Annex 29
to the Procedure for expertise of registration materials for medicinal products submitted for state registration (renewal), as well as expertise of the materials on variations to the registration materials during the marketing authorization validity period
(clause 4 of Section IV)

Report on Non-Clinical Studies

1. Name of the medicinal product (marketing authorization number, if available):	**AKLIEF cream 0,005 %**
1) type of the medicinal product for which the registration was conducted or planned	**Medicinal product with complete dossier**
2) studies conducted	☒ yes ☐ no if no, to justify

2. Pharmacology:

Trifarotene (CD5789) is a potent RARγ agonist (retinoid acid receptor γ agonist) and show a high RAR activity and high selectivity for RARγ (EC50=7.7 nM), compared to a 65- and 16- times lower activity for RARα and RARβ, respectively. The selectivity of trifarotene to RARγ is shown to be more pronounced than that of tretinoin and tazarotene. Trifarotene showed no activity on RXRα or RXRβ.

In human immortalized keratinocytes trifarotene showed a modulation of all retinoid-target genes for keratinization, metabolism and adhesion at concentrations which were approximately 10 times lower than those for tazarotene and about 100-times lower than those for tretinoin. Also, in reconstructed human epidermis and a human skin explant model trifarotene modulated the expression of retinoid target genes involved in e.g. proliferation, differentiation and inflammation and seemed to be more potent than tretionin and tazaratone, respectively.

When the pharmacological activity of metabolites of trifarotene was analysed, using a transactivation assay and stably transfected reporter cell lines, CD09986 exhibited a similar selective RAR gamma agonist activity as trifarotene, CD06700 and CD06530 exhibited RAR alpha/beta antagonist activity and RAR gamma agonistic activity and CD09717 was found to be a poorly active modulator on all three RAR isoforms. It should be noted that there is a low formation of metabolites after topical administration of trifarotene and therefore less likely that the activity of the metabolites have a significant pharmacological effect after administration of trifarotene.

The comedolytic efficacy of trifarotene in cream B was analysed in vivo in the Rhino mouse model and compared with Retacnyl® 0.05% and Zorac®0.1%. Trifarotene induced a marked comedone reduction and increase in epidermal thickness in the Rhino mouse, which were comparable to effects observed with tretinoin or tazarotene but at about 10-times lower dose. The suggested text for section 5.1 includes an acceptable description of the results obtained in the non-clinical studies.

Secondary pharmacology studies show anti-inflammatory effects of trifarotene in the TPA induced ear edema model in mice, similar to those of tazarotene and depigmentation in vivo in a natural pigmentation

model which was stronger and faster than the one induced by tretinoin. Trifarotene also had a depigmenting and anti-pigmenting activity in vivo in an UV induced pigmentation model in mice.

A dose dependent sedative/myorelaxant effect was seen in the Irwin test and lethal effects were observed at 32 mg/kg and at 64 mg/kg with all animals dying after administration of trifarotene. These effects are not considered to be of clinical significance since the exposure at the lowest dose (2 mg/kg) was at least 1000 times higher compared to the expected exposure in the clinic after topical application.

No significant hERG-activity was detected in vitro with an expected IC50 > 10µM and administration of trifarotene up to 2.5 mg/kg giving an exposure of 614 ng/mL did not significantly modify arterial blood pressure and did not significantly modify heart rate, PR interval, QT interval or QTc intervals. No arrhythmia or other changes in the morphology of the electrocardiogram which could be attributed to trifarotene were either observed. It may be noted that a clinical thorough QT/QTc study also has been performed in which trifarotene was concluded not to have any effect on cardiac repolarization (see clinical AR).

Trifarotene did not have any significant effects on any of the respiratory parameters evaluated in rats. No safety pharmacology issues were thus identified in the performed studies at dose levels far in excess to those anticipated in the clinical situation.

No significant risk for systemic pharmacodynamics drug interactions is anticipated based on the negligible plasma concentrations of trifarotene and its metabolites detected following topical administration to humans.

1) primary pharmacodynamics	**1. RDS.03.SRE.48079 - Evaluation of the comedolytic efficacy of CD5789 formulated in cream B and comparison with Retacnyl® 0.05% and Zorac® 0.1% after topical administration in the Rhino mouse model.** OBJECTIVE: The aim of this study was to assess the dose-response effect of CD5789 formulated in cream B at 0.001%, 0.0025%, 0.005% and 0.01% and to compare its activity to the activity of Retacnyl® (tretinoin) 0.05% and Zorac® (Tazarotene) 0.1% in the rhino mouse model. Cream B was a previous name of the acne cream formulation corresponding to this application. MATERIAL AND METHODS: Rhino mice were treated topically once daily for 11 days with Placebo cream, Rectacnyl® 0.05%, Zorac® 0.1% or CD5789 at 0.001%, 0.0025%, 0.005% and 0.01% at 25 mg/mouse. Animal were weighed on days 1 and 11. Trans epidermal water loss (TEWL) was monitored on restrained animals on day 1, day 5, day 8 and day 12 using the Tewameter® device. Clinical score of irritation was recorded every other day starting from the first day of application to 24 h after the last treatment. At the end of the experimental phase, skin biopsies in the treatment area were taken in oder to prepare histological sections. The number of comedones and the epidermis thickness were determined using image analysis (mScope) on scanned slide pictures (NanoZoomer). Statistical analysis was performed using multiple comparisons by Wilcoxon test and Bonferroni p value correction. As Rectanyl® 0.05% and Zorac® 0.1% placebos were not available for testing, results are presented versus the non-treated group. CONCLUSION: CD5789 0.001%, 0.0025%, 0.005% and 0.01% in cream B presented a dose-dependent comedolytic activity that was marked from the dose of 0.0025% (45% reduction) and very strong at 0.01% (98% reduction). When compared to clinical reference drugs, it produced the same effect than Zorac® 0.1% or Retacnyl® 0.05% at about a ten times lower dose. When compared to Zorac® 0.1%, it appeared that irritation following CD5789 topical administration was significant but very transient and disappeared within almost all group at day 12 although Zorac® 0.1% was well tolerated at the beginning of the study but triggered an irritation that seemed to increase with the duration of the treatment. Zorac® 0.1%, strongly reduced the body weight gain and produced a major change of the TEWL that continue to rise at day 12, whereas CD5789

did not modified the body weight gain and induced a more transient modification of the TEWL.

2. RDS.03.SRE.82058 - Review of the In Vitro pharmacological data of CD5789

OBJECTIVE:

The objectives of the study were to characterize the molecular pharmacology of CD5789 using an in vitro molecular screening using a reporter gene assay and to characterize the in vitro cellular pharmacology of CD5789 using human immortalized keratinocytes, reconstructed human epidermis and human skin explant models by studying retinoid-target gene expression.

a. Molecular Pharmacology

MATERIAL AND METHODS:

The pharmacological models used for these studies were based on the reporter gene technology (either Luciferase or Beta-lactamase). These cell-based assays provide a sensitive method for analyzing the intracellular effects of tested compounds on Nuclear Receptors (NRs). These functional assays allow for measurement of receptor agonism or antagonism by compounds and can be used to determine compound potency, efficacy and selectivity.

For the measurement of RAR activation, chimeric RAR (alpha or beta or gamma) ligand-binding domains fused to the GAL4 responsive element were inserted to regulate the expression of the luciferase gene in plasmids transfected in HeLa cells. In the retinoic acid nuclear receptor (RAR) transactivation assays, the activation of RAR receptors by an agonist leads to the expression of the luciferase reporter gene which generates light in presence of its substrate (luciferine). After incubation with CD5789 or reference compounds, cell lysates were prepared and Luc activity was quantified by luminescence.

For the measurement of RXR activation, chimeric RXR (alpha or beta) ligand-binding domains fused to the GAL4 responsive elements were inserted to regulate the expression of the betalactamase (bla) gene in plasmids transfected in UAS-bla HEK293T cells. The models used for this study are based on the GeneBLAzer® technology. After incubation with CD5789 or reference compounds, cell lysates were loaded with an engineered fluorescent substrate and betalactamase (bla) activity was quantified by fluorescence.

CONCLUSION:

CD5789 is a full potent and selective agonist for RARγ (EC50 = 7.7 nM) and has 16 to 65-times weaker affinities for RARα and RARβ respectively.

b. Cellular Pharmacology

- Activity on human immortalized keratinocyte (DK-7)

MATERIAL AND METHODS:

Keratinocytes (DK-7) were seeded in 96-well plates at 60 000 cells per well in complete medium. After four days, cells were treated with serial dilutions of CD5789, tretinoin or tazarotene. DMSO and arotinoid acid ((4-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid or TTNPB, a potent RAR pan agonist) at 125nM, were added as negative and positive controls, respectively. Three days after the start of the incubation, the cells were lysed and the MMP assay (Multiplexed Molecular Profiling from HTG, High Throughput Genomics) was performed according to the manufacturer protocol. Data were normalized using the house keeping gene L-19. Activities were expressed as % of arotinoid acid activity at 125nM.

CONCLUSION:

	CD5789 showed a strong modulation of retinoid target genes for keratinization, metabolism and adhesion and was approximately 10 times more potent than tazarotene and about 100-times more potent than tretinoin. - Activity in reconstructed human epidermis (RHE) MATERIAL AND METHODS: Eighteen-day old reconstructed human epidermis (RHE) from SkinEthic® were topically treated (10 µl/cm2) with ethanol, CD5789, tazarotene (in ethanol) or tretinoin and incubated at 37°C for 24h. Then, samples were collected and stored at -20°C in lysis buffer. RNAs were then eluted and quantified with the Nanodrop spectrophotometer. Forty-five genes involved in different processes (including proliferation, differentiation and inflammation) were analyzed. Gene expression data were obtained using TaqMan® Gene Expression Assays (Applied Biosystems). After reverse transcription, the qPCR reaction was performed using the thermocycler ABI Prism 7900HT Sequence Detection System (Applied Biosystems) according to the manufacturer protocol. Each Ct ("threshold cycle") value (<35Ct) was normalized to the average Ct of housekeeping genes and the comparative □□Ct method was used to calculate the relative gene expression compared to non-treated control. CONCLUSION: CD5789 was a potent as tazarotene to modulate retinoid-target genes related to differentiation process and inflammation process and about 10-times more potent than tretinoin. - Activity in human skin explant model MATERIAL AND METHODS: Human skin biopsies from abdominal plastic surgery were used to prepare 4-mm punch samples treated topically at 5mg/cm2 with ethanol, CD5789 or tazarotene in ethanol at seven concentrations between 3 and 1000 ppm. Compounds were incubated 48h in a humidified CO2 (5%) incubator at 37°C. RNA was prepared and the gene expression was obtained using TaqMan Low Density Arrays (TLDA) (Applied Biosystems). Forty-five genes involved in different processes (including proliferation, differentiation and inflammation) were analyzed. The Ct values not determined by calculation software ('Undetermined'), superior to 35 or aberrant were corrected to 35. Each Ct value was normalized to the average Ct of housekeeping genes to correct any tube-to-tube variations in reverse transcription efficiencies and amount of total RNA added to each reaction. The comparative Ct method was used to calculate the relative gene expression compared to non-treated control. The relative expression (fold modulation) compared to nontreated control was calculated for ten selected genes for CD5789 and tazarotene. The ten genes significantly modulated were selected: KRT4, KRT10 and KTR2 for keratinisation, KLK13 for desquamation, PLAT for cell migration and cicatrisation, ICAM1 for inflammation, DHRS3 for retinoids metabolism, TGM3 and LOR for cornification and DSC1 for cell adhesion. For all seven concentrations of CD5789 and tazarotene tested between 3 and 1000 ppm, the mean expression level was calculated for all ten genes and values were plotted to determine an overall mean EC50 for all retinoid-target genes. CONCLUSION: CD5789 induced retinoid-target genes in the human skin explant model with a slightly higher activity compared to tazarotene.
2) secondary pharmacodynamics	**3. RDS.03.SRE.16654 - Evaluation of the anti-inflammatory activity of CD* after a single topical administration in TPA-induced ear edema mouse model.** OBJECTIVE:

The aim of this study was to evaluate the anti-inflammatory effect of CD5789 formulated in ethanol at 0.1% after a single topical administration in the TPA-induced ear edema mouse model.

MATERIAL AND METHODS:

The ear of each Balb/c ByJIco mice (5 females/group) was treated by a single application of 20 µL of ethanol (negative control) or TPA dissolved in ethanol at 0.01% alone or in association with adapalene, CD5789 or tazarotene all tested at 0.1% in ethanol. Ear thickness was measured before application and then 6h post-application to evaluate the TPA-induced ear edema. Although several compounds were tested in this experiment, only results with CD5789, adapalene and tazarotene as reference compounds are reported hereafter.

CONCLUSION:

CD5789 has a strong anti-inflammatory activity in the TPA-induced ear edema mouse model.

4. **RDS.03.SRE.46005 - Evaluation of the depigmenting activity of CD5789 after 6 weeks of topical application on the tail of SKH2 mouse either alone or in combination with hydroquinone (CD3139). Comparison with adapalene and tretinoine.**

OBJECTIVE:

The aim of this study was to evaluate the depigmenting activity of CD5789 after 6 weeks of topical application on the tail of SKH2 mouse and to compare this effect with those of adapalene (CD0291) and tretinoin (CD0014).

MATERIAL AND METHODS:

SKH2 female mice (5/group) were treated by topical application of different compounds formulated in acetone (see table below) on the tail (20 µl) for 6 weeks (5 days/week).

Group*	Compound	Concentration
Gr 1	acetone (vehicle)	NA
Gr 2	adapalene	0.1%
Gr 3	tretinoin	0.01%
Gr 4	CD5789	0.001%
Gr 5	CD5789	0.01%

*Other groups treated with hydroquinone (CD3139) alone or in combination with other compounds were tested in this study, but the results obtained for these groups were not reported in this document.
NA : Not applicable

The tail of each mouse was observed once a week to score skin pigmentation (scale from 0 (natural pigmentation) to +4 (totally black) or -4 (totally depigmented)) and irritation. In addition, chromametric measurements were performed on mouse tail on three occasions during the study: at day 1, after 3 and 6 weeks. After 6 weeks of treatment, tail skin samples were collected and histopathology evaluations were performed with Fontana Masson and L-DOPA staining.

CONCLUSION:

After 6 weeks of topical application on the tail of SKH2 mouse, CD5789 had a significant depigmentation activity at 0.01%, only which was stronger and faster than the one of tretinoin 0.01%. CD5789 activity was maximal after 4 weeks of application. Both compounds induced a severe epidermal hyperplasia which was stronger with CD5789 0.01% than with tretinoin 0.01%.

5. **RDS.03.SRE.47078 - Evaluation of the activity of 2 tyrosinase inhibitors (CD06972, CD07629) and 1 RAR agonist (CD5789) on natural and UV-**

	induced pigmentation on the tail of SKH2 mouse after 6 weeks of topical application. OBJECTIVE: The aim of this study was to evaluate the depigmentation activity of CD5789 on natural and Uvinduced pigmentation on the tail of SKH2 mouse after 6 weeks of topical application. MATERIAL AND METHODS: SKH2 female mice (5/group) were treated by topical application of CD5789 formulated in acetone at 0.003% on the tail (20 µl) for 6 weeks (5 days/week). In addition, the tail of each animal was irradiated at 90 mJ/cm2 (UVB) 3 days per week. When animals were irradiated and treated on the same day, the treatment was applied after UV irradiation. The tail of each mouse was observed once a week to score skin pigmentation (scale from 0 (natural pigmentation) to +4 (totally black) or -4 (totally depigmented)) and irritation. In addition, chromametric measurements were performed on mouse tail at the beginning and at the end of the study. After 6 weeks of treatment, tail skin samples were collected, and histopathology evaluations were performed with Fontana Masson and L-DOPA staining. CONCLUSION: This study demonstrated that CD5789 has a strong depigmentation and anti-pigmentation activity at 0.003%, visible as early as after 2 weeks and maximum after 4 weeks. This activity is accompanied by a marked to severe epidermal hyperplasia.
3) safety pharmacology	**6. RDS.03.SRE.12657 - Evaluation of CD5789 in The Primary Observation (Irwin) Test in the rat (i.v. administration).** OBJECTIVE: Effect on the central nervous system MATERIAL AND METHODS: The effects of CD5789 (2, 4, 8, 16, 32 and 64 mg/kg) on the behavioral and physiological function in the rat were examined following one intravenous (i.v.) administration in the tail vein using the Irwin test. Four animals per group were used throughout the study. The vehicle Polyethylene glycol (PEG) 400/Ethanol/physiological saline (70/10/20 w/w/w) and physiological saline were used as control substances. CONCLUSION: The maximum tolerated i.v. dose was considered at 16 mg/kg with lethal effects from 32 mg/kg. CD5789 induced clear and dose-dependent sedative/myorelaxant effects associated with increased respiration between 2 to 32 mg/kg. **7. RDS.03.SRE.12528 - In-vitro effects of CD5789 on hERG current (I_KR) expressed in Human Embryonic Kidney (HEK) cells.** OBJECTIVE: Effect on the cardiovascular and respiratory systems MATERIAL AND METHODS: The effects of CD5789 on the delayed rectifier potassium current (IKr) encoded by hERG in stably transfected Human Embryonic Kidney (HEK293) cells were examined following superfusion at cumulative concentrations of 0.01, 0.1, 1 and 10 µM at a stimulation frequency of 6 pulses/min (0.1 Hz). A vehicle group (4 consecutive superfusion periods with the specific vehicle used for CD5789 preparation, i.e. dimethylsulfoxide (DMSO) diluted in extracellular solution) was included in the study

	for comparison. Terfenadine (1 µM) superfused after the fourth vehicle session, was used as reference substance. CONCLUSION: CD5789 showed a very low liability for prolonging QT only at 10 µM. **8. RDS.03.SRE.12617 - Assessment of cardiovascular risk for CD5789 in the conscious dog monitored by telemetry (p.o. administration).** OBJECTIVE: Effect on the cardiovascular and respiratory systems MATERIAL AND METHODS: The effects of CD5789, following single oral administration at 0.25, 1 or 2.5 mg/kg, on arterial blood pressure, heart rate and main parameters of the electrocardiogram were evaluated in the conscious male and female Beagle dogs monitored by telemetry. Dogs (3/sex) received first oral administration of the vehicle (i.e. 0.5% carboxymethylcellulose (CMC) and 0.1% Tween 80 in distilled water), followed by 3 ascending doses of CD5789. Measurements were performed 30 minutes prior to the administration and 24 hours after administration. A washout period of at least 48 hours between each administration was respected. The vehicle (0.5% CMC and 0.1% Tween 80 in distilled water) was used as a control substance. To assess the plasma levels of CD5789, blood samples were collected prior to each administration and 1 and 3 hours after each administration. CONCLUSION: Following oral administration of CD5789 at 0.25, 1 or 2.5 mg/kg in conscious male and female Beagle dogs, maximal plasma concentration of 123, 316 and 614 ng/mL, were measured at 3 hours after each administration. The treatment did not substantially modify arterial blood pressure, heart rate, the PR interval, the QT interval and the QTc (Fridericia's and van de Water's formulae) interval. No arrhythmia or other changes in the morphology of the electrocardiogram which could be attributed to CD5789 were observed. **9. RDS.03.SRE.12981 - Safety pharmacology study for CD5789 on the respiratory function in the conscious rat (whole body plethysmography) (p.o. administration).** OBJECTIVE: Effect on the cardiovascular and respiratory systems MATERIAL AND METHODS: The effects of CD5789 (5, 15 and 45 mg/kg) on respiratory function were examined following oral administration in conscious female Wistar rats (8 rats/dose). For administration, CD5789 was dispersed in 0.5% carboxymethylcellulose and 0.1% Tween® 80 in distilled water which was used as control substance. Theophylline at 100 mg/kg, dispersed in 0.2% hydroxypropylmethylcellulose, was administered as reference substance. The formulation analysis indicated that the mean achieved concentrations of the formulation samples were all satisfactory (within ± 15% of the nominal values). In addition, CD5789 was not detected in the vehicle formulation. CONCLUSION: CD5789, administered orally in the conscious female Wistar rat at the doses of 5, 15 and 45 mg/kg, had no effects on airway function over the 240-minute test period.
4) pharmacodynamic interactions	Not Applicable
3. Pharmacokinetics:	

A complete package of pharmacokinetic studies has been submitted for trifarotene.

Absorption of trifarotene is low after topical application. When penetration was analysed in vitro across non occluded full thickness fresh mouse skin mounted on diffusion cells at a dose of 10 mg/cm² the concentration in the receptor fluid was below LOQ (0.25 ng/mL) after 24 hours. Total penetration of tifarotene represented 21 % of the applied dose and was recovered exclusively from the skin.

Bioavailability after dermal application was low and approximately 5 % in rats and below LOQ (0.05 ng/mL) in minipigs after a single administration. After 12 days repeated topical application of 100 µg/g cream in minipigs, the highest mean Cmax plasma concentrations detected was 1.05 ng/mL, suggesting limited systemic exposure after topical application. (The 50 µg/g cream was not tested.) Oral bioavailability of trifarotene was 17% and 27% in male and female rats, respectively and 26% to 37% in male and female dogs. Clearance was low to moderate and lower than the cardiac output in all three species investigated and the half-life 3, 4 and 6.5 hours in rat, dog and minipig, respectively. The distribution volume was 24 to 42-fold higher in female and male rats respectively, as compared to the corresponding plasma volume, and 20 fold higher in dogs and 32 fold higher in minipigs suggesting high drug distribution.

A marked gender effect was observed in rats, with a Cmax and systemic exposure being higher for females than for males. Female rats were exposed 3.6 times, 5.8-times and 3.3-times more than males after IV, oral and dermal administrations, respectively. No relevant gender effect was seen in the dog. Tissue distribution was studied in Wistar rat (albino) or Lister Hooded rat (pigmented) using doses of 1.5 mg/kg IV or 2 mg/kg PO and Liquid scintillation counting or QWBA for quantification of radioactivity. Trifarotene was widely distributed through the body of rats, following both oral and IV administration. Highest levels of radioactivity were measured in the liver, kidney, preputial gland, adrenal cortex and salivary gland, with the highest concentrations observed in the liver. The distribution to the brain and melanin-containing tissues was relatively low. The radioactivity was not detectable in the majority of tissues at 48 and 72 h post dose for males and females, respectively. The obtained data thus suggest no risk for accumulation or binding to melanin. Trifarotene crossed the blood placenta barrier and seemed to be rapidly eliminated. Plasma protein binding was high (>99.7%) and not saturable in all species analysed (mouse, rat, rabbit, dog, minipig, human) and binding to HSA found to be similarly high. Binding to human α1-glycoprotein seemed to be somewhat less (97.4%).

Trifarotene was the major circulating radioactive constituent in plasma from both rat and dog after IV and oral administration with only two to three metabolites detected. In rat two metabolites were unambiguously identified, CD06530 (Males 5% Females 3%) and CD06700 (< 2% F only) and in addition a tentative structure was assigned to one metabolite, CD09986 (M 11%, F 5%). In dogs, two peaks in addition to the parent compound were detected but their concentrations in plasma were insufficient to enable identification by LC-MS/MS. Trifarotene was also the major radioactive constituent in faeces from rat (~25% in males and ~60% in females) and dog (~70%). Parent compound was a minor component in bile from rats and the most abundant component observed was instead a glucuronide conjugate of trifarotene.

Repeated daily topical application of trifarotene (50 µg/g) cream in adult and pediatric subjects during 4 weeks resulted in low systemic exposure (lower than or close to the LOQ of 5 pg/mL) and only 37% of adult subjects and 18 % of paediatric subjects had quantifiable trifarotene plasma levels. No quantifiable metabolites were detected. When a higher concentration trifarotene cream (100 µg/g) was used 61% adult subjects and 69 % paediatric subjects had quantifiable trifarotene plasma levels, but metabolites were still only detected in few individuals. 2 adult subjects out of 18 displayed quantifiable levels of CD06530 (Cmax= 13 and 15 pg/mL) and one quantifiable level of CD09986 (Cmax=33 pg/mL). Plasma concentrations of CD09717 and CD06700 were below the LOQ (<10 pg/mL). In paediatric subjects treated with Trifarotene 100 µg/g low quantifiable levels of one metabolite (CD06530) was detected (Cmax=19 pg/mL in the highest exposed individual). (See Clinical AR.)

In spite of the low levels of metabolites detected the applicant has presented data that show that the systemic exposure of each metabolite observed during the non-clinical studies cover the exposures observed in human and thus that the non-clinical toxicology studies are sufficient in this respect.

Faeces were the most important route of excretion of total radioactivity after IV and oral dosing in both rat and dog, representing almost 100% of the administered dose and excretion was almost complete within 48 hours. In rat, biliary elimination accounted for a mean of 31% in males and 38% in females. Milk excretion

was observed in lactating rats after single oral administration and mean milk:plasma ratios for total radioactivity increased over the sampling period from 0.53 (1 h post-dose) to 2.42 (8 h post-dose).

The drug-drug interaction (DDI) potential of CD5789 was not studied in animals. Investigations were performed in vitro with human biomaterials, using PBPK modelling and in one clinical DDI study. (See Clinical Pharmacokinetics AR.)

1) analytical methods and their validation reports

Bioanalytical methods and validation reports are listed below :

10. **RDS.03.VRE.34257 - Bioanalytical method for determination of CD5789 in mouse plasma samples – Validation.**
11. **RDS.03.VRE.34162 - Bioanalytical method for determination of CD5789 in rat plasma samples by HPLC with ESI-MS/MS detection – Validation.**
12. **RDS.03.VRE.34185 - Bioanalytical method for determination of CD5789 in rat plasma samples by HPLC with ESI-MS/MS detection – Validation.**
13. **RDS.03.VRE.34206 - Bioanalytical method for determination of CD5789 in rat plasma samples by HPLC with ESI-MS/MS detection – Validation.**
14. **RDS.03.VRE.34305 - Bioanalytical method for determination of CD5789 in rat plasma samples. GLP compliant study.**
15. **RDS.03.VRE.34159 - Bioanalytical method for determination of CD5789 in rabbit plasma samples by HPLC with ESI-MS/MS detection – Validation.**
16. **RDS.03.VPR.34160 - Determination of CD5789 long term stability in rat plasma samples by HPLC with ESI/MS/MS detection.**
17. **RDS.03.SRE.4938 - Bioanalytical and toxicokinetic evaluation of CD5789 metabolites in rat.**
18. **RDS.03.VRE.34180 - Validation of a LC-MS/MS method for the determination of CD5789 in dog plasma.**
19. **RDS.03.ATP.34223 – Bioanalytical method for determination of CD5789 in dog plasma samples by HPLC with ESI-MS/MS detection.**
20. **RDS.03.VRE.34304 - Bioanalytical method for determination of CD5789 in dog plasma samples – validation.**
21. **RDS.03.VRE.34254 - Development of an LC-MS/MS method for the determination of CD5789 in mini pig plasma.**
22. **RDS.03.VRE.111525 - Bioanalytical method for determination of CD5789 in mini pig plasma samples.**
23. **RDS.03.VRE.34275 - Validation of an LC-MS/MS method for the determination of CD5789 in mini pig plasma.**

These methods were used to analyze CD5789 in oral and dermal toxicity studies performed by the Applicant to provide toxicokinetic information in GLP studies. CD5789 metabolites were also determined in the 26-week oral toxicity study in rats (RDS.03.SRE.12863, see section 4. Toxicology 2) multiple-dose toxicity). Some details for these methods are summarized in Table 3 to Table 8.

Table 3 Description of the methods used for CD5789 in mouse plasma samples

Species	Matrix	Sample pre-treatment	Sample size (µL)	Analytical method	Calibration range (ng/mL)	Limit of Quantification (ng/mL)	Report Number
Mouse	Plasma	Protein precipitation	100µL	HPLC-MS/MS	0.1 to 10	0.1	RDS.03.VRE.34257

Table 4 Description of the methods used for CD5789 in rat plasma samples

Species	Matrix	Sample pre-treatment	Sample size (µL)	Analytical method	Calibration range (ng/mL)	Limit of Quantification (ng/mL)	Report Number
Rat	Plasma	Solid phase extraction	100	HPLC-MS/MS	0.25 to 100	0.25	RDS.03.VRE.34162
Rat	Plasma	Solid phase extraction	100	HPLC-MS/MS	0.25 to 100	0.25	RDS.03.VRE.34185
Rat	Plasma	Protein precipitation	100	HPLC-MS/MS	0.25 to 100	0.25	RDS.03.VRE.34206
Rat	Plasma	Protein precipitation	100	HPLC-MS/MS	0.25 to 100	0.25	RDS.03.VRE.34305

Table 5 Description of the methods used for CD5789 metabolites in rat plasma samples

Species	Matrix	Sample pre-treatment	Sample size (µL)	Analytical method	Calibration range (ng/mL)	Limit of Quantification (ng/mL)	Report Number
Rat	Plasma	Liquid-liquid extraction	100	HPLC-MS/MS	0.03 to 3 for all compounds	0.03	RDS.03.VRE.4938*

* Method not validated

Table 6 Description of the methods used for CD5789 in rabbit plasma samples

Species	Matrix	Sample pre-treatment	Sample size (µL)	Analytical method	Calibration range (ng/mL)	Limit of Quantification (ng/mL)	Report Number
Rabbit	Plasma	Solid phase extraction	100	HPLC-MS/MS	0.25 to 100	0.25	RDS.03.VRE.34159

Table 7 Description of the methods used for CD5789 in dog plasma samples

Species	Matrix	Sample pre-treatment	Sample size (µL)	Analytical method	Calibration range (ng/mL)	Limit of Quantification (ng/mL)	Report Number
Dog	Plasma	Liquid-liquid extraction	100	HPLC-MS/MS	0.5 to 100	0.5	RDS.03.VRE.34180
Dog	Plasma	Protein precipitation	100	HPLC-MS/MS	0.5 to 100	0.5	RDS.03.VRE.34223
Dog	Plasma	Protein precipitation	100	HPLC-MS/MS	0.5 to 100	0.5	RDS.03.VRE.34304

Table 8 Description of the methods used for CD5789 in minipig plasma samples

Species	Matrix	Sample pre-treatment	Sample size (µL)	Analytical method	Calibration range (ng/mL)	Limit of Quantification (ng/mL)	Report Number
Minipig	Plasma	Protein precipitation	500	HPLC-MS/MS	0.05 to 5	0.05	RDS.03.VRE.34254
Minipig	Plasma	Liquid-liquid extraction	500	HPLC-MS/MS	0.05 to 5	0.05	RDS.03.VRE.111525
Minipig	Plasma	Liquid-liquid extraction	500	HPLC-MS/MS	0.05 to 5	0.05	RDS.03.VRE.34275

2) absorption

In vitro dermal penetration study

24. RDS.03.SRE.104532 - Assessment of in vitro skin penetration of CD5789 formulated in HE1 Cream and in Cream (Cream B, Cream Simulgel) in ex vivo mouse skin.

OBJECTIVE:

The objective of the present study was to evaluate the penetration of CD5789 formulated in two different formulations (including cream, the formulation developed for acne) in excised mouse skin. Only results with the cream formulation, subject of this application are presented.

MATERIAL AND METHODS:

The in vitro skin penetration of CD5789 in three different formulations: HE1 Cream containing 0.001% or 0.01% CD5789 and Cream containing 0.01% CD5789 was

evaluated across non-occluded full-thickness fresh mouse skin (CD-1® IGS, Male, 20 weeks old) mounted on diffusion cells. A finite dose of 10 mg/cm² of each formulation was applied to the outer surface of the skin with an application area of 2 cm². Each condition was tested in nine replicates over nine different mice. The test system was thermostated at 37°C and the receptor fluid (3 mL of phosphate buffer saline) was continuously stirred at 350 rpm. The treatment duration was 24 hours under static conditions. At the end of treatment, concentrations of CD5789 were measured in total skin, receptor fluid and formulation excess samples using an LC-MS/MS method. The limits of quantification in total skin and receptor fluid matrices were 0.125 ng/mL and 0.25 ng/mL, respectively.

CONCLUSION:

The total penetration of CD5789 when applied at 100 µg/g in cream formulation ex vivo to the skin of mice represented 20.6% of the applied dose and was recovered exclusively in the skin.

25. RDS.03.SRE.4785 - Pharmacokinetics in the Sprague Dawley rat after single oral, dermal or intravenous administration.

OBJECTIVE:

The objective of the present study was to characterize the pharmacokinetic profile of CD5789 in male and female Sprague Dawley rats after a single intravenous (IV), oral or dermal administration.

MATERIAL AND METHODS:

CD5789 was administered to male and female Sprague Dawley rats upon a single IV or oral administrations of 1 mg/kg or upon a single dermal administration of 4.9 mg/kg. Intravenous administration used PEG 400/ethanol (EtOH)/NaCl 0.9% (70/10/20) (w/w/w) at a volume of 1 mL/kg. Oral administration used carboxymethylcellulose 0.5 % + Tween 80 (0.01 %) in purified water (w/w/w) at a volume of 5 mL/kg. Dermal application used propylene glycol (PG)/EtOH (20/80) (w/w) at a volume of 5 mg/cm². Blood samples were drawn from 3 rats/sex/dose/timepoint. Corresponding plasma samples were analyzed by high-performance liquid chromatography (HPLC) with mass spectrometry detection (LOQ: 0.125 ng/mL). Pharmacokinetics parameters were determined by a non-compartmental method from the mean plasma concentrations per sex and groups.

CONCLUSION:

After an IV administration, CD5789 was moderately cleared from plasma and highly distributed. Accumulation of CD5789 should not occur in significant amounts when administered as multiple doses.

26. RDS.03.SRE.31094 - Absorption, Distribution, Metabolism and Excretion of CD5789 in the Wistar rat after a single oral and intra venous dose.

OBJECTIVE:

The objective of the present study was to characterize the pharmacokinetic parameters of total radioactivity after single IV or oral administration of [14C] CD5789. Other results related to distribution, metabolism and excretion parameters are reported in the corresponding sections.

MATERIAL AND METHODS:

Wistar rats received single IV or oral administration of [14C]-CD5789 at respectively 1.5 and 2 mg/kg. Four groups of rats were included in the study: Group 1 (4 rats/sex, IV dose) and Group 2 (4 rats/sex, oral dose) for mass-balance purposes (excretion group). Group 3 (39 rats/sex, i.v. dose) and Group 4 (36 rats/sex, oral dose) for pharmacokinetic (PK) purposes and tissue collection.

In the pharmacokinetic groups, blood was sampled from three rats per sex per time point at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 24, 48, 72 and 96 hours after IV dosing and

at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 24, 48, 72 and 96 hours after oral dosing. Total radioactivity in blood and plasma was determined. In addition, the concentration of the parent compound, CD5789, was determined by HPLC method with tandem mass spectrometry detection (LOQ=0.250 ng/mL).

CONCLUSION:

After a single IV administration of [14C] -CD5789 in rats, a rapid decrease in plasma radioactivity was observed with a terminal half-life of 14.4 hours. By the oral route, maximum plasma radioactivity levels were reached from 1 to 2 hours after dosing. Total radioactivity measured in the whole blood following a single oral or IV administration was lower than in plasma. The mean blood/plasma ratio ranged from 0.61 to 0.78, irrespective of the administration route, indicating no specific affinity of the test substance to red blood cells. CD5789 has no specific affinity to red blood cells of Wistar rats.

The absolute oral bioavailability of radioactivity was 29% in males and 35% in females. Whatever the route, the plasma exposure to CD5789 and to total radioactivity was lower in males than in females.

27. RDS.03.SRE.12599 - CD 5789 Single dose comparative pharmacokinetic study by the oral (gavage) or intravenous (bolus injection) routes followed by a 14-day oral (gavage) dose-range finding toxicity study in the beagle dog.

OBJECTIVE:

The objective of the present study was to evaluate the pharmacokinetic profile of CD5789 administered by the oral or IV route. The dose-range finding toxicity part of the study is reported in the toxicology Section 4. Toxicology of this document.

MATERIAL AND METHODS:

The pharmacokinetic profile of CD5789 was evaluated based on the following design (Table 11).

Table 11 Animals groups

Group/Treatment	Dose level (mg/kg)	Dose volume (mL/kg)	Concentration (mg/mL)	Number of animals	
				Males	Females
PK - single IV administration	1	1	1	1	1
PK - single oral administration	1	5	0.2	1	1

Blood samples were collected at 5 min, 15 min, 30 min, 1, 2, 4, 6, 8 and 24h after i.v. administration and at 15 min, 30 min, 1, 2, 3, 4, 6, 8 and 24h after oral administration. Blood samples were analyzed for unchanged CD5789 and its potential metabolites CD6530 and CD6700 using a non-validated HPLC method with TIS-MS/MS detection with a LoQ of 0.5 ng/mL.

CONCLUSION:

No relevant gender effect was observed, whatever the route of administration. The maximal plasma concentration for CD5789 was reached 5 minutes after dosing by the IV route and between 2 and 4 hours after dosing by the oral route. CD06530 was not quantifiable in plasma from treated animals. The Cmax of CD5789 was markedly higher than that of its metabolite CD06700 (pyrrolidine cycle opening and hydroxylation: N-butanol). The oral bioavailability of CD5789 was low for both genders (26 and 37% in males and females, respectively). CD5789 was moderately cleared from plasma and highly distributed (see Table 12).

Table 12 PK results for CD5789 after a single IV or single oral dose in the dog

Method of Adm.	Dose (mg/kg)	Gender	C_{max} (ng/mL)	T_{max} (hrs)	AUC_{0-24h} (ng.h/mL)	T1/2 (h)	Clearance (L/h/kg)	Vd (L/kg)	Bioavailability (%)
IV	1	Male	1939	0.083	6741	4.44	0.146	0.934	NA
IV	1	Female	1932	0.083	5924	4.31	0.167	1.04	NA
Oral	1	Male	289	2	1750	3.97	0.146	0.835	26
oral	1	Female	280	4	2182	3.67	0.167	0.882	37

NA: Not Applicable

28. RDS.03.SRE.31095 - Absorption, Metabolism and Excretion of [14C]-CD5789 in the male Beagle dog after a single oral and intravenous dose.

OBJECTIVE:

The objective of the present study was to characterize the pharmacokinetics of total radioactivity, to determine the excretion balance in urine and in feces and to identify the main metabolites in Beagle dogs after single IV or oral administration of [14C]-CD5789. Other results related to distribution, metabolism and excretion parameters are reported in the corresponding sections.

MATERIAL AND METHODS:

One group of 4 male Beagle dogs received first a single IV administration of [14C]-CD5789 at 1 mg/kg and after a washout period of 4 weeks an oral administration of [14C]-CD5789 at 1 mg/kg.

For pharmacokinetic purposes, blood was sampled at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 24, 48, 72 and 96 hours after IV dosing and at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 24, 48, 72 and 96 hours after oral dosing. Total radioactivity in blood and plasma was determined. In addition, the concentration of CD5789 was determined by a HPLC method with tandem mass spectrometry detection (LOQ= 1 ng/mL).

CONCLUSION:

After IV administration, radioactivity levels and CD5789 were quantified in blood and plasma up to 96 hours (last sampling time). Total radioactivity measures in the whole blood following a single oral or IV administration was lower than in plasma. The blood/plasma ratio ranged from 0.41 to 0.56 irrespective of the administration route, indicating no specific affinity of parent compound and metabolic pool to red blood cells. CD5789 has no specific affinity to red blood cells of Beagle dogs.

After oral administration, radioactivity levels were quantified in blood and plasma up to 96 hours (last sampling time) and CD5789 was quantified in plasma up to 48 hours. Maximum concentrations of total radioactivity and CD5789 were reached 1 to 2 hours after dosing in blood and in plasma. The terminal half-life of parent compound was 4.35 hours. The absolute bioavailability of total radioactivity after oral administration was 50% in plasma.

29. RDS.03.SRE.4851 - CD5789 pharmacokinetics in the GÖTTINGEN® minipig after single intravenous or topical administration.

OBJECTIVE:

The objective of the present study was to assess the pharmacokinetic profile of CD5789 in the male Göttingen® minipig, after single IV and dermal administrations.

MATERIAL AND METHODS:

CD5789 was administered by i.v. at 1 mg/kg and by the dermal route with two different formulations (gel and cream A) at 0.01% (100 µg/g) at a dose volume of 2 mL/kg (corresponding to 0.171 mg/kg and 0.2 mg/kg, respectively). Each of the 4 male Göttingen® minipigs received a single IV administration, separated by a washout period of 7 days, followed by a single dose of gel at 0.01% and a single dose of cream A at 0.01%, separated by a washout period of 14 days. Treatment sites representing approximately 10% of body surface area were clipped free from hair on both flanks avoiding the spinal column. Dermal formulations were applied topically over the

treatment areas using a suitable administration device. During the exposure period of approximately 6h, large gauzes protected the treatment sites, which were cleaned with lukewarm water. Blood samples were drawn from all animals at the following time points:

- Intravenous administration: 0.16, 0.33, 0.5, 1, 2, 4, 6, 8, 10, 24 and 48h post-dosing
- Dermal administration: before dosing (0h), 0.5, 1, 2, 4, 6, 8, 10, 24 and 48h post-dosing

Plasma concentrations of CD5789 were determined by an HPLC method with ESI-MS/MS detection with a limit of quantification of 0.1 ng/mL. Pharmacokinetic analysis was performed from individual plasma concentrations using a non-compartmental approach.

CONCLUSION:

After a single intravenous administration, CD5789 was slowly eliminated (t1/2= 6.5h) and presented an important volume of distribution (1.26 L/kg) and a low systemic clearance (0.76 L/h/kg).
After a single dermal administration, CD5789 plasma concentrations were below the lower limit of quantification in all animals, whatever the formulation.

30. RDS.03.SRE.4872 - CD5789 pharmacokinetic in the GÖTTINGEN® minipig after repeated topical administration of three different formulations.

OBJECTIVE:

The objective of this study was to compare the pharmacokinetic profiles of CD5789 formulated in 3 different formulations in the male Göttingen® minipig after single and multiple (12 days) daily dermal applications.

MATERIAL AND METHODS:

Four male minipigs/group were treated once daily by topical application with CD5789 formulated at 100 µg/g (0.01%) in 3 different formulations: gel, cream A or cream at a dosing volume of 2 mL/kg/day on a body surface treatment area of approximately 10% (corresponding to approximately 40 mg/cm² of formulation). Formulations were topically applied over both flanks with a suitable device to obtain a thin and uniform film. The sites of application were protected during the exposure period by large gauzes held in place with a body net/bandage and collar. The exposure period lasted approximately 6h post-dosing, except on non-working days where the exposure period lasted approximately 3h post-dosing. After the exposure period, the treatment areas were rinsed with lukewarm water. Blood samples were taken from all animals at the following time points:

- After single dosing (Day 1) : 0.5, 1, 2, 4, 6, 8, 10 and 24h post-dosing
- After multiple dosing (Day 12): before dosing (0h), 0.5, 1, 2, 4, 6, 8, 10, 24, 48 and 72h post-dosing.

Plasma concentrations of CD5789 in minipigs were determined by a HPLC method with ESI-MS/MS detection and a limit of quantification of 0.05 ng/mL. Pharmacokinetic analysis was performed from individual plasma concentrations using a non-compartmental approach.

CONCLUSION:

After a single topical administration in the male minipig of CD5789 at 100 µg/g in either gel, cream A or the cream, CD5789 plasma concentrations were not quantifiable (LOQ = 0.05 ng/mL). After repeated topical applications (12 days) plasma concentrations were very variable, whatever the formulations.

After multiple topical administrations, absorption was variable according to the formulation applied. The most rapid absorption was observed with CD5789 100 µg/g cream followed by CD5789 100 µg/g gel cream A and CD5789 100 µg/g gel.

	CD5789 is characterized by a rapid absorption after topical application with peak plasma concentrations observed 2 hours post dose and a short terminal half-life of about 4 hours with the cream formulation. After multiple topical administrations, the systemic exposure, expressed as AUC0-24h, was determined as the highest for CD5789 100 µg/g cream, followed by CD5789 100 µg/g gel and by CD5789 100 µg/g cream A being the lowest exposure demonstrated in the study. After multiple topical administrations of CD5789 100 µg/g cream, the steady state seemed to be reached on Day 12 and the elimination was relatively rapid. After the multiple topical administrations of CD5789 100 µg/g gel and cream A, the steady state and the elimination could not be evaluated due to pharmacokinetic profiles.
3) distribution	**Tissue distribution studies** **31. RDS.03.SRE.31094 - Absorption, Distribution, Metabolism and Excretion of CD5789 in the Wistar rat after a single oral and intra venous dose.** OBJECTIVE: The objective of the present study was to characterize the pharmacokinetic parameters of total radioactivity after single IV or oral administration of [14C] CD5789. Other results related to absorption, metabolism and excretion parameters are reported in the corresponding sections. MATERIAL AND METHODS: *Detailed in section 3. Pharmacokinetics 2) Absorption.* In the PK Groups used in this study, several tissues (heart, lung, spleen, testes, uterus or ovaries, stomach, liver, kidney, skin, bone marrow and stifle joints) were collected at 4 time-points; 0.083, 2, 4 and 24 after IV dosing and 0.5, 2, 4 and 24 hours after oral dosing. CONCLUSION: The radioactivity in selected tissues was mainly measured in the liver, irrespective of administration route and sampling times. The low remaining radioactivity levels observed in the tissues 24 hours after dosing suggested no risk of accumulation. **32. RDS.03.SRE.102423 - The Tissue Distribution of Total Radioactivity in the Rat Following Single Oral and Intravenous Administration of [14C]-CD5789 (Quantitative Whole Body Autoradiography).** OBJECTIVE: The aim of this study was to assess the tissue distribution of total radioactivity following oral and IV administration of [14C]-CD5789 to male and female albino and pigmented rats. MATERIAL AND METHODS: Wistar (albino) and Lister Hooded (pigmented) rats (1/sex/group) were treated by IV route at 1.5 mg/kg or by oral route at 2 mg/kg with [14C]-CD5789. Groups of albino rats were sacrificed at pre-defined time-points up to 72 h and pigmented rats up to 168h. The tissue distribution was determined by quantitative whole body autoradioactivity (QWBA). CONCLUSION: [14C]-CD5789 was widely distributed through the body of the rats, following both oral and IV administration. Highest levels of radioactivity were measured in the liver, kidney, preputial gland, adrenal cortex and salivary gland, with the highest concentrations observed in the liver. The distribution to the brain and melanin-containing tissues was relatively low. The radioactivity was not detectable at 24 and

	48 h post dose for males and females, respectively in the majority of tissues whatever the route of administration in albino and pigmented rats. **Protein binding and distribution in red blood cells** **33. RDS.03.SRE.31089 - In vitro determination of the plasma protein binding of [14C]-CD5789 in 6 species.** OBJECTIVE: The aim of this study was to determine in vitro the plasma protein binding of [14C]-CD5789 in plasma from male CD1 mouse, male Wistar Han rat, female New Zealand white rabbit, male Beagle dog, male Göttingen minipig and male human. MATERIAL AND METHODS: Plasma protein binding of [14C]-CD5789 was determined using equilibrium dialysis. The binding of [14C]-CD5789 to human serum albumin (HSA) and human □-acid glycoprotein (HAG) was also determined. The concentration of [14C]-CD5789 in buffer and plasma compartments was measured using liquid scintillation counting (LSC). Preliminary experiments were performed to determine the non-specific adsorption of [14C]-CD5789 and the equilibrium time. CONCLUSION: The binding of [14C]-CD5789 to plasma proteins from different species is high (>99.7%) not saturable between 50 ng/mL and 1000 ng/mL and not species-specific. **Placental transfer study in the pregnant rat** **34. RDS.03.SRE.100911 - Placental Transfer Study in the Pregnant Wistar Rat Following Single Oral Administration of [14C]-CD5789.** OBJECTIVE: The aim of this study was to assess the placental transfer of [14C]-CD5789 in pregnant rats. MATERIAL AND METHODS: Fifteen pregnant Wistar female rats were treated by a single oral administration of [14C]-CD5789 at 0.3 mg/kg on gestation day 12. At 1, 2, 4, 8 and 24 h post dose, groups of 3 rats were sacrificed. Maternal blood samples, selected maternal tissues and fetuses were collected and levels of total radioactivity were determined. CONCLUSION: Following oral administration to female pregnant Wistar rats at gestation day 12 at 0.3 mg/kg, [14C]-CD5789 was demonstrated to cross the blood-placenta barrier. The transfer of total radioactivity in fetuses occurred to a minor extent with quantifiable concentration in fetuses up to 8 h post-dose, demonstrating a rapid elimination of the radioactivity. Mean ratios of fetal concentrations to maternal plasma and to placenta were below 0.5, whatever the time point.
4) metabolism	**In vivo metabolism** **35. RDS.03.SRE.31094 - Absorption, Distribution, Metabolism and Excretion of CD5789 in the Wistar rat after a single oral and intra venous dose.** OBJECTIVE: The objective of the present study was to characterize the pharmacokinetic parameters of total radioactivity after single IV or oral administration of [14C] CD5789. Other results related to absorption, metabolism and excretion parameters are reported in the corresponding sections. MATERIAL AND METHODS: *Detailed in section 3. Pharmacokinetics 2) Absorption.*

The metabolite profile was investigated in pooled plasma samples collected in PK groups at different time-points.

CONCLUSION:

In the plasma, CD5789 was the major circulating radioactive constituent whatever the sampling time and four metabolites were evidenced. CD5789 was the major radioactive constituent in feces and nine metabolites were detected.

36. RDS.03.SRE.102754 - The Disposition of [14C]-CD5789 Following Oral and Intravenous Administration in the Bile Duct-Cannulated Rat.

OBJECTIVE:

This study was designed to examine the excretion and biliary kinetics of [14C]-CD5789 total radioactivity in male and female Wistar rats and to identify metabolites of [14C]-CD5789 present in bile.

MATERIAL AND METHODS:

The excretion and biliary kinetics of total radioactivity were determined in male and female bile duct-cannulated Wistar rats following a single oral (2 mg/kg) or IV (1.5 mg/kg) administration of [14C]-CD5789. Excreta (urine, bile and feces), cage wash, gastrointestinal tract and carcass samples were collected at pre-determined time points up to 48 h post dose and levels of total radioactivity determined. The nature and indentification of metabolites of [14C]-CD5789 present in the bile was investigate by radio-LC-MS (limit of reliable determination of 30 d.p.m).

CONCLUSION:

CD5789 (parent) was a minor component in bile, irrespective of the route of administration and the most abundent component observed in bile was a glucuronide conjugate of CD5789. The biotransformation of CD5789 in rat bile was similar in both sexes and proceeded by hydroxylation, reduction/hydrogenation/ring opening, oxidation, sulphate or glucuronide conjugation.
A summary of the transformation and the % administered dose this accounted for in each sex and dose route is presented in Table 16.

Table 16 Summary of the biotransformations and the % administered dose in each sex and dose route

Compound	Reference	Biotransformation	% dose			
			Oral		IV	
			Male	Female	Male	Female
M004	CD5789-01	Hydroxylation, Glucuronidade conjugate	1.5	1.3	2.1	1.2
M001	CD5789-03	Glucuronidade conjugate	ND	0.8	ND	ND
M002	CD5789-04	Glucuronidade conjugate	11.8	20.6	11.4	36.5
M003	CD5789-06	Glucuronidade conjugate	1.3	3.4	3.2	11.9
M005	CD5789-08	Hydroxylation, Glucuronidade conjugate	1.3	1.8	5.3	4.3
M008	CD5789-14	Hydroxylation, Reduction, Sulphate conjugate	0.9	0.7	16.2	3.3
CD06530	CD5789-16	Hydroxylation	1.2	1.1	3.2	2.8
M006	CD5789-28	Hydroxylation, Glucuronidade conjugate	2.6	0.8	9.9	2.5
CD5789	CD5789	No transformation	0.5	0.4	3.4	1.9
CD06700	CD5789-18	Hydroxylation, Reduction	ND	ND	ND	0.3
CD09717	CD5789-19	Hydroxylation, Oxidation	2.7	1.0	9.4	2.6
M007	CD5789-29	Hydroxylation, Oxidation, Glucuronidade conjugate	2.2	2.4	4.6	1.7
CD09986	CD5789-23	Hydroxylation, Oxidation	0.2	0.2	0.4	ND
Total assigned			26.2	34.5	69.1	69
Total unassigned (a)			4.2	2.4	11.6	3.8

(a) = No individual unassigned component exceeded 2.7% of the administered dose; ND = not detected by radio-LC/MS

37. RDS.03.SRE.31095 - Absorption, Metabolism and Excretion of [14C]-CD5789 in the male Beagle dog after a single oral and intravenous dose.

OBJECTIVE:

The objective of the present study was to characterize the pharmacokinetics of total radioactivity, to determine the excretion balance in urine and in feces and to identify the main metabolites in Beagle dogs after single IV or oral administration of [14C]-CD5789. Other results related to absorption, and excretion parameters are reported in the corresponding sections.

MATERIAL AND METHODS:

Detailed in section 3. Pharmacokinetics 2) Absorption.

The metabolite profile was investigated in pooled plasma samples per period.

CONCLUSION:

CD5789 was the major circulating radioactive constituent in the plasma, whatever the sampling time after IV and oral dosing. Only two additional radioactivity peaks were detected in plasma after IV administration, but they could not be identified due to very low concentrations in plasma. In the feces, CD5789 was the major radioactive constituent. Eight metabolites were detected in the feces, of which five were unambiguously identified (CD06530, CD09718, CD06700, CD09717, CD06230). Two others were tentatively identified, the glucuronide of CD5789 and CD5789 with a double bound. One minor metabolite (about 1% of total radioactivity) could not be identified.

38. RDS.03.SRE.4938 - Bioanalytical and toxicokinetic evaluation of CD5789 metabolites in rat.

OBJECTIVE:

The objective of the study was to assess the toxicokinetic parameters of three CD5789 metabolites, CD06530, CD06700 and CD09986 in male and female rats after repeated administration of CD5789.

MATERIAL AND METHODS:

The investigations of CD5789 metabolites were performed using remaining plasma samples of male and female Wistar rats treated at 0.5 mg/kg/day and 0.2 mg/kg/day, respectively, corresponding to the NOAEL doses in males and females, respectively. Samples were collected during the last week of treatment (Day 168) of the 26 weeks oral toxicity study (RDS.03.SRE.12863, see section 4. Toxicology 2) multiple-dose toxicity). Plasma samples were pooled by time-point (0.5, 1, 2, 4, 8 and 24 h post-dosing) and by sex. Plasma concentrations of each metabolite were determined by an LC-MS/MS method with a limit of quantification of 0.05 ng/mL for each compound. Toxicokinetic analysis was performed from plasma concentrations using a non-compartmental approach.

CONCLUSION:

After repeated oral administration of CD5789 at the NOAEL doses, male and female rats were exposed to its three metabolites, CD06530, CD06700 and CD09986. CD09986 was considered as the predominant metabolite.

39. RDS.03.SRE.12516 - CD5789 Embryo-fetal toxicity, dose range-finding study by the oral route (gavage) in the pregnant rat.

OBJECTIVE:

In this study, the systemic exposure of CD5789 and two related metabolites was assessed in pregnant rats following repeated oral administrations, from implantation (day 6 of gestation) to the closure of hard palate (day 17 of gestation). Of note, at the time of completion of this study (*i.e.* 2007), only two potential metabolites were identified.

MATERIAL AND METHODS:

CD5789 was administered to pregnant Sprague-Dawley rats at dose levels of 0.03, 0.1, 0.3 or 1.0 mg/kg/day to satellite animals. Serials blood samples up to 24 hours were

drawn from three rats /dose on gestation day 6 (first dosing day) and gestation day 17 (last dosing day). The determination of unchanged CD5789 and of its two metabolites (CD06530 and CD06700) in the plasma samples was performed by HPLC-MS/MS analysis (LOQ: 0.125 ng/mL).

CONCLUSION:

After oral administration of CD5789 to pregnant rats, systemic exposures on gestation day 17 was very low for CD06530, representing less than 1% of the systemic exposure of CD5789 and was even lower for CD06700.

40. RDS.03.SRE.12517 - CD5789 Embryo-fetal toxicity, dose range-finding study by the oral route (gavage) in the pregnant rabbit.

OBJECTIVE:

In this study, the systemic exposure of CD5789 and two related metabolites was assessed in pregnant rabbits after repeated oral administrations, from implantation (day 6 of gestation) to the closure of hard palate (day 19 of gestation). Of note, at the time of completion of this study (*i.e.* 2007), only two potential metabolites were identified.

MATERIAL AND METHODS:

CD5789 was administered to pregnant rabbits by oral gavage at dose levels of 0.01, 0.05, 0.25 or 1.0 mg/kg/day to satellite animals. Serials blood samples up to 24 hours were drawn from six rabbits/dose on Gestation Day 6 (first dosing day) and on gestation Day 19 (last dosing day). The determination of unchanged CD5789 and of its potential metabolites (CD06530 and CD06700) in the plasma samples was performed by HPLC-MS/MS analysis (LOQ: 0.125 ng/mL).

CONCLUSION:

After oral administration of CD5789 to pregnant rabbits, systemic exposures on gestation day 19 was low for CD5789, very low for CD06530 and almost undetectable for CD06700.

In vitro metabolism studies

41. RDS.03.SRE.31104 - Metabolite profiling/Identification of [14C]-CD5789 in human hepatocytes and in liver microsomes from mouse, minipig and human.

RDS.03.SRE.31104 was conducted to establish experimental conditions for study RDS.03.SRE.31107 by using hepatocytes and to perform some preliminary metabolite structural identifications and analytical set-up. This study is only mentioned for information but is not reported in this application as a full metabolic profiling has been performed in subsequent studies.

42. RDS.03.SRE.31107 - In vitro metabolism of [14C]-CD5789 by hamster, mouse, rat, rabbit, dog, minipig, monkey and human hepatocytes in suspension.

OBJECTIVE:

The aim of this study was to determine the metabolic stability and metabolite profile of [14C]-CD5789 using suspensions of cryopreserved hepatocytes from Golden Syrian hamster, CD1 mouse, Wistar rat, New Zealand white rabbit, Beagle dog, Göttingen® minipig, Cynomolgus monkey and human.

MATERIAL AND METHODS:

[14C]-CD5789 was incubated at 2 concentrations, *i.e.* 10 and 50 µM with suspensions of cryopreserved hepatocytes from Golden Syrian hamster, CD1 mouse, Wistar rat, New Zealand white rabbit, Beagle dog, Göttingen® minipig, Cynomolgus monkey and human. Incubations with 10 µM were used for metabolite profiling and incubations at 50 µM were used for identification purposes. Incubations with [14C]-CD5789 were performed at 1, 30, 60, 90 or 120 minutes in duplicate for all species (10 µM) and

single incubation (50 µM). Incubated samples were analyzed by LC-RAD for metabolic stability and radioactivity profiling purposes and by LC-RAD-MS for metabolite identification purposes.

CONCLUSION:

All phase 1 metabolites detected in human hepatocytes were also detected in at least one of the animal species used for toxicology studies (i.e. CD1 mouse, Wistar rat, New Zealand white rabbit, Beagle dog, Göttingen® minipig). No human-specific metabolites were detected.

43. RDS.03.SRE.4825 - Interspecies comparison of in vitro metabolism of [14C]-CD5789 in rat, dog, minipig, and human fresh hepatocytes in primary culture.

OBJECTIVE:

The aim of this study was to perform qualitative and quantitative interspecies comparison of the *in vitro* metabolism of [14C]-CD5789 using fresh hepatocytes in primary culture prepared from male Wistar rat, male Beagle dog, male Göttingen® minipig and human (males).

MATERIAL AND METHODS:

In vitro metabolism of [14C]-CD5789 by fresh hepatocytes in primary culture was studied at 2 concentrations: 1 µM and 10 µM. The incubation duration with [14C]-CD5789 was 24 hours, with a metabolism assessment after 4 hours and 24 hours. Metabolite profiles were analyzed by HPLC using an on-line radioactive detection method. Structural identification of [14C]-CD5789 main metabolites was performed using an Ion-Trap mass spectrometer. The metabolic activity of fresh hepatocytes in primary culture was confirmed by measuring the metabolism of [4,14C]-Testosterone, used as positive control.

CONCLUSION:

Based on HPLC metabolite profiles, all major [14C]-CD5789 metabolites observed in fresh human hepatocytes were found in hepatocytes from at least one animal species.

44. RDS.03.SRE.4826 - Interspecies comparison of in vitro metabolism of [14C]-CD5789 in mouse, rat, rabbit, dog, minipig, monkey and human liver microsomes.

OBJECTIVE:

The aim of the study was to compare the *in vitro* metabolism of [14C]-CD5789 using liver microsomal fractions prepared from CD-1 mice, Wistar rats, New Zealand White rabbits, Beagle dogs, Göttingen® minipigs, Cynomolgus monkey and Human.

MATERIAL AND METHODS:

Liver microsomal fractions prepared were incubated 5 and 30 minutes with 1 µM and 10 µM of [14C]-CD5789. Samples obtained after 5 and 30 minutes of incubation were used for analysis. Metabolite profiles were determined using a HPLC method with on-line radioactive detection.

CONCLUSION:

All [14C]-CD5789 main metabolites produced by human liver microsomes were also produced in at least one of the animal liver microsomes tested.

45. RDS.03.SRE.38175 - In vitro hepatic and cutaneous metabolic stability of CD5789

OBJECTIVE:

In vitro hepatic and cutaneous metabolic stability of CD5789

MATERIAL AND METHODS:

	CD5789 was incubated separately with human hepatic microsomes with and without glucuronidation cofactors and with human keratinocytes. At different times of experiment, the incubation medium was sampled and analyzed by LC/MS/MS in order to determine the quantity of remaining parent compound. CD5789 half-life time was determined for each type of preparation from the concentrations versus time curves. CONCLUSION: CD5789 is unstable in presence of Phase I metabolic enzymes of hepatic microsomes of different species unlike Adapalene and Tazaroten acid which are both stable. The presence of additional glucuronidation cofactors increase slightly the disappearance of CD5789 only in rabbit species. CD5789 is not metabolized in human keratinocytes like Adapalene and Tazaroten acid
5) excretion	**Excretion study in rats** **46. RDS.03.SRE.31094 - Absorption, Distribution, Metabolism and Excretion of CD5789 in the Wistar rat after a single oral and intra venous dose.** OBJECTIVE: The objective of the present study was to characterize the pharmacokinetic parameters of total radioactivity after single IV or oral administration of [14C] CD5789. Other results related to absorption, distribution and metabolism parameters are reported in the corresponding sections. MATERIAL AND METHODS: *Detailed in section 3. Pharmacokinetics 2) Absorption.* Single doses of [14C]-CD5789 at 1.5 mg/kg (IV) or 2 mg/kg (oral) were administered to Wistar rats. In the excretion groups (4 rats/sex/dose), urine and feces were collected in 0-8, 8-24, 24-48, 48-72 and 72-96 h intervals. Animals were euthanized 96 hours after dose administration and the carcass was collected. Total radioactivity in urine, feces, and carcass was determined. The metabolite profile was investigated in pooled samples of urine and feces per group and sex. CONCLUSION: The excretion of [14C]-CD5789 was rapid and almost complete within 48 hours. Feces was the major elimination pathway after either intravenous or oral administration. Urinary excretion appeared as a very minor process in drug excretion, irrespective of the administration route and the sex. **Excretion study in dogs** **47. RDS.03.SRE.31095 - Absorption, Metabolism and Excretion of [14C]-CD5789 in the male Beagle dog after a single oral and intravenous dose.** OBJECTIVE: The objective of the present study was to characterize the pharmacokinetics of total radioactivity, to determine the excretion balance in urine and in feces and to identify the main metabolites in Beagle dogs after single IV or oral administration of [14C]-CD5789. Other results related to absorption, and metabolism parameters are reported in the corresponding sections. MATERIAL AND METHODS: *Detailed in section 3. Pharmacokinetics 2) Absorption.* One group of 4 male Beagle dogs were included in the study for mass-balance and for pharmacokinetics purposes. They received first a single intravenous (IV) administration of 1 mg/kg [14C]-CD5789, then a single oral administration of 1 mg/kg [14C]-CD5789 after a washout period of four weeks. For mass-balance and excretion analysis, urine and feces were collected in 0-8, 8-24, 24-48, 48-72 and 72-96 h intervals

post-dosing. Total radioactivity in urine and feces was determined. The metabolite profile was investigated in pooled samples of feces per period.

CONCLUSION:

The excretion of CD5789 was rapid and almost complete within 48 hours. Feces was the major elimination pathway after IV or oral route. Excretion in the urine accounted a maximum of 1% of the administered dose (IV or Oral).

Milk excretion in the Wistar rat

48. RDS.03.SRE.102454 - The Secretion of Total Radioactivity in Milk Following Single Oral Administration of [14C]-CD5789 in the Wistar Rat.

OBJECTIVE:

This study was designed to examine the secretion of total radioactivity in milk following a single oral administration of [14C]-CD5789 to female Wistar rats and to determine the levels of unchanged CD5789 and total radioactivity in milk and plasma samples

MATERIAL AND METHODS:

Groups of 3 lactating female Wistar rats were treated on day 20 after parturition with a single oral administration of [14C]-CD5789 at 0.1 mg/kg and sacrificed at pre-determined time points up to 24 h post dose, to collect milk and maternal and pup plasma samples. Levels of total radioactivity were determined by liquid scintillation counting (LSC). The concentration of unchanged CD5789 was determined in milk and plasma samples with LOQ of 0.25 ng/mL.

CONCLUSION:

Following a single oral administration at 0.1 mg/kg, [14C]-CD5789 was quantifiable in milk with mean milk:plasma total radioactivity ratios increasing from 0.53 to 2.42 between 1 h and 8 h post-dose. At 24 h post-dose, the mean total radioactivity concentration in milk was below the limit of reliable measurement (30 d.p.m above background). No milk transfer of total radioactivity or CD5789 to neonate rats occurred over the 24 h period at this dose level since mean concentrations of total radioactivity were below the limit of reliable measurement at each time point in pup plasma. Overall, CD5789 underwent the same distribution and excretion in milk than the total radioactivity.

Biliary excretion

49. RDS.03.SRE.107174 - Determination of in vitro biliary clearance of CD5789 using human and rat sandwich cultured hepatocytes (B-Clear®).

OBJECTIVE:

The aim of this study was to investigate the in vitro clearance of CD5789 in human and rat sandwich cultured hepatocytes, expressing endogenous uptake transporters and having formed bile pockets with functional efflux transporters.

MATERIAL AND METHODS:

The biliary clearance and biliary excretion index of CD5789 was investigated in human and rat sandwich cultured hepatocytes.

For human sandwich cultured hepatocytes assays, 3 donors were selected. On donor one, CD5789 was measured at a single concentration (1 µM) and 3 incubation times (5, 10 and 15 min). On donor two and three, CD5789 was applied at two concentrations (1 and 10 µM) and at one incubation time (10 min).

For rat sandwich cultured hepatocytes assays, three different assays were performed: one at a single concentration (1 µM) and at three incubation times (5, 10 and 15 min), one also at a single concentration (0.1 µM) and at three incubation times (1, 5 and 15 min) and one at a single concentration (1 µM) and at three incubation times (5, 10 and

	15 min), however in this experiment CD5789 was incubated together with 1% BSA in the buffer. CONCLUSION: This study shows that CD5789 accumulates in sandwich cultured human and rat hepatocytes, as evidenced by high intracellular concentrations vs. medium concentrations. CD5789 undergoes biliary clearance in both sandwich cultured human and rat hepatocytes, yet variation observed between donors (in human) as well as low BEI values in rat hepatocytes make it hard to estimate the extent. Based upon the data it is expected that CD5789 may be subject of biliary clearance in vivo. **50. RDS.03.SRE.102754 - The Disposition of [14C]-CD5789 Following Oral and Intravenous Administration in the Bile Duct-Cannulated Rat.** OBJECTIVE: This study was designed to examine the excretion and biliary kinetics of [14C]-CD5789 total radioactivity in male and female Wistar rats and to identify metabolites of [14C]-CD5789 present in bile. MATERIAL AND METHODS: *Detailed in section 3. Pharmacokinetics 4) Metabolism.* CONCLUSION: Following IV administration of [14C]-CD5789, the main excretion route in both male and female rats was via the bile (82% and 74% recovered by 48 h, respectively). Fecal and urinary elimination was minor. Following oral administration, at least 31% of [14C]-CD5789 was absorbed in male rats and 38% in female rats. The main route of excretion via the feces (65% and 59%, over a 48 h period, in males and females respectively). Biliary elimination accounted for a mean of 31% in males and 38% in females and urinary excretion was minor.
6) pharmacokinetic interactions (non-clinical)	The drug-drug interaction (DDI) potential of CD5789 was not studied in animal studies.
7) other pharmacokinetic studies	Not Applicable

4. Toxicology:

Trifarotene has been evaluated in a comprehensive set of nonclinical toxicity studies with the active substance administered by the oral route in rats, rabbits and dogs to maximize systemic exposure. Trifarotene dermal cream was administered by the dermal route (which is the clinically relevant route of administration) in mice and minipigs. It is apparent from the pharmacokinetics section that no human-specific metabolite was detected. All phase 1 metabolites detected in human hepatocytes were also detected in hepatocytes of at least one of the toxicological species used for chronic toxicology studies (i.e. rat, dog or minipig).

Five species (mice, rat, dog, rabbit and mini-pig) have been used in the toxicity characterization, which may be seen as an advantage in that the effects seen can be compared across a number of species. However, the disadvantage of for instance not using the mini-pig for dermal and oral studies is the lack of intra-species comparison possibility. Overall, the lack of systemic exposure in the mini-pig dermal studies, despite (as in the 13-week study) marked dermal toxicities with erythema and scab that required wash-out period, is considered a drawback. This is not reflective of the clinical situation, where exposures are noted after dermal exposure without severe dermal findings.

Skin

The skin was a sensitive target of toxicity throughout all repeated-dose studies, which was expected considering the pharmacology of retinoids. At the application site in the dermal studies in mice and mini-pig, inflammation with hyperplasia, hyperkeratosis and parakeratosis were observed. In mini-pig, severe skin

irritation (erythema) was noted in addition to acanthosis (with spongiosis), inflammatory infiltrates, parakeratosis and minimal or slight crusts. Overall, the local reactions were more marked during the first month of treatment and partly resolved over time. This course of reactions is similar to what is seen in the clinic, where the local reactions usually diminishes over time.

In the oral 13-week study in rats, hyperkeratosis and scabs was noted. In the dog, abdominal skin findings were noted, which consisted of acanthosis, dermal mononuclear cells and lymphocytic exocytosis. Acanthosis was observed in males treated at 0.18 mg/kg/day and in females at 0.02 and 0.18 mg/kg/day. Skin findings were also evident on the head and they consisted of acanthosis, hyperkeratosis, lymphocytic exocytosis and dermal mononuclear cell infiltration. Acanthosis was not observed at 0.02 mg/kg/day. Hyperkeratosis and dermal mononuclear cell infiltration were noted at 0.18 mg/kg/day only. In the ears, acanthosis, hyperkeratosis, lymphocytic exocytosis, spongiosis, dermal mononuclear cell infiltration, and ulceration was seen. All skin findings in the dog were reversible.

The dermal toxicity studies were performed in animals with intact skin. The Applicant agreed that systemic and local absorption will differ on abraded/broken skin (compared to intact skin)and further declared that impact of disease severity was accounted for in the evaluation of the safety of trifarotene. The exposure margins in MUsT study 18237 were based on the systemic exposure of the most exposed patient with moderate to severe acne receiving Trifarotene 50 µg/g cream under maximal use conditions.

Non-glandular stomach

Hyperkeratosis of the mucosa in the non-glandular stomach was evident in the 13-week dermal study in mice, as well as in all the oral studies in rat from 4-week studies an up to 26-weeks. Humans do not have a forestomach, but possess histologically similar organs, including the oral cavity, pharynx, esophagus, and glandular stomach, but the tissue dose in these human organs are not equivalent to that in the forestomach of experimental animals. Thus, overall the human relevance of these findings is unclear.

Spleen

The spleen is a target organ of toxicity in several studies. Thus, an effect in the spleen was noted across studies with increased weight and extramedullary hematopoiesis. The findings were mostly mild, except in the 13-week mouse study where minimal-severe effects were noted. However, overall the findings showed recovery and are attributable to the RAR agonist pharmacological effects of the compound.

Bone

In the rat, main treatment-related finding in the stifle-joints of treated groups consisted of a femoral and/or tibial growth plate disorganization/closure. This was graded minimal to slight and was mainly characterized by tinctorial changes with irregular arrangements/thickness of the dividing cell layers. This finding was shown with increased severity/incidence in females at 0.5g/kg/day (moderate to severe), including a total/partial closure of femoral and/or tibial growth plates. The finding was not reversible in HD females (marked-severe) but signs of recovery were noted in the males. Various changes in bone including growth plate disorganization and increased ossification of epiphyseal cartilage was also noted in the shorter studies in the rat.

Testes

In the testes of treated dogs in the 39-week study, changes consisted of a slight increase in the number of degenerate germ cells (bilateral), which only equivocally involved the low dose group (0.02 mg/kg/day). In the epididymides, there was cell debris as a result of the testicular change. Following the 8-week treatment-free period, these findings were not completely reversible as 1 of 2 recovery-males showed microscopic findings in the testes and epididymides. Therefore, no male NOAEL was set in the dog study. The issue of not setting a NOAEL for both sexes was a matter of discussion. In this case, we have no NOAEL for males as the germ cell degeneration in males was identified also at the lowest dose tested (corresponding to a systemic exposure 1170 times higher than exposures observed in clinical trials). However, it was concluded that the most appropriate approach is to accept that only a LOAEL can be given for the effect and highlight this fact in the SmPC labelling.

Genotoxicity

A test-battery of genotoxicity studies has been performed. As some chemicals may become potent mutagens and clastogens when photosensitized, the mutagenic and clastigenic potential of trifarotene was also evaluated in the absence and presence of UV light.

In the reverse mutation assays, reductions in revertants were noted. This is often regarded as a sign of toxicity, and in study RDS.03.SRE.12526 this effect was only noted above the precipitation limit and without S9 supporting this explanation. In study RDS.03.SRE.12525, a reduction in revertants was only noted in the presence of UV-exposure. This reduction may (also) be due to a shadowing effect of precipitates, reducing the UV-induced increase in revertants expected. Collectively it is agreed the studies do not support a mutagenic effect of trifarotene in the absence or presence of UV-light.

One in vitro (lymphocytes) and one in vivo (bone marrow) micronucleus assay has been performed. The data provided supports a weak but positive signal of clastogenicity in vitro. The in vivo study, which may be considered more important, evidenced an increased frequency of polychromatic erythrocytes in males exposed to 7.5 mg/kg, compared to the control group. However, while significant, the signal is considered weak (in absolute terms) and not considered sufficient to label trifarotene a clastogen.

A test of chromosome aberrations in cultured Chinese hamster ovary (CHO) cells in the absence and presence of ultra violet light structural chromosome aberrations that were similar to those observed in concurrent vehicle controls. A small increase in endoreduplication was observed following exposure in the presence of UV-light. The significance of this finding is unknown.

Carcinogenicity

To evaluate the carcinogenicity potential of trifarotene, two carcinogenicity studies over 104- weeks have been performed in Wistar rats (oral) and CD-1 mice (dermal). In the dermal study in mice, 0.05mg/kg/day and 0.1 mg/kg/day were not tolerated by the animals and these groups were removed from the study and replaced by a 0.01mg/kg/day group from week 14. There was no increased incidence of neoplastic findings in the treated animals. However, scab, ulceration and hyperplasia/hyperkeratosis were noted dose-dependently, which were also correlated with enlargement of lymph nodes and lymphoid hyperplasia in axillary and mandibular lymph nodes as well as the spleen. In the oral study, treatment resulted in mild but dose-dependent reductions in body-weight and food consumption. No neoplastic changes were noted that are considered treatment related. However, gross and histopathologic changes were evident in the stomach, skin and liver. In addition, the growth plate of the femur displayed an irregular thickness in more than half of male and female rats given HD. Thus, overall there is no data to suggest that trifarotene is associated with a carcinogenic potential. The nonneoplastic findings noted after 2 –year exposures are similar to the findings evidenced in the repeated-dose toxicity studies and in accordance with what is expected from a RAR-agonist.

According to the Applicant, through its effect on skin, treatment may have stimulated the lymphoid compartment generally, as the mandibular and inguinal lymph nodes as well as the spleen also showed treatment-related lymphoid hyperplasia. Therefore, the effects noted are considered procedure related rather than a sign of toxicity. It is also the Applicant's view that increased cellularity of the sternal bone marrow was also an indirect procedure-associated finding.

While it may be agreed that the effect on skin resulted in the stimulation of the lymphoid compartment, it is not agreed that the effects are procedure related. If the effects were procedure related, then the same findings with a similar severity would have been seen in the control group. Rather, the effects are an indirect effect of the toxicities seen dermally, and they are induced by trifarotene. In any case the clinical relevance of these findings is limited, as the underlying dermal irritation would be monitorable and thus treatment suspended before progression to clinically relevant lymphoid effects.

Reproductive and developmental toxicity

A full package of required reproductive toxicology studies has been performed, including a preliminary juvenile toxicity study. Rat and rabbit were the species used for the studies. In the fertility study, there was a slight reduction in body-weight at the highest dose in males, but there were no effects on mean sperm count, the mean percentage of motile sperm or the sperm motility parameters. Post-implantation loss was significantly higher in the low-dose group. However, a single female had no viable embryos which influenced the mean value, and since no effects were noted at higher exposures, the finding is not considered related to treatment. Thus collectively, trifarotene did not induce effects on mating performance or fertility in exposed males or females.

In the EFD-study, trifarotene exposure in the pregnant SD rat was associated with dosedependent maternal toxicity from 0.3mg/kg/day manifested as reductions in bodyweight and food consumption. Post-implantation loss (both early and late resorptions) was clear in dams of the 1mg/kg/day dose-group.

However, on closer inspection a difference in percentage postimplantation loss was evident for all dose groups compared to control. According to the Applicant, this is incidentally so and is based on low numbers of resorptions in the concurrent control group compared with historical control data.
Trifarotene is clearly teratogenic. At the highest dose, all fetuses displayed a syndrome of multiple malformations expected from a RAR-agonist and the majority of fetuses exposed at 0.3mg/kg/day were also grossly malformed. At the 0.03 dose-level, one fetus had umbilical hernia and one fetus had cleft palates, findings which were both also noted at the 0.3 and 1mg/kg/day dose groups. No cleft palates were seen in the 0.1mg/kg/day dose group, why the Applicant considers the finding in the 0.03mg/kg/day dose group an incidental and isolated finding. However, while no cleft palates were seen at 0.1mg/kg/day, incomplete ossification of the palate was noted, suggesting effects on bone formation. Cleft palate is one of the most common birth defects in humans, and RA plays important roles during palate development. It is also established that excess RA increases the incidence of cleft palate in rodents as well as humans, why it is curious with findings in low-dose trifarotene exposed fetuses but no findings in controls. However, given the single occurrence in the 0.03mg/kg/day group and the lack of findings at the 0.1mg/kg/day-level, it is not an obvious treatment-related effect.

Severe gross disruptions of the skeleton were noted in the two higher groups. This was expected based on the apparent external malformations. At the 0.1mg/kg/day-level misshapen tibias and fibulas were noted in addition to retarded ossification (and on some places increased ossification) of various bones. Findings at the 0.03 levels were mostly related to effects on ossification. However, there was an increased incidence of unilateral or bilateral rudimentary 14th ribs and incomplete ossification of the 6th sternebra. These findings are considered variations of unclear toxicological significance.

Trifarotene was not tolerated at 50mg/kg/day (the highest dose) in the New Zealand white rabbit. Almost all animals died or were prematurely sacrificed. Treatment was terminated on SD 14-15 in this group, but most animals did not recover. Only one dam in this group had viable fetuses (2♀) which all were severely malformed.

As for HD, treatment with 5mg/kg/day was associated with malformations, mainly acaudia and bent tail. One fetus was also severely malformed with amelia and hemimelia of hindlimbs and gastroschisis. In the low-dose group, one severely malformed (similar findings as the fetus at 5mg/kg/day). In addition, the incidence of bent tail was above both treatment control values and historical control data, suggesting clear treatment causality. Fetal visceral malformations of the urinary system were evident in fetuses to dams exposed to 5 or 50 mg/kg/day and one fetus at 0.5 had a malformed kidney. The skeletal malformations were mainly located to the caudal region of the vertebrae. This correlates well with the tail-related findings in all treated groups.

Thus collectively, the EFD-studies show that trifarotene, as other retinoid receptor agonists, is a teratogen at sufficiently high doses. It is interesting to note the very wide spectrum of malformative effects induced, and the wide dose-spectrum in which they can be produced. It can also be concluded that the selectivity for the RAR-γ over RAR-α and RAR-β, has no apparent influence on the malformative effects of the substance. The labelling in section 4.6 for pregnancy should be the same as for other dermal retinoids.

In the pre-and postnatal development study, there were no treatment related deaths. Pre-birth loss (F1) was increased in the 0.03 and 0.1mg/kg/day dose-groups (11% and 13.4% compared to 6.2% for controls). According to the Applicant, this data was within historical control data. However, the collective trend across the EFD and PPND studies is that post-implantation loss is seen at trifarotene treatment with doses not associated with maternal toxicity. Even so, due the lack of clear data supporting this trend, this issue was considered resolved.

A 4-wek DRF study in juvenile animals was submitted by the Applicant, but no definite study. However, as no definite juvenile toxicity study has been submitted, this study has not been further considered. In addition, according to the PIP provided, the PDCO has agreed to a waiver that applies to the paediatric population from birth to less than 9 years. Further, the agreed PIP does not include any non-clinical studies.

Local tolerance studies
Dermal and ocular irritation studies were performed. The dermal local tolerance studies were not assessed. They were not performed with the clinical formulation, and the dermal irritation potential of the substance has become apparent in the repeated-dose studies in mice and minipigs.

Ocular tolerance studies are considered important, since the application of the product will include the periorbital area of the face. Trifarotene 100 µg/g gel was irritating when administered by the ocular route to rabbits. Trifarotene cream HE1 at up to 400 µg/g was minimally irritating when administered by the ocular route to rabbits. While the relation of the used gel/cream to the clinical formulation is unclear, it can be concluded that trifarotene is an eye-irritant, why appropriate warnings should be included in section4.3 of the SmPC.

Sensitization

Skin sensitization has been evaluated in two studies with trifarotene. Trifarotene cream was studied using the Buehler test and the gel was used in the local lymph-node assay. No sensitization potential was noted in the Buehler assay.

In a Photoirritation and photosensitization study of trifarotene in guinea pig, study data show that a photosensitizing potential of trifarotene was evident, with reactions in 80% of treated animals versus 40% for placebo controls. According to the Applicant, the irritation seen in the induction period may be responsible for the effect noted. While the acute photoirritation observed in these studies is considered relevant to humans, the predictivity of these studies for human photoallergy is unknown. Thus, for regulatory purposes, such nonclinical photoallergy testing is generally not recommended. It is, however, expected that the issue of photosafety assessment is further addressed clinically.

1) single-dose toxicity	**51. RDS.03.SRE.8562 - Extended acute study (dermal route) in the Sprague Dawley rat.** OBJECTIVE: The aim of this study was to assess the toxicity potential of CD5789 in a solution when administered as a single topical (dermal) application. MATERIAL AND METHODS: Groups of Sprague-Dawley rats (12 animals/sex/group) were administered CD5789 in a solution (mixture of propylene glycol - ethanol [20 - 80% w/w]) at 30, 100 or 300 µg/g at a dosage volume of 2 mL/kg/day (corresponding to 0.06, 0.2 and 0.6 mg/kg/day). Control animals were administered the vehicle alone under the same experimental conditions. Application sites were not protected. Animals were observed for mortality/morbidity and clinical signs for a minimum period of 14 days. Body weights and food consumptions were recorded weekly. Blood samples were collected on the day of treatment for toxicokinetic analysis. In addition, blood samples were also collected either from 4 animals/sex/group before an interim sacrifice on the day after treatment or from the remaining 8 animals/sex/group at the end of the observation period, before the final sacrifice for clinical pathology investigations. For each sacrifice period, all animals were subjected to a gross macroscopic examination, selected organs weighed and a range of tissues fixed for microscopic examination. CONCLUSION: CD5789 administered as a single dermal application at dose-levels of 30, 100 or 300 µg/g did not induce any sign of systemic toxicity but signs of slight and reversible skin irritation were noted, with a dose-response relationship. Based on the nature, mild severity and low incidence of these cutaneous signs, the dose level of 30 µg/g (0.06 mg/kg) was considered to be the NOAEL, corresponding to an AUC0-24h of 7.2 and 34.9 ng.h/mL in male and female rats, respectively. **52. RDS.03.SRE.8563 - CD5789 Extended acute study (intra venous route) in the Sprague Dawley rat.** OBJECTIVE: The aim of this study was to assess the toxicity potential of CD5789 when administered as a single intravenous (slow) bolus. MATERIAL AND METHODS:

	Groups of Sprague-Dawley rats (12 animals/sex/group) received CD5789 at 1, 2.5 or 5.5 mg/kg and at a dosing volume of 1.6 mL/kg. Control animals were administered the vehicle alone (mixture of Polyethylene Glycol 400, ethanol and NaCl 0.9% [70 - 10 - 20%] w/w/w). Animals were observed for mortality/morbidity and clinical signs for a minimum period of 14 days. Body weights and food consumptions were recorded weekly. Blood samples were collected on the day of treatment for toxicokinetic analysis. For clinical pathology investigations, blood samples were collected either from 4 animals/sex/group before an interim sacrifice on the day following treatment or from the remaining 8 animals/sex/group at the end of the observation period before the final sacrifice. For each sacrifice period, all animals were subjected to a gross macroscopic examination, selected organs weighed and a range of tissues fixed for microscopic examination. CONCLUSION: No signs of toxicity were observed when CD5789 was administered as a single intravenous dose at 1 mg/kg. Higher dose-levels of 2.5 and 5.5 mg/kg induced signs of toxicity consisting of decreased body weights and food consumption, and histopathological changes in bones and kidneys. The dose of 1 mg/kg was considered to be the NOAEL in this study for both males and females, corresponding to an AUC0-last of 1447 and 4656 ng.h/mL in males and females, respectively.
2) multiple-dose toxicity	**Repeated dose toxicity by the dermal route** *Studies with the CD5789 cream formulation in mice* **53. RDS.03.SRE.12742 - CD5789 Preliminary 4-week dermal toxicity study in the mouse.** OBJECTIVE: The objectives of the study were to assess the local tolerance and potential systemic toxicity of daily dermal administration of CD5789 cream to CD1 mice for at least four weeks. MATERIAL AND METHODS: CD5789 formulated in cream at 10 µg/g, 50 µg/g or 100 µg/g was applied daily by dermal administration to the CD1 mouse for at least four consecutive weeks according to the design presented in Table 3. **Table 3 Design of the 4 week dermal toxicity study (RDS.03.SRE.12742)**

Group/Treatment	Dose level (mg/kg/day)	Dose volume (mL/kg/day)	Dose concentration	Number of animals	
			(%w/w)	Males	Females
1. Control (water)	0	2	0	12 (6)	12 (6)
2 Placebo	0	2	0	12 (6)	12 (6)
3. CD5789 cream 10 µg/g	0.02	2	0.001	12 (18)	12 (18)
4. CD5789 cream 50 µg/g	0.1	2	0.005	12 (18)	12 (18)
5. CD5789 cream 100 µg/g	0.2	2	0.01	12 (18)	12 (18)

Satellite animals used for proof of exposure evaluation are indicated in brackets. These animals were discarded without necropsy after blood sampling. Group 1 animals (control) received water for injection. The test material was applied uniformly to clipped, unprotected area on the back (from the scapular to the lumbar region) representing at least 10 % of the total body surface area. Once a week, the application site was washed with lukewarm water and dried with absorbent paper, approximately 6 hours after the daily application.

Morbidity/mortality checks were performed at least twice daily. Clinical observations, including local tolerance were performed daily. Ophthalmological examinations were performed pretest and on day 26. Individual body weights were recorded twice pretest then weekly during the treatment period. Food consumption was measured weekly for

each animal. Clinical laboratory determinations were performed on Days 28/29. Satellite animals were sampled for proof of exposure at various time-points (1, 4, 8 hours after treatment) after dosing on Day 0 and on Day 28. One animal that died during the study was necropsied; this death was unrelated to treatment. All surviving animals were sacrificed at the end of the treatment period and necropsied. Selected organs were weighed. Organ/tissue samples were fixed and preserved at necropsy for all animals. Selected organs/tissues from group 1 and 5 animals sacrificed at the end of the treatment period, from one animal found dead and the skin from all animals from all groups were examined histopathologically at first instance. Then, histopathological examinations of the low and intermediate dose levels and placebo were also performed for bone (femur) and articulation and bone (sternum) with bone marrow of female animals, spleen and stomach of male and female animals as these organs/tissues were noted with findings at the high dose (group 5).

CONCLUSION:

The daily dermal administration of the test item CD5789 cream at 10, 50 and 100 µg/g (corresponding to 0.02, 0.1 or 0.2 mg/kg/day) for at least 28 days in CD1 mice induced slight local reactions in both sexes at all dose levels, associated with dose-related elevated white blood cells count and hyperplastic and inflammatory changes seen histopathologically in the treated skin. Males were more affected than females. As the severity of local reactions decreased with time and the degree of magnitude was low, these findings were considered not to be adverse. The other histopathology findings noted in the stomach and spleen were of low severity grade at the two lowest dose levels of 0.02 or 0.1 mg/kg/day. Only stromal proliferation in the bone marrow (moderate in the sternum and minimal or slight in the femur) at the highest dose level of 0.2 mg/kg/day is considered as an adverse change.

54. RDS.03.SRE.12754 - CD5789 Cream B 13-week dermal toxicity study in the CD1 mouse.

OBJECTIVE:

The objectives of the study were to assess the local toxicity and potential systemic toxicity of CD5789 cream administered daily by dermal application to CD1 mice for thirteen weeks.

MATERIAL AND METHODS:

CD5789 cream at 10, 50 and 100 µg/g, was administered daily by dermal application to CD1 mice for thirteen consecutive weeks according to the design presented in Table 4.

Table 4 Design of the 13-week dermal toxicity study in the CD1 mouse (RDS.03.SRE.12754)

Group/Treatment	Dose level (mg/kg/day)	Dose volume (mL/kg/day)	Dose concentration	Number of animals	
			(%w/w)	Males	Females
1. Control (water)	0	2	0	12 (6 + 6)	12 (6 + 6)
2. CD5789 Cream Placebo	0	2	0	12 (6 + 6)	12 (6 + 6)
3. CD5789 cream 10 µg/g	0.02	2	0.001	12 (18 + 21)	12 (18 + 21)
4. CD5789 cream 50 µg/g	0.1	2	0.005	12 (18 + 21)	12 (18 + 21)
5. CD5789 cream 100 µg/g	0.2	2	0.01	12 (18 + 21)	12 (18 + 21)

Satellite animals for toxicokinetics are indicated in brackets (day 0 and day 90, respectively).

Group 1 animals (control) received the control item (water for injection). CD5789 cream, placebo or water were topically applied each day on clipped area on the back (from scapular to lumbar region) representing at least 10 % of the total body surface. Application sites were not protected. Morbidity/mortality checks were performed at least twice daily. Clinical observations were performed daily. A full clinical examination was performed weekly. Ophthalmological examinations were performed on pretest and during Week 13. Individual body weights and food consumption were