CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

201153Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Clinical Pharmacology Review

PRODUCT (Generic Name):	Imiquimod Cream 3.75%
PRODUCT (Proposed Brand Name):	ZYCLARA/Marketed 5% name-ALDARA
NDA:	201153
TYPE:	505(b)(2)
PROPOSED INDICATIONS:	external genital warts
SUBMISSION DATES:	12/19/08
SPONSOR:	Graceway Pharmaceuticals
REVIEWER:	CAPT E. Dennis Bashaw, Pharm.D.
OCP DIVISION:	DCP III

TABLE OF CONTENTS

1. EXECUTIVE SUMMARY

Currently a 5% imiquimod cream (ALDARA) is approved for topical use for the following indications and associated treatment durations:

- Clinically typical, nonhyperkeratotic, nonhypertrophic actinic keratoses (AK) on the face or scalp in immunocompetent adults 2 times per week for a full 16 weeks

- Biopsy-confirmed, primary superficial basal cell carcinoma (sBCC) in immunocompetent adults; maximum tumor diameter of 2.0 cm on trunk, neck, or extremities (excluding hands and feet), only when surgical methods are medically less appropriate and patient follow-up can be reasonably assured for a period of : 5 times per week for a full 6 weeks
- External genital and perianal warts/condyloma acuminata in patients 12 years old or older for 3 times per week until total clearance or a maximum of 16 weeks

The development of different formulations/strengths of topical imiquimod has continued in order to address (according to the sponsor) physician and patient needs with regards to dosing and duration. According to the sponsor, recent market surveys, discussions with treating physicians, and data from prescription databases indicated that patients generally did not dose ALDARA beyond 8 weeks for external genital warts (EGW).

As part of the perceived market need the sponsor recently (3/25/10) received approval for a 3.75% imiquimod cream for use in the treatment of atopic keratosis (AK) under the brand name of ZYCLARA. The difference between the 3.75 and 5% products being (beyond the obvious strength difference) a more intense yet shorter administration period.

This NDA consists of the studies needed to validate a similar shorter more intense dosing regimen for the 3.75% product for external genital and perianal warts (EGW).

The clinical pharmacology section of the NDA consisted primarily of the results of two studies, GW01-0804 and GW01-0706, which were designed to characterize the PK profiles of daily applications of 3.75% imiquimod cream under anticipated maximal use conditions in subjects with EGW and AK, respectively. *The results of the GW01-0706 were previously reviewed as part of NDA 22-483*. Serum concentrations of imiquimod and of the two combined major metabolites (S-26704/ S-27700) were measured by the analytical method with a lower limit of quantitation (LLOQ) of 0.05 ng/mL. As part of the 'maximal use' protocols, subjects were required to have disease severity towards the upper end of the anticipated clinical range and had applied study cream to the treatment areas consistent with those of the patients studied in the Phase 3 clinical studies.

1.1 Recommendation

From a Clinical Pharmacology standpoint, the sponsor has met the requirements under 21 CFR 320 and the application is acceptable.

1.2 Post-Marketing Requirements/Commitments

None

1.3 Summary of Important Clinical Pharmacology and Biopharmaceutics Finding

Studies GW01-0804 and GW01-0706 were designed to characterize the PK profiles of daily applications of 3.75% imiquimod cream under anticipated maximal use conditions in subjects with EGW and AK, respectively. In both studies, serum concentrations of

imiquimod and of the two combined major metabolites (S-26704/ S-27700) were measured by the analytical method with a lower limit of quantitation (LLOQ) of 0.05 ng/mL.

GW01-0804 An Open Label, Single Center, Non-Randomized Pharmacokinetic Study to Evaluate Safety of and Systemic Exposure to Multiple Applications of Imiquimod Cream in Subjects with External Genital Warts

GW01-0706 An Open Label, Single Center, Non-Randomized Pharmacokinetic Study to Evaluate Safety of and Systemic Exposure to Multiple Applications of Imiquimod Cream in Subjects with Actinic Keratoses of the Face and/or Balding Scalp

As part of the 'maximal use' protocols, subjects were required to have disease severity towards the upper end of the anticipated clinical range and had applied study cream to the treatment areas consistent with those of the patients studied in the Phase 3 clinical studies.

This review will primarily focus on the results from study GW01-804 as it represents new information for the 3.75% product in a new patient population. As the GW01-706 data has been reviewed previously, it will be summarized briefly here and in the appendices.

GW01-804

The absorption of imiquimod from the to-be-marketed formulation was studied in a single was an open label, single-center, non-randomized pharmacokinetic (PK) study in adult subjects with EGWs (Study GW01-0804.) The study was designed to quantify the PK profile of imiquimod and its metabolites following 3 weeks (21 days) of daily applications of 3.75% imiquimod cream in adult subjects. The study was conducted under maximal use conditions (dose, duration, disease severity, and application areas) in a population with at least 8 warts in the genital/perianal area or a total wart area of $\geq$100 mm^2 applied once daily applications of up to 1 packet of 3.75% imiquimod cream for 3 continuous weeks (21 days). *Note that the proposed regimen is for eight weeks of continuous usage*.

The 3-week treatment duration was selected to confirm that steady-state conditions would exist with a relatively constantly applied dose/wart area. Steady-state conditions for thrice weekly dosing of 5% imiquimod were previously attained within 2 weeks of dosing in subjects with actinic keratosis (AK). A pharmacokinetic (post-marketing) study was conducted in subjects with EGW (Study 1253-IMIQ), during which 12 subjects received 5% imiquimod cream administered 3-times weekly for 16 weeks. While the trough levels in this study were insufficient to determine whether steady-state conditions were achieved (virtually all results were below the lower limit of quantification, LLOQ), the mean C_{max} values at Weeks 4 and 16 were within the range of those observed after the first dose, and the measured half-life values ranged from 3.4 to 33.4 hours. As a result, steady-state conditions would exist after 7 days of treatment at the longest measured half-life value (33.4 hours). Following 21 days of once daily administration, steady-state

conditions would be achieved if the half-life value was ≤100 hours (3 times the highest value observed in Study 1253-IMIQ). Since the measured elimination half-life values previously observed in EGW and AK subjects were consistent with shorter times to steady state (i.e., 1 to 2 weeks), subjects were expected to attain steady-state conditions within the 3 weeks of this study.

A total of 18 subjects, 13 male subjects and 5 female subjects, were enrolled (18 planned) who met the inclusion and exclusion criteria and were able to participate within the time frame of this study. Subjects were required to have either 8 warts at entry or a cumulative involved surface area of 100mm^2. All subjects completed the study.

Serum concentrations of imiquimod (R-837) were low in subjects with EGWs treated with up to one packet of imiquimod 3.75% cream once daily for 21 days. Mean serum concentrations ranged from approximately 0.16 to 0.37 ng/mL on Day 21. The median T_{max} was 12 hours on Days 1 and 21. The mean effective half-life for accumulation, $T_{½EFF}$, was 31.328 hours, and the observed mean elimination half-life, $T_{½}$, was 24.1±12.4 hours on Day 21. Analysis of trough concentrations over time indicated that steady-state conditions were achieved by Day 7, which was consistent with the time to steady state predicted from the observed mean elimination half-life (approximately 5 days) and the mean effective half-life for accumulation (approximately 6 to 7 days), thus validating the use of a 3 week study duration from a pharmacokinetic standpoint.

The in vivo pk characterization included imiquimod's primary metabolites, the isomers S-26704 and S-27700, but due to the low overall systemic absorption, the data were too sparse to assess in a meaningful manner (only 4 subjects had any concentrations above the LLOQ on Day 21).

GW01-0706 (Bridging 5% Data)

In terms of the ability to bridge the data from the 5% to the 3.75% cream, the degree of relative exposure of subjects to systemic imiquimod from the two formulations (5% vs. 3.75%) is unknown given their different dosing regimens in addition to strengths. Ultimately, the issue boils down from a safety point of view as to whether a short term exposure to somewhat higher levels (in certain situations) is more of a risk compared to longer exposure to levels that are lower. This is an unanswered question given the data we have now. Could such a study be performed, possibly, but assessing the long-term safety impacts of dosing regimens that are so different (daily for eight weeks vs. 3 times a week for up to 16 weeks) would likely be an insurmountable goal. Furthermore, the inability of the study to be cross-over in design will introduce the patient variability aspect into the data, ultimately making the study most likely un-interpretable for its purpose given the nature of, site of, and variability in the morphology of EGWs.

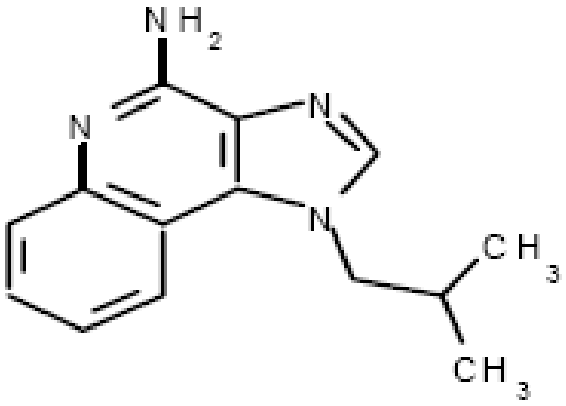
2 QUESTION BASED REVIEW

2.1 General Attributes

2.1.1 What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product

Drug Substance and Formulation

Imiquimod belongs to the chemical class of substances known as imidazoquinolinamines. Chemically, imiquimod is 1-(2-methylpropyl)-1H-imidazo[4,5-c]quinolin-4-amine. Imiquimod has a molecular formula of $C_{14}H_{16}N_4$ and a molecular weight of 240.3. Its structural formula is:



The drug product is a white to faintly yellow topical cream with a uniform appearance, packaged in a form, fill and seal (b) (4) (b) (4) single dose sachet. Each sachet contains 250 mg of imiquimod 3.75% topical cream (9.4mg of active drug).

(b) (4)

The composition of imiquimod 3.75% topical cream, relative to the placebo formulation used in the clinical trials and the currently approved 5% product is presented below:

	GW030	GW030P (placebo)	Aldara 5%
	%w/w	%w/w	%w/w
Excipients			
Isostearic acid	[redacted] (b) (4)		
Cetyl alcohol			
Stearyl alcohol			
White petrolatum			
Polysorbate 60			
Sorbitan Monostearate			
Glycerin			
Xanthan gum			
Purified water			
Benzyl alcohol			
Methylparaben			
Propylparaben			
Imiquimod	3.75	0.00	5.00

2.1.2 What are the proposed mechanisms of action and therapeutic indications?

Genital warts are exophytic (growing outward) lesions that are usually asymptomatic, but, depending on the size and anatomic location, they can be painful, friable, or pruritic. Characteristically, genital warts appear as flesh-colored, flat, papular, or pedunculated growths on the genital mucosa. In addition to the external genitalia (ie, penis, vulva, scrotum, perineum, and perianal skin), genital warts can occur on the uterine cervix and also in the vagina, urethra, anus, and mouth. The approved indication for 5% imiquimod and that being sought here for the 3.75% product is for EXTERNAL genital warts.

Imiquimod is a toll-like receptor (TLR) agonist that stimulates the innate and adaptive immune systems. Among its actions, imiquimod induces the production of interferon alpha (IFN-α), interleukin-12 (IL-12), and tumor necrosis factor-alpha (TNF-α), with a resulting cytokine cascade that may induce and/or support a cytotoxic T-lymphocyte (Th1) immune response. Although the exact mechanism of action is not fully elucidated, imiquimod appears to mediate its effects via the activation of TLR7. This interaction

stimulates effector cells such as monocytes/macrophages, and dendritic cells to produce cytokines and chemokines.

2.1.3 What are the proposed dosage and route of administration?

As noted previously, 5% imiquimod cream (ALDARA) is approved for the treatment of external genital and perianal warts/condyloma acuminata in patients 12 years old or older for 3 times per week until total clearance or a maximum of 16 weeks. The development of different formulations/strengths of topical imiquimod has continued in order to address what is perceived by the sponsor to be physician and patient needs for effective treatment regimens that work in a shorter time with a simpler dosing schedule. This submission is a logical extension of the sponsors development program as they have previously obtained approval for the 3.75% imiquimod cream product applied under a more frequent but shorter duration for the successful treatment of AK lesions.

2.2 General Clinical Pharmacology

2.2.1 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

The sponsor has conducted 3 clinical studies with 2 investigational formulations (3.75% and 2.5% imiquimod creams) to support a marketing application for the treatment of external genital warts.

The clinical program consists of the following 3 studies as follows:

- One pharmacokinetic (PK) study conducted under maximal use conditions with 3.75% imiquimod cream (Study GW01-0804). The study was performed primarily to determine the pharmacokinetics of 3.75% imiquimod cream during 3 weeks of once daily applications in subjects with EGW.

- Two identical randomized, double-blind, placebo-controlled multi-center Phase 3 clinical studies (GW01-0801 and GW01-0805). Both studies included 2 active dose groups of investigational imiquimod formulations and a placebo group. The 2 studies were conducted at separate study sites in order to provide independent confirmatory evidence of efficacy and safety.

The design of the Clinical Pharmacology studies were discussed with the sponsor during the IND phase and were specifically discussed and agreed to in meetings on July 27th 2007 and Feb. 20th, 2008. The protocol and study execution as described in this NDA is consistent with the advice given at that time as to maximal use, duration, etc.

2.2.2 Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

Yes, as part of the development of the 5% product the sponsor undertook a detailed evaluation of the metabolic schema of imiquimod. This report was re-submitted to this

NDA from the original 5% application to inform the metabolic profile and the analytical section below as to the structure and relationship of two metabolites S-26704 and S-27700. These metabolites in fact isomers and represent approximately 45% of the metabolites generated in the in vitro system. No deviations are to be expected with this indication.

2.2.3 What is the basis for selecting the response endpoints (i.e., clinical or surrogate endpoints) or biomarkers (collectively called pharmacodynamics (PD)) and how are they measured in clinical pharmacology and clinical studies?

The evidence for the efficacy of 3.75% imiquimod cream in the treatment of EGW is supported by data from 2 identical randomized, double-blind, multicenter, placebo-controlled Phase 3 studies (GW01-0801 and GW01-0805). Each study compared the efficacy and safety of 2 formulations of imiquimod, 2.5% imiquimod cream and 3.75% imiquimod cream, with that of placebo in the treatment of EGW of the following anatomic areas: in the inguinal, perineal, and perianal areas (both sexes); over the glans penis, penis shaft, scrotum, and foreskin (men only); and on the vulva (women only). The 2 studies were conducted concurrently.

In each of the two Phase 2 studies, the primary efficacy variable was the subject status with respect to complete clearance of all warts (Baseline and new) in all anatomic areas prior to or at Week 16/EOS.

Other efficacy variables included the following:

- Subject status with respect to partial clearance of baseline warts, defined as at least a 75% reduction in the number of baseline warts at EOS/Week 16;
- Percent change from Baseline to EOS in total number of warts;
- Subject status with respect to complete clearance of all warts at EOS that was sustained, as determined by the investigator, through the end of the follow-up for recurrence period;
- Time from Baseline to complete clearance as determined by the investigator;
- Subject status with respect to complete clearance of all warts (baseline and new) in all anatomic areas at End of Treatment (EOT)/Week 8;
- Subject status with respect to at least 50% reduction in the number of baseline warts at EOS/Week 16.

These endpoints were selected and were agreed to by the FDA following an End of Phase 2 meeting.

2.2.4 Exposure-Response

2.2.4.1 Does this drug prolong the QT or QTc interval?

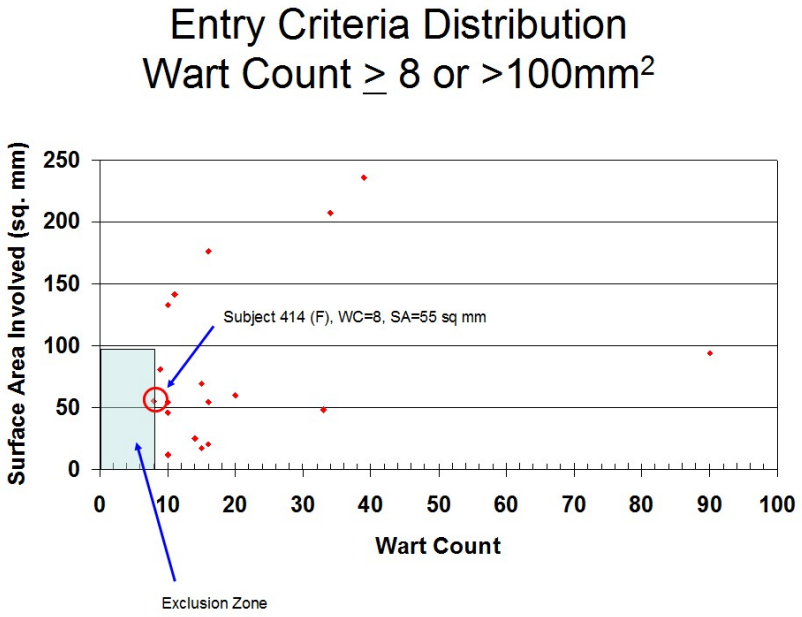
This issue was addressed as part of the recent approval of the 3.75% topical cream for AKs. No evidence of prolongation was found.

2.2.5 Pharmacokinetic characteristics of the drug and its major metabolites

2.2.5.1 What are the single dose (SD) and multiple dose (MD) PK parameters?

Maximal Usage Trial Study GW01-0706

The study was designed to evaluate the 3.75% imiquimod formulation under maximal use conditions a total of 18 subjects were enrolled who met the inclusion and exclusion criteria and were able to participate within the time frame of this study. A detailed breakdown of subject involvement by location, gender, number of warts and involved surface area is contained in the individual study report. The following graphic shows the subject data at screening with the exclusion area highlighted.



One subject was at the cut-off of 8 lesions with an inferior surface area. Of the 18 subjects only 5 had an involved surface area greater than 100mm at screening, and at study enrollment. By comparison, in the clinical trials, the surface area involvement was greater, but this also reflects a much larger study population as the ratio of subjects is 3:1 in the clinical studies (≤ 70 to >70 and <150) while it is 5:1 in study 804.

Clinical Study Summary-Baseline Surface Area				
Baseline Wart Area	Sub-Population	3.75% Subjects	5% Subjects	Placebo
Overall	≤ 70	235	214	104
	>70 and <150	86	78	52
	≥150	78	87	46
		399	379	202
Study 804				
	≤ 70	11		
	>70 and <150	2		
	>150	3		

Over the 21 days of treatment as reported in a subject diary, 14 of 18 subjects (77.8%) applied all the daily applications of study drug cream; no dropouts or discontinuations were reported. Applications were missed by 4 of 18 subjects (22.2%); each of these 4 subjects assessed at least one application during the third week of the study. Subject 001-403 missed applications on Days 17 and 18 due to resting from the study drug, but this subject later applied an additional seven applications, making up for the missed applications (total of 23 applications); Subject 001-404 missed applications on Days 8 and 18 (total of 19 applications); Subject 001-407 missed an application on Day 20 (total of 20 applications); and Subject 001-416 missed an application on Day 17 (total of 20 applications).

During the 3-week treatment period, blood samples for determination of the concentrations of imiquimod (R-837) and two metabolites combined (S-26704 and S-27700) were collected at 9 time points on Day 1 (first application) within approximately 30 minutes of pre-application (0 hour) and 1, 2, 4, 6, 9, 12, 16, and 24 hours after application of study cream and on Day 21 (last application) at pre-application and 1, 2, 4, 6, 9, 12, 16, and 24 hours after application of study cream. Pharmacokinetic (PK) blood samples were also collected 48 hours after the last application on Day 21, and End-of-Study (EOS) PK blood samples were collected 72 hours after the final application. In addition, single blood draws for PK analysis of trough concentrations to determine steady state were obtained on Day 7 and Day 14 (in the evening prior to application).

Table 9-2 Pharmacokinetic Blood Sample Collection Times

	Pre-dose	Dose	Post Dose									
Time Relative to Dose (Hour)	-0.5[a]	0	1	2	4	6	9	12	16	24	48[b]	72[b]
24-Hr Clock Time Example	1930	2000	2100	2200	0000	0200	0500	0800	1200	2000	2000	2000
Day 1 (first application)	X		X	X	X	X	X	X	X	X	–	–
Day 21 (last application)	X		X	X	X	X	X	X	X	X	X	X

[a] Pre-application blood samples were collected within approximately 30 minutes prior to study cream application.
[b] Subjects returned to the clinic to have a blood sample collected at approximately 48 and 72 hours post last application on Day 21.

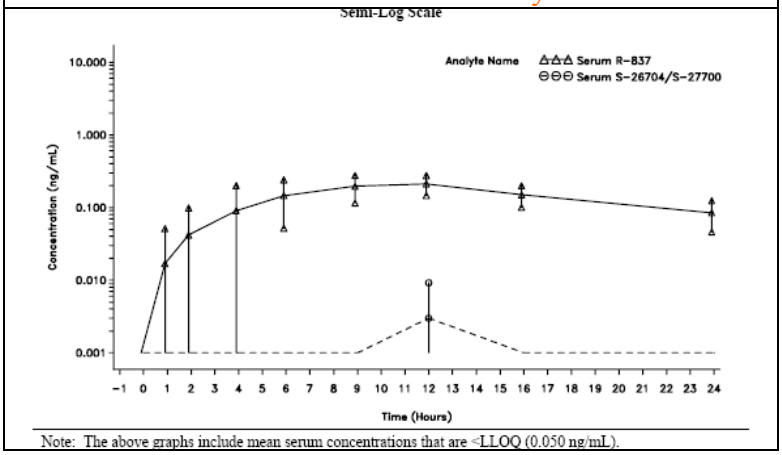
The pharmacokinetics (PK) population included all subjects who completed PK sampling and who had sufficient concentrations to obtain *reliable* estimates of PK parameters in the opinion of the sponsor. In addition, PK tables for Day 21 were prepared without 3 subjects (Subjects 404, 407, and 416) who each missed one application during the last week of treatment; this provided comparative data to those subjects who applied all applications. This reviewer has chosen to report all of the data without censoring, unless otherwise indicated due to the effect of 1 subject on the data (#418)

As shown below, mean serum concentration of imiquimod on Day 1 increased steadily until reaching a peak concentration of approximately 0.20 ng/mL at approximately 12 hours after the first application of imiquimod 3.75% cream. By 24 hours after application, the mean serum concentration of imiquimod had decreased to approximately half the peak concentration. Subject 001-411 had no concentrations above BLQ on Day 1;

consequently, no pharmacokinetic parameters could be calculated for this subject on Day 1. Subject 001-408 had an imiquimod concentration above BLQ (0.058 ng/mL) only at 12 hours on Day 1, limiting the pharmacokinetics that could be calculated.

Serum concentrations of the two imiquimod metabolites (S-26704 and S-27700 combined) were undetectable on Day 1 except for Subject 001-418 who had a concentration of 0.056 ng/mL at 12 hours after application.

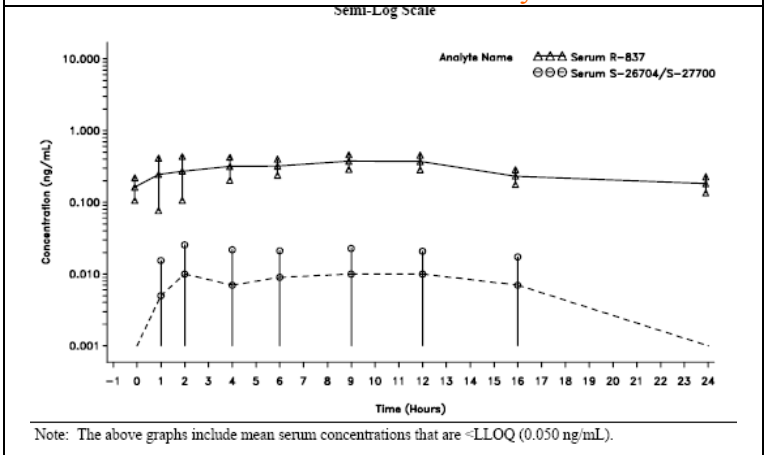
Mean Concentrations Day 1



Note: The above graphs include mean serum concentrations that are <LLOQ (0.050 ng/mL).

As has been seen with other topical administrations of imiquimod, mean serum concentrations of imiquimod on Day 21 (shown below) ranged from approximately 0.16 to 0.37 ng/mL over the 24-hour period after study drug application. Serum concentrations of two imiquimod metabolites (S-26704 and S-27700 combined) were reported for only 4 subjects on Day 21; the few concentrations that were reported tended to be low (0.050 ng/mL to 0.133 ng/mL).

Mean Concentrations Day21



Note: The above graphs include mean serum concentrations that are <LLOQ (0.050 ng/mL).

One concern over the design of this trial was the fact that the final pk evaluation occurred on day 21 for a dosing regimen designed for eight weeks of daily dosing (day 56). As part

of the demonstration of steady-state, the day 7, 14, 21, and 22 trough values were compared (day 14 vs 7, 21 vs 14, and 22 vs 21).

The primary analysis of steady state only included those subjects with paired and non-zero serum concentration data on the days being compared who applied all 7 applications in the last week of treatment and also applied at least 80% of the prescribed applications in all prior weeks. The secondary analysis of steady state only included those subjects with paired serum concentration data that replaced BLQ values with LLOQ/2 on the days being compared who applied all 7 applications in the last week of treatment and also applied at least 80% of the prescribed applications in all prior weeks

Table 11-4 Primary Analysis of Steady State for Imiquimod (R-837) Trough Serum Concentrations (PK Population)

Trough (Pre-Dose) Comparison	N	Geometric LS Mean[a]		Geometric Mean Ratio[b]	90% Confidence Interval
		Test	Reference		
Day 14 vs. Day 7	13	0.1749	0.1543	1.1335	0.7364-1.7446
Day 21 vs. Day 14	11	0.1571	0.1874	0.8384	0.5308-1.3243
Day 22 vs. Day 21	12	0.1839	0.1643	1.1194	0.7640-1.6402

Note: Primary steady-state analysis only included subjects with paired and non-zero serum concentration data on the days being compared and subjects who applied all 7 applications in the preceding week and applied at least 80% of the prescribed applications in all prior weeks.

[a] Point estimate for geometric least-squares (LS) mean was based on an ANOVA model, including study day as a fixed effect.

[b] Steady-state conditions were considered to exist during an interval if the point estimate for the geometric mean ratio was <1.43.

In the primary analysis, the geometric mean ratio during the Day 14 versus Day 7 suggests that plasma levels are still increasing, however by the second comparison 21 vs 14 the interval is now dropping (although all are quite variable). The sponsor has concluded that steady-state occurred by day 7 and cites the GMR approximating 1 as evidence supporting this. This reviewer is not totally convinced by their finding. Based on the observed data, it appears that steady-state could occur somewhere between days 7 and 14 (at its latest). In either event, these estimates do validate the use of a shorter dosing period (21 days vs. 56 days) in this study.

Whether or not this study represents "maximal use" is a valid question. The principle behind the maximal usage trials is to drive absorption of topically applied drugs to levels that would be detectable. In the case of imiquimod, even in the subjects at the low end of the treatment range, all plasma samples were above the LLOQ (testament to a good assay). In addition, approximately 1/3 of the subjects in the clin pharm trial do have surface areas in excess of 100mm2, (133, 141, 176, 206, and 236 mm2 respectively). Thus while more subjects with more severe disease could have been enrolled, there is a wide range of use in this trial and given the robust analytical method, the study is sufficient for our purposes of defining systemic availability-which is the goal of a "max use" trial.

2.2.5.2 How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?

The dermal absorption of a topical product is dependent upon the interplay of drug substance, formulation, and disease state. Given this, dermal absorption in normal volunteers is a poor predictor of absorption in diseased skin and it thus not relevant.

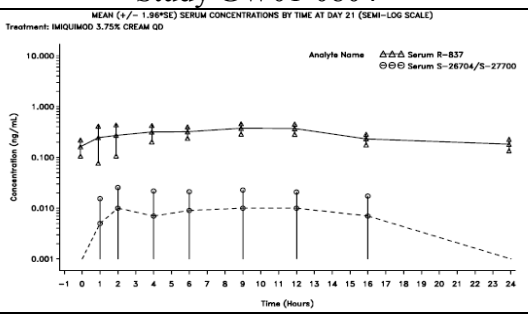
2.2.5.3 What are the characteristics of drug absorption?

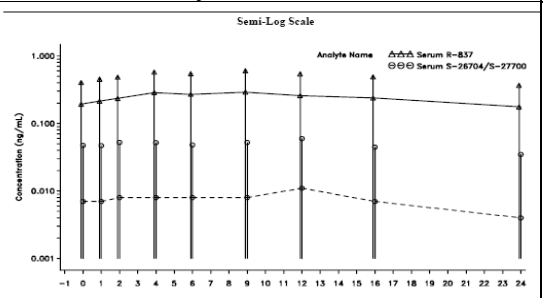
Bioavailability was not estimated for the 3.75% imiquimod cream product as part of the EGW indication in this NDA. In a previously reviewed study 1402-IMIQ that was part of the 5% and 3.75% AK indications, urinary exposure data was used to estimate topical bioavailabilty. The results of that analysis showed estimated a bioavailabilty of less than 0.6% in any individual using combined urinary recovery of imiquimod and its metabolites.

Using earlier data generated by 3M (the original IND sponsor) using a comparison of topical to subcutaneous dosing, AUC comparisons gave estimated topical imiquimod bioavailabilities (median values) in patients with AK lesions of approximately 0.4 to 1% in the face and scalp groups, and between 2% and 3.5% in the hands/arms group.

The relevancy of these estimates to the current situation is unknown given that the lesions are morphologically quite distinct, in addition to the sites of application. What can be said is that the overall comparison of exposure following 21 days of dosing between subjects with AKs and EGWs is qualitatively similar, thus we should expect a similar (but not bioequivalent) bioavailabilty.

Current NDA Study GW01-0804	NDA 22-483 Study GW01-0706





Mean Pk Parameters

Table 2.7.2-3 Study GW01-0804: Summary of Serum PK for Imiquimod on Day 1 and Day 21 (EGW patients)

	Mean (SD)			
Parameter	N	Day 1[a]	N	Day 21[b]
C_{max} (ng/mL)	18	0.259 (0.223)	15	0.488 (0.368)
C_{min} (ng/mL)	–	NA	15	0.158 (0.121)
T_{max} (hr)[c]	17	12.00 (4.00-16.00)	15	12.00 (1.00-16.00)
AUC_{0-24} (ng•hr/mL)	15	3.748 (2.541)	15	6.795 (3.591)
AUC_{0-t} (ng•hr/mL)	18	3.123 (2.665)	–	NA
AUC_{0-inf} (ng•hr/mL)	12	5.352 (2.636)	–	NA
λ_z (1/hr)	12	0.0756 (0.0416)	14	0.0370 (0.0222)
$T_{½}$ (hr)	12	12.450 (8.249)	14	24.135 (12.402)
R_{Cmax}	–	NA	14	2.260 (1.579)
R_{AUC}	–	NA	12	2.169 (1.752)
λ_{zEFF} (hr^{-1})	–	NA	10	0.0380 (0.0261)
$T_{½EFF}$ (hr)	–	NA	10	31.328 (30.308)

NA=Not applicable
a: Day 1 results include all PK population subjects.
b: Day 21 PK results include all PK population subjects except Subject 001-404 (missed applications on Days 8 and 18), Subject 001-407 (missed an application on Day 20), and Subject 001-416 (missed an application on Day 17).
c: Median (minimum-maximum)

Table 11-2 Summary of Serum Pharmacokinetics for Imiquimod (R-837) on Day 1 and Day 21

	Mean (SD)			
Parameter	N	Day 1[a]	N	Day 21[b]
C_{max} (ng/mL)	17	0.136 (0.059)	17	0.323 (0.159)
C_{min} (ng/mL)[c]	–	NA	17	0.199 (0.109)
T_{max} (hr)[d]	17	9.0 (4.0-24.03)	17	9.0 (4.0-16.0)
AUC_{0-24} (ng•hr/mL)	17	1.831 (0.889)	17	5.974 (3.088)
AUC_{0-t} (ng•hr/mL)	17	1.679 (1.056)	–	NA
AUC_{0-inf} (ng•hr/mL)	11	4.443 (1.309)	–	NA
λ_z (1/hr)	11	0.0450 (0.0219)	15	0.0294 (0.0142)
$T_{½}$ (hr)	10	19.818 (10.125)	15	29.260 (16.979)
R_{AUC}	–	NA	15	3.873 (2.153)
R_{Cmax}	–	NA	15	2.810 (1.514)
λ_{zEFF} (hr^{-1})	–	NA	15	0.0235 (0.0229)
$T_{½EFF}$ (hr)	–	NA	15	55.339 (36.380)

NA=Not applicable
a Serum concentrations for Subjects 001-601 and 001-618 were BLQ; consequently, PK parameters could not be calculated.
b Subject 001-619 did not have concentration data on Day 21; Subject 001-608 was excluded for missing the dose on Day 20.
c Pre-dose concentration (t=0)
d Median (minimum-maximum)

2.2.5.4 What are the characteristics of drug distribution?

The binding of [14C]imiquimod and [14C]R-842 (S-26704) to human plasma proteins was assessed in vitro with heparinized plasma from healthy adult donors using the Amicon Centrifree Micropartition System (Study No. R-837-DM-60). The extent of binding of both compounds of interest to plasma protein was independent of the concentration of drug over the concentration ranges studied. [14C]Imiquimod was bound more extensively (90-95%) than [14C]R-842 (60-67%). The extent of binding to isolated plasma proteins, high and low density lipoproteins, albumin, and α1-acid glycoprotein was also assessed. The rank order of binding to these proteins was the same for the 2 compounds. The extent of binding to the isolated proteins was greater for imiquimod than for R-842.

2.2.5.5 Does the mass balance study suggest renal or hepatic as the major route of elimination?

The primary excretion route for imiquimod is the urine.

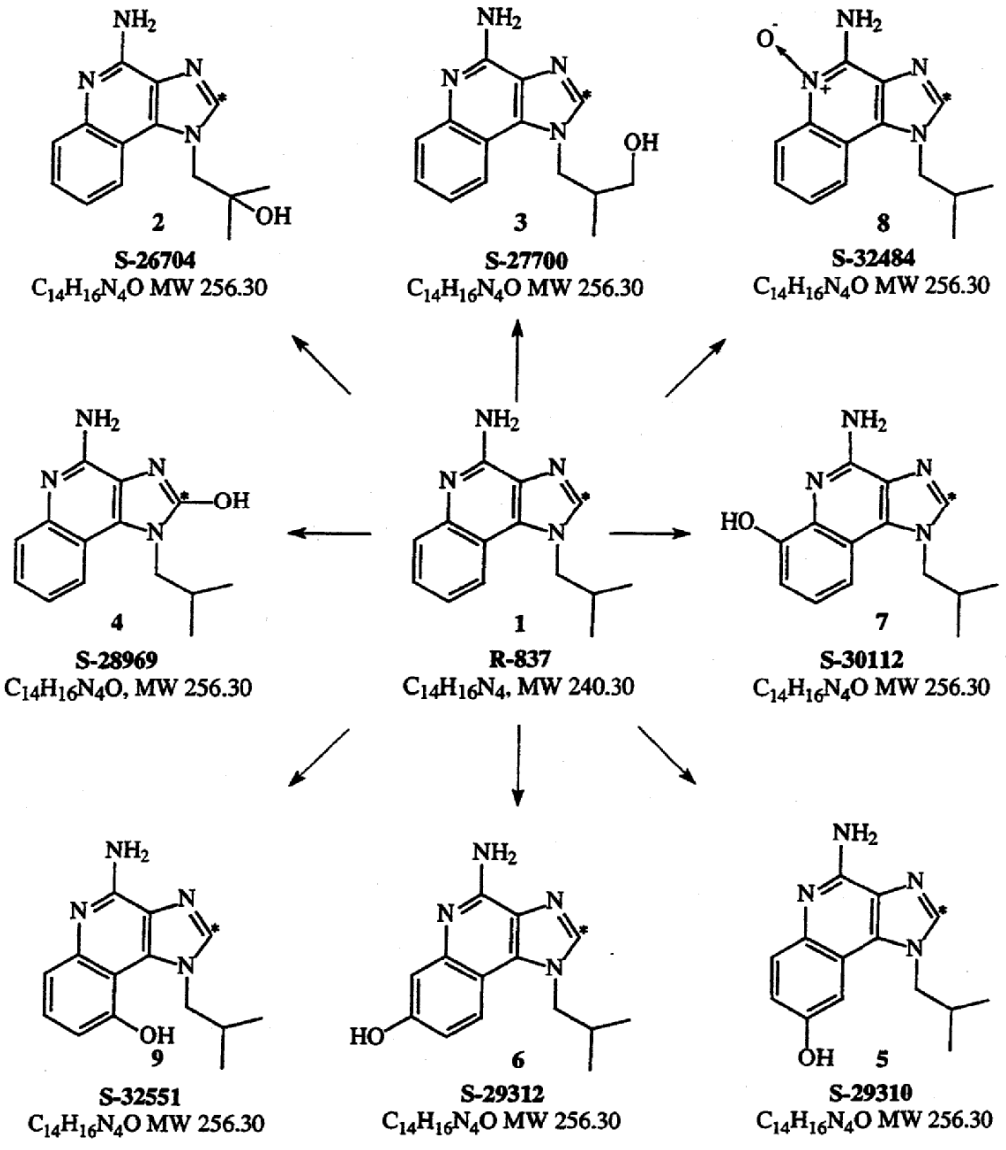
2.2.5.6 What are the characteristics of drug metabolism?

As part of the development of the original 5% imiquimod gel formulation, the human urinary metabolites of imiquimod were identified by LC/MS and H NMR analysis. The in vitro metabolism of imiquimod performed previously in the presence of human liver

microsomes, yielded seven metabolites that were identified by LC/MS analysis with a thermospray interface. The objective of this study (R-837-DM-79) by 3M was to confirm, and possibly enhance, the identification of the in vitro metabolites of imiquimod using newer MS techniques, atmospheric pressure chemical ionization (APCI) and electrospray (ES). This report describes the new HPLC-UV-radiometric and LC/MS methods and the identification of the chemical structures of imiquimod metabolites formed in the presence of human liver microsomes as determined by the new LC/MS methods.

Imiquimod Metabolic Profile

(* = Position of C-14 label)



2.2.5.7 What are the characteristics of drug excretion?

Median total urinary recoveries of unchanged drug plus five metabolites (S-26704, S-27700, S-29310, S-29312 and S-30112) were less than 0.25% of the applied dose (5% imiquimod cream) for all treatment groups in study 1402-IMIQ.

Mean ± SD and (Median) Total % Dose Excreted in the Urine from Study 1402.-IMIQ in Subjects with Actinic Keratosis given 5% Imiquimod Cream

Application Site	Dose, mg	Sex	Total % Dose in Urine [b]	
			Day 1	Week 16
Face	12.5	male	0.12 ± 0.09 (0.09)	0.19 ± 0.13 (0.14)
		female	0.12 ± 0.07 (0.10)	0.17 ± 0.14 (0.14)
		total	0.12 ± 0.08 (0.10)	0.18 ± 0.13 (0.14)
Scalp	25	male	0.08 ± 0.05 (0.07)	0.24 ± 0.17 (0.24)
Hands/Arms	75	male	0.04 ± 0.01 (0.04)	0.08 ±0.05 (0.07)
		female	0.05 ± 0.06 (0.03)	0.15 ±0.13 (0.10)
		total	0.05 ± 0.05 (0.04)	0.12 ±0.11 (0.09)

[a] Medians also presented because of highly variable data
[b] Imiquimod, S-26704, S-27700, S-29310, S-29312, and S-30112

2.3 Intrinsic Factors

2.3.1 What intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction) influence exposure (PK usually) and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

2.3.1.2 Effect of Gender

Although caused by the same human papilloma virus and general anatomical location, there are differences in the distribution of EGW between males and females. As indicated earlier the site of the warts treated in this study covered a variety of locations: glans penis, penis shaft, scrotum, foreskin, vulva, inquinal, perineal, and perianal.

Because of the underlying gender based anatomical distribution and small number of subjects present, unlike the previous approval of the AK indication, it was not possible to select out or do an analysis of absorption based on anatomical site (eg., scalp vs. arms) .

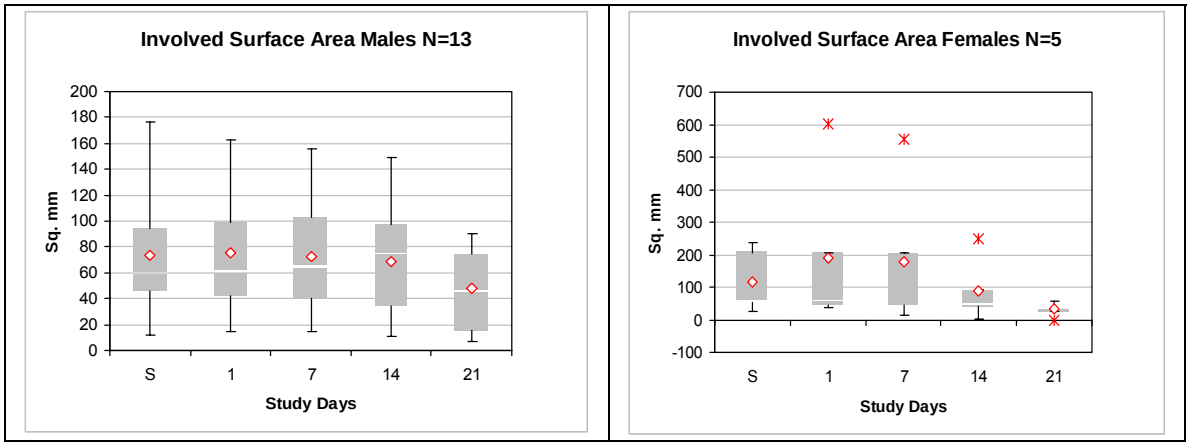
A general analysis split along the lines of males vs. females was done and is summarized in the following table.

Table 11-3 Comparison of Female and Male Subject Non-Dose-Normalized and Dose-Normalized Pharmacokinetic Parameters on Day 21

	Mean (SD) Day 21							
	Female[a]				Male[b]			
Parameter	N	Not Dose Normalized	N	Dose Normalized	N	Not Dose Normalized	N	Dose Normalized
C_{max} (ng/mL)	4	0.676 (0.656)	4	0.583 (0.484)	11	0.420 (0.203)	11	0.431 (0.198)
AUC_{0-24} (ng•hr/mL)	4	7.192 (4.796)	4	6.428 (3.791)	11	6.651 (3.327)	11	6.858 (3.351)
T_{max} (hr)[c]	4	6.50 (1.00-12.00)	–	–	11	12.00 (4.00-16.00)	–	–

[a] Results do not include Subject 001-416 (missed an application on Day 17).
[b] Results do not include Subject 001-404 (missed applications on Days 8 and 18) and Subject 001-407 (missed an application on Day 20).
c Median (minimum-maximum)
Data Source: Table 14.2.1.14, Table 14.2.1.18, Table 14.2.1.16, and Table 14.2.1.20

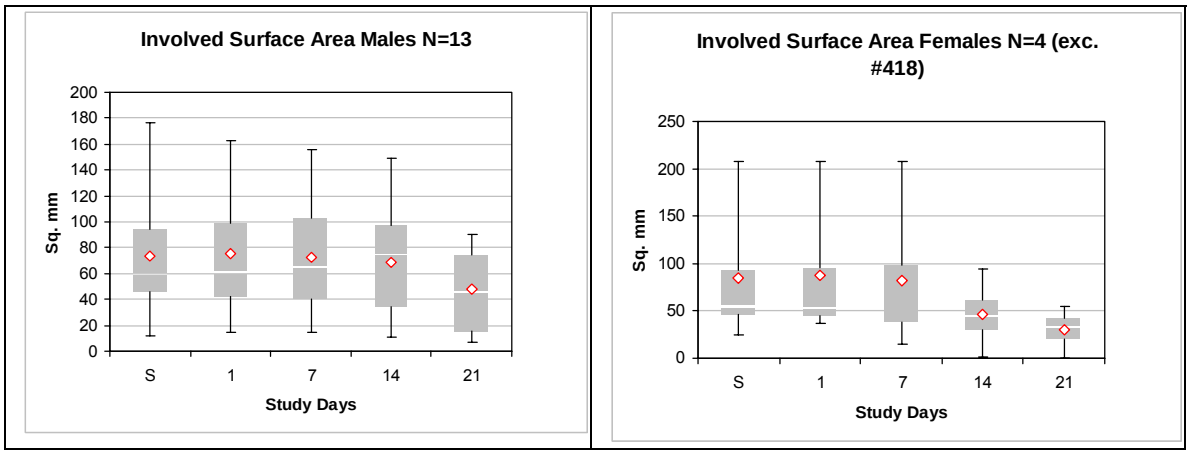
Comparison of pharmacokinetic parameters by gender on Day 21 was limited by wide variability in the data, small overall numbers, a large disparity in group sizes female/male (4 versus 11 subjects). In the provided analysis the sponsor removed subjects for missed doses as noted in the footnotes to the above table. The sponsor utilized a dose normalization to adjust for differences in dosage as much as possible, although ultimately such an approach is impossible to validate given the disparities in the data. The following figures are a representation of the involved surface areas at screening, day 1 (study entry), days 7, 14, and 21. From this one can see that one subject in the female arm (#418) had a dramatic impact as at screening and days 1, 7, and 14 this one subject had the highest surface area and exposure.



The red "x"s in the figure on the right refer to one subject (#418) who had the following surface area involvement.

Patient 418	Screening	Day 1	Day 7	Day 14	Day 21
Surface Area	236	603	554	248	38
Wart Count	39	67			

Given the magnitude of her disease, the sponsor provided a secondary analysis without here data as it directly skews the data. Without her the male to female distribution of surface area involvement is closer:



In general, although there may be some difference between the levels produced between males and females, the population in this study was too small, given the distribution of disease to identify a purely gender basis for it beyond degree of disease and location. Previously no meaningful difference in gender was seen for AKs with either the 5% or 3.75% product, such a finding is unlikely here.

2.3.1.3 Effect of Site of Application

This topic is subsumed into the discussion of gender as the anatomical sites of application are by their nature gender based.

2.3.2.1 Pediatric patients

During the Zyclara development meeting on July 27, 2007, Graceway requested DDDP advice on the requirements for a pediatric waiver. DDDP responded:

It was recommended that the sponsor consider lowering the age of inclusion to 12 years for the EGW trials. They would not need to target enrollment of a particular number of younger pediatric subjects. The sponsor should provide the rationale should they propose to extrapolate data from older pediatric subjects to younger ones, e.g.extrapolated from 14 years to 12 years of age.

In the Zyclara clinical development program, 3 subjects enrolled between 15 and 17 years of age. The following rationale for extrapolation was provided within Module 2.7.4 Summary of Clinical Safety, Section 2.7.4.5.2 Pediatric Use:

In the Phase 3 studies, no subjects were under 15 years old although the protocol permitted enrollment of subjects as young as 12 years of age. Three subjects between age of 15 and 17 were enrolled.

Aldara is approved for the treatment of EGWs in children age 12 years and older. In addition, pediatric use and pharmacokinetics have been studied for 5% imiquimod cream in studies of molluscum contagiosum in children ages 2-12 years (previously submitted to NDA 20-723).

In two randomized, vehicle-controlled, double-blind trials involving 702 pediatric subjects with molluscum contagiosum, 470 subjects exposed to 5% imiquimod cream. Median age was 5 years with a range from 2 to 12 years. Subjects applied the cream or vehicle 3 times weekly for up to 16 weeks.(Aldara Package Insert) Similar to the studies conducted in adults with the 5% imiquimod cream, the most frequently reported adverse reaction was application site reaction. Erythema was the most frequently reported local skin reaction.(Aldara Package Insert)

Systemic absorption of imiquimod across the affected skin of 22 subjects aged 2 to 12 years with extensive molluscum contagiosum involving at least 10% of the total body surface area was observed after single and multiple doses at a dosing frequency of 3 applications per week for 4 weeks. The dose applied, either 1, 2, or 3 packets per dose, was based on the size of the treatment area and the subject's weight. The overall systemic drug levels were low and the median peak serum drug concentrations at the end of Week 4 were between 0.29 and 1.06 ng/mL.(Aldara Package Insert)

Therefore, in different pediatric populations (patients ≥12 years of age with EGW; patients 2-12 years of age with molluscum contagiosum), the safety of Aldara has been determined to be acceptable. In studies of children 12 years of age and younger, the most common treatment-related adverse events were application site reactions, which is consistent with observations in adults for other indications. The prior safety experience suggests that the safety profile of 3.75% imiquimod for the treatment of EGW in patients aged 12-17 years would be similar to that observed in adults. **Graceway is unaware of any clinical factors that would indicate that the effects of treatment of EGWs with topical imiquimod would be different for patients between age 12-15 years versus those older than 15 years of age, or between those age 12-17 years and adults. [*note emphasis added by sponsor*]**

.

Discussion as the the appropriateness of this waiver is still under discussion at this time. In Study 804, no subject below the age of 19 was enrolled in the trial, as seen in the following table:

AGE (year)	N	18
	MEAN	32.33
	STD	12.029
	MIN	19.0
	MEDIAN	28.50
	MAX	64.0

The request for a partial pediatric waiver for Zyclara (imiquimod) Cream, 3.75% is not only based on the overall very low incidence of genital warts in the pediatric population, but also on the legal issues involved in conducting an interventional study involving treatment of the genitalia in sexually abused children as genital warts are a venereal disease.

2.3.2.2 Renal impairment

No clinical studies have been conducted to evaluate the effect of renal impairment on the PK of imiquimod, nor are they required given the low level of absorption and the indication.

2.3.2.3 Hepatic impairment

No clinical studies have been conducted to evaluate the effect of hepatic impairment on the PK of imiquimod, nor are they required given the low level of absorption and the indication.

2.3.2.4 What pregnancy and lactation use information is there in the application?

No information is provided; the currently marketed 3.75% product is labeled as a Pregnancy Class C. Its use in pregnant or lactating women should be done only if the potential benefit justifies the potential risk to the fetus.

2.4 Extrinsic Factors

2.4.1 What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence dose-exposure and/or -response and what is the impact of any differences in exposure on response?

The extrinsic factor influence on dose-exposure and/or –response was not explored

2.4.2 Drug-drug interactions

Drug-drug interactions were not and are normally not evaluated for topically applied products.

2.5 General Biopharmaceutics

2.5.1 Based on the biopharmaceutics classification system (BCS) principles, in what class is this drug and formulation? What solubility, permeability, and dissolution data support this classification?

Not Applicable

2.5.2 What is the relative bioavailability of the proposed to-be-marketed formulation to the pivotal clinical trial?

The proposed-to-be-marketed formulation is the same as the formulation used in pivotal Phase 3 trials.

2.5.2.1 What data support or do not support a waiver of in vivo BE data?

A waiver of in vivo BE data is not necessary, as the proposed-to-be-marketed formulation is the same as the formulation used in pivotal Phase 3 trials.

2.5.3 What is the effect of food on the bioavailability (BA) of the drug from the dosage form? What dosing recommendation should be made, if any, regarding administration of the product in relation to meals or meal types?

Not Applicable

2.6 Analytical Section

2.6.1 How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies?

Imiquimod (R-837) and its two metabolites combined (S-26704 and S-27700) were extracted by protein precipitation from human serum using a validated analytical method. The samples were injected into a liquid chromatography with tandem mass spectrometer detection (LC/MS/MS) system of analysis. The lower limit of quantitation (LLOQ) was 0.05 ng/mL, and the upper limit of quantitation (ULOQ) was 10 ng/mL for R-837 in human serum. For S-26704/S-27700 in human serum, the LLOQ was 0.05 ng/mL and the ULOQ was 20 ng/mL. A detailed discussion of the analytical methods including its performance characteristics is presented in the study appendix. As performed the assay method may be considered to be adequately validated for the purposes of this study.

2.6.2 Which metabolites have been selected for analysis and why?

Summarizing work that was originally submitted in support of the approved 5% product, in vitro metabolism studies were performed by incubating [14C]imiquimod in the presence of human liver microsomes. The chemical structures of the metabolites produced in the incubations were determined by analyzing the incubation samples using LC-MS methods. All in vitro metabolite formation was microsome- and NADPH-dependent. Eight metabolites were identified. The primary imiquimod metabolites from these incubations were the 8-hydroxy derivative, representing 45% of all metabolites; and the 5-N-oxide derivative, representing 25% of all metabolites.

2.6.3 For all moieties measured, is free, bound, or total measured? What is the basis for that decision, if any, and is it appropriate?

Imiquimod and its two primary metabolites (S-26704/S-27700) were appropriately measured as total (i.e., unbound and bound) drug.

2.6.4 What bioanalytical methods are used to assess concentrations?

The liquid chromatography (LC) system employed a reversed-phase gradient method with triple-quadrupole mass spectrometric (MS/MS) detection. Sample preparation involved organic solvent precipitation of serum proteins.

2.6.4.1 What is the range of the standard curve? How does it relate to the requirements for clinical studies?

The linear range of the method was 0.0500 to 10.0 ng/mL for R-837 and S-26704/S-27700 using 0.100 mL of human serum. Given the experience gained in the development of the 5% cream, the working range was adequate.

2.6.4.2 What are the lower and upper limits of quantification (LLOQ/ULOQ)?

The lower limit of quantitation (LLOQ) was 0.05 ng/mL, and the upper limit of quantitation (ULOQ) was 10 ng/mL for R-837 in human serum. For S-26704/S-27700 in human serum, the LLOQ was 0.05 ng/mL and the ULOQ was 20 ng/mL.

2.6.4.3 What are the accuracy, precision, and selectivity at these limits?

The precision (%CV) ranged from 3.39% to 7.35%, and the accuracy (%RE) at all concentrations ranged from -9.33% to 6.89% for R-837. The precision (%CV) ranged from 5.21% to 7.89%, and the accuracy (%RE) at all concentrations ranged from -4.80% to 3.00% for S-26704/27700.

2.7 Labeling

Aldara Cream (5%) is currently indicated for the topical treatment of:

- Clinically typical, nonhyperkeratotic, nonhypertrophic actinic keratoses (AK) on the face or scalp in immunocompetent adults
- Biopsy-confirmed, primary superficial basal cell carcinoma (sBCC) in immunocompetent adults; maximum tumor diameter of 2.0 cm on trunk, neck, or extremities (excluding hands and feet), only when surgical methods are medically less appropriate and patient follow-up can be reasonably assured
- External genital and perianal warts/condyloma acuminata in patients 12 years old or older

As noted previously, the approved 3.75% cream is approved for:

- The topical treatment of clinically typical visible or palpable actinic keratoses of the face or balding scalp in immunocompetent adults

The proposed indication in this NDA is:

- Zyclara Cream is indicated for the treatment of external genital and perianal warts/condyloma acuminata, (b) (4) in patients 12 years or older.

Indicative of the lack of systemic availability of the 3.75% product the proposed package insert contains minimal Clinical Pharmacology information (b) (4)
(b) (4)

(b) (4)

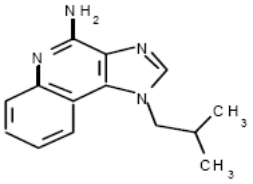
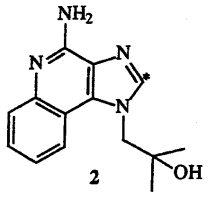
FDA Proposed Labeling

(b) (4)

1 page of draft labeling has been withheld in full as B(4) CCI/TS immediately following this page

"LC/MS/MS Quantitation of R-837 and S-26704/S-27700 in Human Serum."

The liquid chromatography (LC) system employed a reversed-phase gradient method with triple-quadrupole mass spectrometric (MS/MS) detection. Sample preparation involved organic solvent precipitation of serum proteins.

	Imiquimod, code #R-837
	Metabolites S-26704 and S-27700 are isomers and elute from the column as one completely merged peak as the column is not stereospecific. Thus, as isomers, the two metabolites have the same exact mass, fragmentation pattern and product ion, they can be analyzed as a combined peak. To assess the technical merit of this approach at the time of initial assay development, the two metabolites were assayed separately and the measured concentrations were compared to theoretical values at levels of 100 pg/mL and 1000 pg/mL. The measured concentrations of each individual metabolite were within -8.0 to 6.0% of the theoretical values, indicating that S-26704 and S-27700 can be measured as one combined peak.

A total of 396 human serum samples were received in four shipments on Jan. 7th, March 4th, March 19th, and April 21st 2009. Samples were received frozen and in good condition, and were stored at -20ºC at the analytical site. According to the sponsor all samples were received in good condition and no samples were hemolyzed.

Results of Quality Control Samples

The precision (%CV) ranged from 5.7% to 12% and the accuracy (%RE) at all concentrations ranged from 3.3% to 12.9% for R-837. The precision (%CV) ranged from 4.8% to 17.9% and the accuracy (%RE) at all concentrations ranged from 1.46% to 10.6% for S-26704/27700.

Watson Run ID	R-837 Concentration, ng/mL		
	0.15	1.5	7.5
1	0.166 0.155	1.664 1.724	8.060 9.594*
2	0.164 0.171	1.509 1.535	8.357 8.443
3	0.153 0.154	1.622 1.621	9.410* 8.506
4	0.117* 0.171	1.440 1.599	7.150 10.564*
6	0.141 0.146	1.513 1.587	8.328 8.562
8	0.165 0.151	1.485 1.444	7.687 6.955
Mean	0.155	1.562	8.468
SD	0.015	0.089	1.018
%CV	9.7	5.7	12.0
%Nominal	103.3	104.1	112.9

Notes:
*Value is out of acceptable tolerance range and included in statical calculations.
Refer to the run information listed in Table 7.

Table 3. Performance of S-26704/S-27700 QC Samples

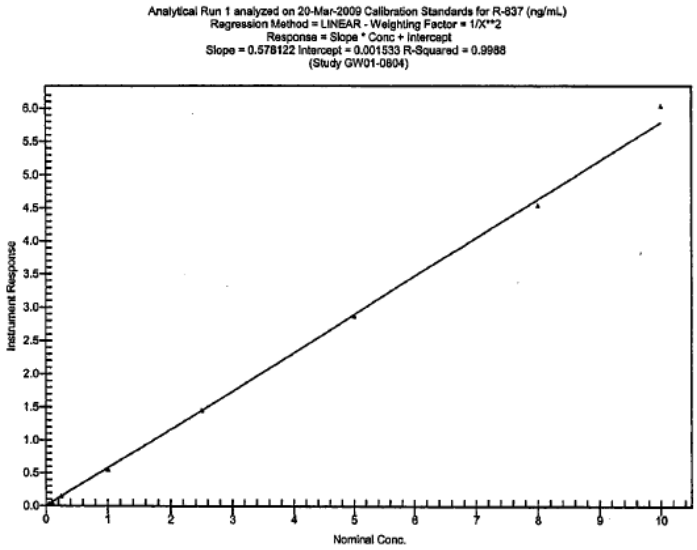
Watson Run ID	S-26704/S-27700 Concentration, ng/mL			
	0.15	0.3	3	15
1	0.151 0.098*	0.267 0.235*	3.154 3.026	15.515 15.948
2	0.132 0.153	0.256 0.325	2.824 2.868	15.630 15.473
3	0.134 0.098*	0.280 0.278	2.812 2.815	16.078 14.478
4	0.115* 0.133	0.264 0.301	2.828 2.973	14.694 16.487
5	0.129 0.130	0.296 0.299	3.066 3.308	15.968 17.078
8	0.169 0.170	0.334 0.359*	2.821 2.975	15.196 14.950
Mean	0.134	0.291	2.956	15.625
SD	0.024	0.035	0.160	0.747
%CV	17.9	12.0	5.4	4.8
%Nominal	89.3	97.0	98.5	104.2

Notes:
*Value is out of acceptable tolerance range and included in statical calculations.
Refer to the run information listed in Table 7.

Calibration/Standard Curve

The linear range of the method was 0.0500 to 10.0 ng/mL for R-837 and 0.05-20 ng/mL S-267041S-27700 using 0.100 mL of human serum. The lower limit of quantitation (LLOQ) is defined as the lowest calibration standard that meets the validation criteria for linearity, as well as precision and accuracy. The upper limit of quantitation (ULOQ) is

the highest calibration standard that meets the validation criteria for linearity, as well as precision and accuracy. The precision (%CV) ranged from 2.0% to 5.6% of the back-calculated values of the calibration standards for R-837. The precision (%CV) ranged from 2.6% to 6.6% for S-26704/S-27700. The mean coefficients of determination (r2) for R-837 and S-26704/S-27700 were 0.997 and 0.996, respectively.



Watson Run ID	Analyte	Parameters		
		Slope	Intercept	r^2
1	R-837	0.578122	0.001533	0.9988
	S-26704/S-27700	0.159153	0.001015	0.9987
2	R-837	0.539405	-0.000178	0.9985
	S-26704/S-27700	0.156280	-0.001115	0.9975
3	R-837	0.518379	-0.000525	0.9981
	S-26704/S-27700	0.156971	-0.001816	0.9952
4	R-837	0.464833	-0.000806	0.9958
	S-26704/S-27700	0.154829	0.000052	0.9922
5	S-26704/S-27700	0.165530	0.003316	0.9951
6	R-837	0.294974	0.002586	0.9968
8	R-837	0.576318	-0.005841	0.9993
	S-26704/S-27700	0.265649	0.005986	0.9983

Note: Refer to the run information listed in Table 7.

Analytical Specificity

Specificity was demonstrated by analyzing different lots of blank human serum with and without the addition of IS and LLOQ concentrations of R-837 and S-267041S-27700. the figures below display the LC/MS/MS chromatographic profile of R-837 spiked at the LLOQ level, as well as for unspiked human serum, respectively.

Figure 3. Typical Chromatogram of Blank Human Serum for R-837

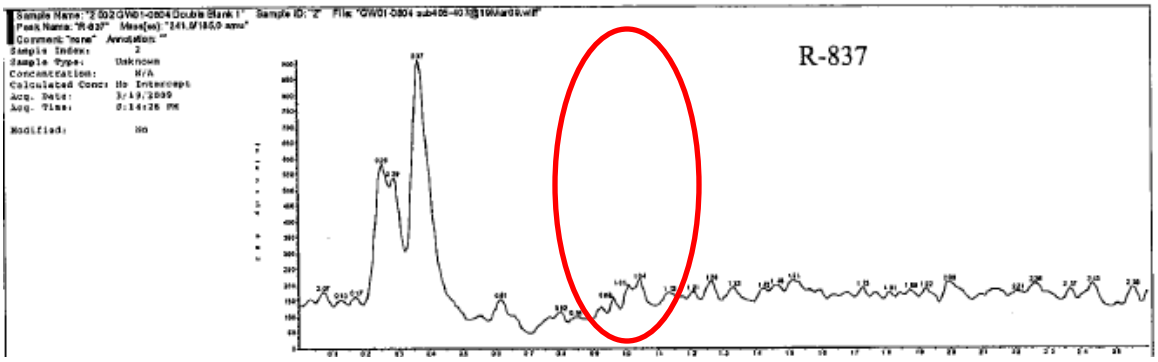
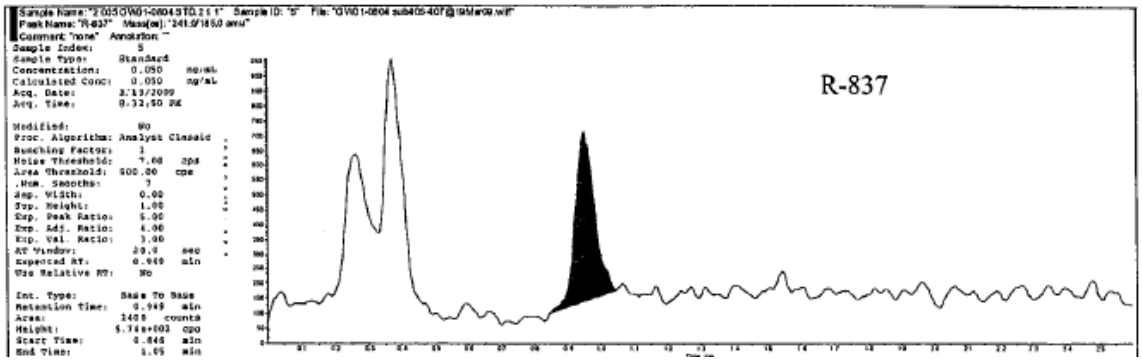


Figure 5. Typical Chromatogram of Lowest Calibration Standard for R-837



As can be seen the blank matrices tested did not have significant interference in the chromatographic regions of interest for R-837 (nor for S-26704/S-27700 (>25% of the LLOQ response)) or the internal standard (>5% of internal standard response). Thus, the blank matrices were determined to be free of interference that would adversely affect quantitation of R-837 or S-26704/S-27700.

Analytical Conclusion

The assay as performed was adequately validated over the working range and no meaningful problems with regards to either accuracy, specificity, linearity, etc were noted.

SPONSOR's SUMMARY

Title:	An Open Label, Single Center, Non-Randomized Pharmacokinetic Study to Evaluate Safety of and Systemic Exposure to Multiple Applications of Imiquimod Cream in Subjects with External Genital Warts
Sponsor:	Graceway Pharmaceuticals, LLC
Study Number:	GW01-0804
Name of Study Drug:	Imiquimod
Phase:	I
Study Initiation Date:	11-Nov-2008 (first subject screened)
Study Completion Date:	16-Apr-2009 (last subject contact)
Conduct Statement:	This study was conducted in compliance with the Code of Federal Regulations of the United States Food and Drug Administration (21 CFR Part 56, Institutional Review Boards and 21 CFR Part 50, Protection of Human Subjects) and ICH E6, Guideline for Good Clinical Practice
Principal Investigator:	Robert Feldman, MD, Miami Research Associates 6280 Sunset Drive, Suite 600 South Miami, FL 33143
Sponsor Signatory:	Sharon Levy, MD Senior Vice President, Product Development Graceway Pharmaceuticals, LLC
Date of Report:	14-Oct-2009

Objectives: The objective of this study was to quantify the pharmacokinetics of imiquimod and its metabolites during 3 weeks of daily applications of 3.75% imiquimod cream in subjects with external genital warts (EGW) under maximal use conditions. Secondary objectives include subject tolerability and safety assessments.

Methodology: In this open-label, single-center, non-randomized, pharmacokinetic (PK) study, approximately 18 adult subjects (a target of at least 5 subjects of each gender) with at least 8 EGW in the genital/perianal area or a total wart area of $\geq$100 mm^2 applied once daily applications of up to 1 packet of 3.75% imiquimod cream for 3 continuous weeks (21 days). The study was conducted under the maximal use conditions (dose, disease severity, and wart area) anticipated in Phase III studies.

Subjects stayed at the study center overnight during the treatment initiation visit (Day 1, first evening application) and the end-of-treatment visit (Day 21, last evening application). On Days 1 and 21, serum PK samples were collected pre-application and at planned time points for 24 hours post application; samples were also collected at 48 and 72 hours after application on Day 21. In addition, serum PK samples were collected in the evening prior to application on Days 7 and 14 to determine trough concentrations for steady-state analysis.

Adverse events (AEs), local skin reactions (LSRs), wart area measurements, concomitant medication use, study medication accountability, and subject compliance were reviewed at each visit. Routine clinical laboratory assessments (serum chemistry, hematology, and urinalysis) were performed at screening and 72 hours after the last application on Day 21.

Duration of Treatment: 21-day treatment period with 3-day follow-up; 4-week screening period

Criteria for Evaluation:

Pharmacokinetics: Blood samples to determine the concentrations of imiquimod and two metabolites combined (S-26704 and S-27700) were obtained at 9 time points each on Days 1 and 21 and also at 48 and 72 hours after the last application. In addition, single blood samples for PK analysis of trough concentrations were obtained on Days 7 and 14. The following parameters were calculated: maximum serum concentration (C_{max}), minimum serum concentration (C_{min}), time C_{max} was observed (T_{max}), area under the concentration versus time curve (AUC_{0-24} and AUC_{0-t}), accumulation ratios (R_{Cmax} and R_{AUC}), effective elimination rate constant (λ_{zEFF}), and effective half-life for accumulation ($T_{½EFF}$). If sufficient data were available, apparent elimination rate constant (λ_z), apparent half-life ($T_{½}$), and AUC_{0-inf} were also calculated.

Safety and Tolerability: Safety and tolerability were evaluated through the monitoring and recording of AEs, assessment of LSRs, clinical laboratory results, vital signs, and physical examination results.

Pharmacokinetic Results:

Serum concentrations of imiquimod (R-837) were low in subjects with EGWs treated with up to one packet imiquimod 3.75% cream once daily for 21 days. Mean serum concentrations ranged from approximately 0.16 to 0.37 ng/mL on Day 21. Serum concentrations of two imiquimod metabolites (S-26704 and S-27700 combined) were measured, but the data were too sparse to assess (only 4 subjects had any concentrations above the LLOQ on Day 21).

In the pharmacokinetic population, imiquimod (R-837) mean peak (C_{max}) and total exposure (AUC_{0-24}) increased between Day 1 and Day 21. The accumulation ratios based on peak exposure, R_{Cmax}, and overall systemic exposure, R_{AUC}, indicated an approximate 2-fold accumulation (2.260 and 2.169, respectively) at steady state. Imiquimod (R-837) median T_{max} was 12 hours on Days 1 and 21. The mean effective half-life for accumulation, $T_{½EFF}$, was 31.328 hours and the observed mean half-life, $T_{½}$, was 24.1±12.4 hours on Day 21. Analysis of trough concentrations over time indicated that steady-state conditions were achieved by Day 7, which was consistent with the time to steady state predicted from the observed mean elimination half-life (approximately 5 days) and the mean effective half-life for accumulation (approximately 6 to 7 days).

On Day 21, non-dose-normalized mean peak exposure, C_{max}, was 61% higher in female subjects than in male subjects and dose-normalized (adjustment for differences in dosage) mean C_{max} was 35% higher in female subjects. Non-dose-normalized mean total systemic exposure, AUC_{0-24}, was 8% higher in female subjects than in male subjects while dose-normalized mean AUC_{0-24} was 6% lower in female subjects on Day 21. Median T_{max} occurred approximately twice as quickly in female subjects (6.5 hours) as in male subjects (12.0 hours). Due to the controlling influence of a single female subject and the disparity in the number of female and male subjects (4/11), female/male comparative results appeared somewhat skewed, but mean C_{max} values were low for both female and male subjects (<1.0 ng/mL). Overall, peak exposure (C_{max}) appeared higher and reached more quickly (T_{max}) in female subjects than in male subjects, and total systemic exposure (AUC_{0-24}) appeared comparable in female and male subjects.

Safety Results:

Imiquimod 3.75% cream applied once daily for up to 21 days was well tolerated.

Treatment-emergent adverse events (TEAEs) were experienced by 10 of 18 subjects (55.6%). TEAEs considered probably related or related to treatment included 4 TEAEs reported by 3 of 18 subjects (16.7%): application site ulcer experienced by 2 subjects (11.1%) and application site irritation and application site pruritus experienced by the same subject (5.6%). Dosing was interrupted for 2 days for 1 subject (5.6%) due to an application site ulcer. All TEAEs were mild in intensity except for moderate application site ulcer experienced by 2 subjects (11.1%) and moderate upper respiratory tract infection experienced by 1 subject (5.6%). No deaths, SAEs, or discontinuations due to AEs were reported.

Expected local skin reactions were generally mild to moderate and were observed primarily on or after Day 14. Erythema was the most frequently reported local skin reaction (13 of 18 subjects, 72.2%), followed by edema (9 subjects, 50%); weeping/exudate and scabbing/crusting (7 subjects each, 38.9%); flaking/scaling/dryness (6 subjects, 33.3%), erosion (6 subjects, 33.3%), and ulceration (5 subjects, 27.8%). Overall, 7 of 18 subjects (38.9%) experienced all or most of the local skin reactions, with 4 of these subjects (22.2%) experiencing severe reactions. LSRs generally resolved or lessened in severity during the 72 hours after the last application of the study drug was applied.

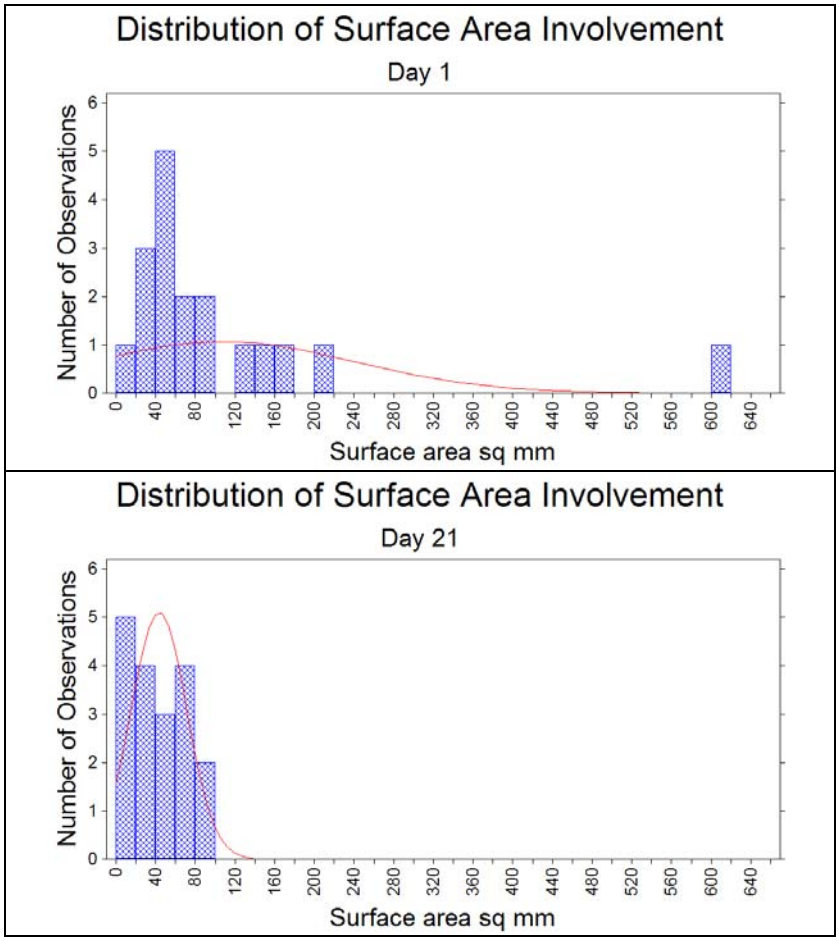
Table 11-1 Demographics and Baseline Characteristics

Demographic or Baseline Characteristic	N	Parameter	Imiquimod 3.75% Cream QD	
General				
Gender	18	Male - n (%)	13 (72.2)	
		Female - n (%)	5 (27.8)	
Race	18	White - n (%)	16 (88.9)	
		Black or African American – n (%)	2 (11.1)	
Ethnicity	18	Hispanic or Latino - n (%)	14 (77.8)	
		Not Hispanic or Latino - n (%)	4 (22.2)	
Age (years)	18	Mean (SD)	32.33 (12.029)	
		Median (min-max)	28.50 (19.0-64.0)	
Height (in)	18	Mean (SD)	68.17 (4.489)	
		Median (min-max)	68.00 (59.0-76.0)	
Weight (lbs)	18	Mean (SD)	175.89 (34.067)	
		Median (min-max)	178.00 (123.0-234.0)	
EGWs – Day 1			Wart Area (mm^2)[a]	Wart Count[a]
Inguinal	18	Mean (SD)	1.17 (2.915)	0.39 (0.698)
		Median (min-max)	0.00 (0.0-12.0)	0.00 (0.0-2.0)
Perineal	18	Mean (SD)	1.83 (5.659)	0.39 (1.243)
		Median (min-max)	0.00 (0.0-22.0)	0.00 (0.0-5.0)
Perianal	18	Mean (SD)	11.28 (32.911)	2.00 (4.379)
		Median (min-max)	0.00 (0.0-137.0)	0.00 (0.0-15.0)
Glans penis	13	Mean (SD)	3.23 (11.649)	0.85 (3.051)
		Median (min-max)	0.00 (0.0-42.0)	0.00 (0.0-11.0)
Penis shaft	13	Mean (SD)	49.08 (51.489)	11.69 (8.567)
		Median (min-max)	27.00 (0.0-153.0)	13.00 (0.0-32.0)
Scrotum	13	Mean (SD)	10.54 (27.494)	7.77 (25.636)
		Median (min-max)	0.00 (0.0-99.0)	0.00 (0.0-93.0)
Foreskin	13	Mean (SD)	0.54 (1.941)	0.23 (0.832)
		Median (min-max)	0.00 (0.0-7.0)	0.00 (0.0-3.0)
Vulva	5	Mean (SD)	173.80 (253.613)	22.20 (28.429)
		Median (min-max)	37.00 (0.0-603.0)	6.00 (0.0-67.0)
Total	18	Mean (SD)	108.33 (138.682)	23.78 (23.376)
		Median (min-max)	60.00 (15.0-620.0)	16.00 (8.0-93.0)

EGW=External genital wart; Max=maximum; Min=minimum

[a] Subjects were required to have at least 8 warts in the genital/perianal area or a total wart area of ≥100 mm^2 to be enrolled in the study.

Data Source: Table 14.1.2


Distribution of Surface Area Involvement
Day 1
Number of Observations
0
1
2
3
4
5
6
0
40
80
120
160
200
240
280
320
360
400
440
480
520
560
600
640
Surface area sq mm
Distribution of Surface Area Involvement
Day 21
Number of Observations
0
1
2
3
4
5
6
0
40
80
120
160
200
240
280
320
360
400
440
480
520
560
600
640
Surface area sq mm

SUMMARY OF SERUM PHARMACOKINETIC PARAMETERS AT DAY 1 FOR IMIQUIMOD, PK POPULATION (FEMALE SUBJECTS)

TREATMENT: IMIQUIMOD 3.75% CREAM QD
ANALYTE: Serum R-837

SUBJECT/ STATISTICS	Cmax (ng/mL)	Tmax (hr)	AUC(0-24) (hr*ng/mL)	AUC(0-t) (hr*ng/mL)	AUC(0-inf) (hr*ng/mL)	Lz (1/hr)	T½ (hr)
001-401	0.150	12.000	1.918	1.918	2.625	0.0799	8.680
001-414	0.257	9.000	3.424	2.802	3.861	0.1139	6.085
001-415	0.348	9.000	4.934	4.934	8.060	0.0480	14.435
001-416	0.152	6.000	2.125	2.119	4.802	0.0282	24.575
001-418	1.022	4.000	11.453	11.453	11.814	0.1773	3.910
N	5	5	5	5	5	5	5
MEAN	0.386	8.000	4.771	4.645	6.232	0.0895	11.537
SD	0.365	3.082	3.925	3.989	3.714	0.0589	8.283
CV%	94.620	38.528	82.273	85.878	59.595	65.8313	71.797
GEOMETRIC MEAN	0.291	7.474	3.796	3.645	5.410	0.0738	9.397
MIN	0.150	4.000	1.918	1.918	2.625	0.0282	3.910
MEDIAN	0.257	9.000	3.424	2.802	4.802	0.0799	8.680
MAX	1.022	12.000	11.453	11.453	11.814	0.1773	24.575

SUMMARY OF DOSE NORMALIZED SERUM PHARMACOKINETIC PARAMETERS AT DAY 1 FOR IMIQUIMOD PK POPULATION (FEMALE SUBJECTS)

TREATMENT: IMIQUIMOD 3.75% CREAM QD
ANALYTE: Serum R-837

SUBJECT/ STATISTICS	Cmax (ng/mL)	AUC(0-24) (hr*ng/mL)
001-401	0.167	2.132
001-414	0.241	3.216
001-415	0.380	5.388
001-416	0.188	2.624
001-418	0.907	10.159
N	5	5
MEAN	0.376	4.704
SD	0.308	3.293
CV%	81.755	70.016
GEOMETRIC MEAN	0.304	3.969
MIN	0.167	2.132
MEDIAN	0.241	3.216
MAX	0.907	10.159

SUMMARY OF SERUM PHARMACOKINETIC PARAMETERS AT DAY 1 FOR IMIQUIMOD, PK POPULATION (MALE SUBJECTS)

TREATMENT: IMIQUIMOD 3.75% CREAM QD
ANALYTE: Serum R-837

SUBJECT/ STATISTICS	Cmax (ng/mL)	Tmax (hr)	AUC(0-24) (hr*ng/mL)	AUC(0-t) (hr*ng/mL)	AUC(0-inf) (hr*ng/mL)	Lz (1/hr)	T½ (hr)
001-402	0.091	16.000	1.123	1.123			
001-403	0.122	12.000	1.657	1.657	2.591	0.0623	11.127
001-404	0.279	12.000	3.914	3.914	6.660	0.0518	13.384
001-405	0.434	16.000	6.165	6.165			
001-406	0.140	9.000	2.095	1.254	6.447	0.0213	32.595
001-407	0.230	12.000	2.829	2.829	4.095	0.0720	9.625
001-408	0.058	12.000		0.087			
001-409	0.322	16.000	4.666	4.666			
001-410	0.150	9.000		1.381			
001-411	0.000			0.000			
001-412	0.347	12.000	3.630	3.630	4.681	0.0979	7.078
001-413	0.215	9.080	2.356	2.356	3.153	0.0756	9.164
001-417	0.341	12.000	3.934	3.934	5.430	0.0793	8.746
N	13	12	10	13	7	7	7
MEAN	0.210	12.257	3.237	2.538	4.722	0.0657	13.103
SD	0.130	2.589	1.525	1.849	1.562	0.0243	8.821
CV%	61.720	21.124	47.108	72.871	33.066	36.9516	67.320
GEOMETRIC MEAN	0.195	12.009	2.898	1.949	4.485	0.0603	11.494
MIN	0.000	9.000	1.123	0.000	2.591	0.0213	7.078
MEDIAN	0.215	12.000	3.230	2.356	4.681	0.0720	9.625
MAX	0.434	16.000	6.165	6.165	6.660	0.0979	32.595

SUMMARY OF DOSE NORMALIZED SERUM PHARMACOKINETIC PARAMETERS AT DAY 1 FOR IMIQUIMOD PK POPULATION (MALE SUBJECTS)

TREATMENT: IMIQUIMOD 3.75% CREAM QD
ANALYTE: Serum R-837

SUBJECT/ STATISTICS	Cmax (ng/mL)	AUC(0-24) (hr*ng/mL)
001-402	0.081	1.005
001-403	0.144	1.953
001-404	0.440	6.173
001-405	0.395	5.612
001-406	0.134	2.008
001-407	0.206	2.530
001-408	0.058	
001-409	0.296	4.295
001-410	0.143	
001-411	0.000	
001-412	0.288	3.013
001-413	0.298	3.263
001-417	0.454	5.239
N	13	10
MEAN	0.226	3.509
SD	0.148	1.743
CV%	65.518	49.672
GEOMETRIC MEAN	0.205	3.078
MIN	0.000	1.005
MEDIAN	0.206	3.138
MAX	0.454	6.173

SUMMARY OF SERUM PHARMACOKINETIC PARAMETERS AT DAY 21 FOR IMIQUIMOD, SAFETY POPULATION (FEMALE SUBJECTS)

TREATMENT: IMIQUIMOD 3.75% CREAM QD
ANALYTE: Serum R-837

SUBJECT/ STATISTICS	Cmax (ng/mL)	Tmax (hr)	AUC(0-24) (hr*ng/mL)	RAUC	RCmax	Lz (1/hr)	Lzeff (hr^{-1})	T½ (hr)	T½ eff (hr)
001-401	0.140	12.000	2.201	1.148	0.933	0.0516	0.0855	13.436	8.111
001-414	0.446	4.000	6.232	1.820	1.735	0.0408	0.0332	16.971	20.867
001-415	0.485	9.000	6.599	1.337	1.394	0.0506	0.0574	13.712	12.077
001-416	0.281	1.000	3.603	1.695	1.849	0.0337	0.0371	20.594	18.659
001-418	1.632	1.000	13.735	1.199	1.597	0.0274	0.0748	25.337	9.270
N	5	5	5	5	5	5	5	5	5
MEAN	0.597	5.400	6.474	1.440	1.502	0.0408	0.0576	18.010	13.797
SD	0.595	4.930	4.453	0.301	0.360	0.0105	0.0228	5.021	5.688
CV%	99.669	91.287	68.781	20.935	23.983	25.8004	39.6273	27.879	41.225
GEOMETRIC MEAN	0.425	3.366	5.373	1.415	1.461	0.0397	0.0538	17.479	12.873
MIN	0.140	1.000	2.201	1.148	0.933	0.0274	0.0332	13.436	8.111
MEDIAN	0.446	4.000	6.232	1.337	1.597	0.0408	0.0574	16.971	12.077
MAX	1.632	12.000	13.735	1.820	1.849	0.0516	0.0855	25.337	20.867

SUMMARY OF DOSE NORMALIZED SERUM PHARMACOKINETIC PARAMETERS AT DAY 21 FOR IMIQUIMOD
SAFETY POPULATION (FEMALE SUBJECTS)

TREATMENT: IMIQUIMOD 3.75% CREAM QD
ANALYTE: Serum R-837

SUBJECT/ STATISTICS	Cmax (ng/mL)	AUC(0-24) (hr*ng/mL)
001-401	0.137	2.157
001-414	0.320	4.471
001-415	0.635	8.637
001-416	0.353	4.530
001-418	1.241	10.448
N	5	5
MEAN	0.537	6.049
SD	0.432	3.391
CV%	80.390	56.062
GEOMETRIC MEAN	0.414	5.238
MIN	0.137	2.157
MEDIAN	0.353	4.530
MAX	1.241	10.448

SUMMARY OF SERUM PHARMACOKINETIC PARAMETERS AT DAY 21 FOR IMIQUIMOD, SAFETY POPULATION (MALE SUBJECTS)

TREATMENT: IMIQUIMOD 3.75% CREAM QD
ANALYTE: Serum R-837

SUBJECT/ STATISTICS	Cmax (ng/mL)	Tmax (hr)	AUC(0-24) (hr*ng/mL)	RAUC	RCmax	Lz (1/hr)	Lzeff (hr^{-1})	$T_{½}$ (hr)	$T_{½}$ eff (hr)
001-402	0.492	12.000	8.096	7.209	5.407		0.0062		111.398
001-403	0.202	12.000	3.244	1.958	1.656	0.0293	0.0298	23.686	23.273
001-404	0.511	12.000	8.906	2.275	1.832	0.0495	0.0241	14.010	28.735
001-405	0.215	12.000	3.949	0.641	0.495	0.0128		53.990	
001-406	0.444	9.000	4.682	2.235	3.171	0.1022	0.0247	6.784	28.044
001-407	0.411	12.250	6.195	2.190	1.787		0.0254		27.271
001-408	0.281	4.000	3.723		4.845	0.0454		15.275	
001-409	0.518	12.000	8.606	1.844	1.609	0.0252	0.0326	27.514	21.294
001-410	0.659	9.000	10.296		4.393	0.0219		31.695	
001-411	0.359	9.000	6.732			0.0262		26.470	
001-412	0.651	12.000	11.536	3.178	1.876	0.0374	0.0157	18.515	44.024
001-413	0.107	16.000	1.924	0.817	0.498	0.0163		42.622	
001-417	0.692	12.000	10.379	2.639	2.029	0.0317	0.0199	21.887	34.919
N	13	13	13	10	12	11	8	11	8
MEAN	0.426	11.019	6.790	2.499	2.466	0.0362	0.0223	25.677	39.870
SD	0.187	2.807	3.105	1.822	1.626	0.0246	0.0083	13.416	29.764
CV%	43.792	25.475	45.734	72.931	65.909	68.0447	37.4185	52.249	74.652
GEOMETRIC MEAN	0.379	10.567	6.022	2.044	1.948	0.0308	0.0203	22.478	34.185
MIN	0.107	4.000	1.924	0.641	0.495	0.0128	0.0062	6.784	21.294
MEDIAN	0.444	12.000	6.732	2.212	1.854	0.0293	0.0244	23.686	28.389
MAX	0.692	16.000	11.536	7.209	5.407	0.1022	0.0326	53.990	111.398

SUMMARY OF DOSE NORMALIZED SERUM PHARMACOKINETIC PARAMETERS AT DAY 21 FOR IMIQUIMOD SAFETY POPULATION (MALE SUBJECTS)

TREATMENT: IMIQUIMOD 3.75% CREAM QD
ANALYTE: Serum R-837

SUBJECT/ STATISTICS	Cmax (ng/mL)	AUC(0-24) (hr*ng/mL)
001-402	0.381	6.263
001-403	0.278	4.469
001-404	0.639	11.130
001-405	0.184	3.383
001-406	0.484	5.104
001-407	0.376	5.666
001-408	0.273	3.613
001-409	0.418	6.937
001-410	0.569	8.889
001-411	0.661	12.392
001-412	0.541	9.579
001-413	0.170	3.062
001-417	0.784	11.752
N	13	13
MEAN	0.443	7.095
SD	0.190	3.307
CV%	43.014	46.604
GEOMETRIC MEAN	0.401	6.380
MIN	0.170	3.062
MEDIAN	0.418	6.263
MAX	0.784	12.392

Table 2.7.2-10 Comparison of Exposures between Each Study and Study GW01-0804

Study (formulation)	Dose of imiquimod	Duration	N	Site	Mean (SD) C_{max} [ng/mL]	Mean (SD) AUC [ng·hr/mL]	Ratio of Mean GW01-0804 C_{max} / Study C_{max}	Ratio of Mean GW01-0804 AUC / Study AUC
Study GW01-0804 (3.75% imiquimod cream)	Up to 1 packet (9.375 mg) daily	21 days	15 [a]	Genital area	0.488 (0.368)	6.795 (3.591)	NA	NA
Study GW01-0706 (3.75% imiquimod cream)	2 packets (18.75 mg) daily	21 days	17 [a]	Face or Scalp	0.323 (0.159)	5.974 (3.088)	1.51	1.14
Study 1253-IMIQ (5% imiquimod cream)	As needed to cover the wart areas; 3x/week	16 weeks	12 [b]	Genital area	0.437 (0.517) [b]	5.324 (3.256) [b]	1.12	1.28
Study 1402-IMIQ (5% imiquimod cream)	1 packet (12.5 mg) 3x weekly 2 packets (25 mg) 3x weekly 6 packets (75 mg) 3x weekly	16 weeks	23 [a] 11 [a] 24 [a]	Face Scalp Hands/Arms	0.120 (0.063) 0.214 (0.097) 1.35 (0.841) [c] 3.53 (6.52) [d]	2.06 (1.70) 4.98 (4.41) 29.1 (17.1) [c] 55.4 (76.0) [d]	4.06 2.28 0.36 0.14	3.30 1.36 0.23 0.12
Study 1520-IMIQ (5% imiquimod cream)	6 packets (75 mg) 2x weekly	16 weeks	13 [a]	> 25% of BSA	0.958 (1.18)	24.3 (26.9)	0.51	0.28
Study R-837-009 (imiquimod capsules)	Single dose of 100 mg 200 mg 250 mg 300 mg	1 dose	 6 (100 mg) 6 (200 mg) 6 (250 mg) 12 (300 mg)	 NA	 120 (60) 281 (138) 359 (230) 528 (128)	 573 (301) 1728 (1183) 2059 (1405) 5072 (2294)	 0.0041 0.0017 0.0014 0.0009	 0.0119 0.0039 0.0033 0.0013
Study R-837-019 (imiquimod capsules)	Single dose of 100 mg 200 mg 300 mg	1 dose	 6 (100 mg) 6 (200 mg) 6 (300 mg)	 NA	 126 (90) 272 (51) 424 (146)	 585 (520) 1723 (825) 4486 (3206)	 0.0039 0.0018 0.0012	 0.0116 0.0039 0.0015

BSA = Body surface area; NA = Not applicable

Note: All other studies were compared with Study GW01-0804 (bolded).

a: Number of subjects in PK population at steady state.

b: Data from Week 4 – Note that overall mean imiquimod Cmax and AUC for all patients over the 3 doses monitored in Study 1253-IMIQ were 0.405 ng/mL and 5.504 ng·hr/mL, respectively.

c: Data from Harrison et al, 2004 [9] (rejecting outliers that were > 5X the SD of their respective means).

d: Data from the 1402-IMIQ report that includes outliers.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

EDWARD D BASHAW
10/06/2010