visit 3		Visit 4		Visit 5		Visit 6		Visit 7		Visit 8		Follow up 1		Follow up 2		Follow up 3	
	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)
003 (103)	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
007 (107)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
008 (108)	1	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0
009 (109)	1	0	0	1	0	0	-	-	-	-	-	-	0	0	0	0	0	0
010 (111)	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
013 (113)	0	1	1	1	0	1	-	-	-	-	-	-	0	0	0	0	0	0
016 (116)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
017 (117)	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
018 (118)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
021 (121)	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
022 (122)	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
026 (126)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
028 (128)	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
029 (129)	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
032 (132)	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	1	0	0
035 (137)	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
036 (136	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
037 (138)	1	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
040 (141)	2	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
041 (142)	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
042 (143)	1	1	2	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0
046 (147)	1	-	2	-	0	-	-	-	-	-	-	-	0	-	0	-	0	-
048 (149)	2	1	3	3	0	0	-	-	-	-	-	-	0	0	0	0	0	0
049 (150)	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
051 (152)	1	1	1	1	0	0	-	-	-	-	-	-	0	0	0	0	0	0
055 (156)	1	1	2	3	0	0	-	-	-	-	-	-	0	0	0	0	0	0
059 (160)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
061 (162)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
062 (163)	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
067 (168)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd
068 (169)	1	1	2	2	0	0	-	-	-	-	-	-	0	0	0	0	tbd	tbd
070 (171)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd	tbd	tbd
071 (172)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd
073 (173)	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd
076 (177)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	tbd	tbd	tbd	tbd
077 (178)	0	0	0	0	0	0	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd

Supplement Table S20. Ulcerations rating (0 = none, 1 = mild, 2 = moderate, 3 = severe), Aldara™ group, data in red missing and replaced according to LOFC, tbd = to be determined, patient in follow-up

Aldara™ Ulcerations																		
	Baseline/Visit 3		Visit 4		Visit 5		Visit 6		Visit 7		Visit 8		Follow up 1		Follow up 2		Follow up 3	
Patient Nr.	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)
002 (102)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
004 (104)	1	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
005 (105)	2	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
011 (112)	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
012 (110)	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
014 (114)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
015 (115)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
020 (120)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
023 (123)	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
024 (124)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
027 (127)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0
031 (131)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
033 (133)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
034 (134)	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
039 (140)	1	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
043 (144)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
045 (146)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
047 (148)	0	0	1	1	0	0	-	-	-	-	-	-	0	0	0	0	0	0
050 (151)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
052(153)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
053 (154)	0	0	1	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
057 (158)	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
058 (159)	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
064 (165)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
065 (166)	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd
066 (167)	0	0	2	0	0	0	-	-	-	-	-	-	0	0	0	0	tbd	tbd
069 (170)	0	0	1	3	0	0	-	-	-	-	-	-	0	0	0	0	0	0
072 (173)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	tbd	tbd
074 (175)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd	tbd	tbd
075 (176)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	tbd	tbd	tbd	tbd

Supplement Table S21. Ulcerations rating (0 = none, 1 = mild, 2 = moderate, 3 = severe), IMI-Gel group, data in red missing and replaced according to LOFC, tbd=to be determined, patient in follow-up

IMI-Gel Ulcerations

Patient Nr.	Baseline/Visit 3		Visit 4		Visit 5		Visit 6		Visit 7		Visit 8		Follow up 1		Follow up 2		Follow up 3	
	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)
003 (103)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
007 (107)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
008 (108)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
009 (109)	0	0	0	0	0	0							0	0	0	0	0	0
010 (111)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
013 (113)	0	0	1	1	0	0							0	0	0	0	0	0
016 (116)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
017 (117)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
018 (118)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
021 (121)	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
022 (122)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
026 (126)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
028 (128)	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
029 (129)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
032 (132)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
035 (137)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
036 (136	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
037 (138)	0	0	0	0	0	0							0	0	0	0	0	0
040 (141)	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
041 (142)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
042 (143)	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
046 (147)	0	-	1	-	0	-	-	-	-	-	-	-	0	-	0	-	0	-
048 (149)	0	0	2	2	0	0							0	0	0	0	0	0
049 (150)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
051 (152)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
055 (156)	1	1	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
059 (160)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
061 (162)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
062 (163)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
067 (168)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd
068 (169)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	tbd	tbd
070 (171)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd	tbd	tbd
071 (172)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd
073 (173)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd
076 (177)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	tbd	tbd	tbd	tbd
077 (178)	1	0	0	0	0	0	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd

Supplement Table S22. Scaling/Flaking rating (0 = none, 1 = mild, 2 = moderate, 3 = severe), Aldara™ group, data in red missing and replaced according to LOFC, tbd = to be determined, patient in follow-up

Aldara™ Scaling/Flaking

	Baseline/Visit 3		Visit 4		Visit 5		Visit 6		Visit 7		Visit 8		Follow up 1		Follow up 2		Follow up 3	
Patient Nr.	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)
002 (102)	1	0	0	0	0	0	1	3	2	1	2	2	0	0	0	0	2	2
004 (104)	1	0	1	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
005 (105)	1	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
011 (112)	0	0	0	0	2	1	1	1	1	1	2	1	0	0	0	0	1	1
012 (110)	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
014 (114)	1	0	1	1	0	0	0	1	0	1	0	1	0	1	0	0	1	2
015 (115)	0	0	1	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
020 (120)	0	0	1	1	0	0	1	1	1	1	0	0	1	0	0	0	1	1
023 (123)	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
024 (124)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
027 (127)	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	1	2	1
031 (131)	1	1	1	1	0	0	-	-	-	-	-	-	0	0	0	0	0	0
033 (133)	0	0	0	0	1	1	1	1	0	1	0	1	1	2	1	1	1	1
034 (134)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
039 (140)	1	1	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
043 (144)	1	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0	1	0
045 (146)	1	1	1	1	0	0	-	-	-	-	-	-	2	1	3	2	0	1
047 (148)	1	1	1	1	0	0	-	-	-	-	-	-	0	0	1	0	1	1
050 (151)	1	1	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
052(153)	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
053 (154)	0	0	0	0	0	0	-	-	-	-	-	-	1	0	0	0	0	0
057 (158)	0	0	1	1	3	3	2	2	2	1	1	1	1	2	2	2	2	1
058 (159)	1	1	1	1	2	1	1	0	2	1	1	0	0	0	0	0	1	1
064 (165)	1	1	0	0	1	1	1	1	1	1	1	0	1	1	0	1	0	0
065 (166)	1	2	3	3	2	2	2	2	2	2	3	3	3	3	1	1	tbd	tbd
066 (167)	0	0	2	1	0	0	-	-	-	-	-	-	0	0	0	0	tbd	tbd
069 (170)	1	1	1	1	0	0	-	-	-	-	-	-	0	0	0	1	0	1
072 (173)	1	1	0	0	0	0	-	-	-	-	-	-	0	1	2	1	tbd	tbd
074 (175)	2	1	2	1	1	1	0	1	0	1	0	1	0	1	tbd	tbd	tbd	tbd
075 (176)	1	1	1	1	0	0	-	-	-	-	-	-	1	0	tbd	tbd	tbd	tbd

Supplement Table S23. Scaling/Flaking rating (0 = none, 1 = mild, 2 = moderate, 3 = severe), IMI-Gel group, data in red missing and replaced according to LOFC, tbd = to be determined, patient in follow-up

IMI-Gel Scaling/Flaking

	Baseline/Visit 3		Visit 4		Visit 5		Visit 6		Visit 7		Visit 8		Follow up 1		Follow up 2		Follow up 3	
Patient Nr.	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)
003 (103)	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
007 (107)	0	0	0	0	1	1	0	0	2	2	1	1	0	0	0	0	0	0
008 (108)	0	0	1	0	1	0	2	1	0	0	0	0	0	0	0	0	0	0
009 (109)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
010 (111)	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0
013 (113)	1	2	1	1	2	3	-	-	-	-	-	-	2	2	1	1	0	0
016 (116)	1	1	1	1	0	0	1	1	1	1	0	0	0	0	0	0	0	0
017 (117)	1	2	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
018 (118)	1	1	1	1	2	1	0	2	2	1	1	0	0	0	0	0	0	0
021 (121)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
022 (122)	1	1	2	1	2	1	0	0	1	1	0	0	0	0	1	1	0	0
026 (126)	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
028 (128)	0	0	0	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0
029 (129)	1	1	2	0	2	2	2	2	1	0	0	0	0	0	0	0	0	0
032 (132)	2	2	1	1	2	2	2	1	2	1	0	0	0	0	1	1	1	1
035 (137)	1	0	1	0	0	0	0	0	0	0	1	1	1	1	0	0	1	1
036 (136	1	0	0	0	1	1	1	1	1	1	1	1	0	0	1	1	1	1
037 (138)	0	0	1	1	0	0	-	-	-	-	-	-	0	0	0	0	0	0
040 (141)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
041 (142)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
042 (143)	1	1	1	1	1	1	1	1	0	1	0	0	0	0	0	0	0	1
046 (147)	1	-	2	-	0	-	-	-	-	-	-	-	0	-	1	-	1	-
048 (149)	1	1	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
049 (150)	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
051 (152)	0	0	0	1	0	0	-	-	-	-	-	-	0	0	0	0	0	0
055 (156)	0	0	1	1	0	0	-	-	-	-	-	-	0	0	0	0	0	0
059 (160)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	1
061 (162)	1	1	1	0	1	1	0	0	0	0	0	0	1	1	0	1	1	1
062 (163)	1	1	1	1	0	1	0	1	0	1	0	1	0	1	0	0	1	1
067 (168)	1	0	1	1	1	0	0	0	1	1	1	1	1	0	0	1	tbd	tbd
068 (169)	1	1	1	1	0	0	-	-	-	-	-	-	1	0	0	0	tbd	tbd
070 (171)	1	1	1	1	0	0	0	0	0	0	0	0	1	0	tbd	tbd	tbd	tbd
071 (172)	1	1	1	1	1	2	0	0	0	0	0	0	1	0	1	0	tbd	tbd
073 (173)	1	1	1	1	1	0	1	0	1	0	1	1	0	0	0	0	tbd	tbd
076 (177)	1	1	0	0	0	1	-	-	-	-	-	-	0	0	tbd	tbd	tbd	tbd
077 (178)	0	0	0	0	1	1	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd

Supplement Table S24. Scabbing/Crusting rating (0 = none, 1 = mild, 2 = moderate, 3 = severe), Aldara™ group, data in red missing and replaced according to LOFC, tbd = to be determined, patient in follow-up

Aldara™ Scabbing/Crusting

Patient Nr.	Baseline/Visit 3		Visit 4		Visit 5		Visit 6		Visit 7		Visit 8		Follow up 1		Follow up 2		Follow up 3	
	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)
002 (102)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
004 (104)	1	0	1	1	0	0	-	-	-	-	-	-	0	0	0	0	0	0
005 (105)	1	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
011 (112)	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
012 (110)	0	1	0	1	1	0	1	0	1	1	0	1	0	0	0	0	0	0
014 (114)	0	0	1	1	0	1	0	1	0	1	0	2	0	1	0	2	0	1
015 (115)	1	1	1	2	0	0	-	-	-	-	-	-	0	0	0	0	0	0
020 (120)	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	1
023 (123)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
024 (124)	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
027 (127)	1	0	0	0	0	0	1	2	0	0	2	0	1	1	2	1	2	1
031 (131)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
033 (133)	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
034 (134)	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
039 (140)	1	1	1	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
043 (144)	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
045 (146)	1	1	2	1	0	0	-	-	-	-	-	-	2	1	0	0	0	0
047 (148)	1	1	3	3	0	0	-	-	-	-	-	-	0	0	0	0	0	0
050 (151)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
052(153)	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
053 (154)	0	0	1	1	0	0	-	-	-	-	-	-	0	0	0	0	0	0
057 (158)	1	1	1	1	0	0	2	2	2	1	0	0	0	0	0	0	2	1
058 (159)	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	1	1
064 (165)	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
065 (166)	1	3	3	3	0	0	0	0	0	0	0	1	0	0	1	1	tbd	tbd
066 (167)	0	0	2	0	0	0	-	-	-	-	-	-	0	0	0	0	tbd	tbd
069 (170)	1	1	3	3	0	0	-	-	-	-	-	-	0	0	0	0	0	0
072 (173)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	3	1	tbd	tbd
074 (175)	1	1	1	1	1	0	1	0	0	0	0	0	0	0	tbd	tbd	tbd	tbd
075 (176)	1	1	1	1	0	0	-	-	-	-	-	-	0	0	tbd	tbd	tbd	tbd

Supplement Table S25. Scabbing/Crusting rating (0 = none, 1 = mild, 2 = moderate, 3 = severe), IMI-Gel group, data in red missing and replaced according to LOFC, tbd = to be determined, patient in follow-up

IMI-Gel Scabbing/Crusting

Patient Nr.	Baseline/Visit 3		Visit 4		Visit 5		Visit 6		Visit 7		Visit 8		Follow up 1		Follow up 2		Follow up 3	
	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)
003 (103)	1	0	1	1	2	0	2	1	0	2	0	1	0	0	0	1	0	0
007 (107)	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
008 (108)	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0
009 (109)	1	0	0	1	0	0	-	-	-	-	-	-	0	0	0	0	0	0
010 (111)	0	0	0	0	0	0	1	0	0	0	1	1	1	1	1	0	0	0
013 (113)	1	2	1	1	1	2	-	-	-	-	-	-	2	2	0	0	0	0
016 (116)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
017 (117)	2	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
018 (118)	0	0	1	2	0	0	0	0	2	1	0	0	0	0	0	0	0	0
021 (121)	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
022 (122)	1	1	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
026 (126)	1	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
028 (128)	1	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
029 (129)	0	0	2	0	2	2			0	0	0	0	0	0	0	0	0	0
032 (132)	0	0	0	0	0	0	2	1	0	0	0	0	1	1	0	0	0	0
035 (137)	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
036 (136	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0
037 (138)	1	1	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
040 (141)	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
041 (142)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
042 (143)	2	1	1	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0
046 (147)	0	-	2	-	0	-	-	-	-	-	-	-	0	-	0	-	0	-
048 (149)	2	1	3	3	0	0	-	-	-	-	-	-	0	0	0	0	0	0
049 (150)	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
051 (152)	1	1	0	1	0	0	-	-	-	-	-	-	0	0	0	0	0	0
055 (156)	1	1	1	1	0	0	-	-	-	-	-	-	0	0	0	0	0	0
059 (160)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
061 (162)	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
062 (163)	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0
067 (168)	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	tbd	tbd
068 (169)	0	0	1	1	0	0	-	-	-	-	-	-	0	0	0	0	tbd	tbd
070 (171)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd	tbd	tbd
071 (172)	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	tbd	tbd
073 (173)	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd
076 (177)	0	0	2	2	0	0	-	-	-	-	-	-	0	0	tbd	tbd	tbd	tbd
077 (178)	0	0	0	0	0	0	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd

Supplement Table S26. Weeping/Exudate (0 = none, 1 = mild, 2 = moderate, 3 = severe), AldaraTM group, data in red missing and replaced according to LOFC, tbd = to be determined, patient in follow-up

AldaraTM Weeping/Exudate

	Baseline/Visit 3		Visit 4		Visit 5		Visit 6		Visit 7		Visit 8		Follow up 1		Follow up 2		Follow up 3	
Patient Nr.	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)
002 (102)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
004 (104)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
005 (105)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
011 (112)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
012 (110)	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
014 (114)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
015 (115)	1	2	2	2	0	0	-	-	-	-	-	-	0	0	0	0	0	0
020 (120)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
023 (123)	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
024 (124)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
027 (127)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
031 (131)	1	1	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
033 (133)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
034 (134)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
039 (140)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
043 (144)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
045 (146)	0	0	1	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
047 (148)	1	0	1	1	0	0	-	-	-	-	-	-	0	0	0	0	0	0
050 (151)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
052(153)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
053 (154)	0	0	1	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
057 (158)	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
058 (159)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
064 (165)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
065 (166)	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd
066 (167)	0	0	2	0	0	0	-	-	-	-	-	-	0	0	0	0	tbd	tbd
069 (170)	0	1	2	3	0	0	-	-	-	-	-	-	0	0	0	0	0	0
072 (173)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	tbd	tbd
074 (175)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd	tbd	tbd
075 (176)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	tbd	tbd	tbd	tbd

Supplement Table S27. Weeping/Exudate rating (0 = none, 1 = mild, 2 = moderate, 3 = severe), IMI-Gel group, data in red missing and replaced according to LOFC, tbd = to be determined, patient in follow-up

IMI-Gel Weeping/Exudate

	Baseline/Visit 3		Visit 4		Visit 5		Visit 6		Visit 7		Visit 8		Follow up 1		Follow up 2		Follow up 3	
Patient Nr.	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)	Lesion 1 (L1)	Lesion 2 (L2)
003 (103)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
007 (107)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
008 (108)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
009 (109)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
010 (111)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
013 (113)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
016 (116)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
017 (117)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
018 (118)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
021 (121)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
022 (122)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
026 (126)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
028 (128)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
029 (129)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
032 (132)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
035 (137)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
036 (136	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
037 (138)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
040 (141)	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
041 (142)	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
042 (143)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
046 (147)	1	-	0	-	0	-	-	-	-	-	-	-	0	-	0	-	0	-
048 (149)	1	1	2	2	0	0	-	-	-	-	-	-	0	0	0	0	0	0
049 (150)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
051 (152)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
055 (156)	1	1	1	1	0	0	-	-	-	-	-	-	0	0	0	0	0	0
059 (160)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0
061 (162)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
062 (163)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
067 (168)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd
068 (169)	0	0	1	1	0	0	-	-	-	-	-	-	0	0	0	0	tbd	tbd
070 (171)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd	tbd	tbd
071 (172)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd
073 (173)	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	tbd	tbd
076 (177)	0	0	0	0	0	0	-	-	-	-	-	-	0	0	tbd	tbd	tbd	tbd
077 (178)	0	0	0	0	0	0	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd	tbd

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