PROTOCOL SYNOPSIS

Title

Immunological characterization of TLR-7 mediated inflammation and complement activation after prolonged imiquimod exposure in healthy volunteers

Short Title

In vivo immune activation after prolonged TLR-7 inflammatory challenge

Principal investigator & Trial Site

M. Moerland, Centre for Human Drug Research, Zernikedreef 8, 2333 CL Leiden, The Netherlands

Background & Rationale

Inflammation is a response to damaged tissue and/or pathogens resulting in cellular activation and a release of cytokines. Although inflammation is in principle a physiological process, in some cases an excessive and/or poorly regulated inflammatory response can be harmful to the host, which is the case in many inflammatory disorders.

Toll-like receptors belong to the family of pattern recognition receptors (PRRs). These highly conserved receptors recognize pathogen-associated molecular patterns (PAMPs) and danger associated molecular patterns (DAMPs). Detection of PAMPs by mediators of innate immunity brings multiple components of immunity into play, including the complement system. One part of the complement system is a collection of proteins (C5-C9) that, when activated, form aggregates that punch holes in the cell membranes of targeted microbes, killing the cells by lysis. The complement system also includes serum glycoproteins that, when activated, promote uptake of microorganisms by phagocytes (opsonization). As such, the complement system is a first line of defence for fighting pathogens and clearing apoptotic cells. However, when hyperactivated, it is a driver of a variety of autoimmune and inflammatory diseases, making it an interesting target for drug development. An *in vivo* complement activation model would be of great benefit for clinical evaluation of the pharmacological activity of novel complement-targeting investigational compounds, but such a model is not readily available.

Toll-like receptor 7 (TLR7) is an intracellular, endosomal TLR recognizing single stranded (ss)RNA from viruses. Activation of TLR7 results in the production of a variety of pro-inflammatory cytokines via activation of the central transcription factor, nuclear factor-kB (NFkB), and interferon regulatory factor 3 (IRF3). Imiquimod is an imidazoquinolone drug acting as TLR7 agonist, exhibiting tumoricidal and anti-viral effects both *in vitro* and *in vivo*[1]. Aldara (imiquimod 5%) cream is on the market for treatment of (pre)malignant and HPV-induced skin lesions (see SPC Aldara). Mouse studies by Giacomassi et al with 7-day Imiquimod application suggest that complement factor 3 is involved in the imiquimod induced inflammatory response, as C3-knockout mice showed reduced expression of psoriasis-relevant genes in the skin, reduced neutrophil infiltration and reduced IL-17 production[2]. In this model, clinical inflammation (ear thickness, erythema and scaling) peaked on day 7 and a significant reduction in neutrophil influx was seen in C3-knockout mice.

In recent studies performed by CHDR, topical application of imiquimod (2 or 3 days) resulted in erythema and a significant increase in skin perfusion, peaking 48 hours after start of the imiquimod treatment, and clear IRF signalling (MXA release). Interestingly, the cellular and molecular responses to imiquimod were relatively mild, with moderate monocyte, NK cell, and DC responses, a mild IL-6 response, and no significant deposition of complement factors C3c and C3d. The involvement of neutrophils and T cells was limited or absent. These data suggest that the imiquimod exposure in these studies may have been too limited, or too short, to drive complement activation. CHDR never performed studies with imiquimod application exceeding 3 days.

In this study, we aim to characterize TLR7-mediated inflammation, including complement involvement, after 7-day imiquimod exposure in healthy volunteers. Readouts will be based on non-invasive measures (local erythema, perfusion, temperature) and invasive measures (IHC and mRNA analysis of skin punch biopsies, for cytokines/chemokines, immune cells, and complement factors). Further characterization of the Imiquimod induced inflammation including the role of complement

could provide valuable insights for the development of an in vivo innate immune activation model. This model could be used for the early clinical evaluation of the pharmacological activity of novel complement-targeting investigational compounds as well as for other innate immune-targeting compounds.

Objective(s) and endpoints

Objectives	Endpoints
Primary	
• To immunologically characterize imiquimod-induced inflammation after 7-day exposure of healthy skin; • To evaluate local complement activation/depositions after a prolonged topical imiquimod challenge; • To evaluate systemic activation of complement after imiquimod challenge	• Cytokines and immune cells in skin biopsies • Local complement factors in skin biopsies • Complement factors and activation markers in blood samples
Secondary	
• To characterize the clinical response to prolonged imiquimod challenge over a 7-day imiquimod treatment period;	• Perfusion by LSCI • Erythema by Antera 3D and clinical evaluation

Design

This is a single-center, inflammatory challenge study in healthy volunteers, to evaluate immune cell and complement activation by prolonged exposure to imiquimod. 10 volunteers will receive imiquimod as a challenge agent on tape-stripped skin, followed by serial biopsies of the challenge sites. In addition, one area will treated with imiquimod for 7 days and only be followed non-invasively over time (local perfusion and erythema).

Timelines

- Screening: 1 hour visit
- Study day (day 1): 3 hours visit (baseline biopsy and first application)
- Follow-up visit 1 (day 2): 1 hour visit (second application)
- Follow-up visit 2 (day 3): 2 hours visit (48h biopsy and fourth application)
- Follow-up visit 3 (day 4): 2 hours visit (72h biopsy and fourth application)
- Follow-up visit 4 (day 5): 1 hour visit (fifth application)
- Follow-up visit 5 (day 6): 2 hours visit (120h biopsy and sixth application)
- Follow-up visit 6 (day 7): 1 hour visit (seventh application)
- Follow-up visit 7 (day 8): 2 hours visit (168h biopsy)

- Follow-up visit 8 (day 14): 2 hours visit (final measurements + end of study)

Non-Investigational drug

Aldara®/imiquimod

Aldara 5% is a cream containing the active ingredient imiquimod (50 mg/g). The maximum application duration is up to 16 weeks with 3-5 applications per week, depending on the indication (see SPC). In previous CHDR studies (CHDR1430, CHDR 1631, CHDR1912 and CHDR2036), a dosage of 5 mg IMQ (100 mg Aldara®) was applied with a maximum of 15 mg and 60 mg IMQ per treatment day for 3 days. In all studies the applied dosage led to a mild to moderate reversible skin inflammation and was well-tolerated by healthy volunteers. Although CHDR does not have experience yet with 7 day treatment of IMQ, studies in cancer patients have shown that 7 day treatment (both once daily and twice daily) or even 12 weeks treatment (once daily or 5 times a week) are well tolerated[3,4]. In the treatment of Lentigo Maligna, imiquimod is applied daily for 12 consecutive weeks[6,7].

Mouse studies by Giacomassi et al (2017) with 7-day Imiquimod application suggest that complement factor 3 is involved in the inflammatory response, as C3-knockout mice showed reduced expression of psoriasis-relevant genes in the skin, reduced neutrophil infiltration and reduced IL-17 production[2]. In this model, clinical inflammation (ear thickness, erythema and scaling) peaked on day 7 and a significant reduction in neutrophil influx was seen in C3-knockout mice.

In this study, IMQ will be topically administered to the subjects as detailed in the study design tables. This will be done by trained medical staff. A dosage of 5 mg IMQ (100 mg Aldara®) per treatment site will be applied, for 7 consecutive days.

Subjects / Groups

A total of 10 healthy male volunteers will be enrolled. All subjects will undergo a 7-day imiquimod challenge.

Inclusion criteria

1. Healthy male and female subjects, 18 to 45 years of age, inclusive. Healthy status is defined by absence of evidence of any active or chronic disease following a detailed medical and surgical history, a complete physical examination including vital signs, 12-lead ECG, hematology, blood chemistry, blood serology and urinalysis. In the case of uncertain or questionable results, tests performed during screening may be repeated before randomization to confirm eligibility or judged to be clinically irrelevant for healthy subjects;

2. Body mass index (BMI) between 18 and 30 kg/m^2 and a minimum weight of 50 kg, inclusive;

3. Fitzpatrick skin type I-III (Caucasian);

4. Subjects and their partners of childbearing potential must use effective contraception for the duration of the study;

5. Able and willing to give written informed consent and to comply with the study restrictions.

Exclusion criteria

Eligible subjects must meet none of the following exclusion criteria at screening:

1. History of pathological scar formation (keloid, hypertrophic scar) or keloids or surgical scars in the target treatment area that in the opinion of the investigator, would limit or interfere with dosing and/or measurement in the trial;

2. Diagnosed with psoriasis or family history of psoriasis

3. History of skin cancer (basal cell carcinoma, squamous cell carcinoma, melanoma);

4. Have any current and / or recurrent clinically significant skin condition at the treatment area (e.g. atopic dermatitis); including tattoos;

5. Using immunosuppressive or immunomodulatory medication within 30 days prior to enrolment or planned to use during the course of the study;

6. Use of topical medication (prescription or over-the-counter [OTC]) within 30 days of study drug administration, or less than 5 half-lives (whichever is longer) in local treatment area;

7. Participation in an investigational drug or device study within 3 months prior to screening or more than 4 times a year;

8. Loss or donation of blood over 500 mL within three months prior to screening or donation of plasma within 14 days of screening;

9. Any (medical) condition that would, in the opinion of the investigator, potentially compromise the safety or compliance of the patient or may preclude the patient's successful completion of the clinical trial;

10. Any vaccination within 30 days prior to initial IMQ dosing or planned during the course of the study with exception of vaccination for SARS-CoV-2;
11. Vaccination for SARS-CoV-2 within 14 days prior to initial IMQ dosing, or planned during the course of the study;
12. Chronic infection with HIV, hepatitis B (HBV) or hepatitis C (HCV). A positive HBV surface antigen (HBsAg) test at screening excludes a subject;

13. A history of ongoing, chronic or recurrent infectious disease;

14. Current smoker and/or regular user of other nicotine-containing products (e.g., patches);

15. History of or current drug or substance abuse considered significant by the PI (or medically qualified designee), including a positive urine drug screen.

16. Previous use of Aldara (imiquimod cream) 3 months prior to the baseline visit;

17. Volunteers with clinically relevant infections

18. Hypersensitivity for dermatological marker at screening

19. Tanning due to sunbathing, excessive sun exposure or a tanning both within 3 weeks of enrollment.

20. Pregnant, a positive pregnancy test, intending to become pregnant, or breastfeeding

Concomitant medications

No prescription medications, OTC medications, vitamin, herbal and dietary supplements will be permitted within 7 days prior to study drug administrations, or less than 5 half-lives (whichever is longer), and during the course of the study. Exception is paracetamol (up to 4 g/day) in case of local pain. Use of pain medication will be determined by the investigator individually. Allowance of use of other medications will be determined by the investigator individually.

Sample Size Justification

This is an exploratory study, and the sample size is not based on formal statistical considerations. 10 subjects per treatment group is a conventional sample size for early phase clinical pharmacology

trials, which is the type of trial for which the current methodology is being developed. Earlier studies with shorter imiquimod exposure showed that sample sizes of 6-12 per treatment group are sufficient to identify imiquimod-responsive endpoints.

Statistical methodology

Data listings and averages will be presented for pharmacodynamics and safety measures. Given the exploratory character of the study, pharmacodynamic endpoints will be primarily analysed using descriptive statistics. All pharmacodynamic endpoints will be summarized with at least mean and standard deviation of the mean, median, minimum, and maximum values, by treatment and time, and will also be presented graphically as mean over time, with standard deviation as error bars. All categorical efficacy endpoints will be summarised by frequencies.

Table 1 Visit and Assessment Schedule

	SCR	Day 1		Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	EOS
Time point / Assessment	Up to -42 d	Pre-dose	0	24hr	48hr	72hr	96 hr	120hr	144hr	168hr	Day 14 ± 2 d 312h
Informed consent	X										
Demography	X										
Skin marker test	X										
Inclusion and exclusion criteria	X	X									
Medical history	X										
Height	X										
Weight	X										
Physical examination	X										X[1]
Fitzpatrick skin type (I-III)	X										
Serology	X										
Covid rapid antigen test[5]		X		X	X	X	X	X	X	X	X
BsHaem, BsChem, BsCoag, BsGluc[2]	X										X
BsCompl		X			X	X		X		X	X
Urinalysis, Urpregnancy[3]	X										X

[1] Symptom-guided physical + skin site reaction

[2] Samples are taken after at least 4 hours of fasting

[3] Pregnancy test for women of childbearing potential will be performed at screening, EOS and if pregnancy is suspected during the study

[4] TEWL assessment will be performed on day 1, before and after tape stripping of skin

[5] Depending on the current guidelines this might be performed for all participants or non-protected participants only

[6] target area D and E

[7] target area D, E and one overview of both areas

UrDrug, BrAlc	X	X									
Temperature	X	X		X	X	X	X	X	X	X	X
ECG	X										X
General symptoms		X	X	X	X	X	X	X	X	X	X
Vital Signs (HR, BP)	X	X		X	X	X	X	X	X	X	X
Tape stripping of skin		X									
TEWL assessment[4]		X									
Imiquimod administration			X	X	X	X	X	X	X		
Clinical evaluation (erythema grading scale)		X			X	X		X		X	X
LSCI[7]		X			X	X		X		X	X
Antera 3D camera[6]		X			X	X		X		X	X
2D photography (overview photo of whole back)		X			X	X		X		X	X
Skin punch biopsy		X			X	X		X		X	
Local NRS itch and local pain		X		X	X	X	X	X	X	X	X
(S)AE/Con-meds	<--- continuous --->										

BP = Blood Pressure, HR = Heart Rate, SCR = Screening, EEG = Electroencephalogram, AE = Adverse Event, UrDrug = Urine Drug SCR = Screen, UrPregnancy = Urine pregnancy test for women of childbearing potential, BsHaem = Blood Sample Haematology, BsChem = Blood Sample Chemistry, BsCompl = Blood Sample Complement, BsGluc= Blood Sample Glucose, BrAlc = Breath Alcohol Test.