

Draft Guidance on Tacrolimus

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA, or the Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the Office of Generic Drugs.

- Active Ingredient:** Tacrolimus
- Dosage Form; Route:** Ointment; topical
- Recommended Studies:** Two options: in vitro or in vivo study

1. In Vitro Option:

To qualify for the in vitro approach for this drug product the following criteria should be met:

- A. The test and Reference Listed Drug (RLD) products should be qualitatively (Q1) and quantitatively (Q2) the same as defined in the Guidance for Industry: *ANDA Submissions – Refuse-to-Receive Standards*, Revision 2 (December 2016).¹
- B. The test and RLD products should be physically and structurally similar based upon an acceptable comparative physicochemical characterization of a minimum of three batches of the test and a minimum of three batches (as available) of the RLD product. The characterization of the test and RLD products should include the following comparisons of physical and structural attributes between the test and RLD products:
 - i. Assessment of appearance.
 - ii. Microscopic characterization of the ointment including globule size distribution with representative images at multiple magnifications.
 - iii. Analysis of the rheological behavior which may be characterized using a rheometer that is appropriate for monitoring the non-Newtonian flow behavior of semi-solid dosage forms. The following evaluations are recommended:
 - A complete flow curve of shear stress (or viscosity) vs. shear rate should consist of multiple data points across the range of attainable shear rates, until low or high shear plateaus are identified.
 - Yield stress values should be reported if the material tested exhibits plastic flow behavior.
 - The linear viscoelastic response (storage and loss modulus vs. frequency) should be measured and reported.

¹ Guidance for Industry: *ANDA Submissions – Refuse-to-Receive Standards*, Revision 2 (December 2016)

- iv. Analysis of any other potentially relevant physical and structural similarity characterizations
 - C. The test and RLD products should have an equivalent rate of tacrolimus release based upon an acceptable in vitro release test (IVRT) comparing a minimum of one batch each of the test and RLD products using an appropriately validated IVRT method. Refer to the *Guidance on Acyclovir* (for acyclovir topical cream, 5%)² for additional information regarding the development, validation, conduct and analysis of acceptable IVRT methods/studies.
 - D. The test and RLD products should have an equivalent rate and extent of tacrolimus permeation through excised human skin based upon an acceptable in vitro permeation test (IVPT) comparing a minimum of one batch each of the test and RLD products using an appropriately validated IVPT method. Refer to the *Guidance on Acyclovir* (for acyclovir topical cream, 5%)² for additional information regarding the development, validation, conduct and analysis of acceptable IVPT methods/studies. The batches of test and RLD products evaluated in the IVPT study should be the same as those evaluated in the IVRT study, and these batches should be included among those for which the physical and structural similarity is characterized and compared.
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2. In Vivo Option:

Type of study: Bioequivalence (BE) study with clinical endpoint

Design: Randomized, double blind, parallel, placebo controlled, in vivo

Strength: 0.03%

Subjects: Non-immunocompromised male and female adults with clinical diagnosis of moderate to severe atopic dermatitis

Additional comments: Specific recommendations are provided below.

Analytes to measure (in appropriate biological fluid): Not applicable

Bioequivalence based on (90% CI): See additional comments below for the bioequivalence study with clinical endpoint

Waiver request of in vivo testing: Not applicable

Dissolution test method and sampling times: Not applicable

Additional comments regarding the BE study with clinical endpoint:

² Draft Guidance on Acyclovir for acyclovir topical cream, 5% (recommended Dec 2014; revised Dec 2016).

1. The Office of Generic Drugs (OGD) recommends conducting a bioequivalence study with a clinical endpoint in the treatment of moderate to severe atopic dermatitis (AD) comparing the 0.03% test product versus the 0.03% reference listed drug (RLD) and vehicle control, each applied as a thin layer twice daily to the affected area(s) for 28 days (4 weeks). The primary endpoint is the proportion of subjects with treatment success (a grade of clear or almost clear; a score of 0 or 1, within the treatment area) based on the Investigator’s Global Assessment of Disease Severity (see Table 1) at the end of treatment (study Day 29).
2. A placebo control arm (vehicle of test product) is recommended to demonstrate that the test product and RLD are active and as a parameter to establish that the study is sufficiently sensitive to detect differences between products.
3. Inclusion Criteria (the sponsor may add additional criteria)
 - a. Non-immunocompromised male or female aged 12 years and older with a clinical diagnosis of moderate to severe atopic dermatitis that has failed to respond adequately to other topical prescription treatments for atopic dermatitis, or for whom those treatments are not advisable. If the enrollment of subjects younger than aged 12 years is necessary due to the difficulty of recruiting sufficient subjects into the study, the OGD recommends limiting the subject’s age to 8 years and older. If pediatric subjects are included, ensure that the age distribution is similar in all treatment groups.
 - b. Had a diagnosis of AD for at least 3 months.
 - c. An Investigator’s Global Assessment (IGA) of disease severity of at least moderate at baseline (per Table 1, a score of 3 or 4).
 - d. Affected area of AD involvement at least 20% body surface area (BSA) at baseline as defined by the criteria of Hanifin and Rajka³.

Table 1: Investigator’s Global Assessment (IGA) of Disease Severity

Score	Category	Definition
0	Clear	Minor, residual discoloration, no erythema or induration/papulation, no oozing/crusting
1	Almost Clear	Trace, faint pink erythema with almost no induration/papulation and no oozing/crusting
2	Mild disease	Faint pink erythema with mild induration/papulation and no oozing/crusting
3	Moderate disease	Pink-red erythema with moderate induration/papulation and there may be some oozing/crusting
4	Severe disease	Deep/bright red erythema with severe induration/papulation with oozing/crusting

³ Hanifin JM and Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol. 1980; Suppl. 92: 44-7

4. Exclusion Criteria (the sponsor may add additional criteria)
 - a. Females who are pregnant, breast feeding, or who wish to become pregnant during the study period.
 - b. Active cutaneous bacterial or viral infection in any treatment area at baseline (e.g., clinically infected atopic dermatitis).
 - c. Sunburn, extensive scarring, or pigmented lesion(s) in any treatment area at baseline, which would interfere with evaluations.
 - d. History of confounding skin conditions, e.g., psoriasis, rosacea, erythroderma, or ichthyosis.
 - e. History or presence of Netherton's Syndrome, immunological deficiencies or diseases, HIV, diabetes, malignancy, serious active or recurrent infection, clinically significant severe renal insufficiency or severe hepatic disorders.
 - f. Use within one month prior to baseline of 1) oral or intravenous corticosteroids, 2) UVA/UVB therapy, 3) PUVA (psoralen plus ultraviolet A) therapy, 4) tanning booths, 5) nonprescription UV light sources, 6) immunomodulators or immunosuppressive therapies, 7) interferon, 8) cytotoxic drugs, 9) tacrolimus, or 10) pimecrolimus.
 - g. Use within 14 days of baseline of: 1) systemic antibiotics, 2) calcipotriene or other vitamin D preparations, or 3) retinoids.
 - h. Use within 7 days prior to baseline of: 1) antihistamines, 2) topical antibiotics, 3) topical corticosteroids or 4) other topical drug products.
 - i. Use within 24 hours prior to baseline of any topical product (e.g., sunscreens, lotions, creams, emollients, moisturizers) in the areas to be treated.
 - j. Known allergy or hypersensitivity to tacrolimus or any other component of the test product or RLD.
 - k. Not willing to minimize or avoid natural and artificial sunlight exposure during treatment.
5. The protocol should include a list of the prescription and over-the-counter drug products, procedures, and activities that are prohibited during the study, such as:
 - a. Treatment for atopic dermatitis, other than assigned treatment.
 - b. Topical or systemic corticosteroid, topical or systemic antibiotic, topical or systemic antifungal, oral or topical antihistamine, immunosuppressive drugs, immunomodulator (e.g., pimecrolimus), calcipotriene or other vitamin D preparations, retinoids, interferon, cyclosporine, methotrexate, azathioprine or antihistamines (e.g., diphenhydramine, hydroxyzine).
 - c. CYP3A inhibitor, e.g., erythromycin, itraconazole, ketoconazole, fluconazole, calcium channel blockers cimetidine, grapefruit or grapefruit juice.
 - d. Topical product, other than assigned treatment, (e.g., sun screen, new brand of cosmetic or cleanser, cream, lotion, ointment, or powder) applied on or near the treatment area(s).
 - e. Phototherapy, e.g., PUVA, UVA or UVB therapy.
 - f. Bathing, showering or swimming right after applying study treatment.
 - g. Prolonged baths (i.e., longer than 5 minutes), excessive exposure to sunlight, or use of tanning booths, sun lamps or nonprescription UV light sources.
 - h. Covering any treated area with bandage(s), dressing(s) or wrap(s).
 - i. Allowing the study treatment to come in contact with the eyes or mouth.

6. When applying assigned study treatment after a bath or shower, the skin should be dry. Caregivers applying study treatment to a subject, or subject who is not treating their hands should wash their hands with soap and water after applying study treatment.
7. It is the sponsor's responsibility to include a provision in the protocol and subject consent form to ensure appropriate referral for continued therapy and follow-up of subjects according to the standard of care after the end of the study. If there is worsening during the treatment period, no improvement in the follow-up period, or signs and symptoms persist beyond the treatment period, subjects must be evaluated by a healthcare provider for careful re-evaluation, and consideration should be given to performing a skin biopsy in such cases to rule out malignancy.
8. The primary endpoint is the proportion of subjects in the Per Protocol (PP) population in each treatment group with treatment success (i.e., a grade of clear or almost clear; a score of 0 or 1, within all treatment areas) based on the Investigator's Global Assessment of Disease Severity (see Table 1) at the end of treatment (week 4 visit; study Day 29). This is the earliest time at which a significant success proportion is expected. This shorter treatment duration would be most likely to detect differences between test and reference products and is intended to minimize systemic exposure to the drug and the potential cancer risk.
9. The secondary endpoints are change in severity from baseline to week 4 (study Day 29) of four individual signs and symptoms of AD (i.e., erythema, induration/papulation, lichenification and pruritus; see Table 2) and are considered supportive information. It is recommended that pruritus be assessed by questioning the subject or the subject's parent/legal guardian regarding the intensity of overall itching/scratching/discomfort in the 24 hours prior to the visit.

Table 2: Individual Signs and Symptoms of AD

Erythema		
0	None	No erythema present
1	Mild	Slight erythema: very light-pink
2	Moderate	Dull red, clearly distinguishable
3	Severe	Deep/dark red

Induration/Papulation		
0	None	None
1	Mild	Slightly perceptible elevation
2	Moderate	Clearly perceptible elevation but not extensive
3	Severe	Marked and extensive elevation
Lichenification		
0	None	None
1	Mild	Slight thickening of the skin discernible only by touch and with skin markings minimally exaggerated
2	Moderate	Definite thickening of the skin with skin marking exaggerated so that they form a visible criss-cross pattern
3	Severe	Thickened indurated skin with skin markings visibly portraying an exaggerated criss-cross pattern
Pruritus		
0	None	None
1	Mild	Occasional, slight itching/scratching
2	Moderate	Constant or intermittent itching/scratching/discomfort which is not disturbing sleep
3	Severe	Bothersome itching/scratching/discomfort which is disturbing sleep

10. If the signs and symptoms of atopic dermatitis resolve during treatment, subjects should continue the application of the study drug for at least 4 weeks and should not stop treatment. Subjects should not be discontinued early from the study due to lack of treatment effect. Subjects who do not show complete clearing of all lesions by the end of the study (study Day 29) should receive continuing treatment with the RLD and appropriate follow-up according to the standard of care. Per the RLD labeling, if signs and symptoms of atopic dermatitis do not improve within 6 weeks, subjects should be re-examined.
11. The OGD recommends blood sampling for tacrolimus trough serum concentrations on study Day 4 (prior to application of 8th dose of study treatment) as a safety measure to assure that the frequency and magnitude of measurable serum concentrations is not apparently greater with the generic than with the reference product. Based on previous PK data for tacrolimus, steady state concentrations are expected to be reached by study Day 4, and sampling at this time would likely reflect any systemic accumulation of tacrolimus with repeated applications to the same treatment area before significant healing takes place. Available data suggest that systemic exposure decreases as the lesions heal.

For measuring trough levels, the OGD recommends the following procedures on study Day 4

- 1) The blood should be sampled at the same time point for all patients.
- 2) The blood samples should not be taken from areas treated with tacrolimus ointment.
- 3) The study tube should be weighed (dose) before and after the morning dose on study Day 4.
- 4) The blood should be sampled just before the evening dose (i.e., 12 hours after the 7th dose was administered in the morning) on study Day 4.

- 5) To maintain blinding, tacrolimus trough concentrations should be taken from all study patients in the test, reference and placebo groups. Blood samples from the placebo group need not be analyzed to determine tacrolimus concentrations.

For serum concentrations obtained as a safety measure, the OGD does not require the sponsor to meet the usual pharmacokinetic bioequivalence limits used to determine equivalence.

12. Application site reactions such as dryness, burning/stinging, erosion, edema, and pain are to be recorded at each visit to allow a comparison between treatment groups. A descriptive analysis comparing the application site reactions for each treatment group is recommended. It is important to ensure that the test product is not worse than the reference product with regard to the expected and unexpected application site reactions.
13. The size of the treatment area and the site of the treatment area should be compared and tabulated for each treatment group.
14. The protocol should clearly define the PP, modified intent-to-treat (mITT) and safety populations.
 - a. The accepted PP population used for bioequivalence evaluation includes all randomized subjects who:
 - i. Meet all inclusion/exclusion criteria
 - ii. Are dosed a pre-specified proportion of the scheduled doses (Generally At least 75% and no more than 125%) of the assigned product for the specified duration of the study. The protocol should specify how compliance will be verified, (e.g., using subject diaries).
 - iii. Do not miss a pre-specified number of scheduled doses for more than pre-specified number of consecutive days.
 - iv. Complete the evaluation within the designated visit window with no protocol violations that would affect the treatment evaluation.
 - b. The mITT and safety populations include all randomized subjects who use at least one dose of product.
15. Subjects who are discontinued early from the study due to lack of treatment effect should be included in the PP population using Last Observation Carried Forward (LOCF). Subjects whose condition worsens and who require alternate or supplemental therapy for the treatment of their condition during the treatment phase of the study should be discontinued, included in the mITT and PP population analyses using LOCF, and provided with effective treatment. Subjects discontinued early for other reasons should be excluded from the PP population, but included in the mITT population, using LOCF. Applicants should provide a pre-specified definition of lack of treatment effect. The start and stop date of concomitant medication use during the study should be provided in the data set in addition to the reason for the medication use. The sponsor should clearly explain whether the medication was used prior to baseline visit, during the study, or both.

16. The start and stop calendar date (e.g., mm/dd/yyyy) and study day (e.g. Day X) of concomitant medication use should be provided in the data set in addition to the reason for the medication use. The Applicant should clearly note whether the medication was used prior to baseline visit, during the study, or both.
17. All adverse events (AEs) should be reported, whether or not they are considered to be related to the treatment. The report of AEs should include date of onset, description of the AE, severity, relation to study medication, action taken, outcome and date of resolution. This information is needed to determine if the incidence and severity of adverse reactions is different between the test product and RLD.
18. All pregnancies should be reported, including outcome information.
19. If the inactive ingredients are different than those contained in the RLD or in significantly different amounts, then the Applicant is to clearly describe the differences and provide information to show that the differences will not affect the safety, efficacy, or systemic or local availability of the drug. Inactive ingredients used should provide adequate margins of safety for the proposed clinical exposure in the target population (e.g., 2 months and older).
20. The method of randomization should be described in the protocol. It is recommended that an independent third party generate and hold the randomization code throughout the conduct of the study to minimize bias. The sponsor may generate the randomization code if not involved in the packaging and labeling of the study medication. A sealed copy of the randomization scheme should be retained at the study site and should be available to FDA investigators at the time of site inspection to allow for verification of the treatment identity of each subject.
21. A detailed description of the blinding procedure is to be provided in the protocol. The packaging of the test, reference and placebo products should be similar in appearance to make differences in treatment less obvious to the subjects and to maintain adequate blinding of evaluators. When possible, neither the subject nor the investigator should be able to identify the treatment. The containers should not be opened by the subject at the study center.
22. Please refer to 21 CFR 320.38, 320.63 and the Guidance for Industry, “Handling and Retention of BA and BE Testing Samples”, regarding retention of study drug samples and 21 CFR 320.36 for requirements for maintenance of records of bioequivalence testing. In addition, the investigators should follow the procedures of 21 CFR 58 and ICH E6, “Good Clinical Practice: Consolidated Guideline”, for retention of study records and data in order to conduct their studies in compliance with Good Laboratory Practices (GLP) and Good Clinical Practices (GCP). Retention samples should be randomly selected from the drug supplies received prior to dispensing to subjects. Retention samples should not be returned to the sponsor at any time.
23. It is the sponsor's responsibility to enroll sufficient subjects for the study to demonstrate bioequivalence between the products.
24. To establish bioequivalence for a dichotomous endpoint, it is recommended the following compound hypotheses be tested using the per protocol population:

$H_0: \pi_T - \pi_R < \Delta_1$ or $\pi_T - \pi_R > \Delta_2$ versus $H_A: \Delta_1 \leq \pi_T - \pi_R \leq \Delta_2$

where π_T = the success rate of the primary endpoint for the treatment group, and
 π_R = the success rate of the primary endpoint for the reference group.

The null hypothesis, H_0 , is rejected with a type I error (α) of 0.05 (two one-sided tests) if the estimated 90% confidence interval for the difference of the success rates between test and reference products ($\pi_T - \pi_R$) is contained within the interval $[\Delta_1, \Delta_2]$, where $\Delta_1 = -0.20$ and $\Delta_2 = 0.20$. Rejection of the null hypothesis supports the conclusion of equivalence of the two products.

25. To establish sensitivity within the study for either a dichotomous or continuous primary endpoint, the test and reference products should both be statistically superior to the placebo. Conduct an appropriate two-sided inferential test with a type I error (α) of 0.05, using the mITT population.
26. The study data should be submitted in a standardized format. Please refer to study data standards published at www.FDA.gov⁴.
27. The protocol should include a section with fully detailed statistical analysis plan.
28. Please provide Subject-Level Analysis Dataset (ADSL), one record per subject, using the following headings, if applicable:
 - a. Study identifier
 - b. Unique subject identifier
 - c. Subject identifier
 - d. Study site identifier
 - e. Age
 - f. Age units (years)
 - g. Sex
 - h. Race
 - i. Name of planned treatment
 - j. Name of actual treatment
 - k. Safety population flag (yes/no)
 - l. Reason for exclusion from safety population
 - m. Modified Intent-to-Treat (mITT) population flag (yes/no)
 - n. Reason for exclusion from mITT
 - o. Per-Protocol (PP) population flag (yes/no)
 - p. Reason for exclusion from PP population
 - q. Randomized population flag (yes/no)
 - r. Date/time of first exposure to treatment

⁴ Study Data Standards for Submission to CDER available at:
<http://www.fda.gov/Drugs/DevelopmentApprovalProcess/FormsSubmissionRequirements/ElectronicSubmissions/ucm248635.htm>

- s. Date/time of last exposure to treatment
 - t. End of study date
 - u. End of study status
 - v. Subject required additional treatment due to unsatisfactory treatment response (yes/no)
 - w. Specific reason for use of this product (e.g., A= failure to respond adequately to other topical prescription treatments for atopic dermatitis, B=when those treatments are not advisable)
 - x. Location of Treatment Area (i.e., neck, elbow, knee, hand, wrist, ankle)
 - y. Size of Treatment Area (e.g., cm²)
 - z. Previous use of AD treatment (yes/no)
 - aa. Reason for premature discontinuation of subject
 - bb. Percent (%) Body Surface Area (BSA) involvement at baseline
 - cc. Percent (%) Body Surface Area (BSA) involvement at study Day 29
 - dd. IGA score at baseline
 - ee. IGA score at study Day 29
 - ff. Date/time of tacrolimus trough blood sample
 - gg. Tacrolimus trough blood concentration (on study Day 4)
 - hh. Weight of study drug before and after the morning dose on study Day 4
 - ii. Final designation of treatment outcome (success/failure) based on IGA
 - jj. Compliance rate (%)
 - kk. Subject missed pre-specified number of scheduled doses for more than pre-specified number of consecutive days (yes/no)
 - ll. Adverse event reported (yes/no)
 - mm. Concomitant medication (yes/no)
29. Please provide the basic data structure (BDS) dataset with records per subject, per visit, per analysis timepoint, using the following headings, if applicable:
- a. Study identifier
 - b. Unique subject identifier
 - c. Subject identifier for the study
 - d. Study site identifier
 - e. Name of planned treatment
 - f. Name of Actual Treatment (exposure): test product, RLD, placebo
 - g. Location of Dose Administration: application site
 - h. Safety population flag (yes/no)
 - i. Modified ITT population flag (yes/no)
 - j. Per-protocol (PP) population flag (yes/no)
 - k. Analysis visit
 - l. Analysis date
 - m. Study visit within designated window (yes/no)
 - n. IGA score
 - o. Individual signs and symptoms of AD score for erythema, induration/papulation, lichenification, and pruritus
 - p. Skin reaction score for each sign and symptom evaluated (e.g., dryness, burning/stinging, erosion, edema, pain)

- q. Additional treatment required during the visit (yes/no)
- r. Concomitant medication during the visit (yes/no)
- s. Adverse event reported during the visit (yes/no)
- t. Laboratory testing during the visit (yes/no)