

Fig. S1. Patient disposition. Discontinuations are from Baseline to Week 8. The first visit of the first patient was on 28/12/2011, and the last follow-up visit occurred on 17/9/2012.

*Supplementary material to article by D. Roblin et al. "Topical TrkA Kinase Inhibitor CT327 is an Effective, Novel Therapy for the Treatment of Pruritus due to Psoriasis: Results from In vitro and Tissue Experimental Studies, and Efficacy and Safety of CT327 in a Phase 2b, Randomised, Double-blind, Vehicle-controlled Clinical Trial in Patients with Psoriasis"*

## Appendix S1

### MATERIALS AND METHODS

#### Experimental studies – in vitro and tissue studies

For the *in vitro* and human tissue studies, avulsed human cervical Dorsal Root Ganglia (DRG) were removed as a necessary part of the surgical brachial plexus repair procedure from 3 patients with informed consent and approval of the Research Ethics Committee (NRES Reference, 11/LO/1011, and Material Transfer Agreement). Post-mortem human DRG were obtained from the Netherlands Brain Bank. Bilateral DRG from 11 adult rats were micro-dissected and neuronal cultures prepared as previously described (18).

#### In vitro studies and immunostaining

Dose-related effects of CT327 on capsaicin responses were quantified in cultured rat and human DRG neurons using calcium imaging, as described in our previous publications (11, 18, 19). For calcium imaging, neurons were plated with 100 ng/ml NGF for 48 h, in a model of sensitization, and dose related effects of CT327 were determined acutely on capsaicin responses in cultured rat and human DRG neurons. Morphological effects were determined by treating neuronal cultures with CT327 when neurites were well established, for 24 h. Neurons were treated with 1 nM, 10 nM, 100 nM, 1 mM and 10 mM doses of CT327. Cultures were fixed with 4% paraformaldehyde and immunostained with primary mouse monoclonal antibody to Gap43 (1: 200, Sigma, UK), visualised with secondary antibody Alexa 488 (1: 200, Molecular Probes). The glass bottom coverslips were detached and mounted on glass slides, and TIFF images were acquired with a Zeiss inverted microscope equipped with standard FITC optics, for image analysis and neurite measurement using Metamorph software. Neurons with neurites greater than twice the cell body diameter were identified, and only those neurons which were clearly identifiable were used for analysis. The longest neurite length was measured for each neuron and the mean length calculated for each group using Excel software. 30–50 neurons were analyzed for each concentration in each of 3 experiments, and mean neurite length for each group was expressed as mean  $\pm$  SEM. Student's *t*-test was used to compare between groups and a value of  $p < 0.05$  was considered statistically significant.

#### Human tissue immunohistochemistry

Tissue specimens were immersed in Zamboni's fixative (2% w/v formalin, 0.1 M phosphate and 15% v/v saturated picric acid) for 2 h and stored in a solution containing 15% sucrose, 0.1% azide in PBS (pre-fixed). Tissue sections (15 mm thick) were collected onto poly-L-lysine (Sigma, Poole, UK) coated glass slides. Endogenous peroxidase was blocked by incubation in methanol containing 0.3% w/v hydrogen peroxide for 30 min. After rehydration with PBS buffer, sections were incubated overnight with primary antibody (3 anti-TrkA antibodies (Santa Cruz 379-sc118, Kaplan 32, Abcam 520-ab8871)) to determine TrkA expression. Sites of primary antibody attachment were revealed using nickel-enhanced avidin-biotin peroxidase (ABC Vector Laboratories, Peterborough UK). Sections were counterstained for nuclei in 0.1% w/v aqueous neutral red, dehydrated and mounted in xylene-based mountant (DPX, BDH/Merck, Poole, UK), prior to photomicrography.

#### Phase 2b clinical trial

This study (EudraCT Number: 2011-004640-21; clinicaltrials.gov Number: NCT01465282) was sponsored by Creabilis SA

and conducted at 8 centres in the USA and 5 centres in the UK, in compliance with the aethical principles that have their origin in the Declaration of Helsinki (2008 version) on biomedical research involving human volunteers, the International Conference on Harmonisation (ICH) Guidelines for Good Clinical Practice (GCP) (CPMP/ICH/135/95). This study conforms to the CONSORT guidelines.

#### Patients

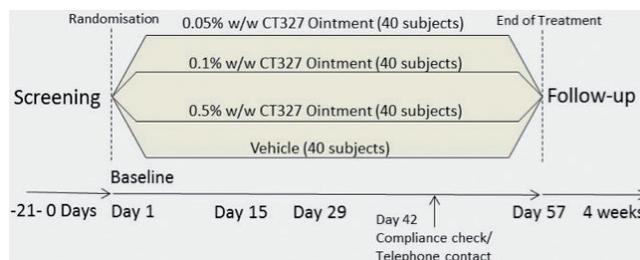
Patients aged 18 years and older with stable, mild-moderate psoriasis affecting up to 10% body surface area (excluding face and scalp) were included in the trial. Subjects were excluded if they had a current diagnosis of guttate, erythrodermic, exfoliative or pustular psoriasis, had used topical anti-psoriatic treatments (including corticosteroids, retinoids and vitamin D derivatives) in the two weeks prior to study start, received monoclonal antibody therapy in the 4 months prior to the study, received systemic anti-psoriatic treatment, PUVA therapy within 4 weeks or UVB therapy within 2 weeks of study start, or were taking or scheduled to start non-antipsoriatic concomitant medication that could affect psoriasis (e.g. immunosuppressants, beta blockers, lithium) during the study. Subjects who were pregnant, had clinically significant abnormal clinical laboratory test results at screening, or had received any investigational drug or taken part in any clinical study within 3 months prior to the study start were also excluded.

#### Study design

This was an international, multi-centre, randomised, double-blind, vehicle-controlled Phase 2b trial (App S1: Fig. 1). 160 subjects were enrolled across 4 treatment groups, each containing 40 subjects. Subjects received twice daily applications of CT327 ointment or vehicle for up to 8 weeks.

The treatment groups were 0.05% (w/w) CT327 ointment, 0.1% (w/w) CT327 ointment, 0.5% (w/w) CT327 ointment, and vehicle only (no active ingredient; petrolatum-based). Subjects treated all psoriatic plaques with CT327 or a blinded emollient vehicle ointment twice daily at approximately the same time each day, after washing.

Psoriasis severity was assessed using the validated endpoints of IGA, and mPASI; modified as psoriasis of the face and scalp was not treated. Pruritus severity was assessed using a 100 mm VAS, with 100 mm corresponding to the 'worst possible pruritus', and 0 mm corresponding to 'no pruritus'. All assessments of the subjects' psoriasis were made at least 4 h after application of study medication to ensure emollient did not mask any of the signs or symptoms of psoriasis. Subjects who did not



App S1: Fig. 1. Phase 2b study design. 160 patients were treated with 0.05% w/w, 0.1% w/w, or 0.5% w/w CT327 ointment, or vehicle ointment, across 4 treatment groups that each contained 40 subjects. Patients received twice daily applications of CT327 ointment for up to 8 weeks. Subjects who did not resume treatment for their psoriasis attended a follow-up visit 28 days after the last study treatment.

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Vital signs (blood pressure, pulse) were taken on each of the study visits (4–6 h after application of study medication). Blood samples were taken pre-dose and 4 h post-dose on the first study visit, and on the Week 2 and Week 8 study visit.

#### Randomisation and marking

Subjects were randomised into the study provided they satisfied all of the subject selection criteria. An Interactive Web Response System (IWRS) used a computer-generated randomisation schedule to assign subjects to a treatment sequence. The randomisation was implemented centrally without stratification. HMD Clinical generated the random allocation sequence and the study software assigned participants to treatment interventions in a blinded fashion. Participants were enrolled by the study sites.

#### Study assessments

The endpoints were defined as:

- Controlled disease (Primary Endpoint) – Binary response defined as ‘none’ or ‘minimal’ disease on the IGA and a minimum improvement of 2 categories from baseline at the assessment time-point, or ‘none’ on the IGA resulting in discontinuation of study medication at either the assessment or any previous time-point. The IGA was measured by the investigator at each visit, using a 6-point scale from 0–5, where 0=none, and 5=very severe (see App. S1: Table I).
- Pruritus VAS (Secondary Endpoint) – change from baseline in pruritus VAS score, in subjects with at least moderate pruritus at baseline (VAS  $\geq 40$  mm) (20).
- mPASI (Secondary Endpoint) – change from baseline in the mPASI score. The mPASI score is a weighted sum of

erythema, scaling and plaque elevation scores over different regions of the body. It was determined, at each visit, using the algorithm:

$$\bullet \text{ mPASI} = [0.2 \times (E_{\text{arms}} + T_{\text{arms}} + S_{\text{arms}}) \times \text{Extent}_{\text{arms}}] + [0.3 \times (E_{\text{trunk}} + T_{\text{trunk}} + S_{\text{trunk}}) \times \text{Extent}_{\text{trunk}}] + [0.4 \times (E_{\text{legs}} + T_{\text{legs}} + S_{\text{legs}}) \times \text{Extent}_{\text{legs}}]$$

- Each symptom was scored on a 5-point scale from 0–4, where 0=no symptoms and 4=very marked. The PASI score was modified, as psoriasis of the face and scalp was not treated.

Adverse events were recorded, and blood pressure, pulse, electrocardiogram (ECG), laboratory variables and physical examination were monitored. Blood samples were taken to determine the systemic exposure of CT327.

#### Sample size and statistical analyses

Sample size determinations were made using the binary endpoint of controlled disease. Based on simulation studies, assuming a 7.5% vehicle response rate, a sample size of 40 evaluable patients per treatment group had at least 90% power to detect a dose-response relationship, where active response rates varied between 7.5% and 50%, and at least 80% power to show the CT327 0.5% dose group was different from vehicle.

For the controlled disease response rate and other binary endpoints, differences between active doses of CT327 and vehicle were investigated using Fishers Exact test and a fixed-sequence step-down testing procedure to control for multiplicity, where each test was conducted at the 5% significance level. Dose-response was tested for the controlled disease endpoint using the Cochran-Armitage trend test. Continuous endpoints were analysed using analysis of covariance with baseline as a covariate. *Post-hoc* analyses were also performed on the full analysis set (all randomised subjects who received at least one administration of study medication) and the subgroup of full analysis set with baseline pruritus VAS scores  $\geq 40$  mm.

App. S1: Table I. Investigator's Global Assessment grade definitions

Score	Grade	Definition
0	None	No plaque elevation above normal skin level; may have residual non-erythematous discoloration; no psoriatic scale
1	Minimal	Essentially flat with possible trace elevation; faint erythema; no psoriatic scale
2	Mild	Slight but definite elevation of plaque above normal skin level; may have up to moderate erythema (red coloration); fine scales with some lesions partially covered
3	Moderate	Moderate elevation with rounded or sloped edges to plaque; moderate erythema (red coloration); somewhat coarse scales with most lesions partially covered
4	Severe	Marked elevation with hard, sharp edges to plaques; severe erythema (very red coloration); coarse thick scales with virtually all lesions covered and a rough surface
5	Very severe	Very marked elevation with very hard, sharp edges to plaque; very severe erythema (extreme red coloration); very coarse thick scales with all lesions covered and a very rough surface

Table SI. Study demographics

	CT327 0.05%	CT327 0.1%	CT327 0.5%	Vehicle
Total study population (full analysis set)	<i>n</i> =40	<i>n</i> =40	<i>n</i> =40	<i>n</i> =40
Age, years, mean ± SD	48.7 ± 14.4	48.3 ± 13.5	49.4 ± 15.6	50.7 ± 14.4
Male, <i>n</i> (%)	25 (63)	26 (65)	25 (63)	29 (73)
Female, <i>n</i> (%)	15 (38)	14 (35)	15 (38)	11 (28)
Time since diagnosis of psoriasis, years, mean ± SD	19.1 ± 12.2	16.8 ± 13.0	17.0 ± 13.1	20.0 ± 14.0
Baseline modified Psoriasis Area and Severity Index score, mean ± SD	8.7 ± 2.7	9.0 ± 2.6	9.3 ± 2.6	8.5 ± 2.4
Baseline pruritus Visual Analogue Scale score (mm), mean ± SD <sup>a</sup>	51.9 ± 24.6	50.6 ± 25.3	57.3 ± 22.5	43.6 ± 29.8
Subjects with baseline Visual Analogue Scale ≥ 40 mm (full analysis set)	<i>n</i> =29	<i>n</i> =26	<i>n</i> =31	<i>n</i> =22
Age, years, mean ± SD	48.3 ± 14.0	45.5 ± 12.8	47.5 ± 15.5	51.6 ± 14.2
Male, <i>n</i> (%)	16 (55)	17 (65)	23 (74)	16 (73)
Female, <i>n</i> (%)	13 (45)	9 (35)	8 (26)	6 (27)
Time since diagnosis of psoriasis, years, mean ± SD	17.8 ± 11.4	14.2 ± 10.3	14.8 ± 10.6	18.2 ± 13.2
Baseline modified Psoriasis Area and Severity Index score, mean ± SD	9.1 ± 2.8	9.6 ± 2.1	9.5 ± 2.6	8.9 ± 2.6
Baseline pruritus Visual Analogue Scale score (mm), mean ± SD	64.3 ± 14.2	64.8 ± 17.0	66.3 ± 14.6	65.5 ± 18.7

<sup>a</sup>*n*=39 for CT327 0.1%, CT327 0.5% and vehicle groups.

Table SII. Pruritus visual analogue scale (VAS) scores at Weeks 2, 4 and 8 for patient subgroup with pruritus VAS  $\geq 40$  mm at baseline

	CT327 0.05% n=29 Mean (min, max)	CT327 0.1% n=26 Mean (min, max)	CT327 0.5% n=31 Mean (min, max)	Vehicle n=22 Mean (min, max)
Baseline	64.3 (40, 91)	64.8 (40, 98)	66.3 (40, 100)	65.5 (40, 100)
Week 2	36.7 (0, 91)	38.3 (0, 82)	41.9 (1, 80)	42.5 (0, 100)
Week 4	33.6 (0, 100)	32.4 (0, 83)	37.9 (0, 100)	46.0 (1, 95)
Week 8	27.2 (0, 91)	33.3 (0, 80)	29.9 (0, 100)	49.4 (0, 100)
Change at Week 2	-27.7 (-66, 15)	-26.5(-71, 16)	-24.3 (-70, 26)	-23.0 (-70, 55)
Change at Week 4	-30.7 (-68, 50)	-32.5 (-90, 39)	-28.4 (-70, 20)	-19.5 (-70, 46)
Change at Week 8	-37.1** (-73, 20) <i>p</i> =0.0067	-31.5 (-91, 25) <i>p</i> =0.0523	-36.4* (-95, 20) <i>p</i> =0.0124	-16.1 (-88, 50)
Change at Week 2, %	-44.9 (-100, 26.0)	-41.3 (-100, 30.2)	-36.8 (-98.6, 50.0)	-33.1 (-100, 122.2)
Change at Week 4, %	-49.4 (-100.0, 100.0)	-48.0 (-100.0, 88.6)	-44.0 (-100, 28.8)	-27.0 (-98.2, 102.2)
Change at Week 8, %	-59.2 (-100.0, 33.3)	-43.3 (-100.0, 56.8)	-55.0 (-100.0, 26.8)	-21.2 (-100.0, 111.1)

\**p*<0.02; \*\**p*<0.01.

Table SIII. Summary of pruritus visual analogue scale scores (full analysis set)

	CT327 0.05% <i>n</i> =40 Mean (min, max)	CT327 0.1% <i>n</i> =40 Mean (min, max)	CT327 0.5% <i>n</i> =40 Mean (min, max)	Vehicle <i>n</i> =0 Mean (min, max)
Baseline <sup>a</sup>	51.9 (0, 91)	50.6 (0, 98)	57.3 (5, 100)	43.6 (0, 100)
Week 2	29.1 (0, 91)	34.3(0, 89)	38.3 (1, 100)	30.1 (0, 100)
Week 4	26.0 (0, 100)	27.2 (0, 83)	35.6 (0, 100)	30.2 (0, 95)
Week 8	20.9 (0, 91)	26.7 (0, 80)	28.4 (0, 100)	34.3 (0, 100)
Change at Week 2 <sup>a</sup>	-22.8 (-66, 15)	-17.7 (-71, 22)	-20.6 (-70, 26)	-14.6 (-70, 55)
Change at Week 4 <sup>a</sup>	-25.9 (-68, 50)	-24.5 (-90, 39)	-23.4 (-70, 40)	-14.1 (-70, 46)
Change at Week 8 <sup>a</sup>	-31.0** (-73, 20)	-24.9 (-91, 25)	-30.8* (-95, 20)	-10.5 (-88, 71)
	<i>p</i> =0.0040	<i>p</i> =0.0537	<i>p</i> =0.0194	
Change at Week 2, % <sup>a</sup>	-43.3 (-100, 42.9)	-29.5 (-100, 95.7)	-35.1 (-98.6, 50.0)	-28.3 (-100, 163.2)
Change at Week 4, % <sup>a</sup>	-50.4 (-100, 100)	-45.3 (-100, 88.6)	-37.6 (-100, 200)	-31.7 (-100, 110.5)
Change at Week 8, % <sup>a</sup>	-60.0 (-100, 33.3)	-47.4 (-100, 56.8)	-50.3 (-100, 80)	-20.4 (-100, 355)

<sup>a</sup>*n*=39 for CT327 0.1%, CT327 0.5% and vehicle groups.

\**p*<0.02; \*\**p*<0.005.

Table SIV. List of treatment emergent adverse events (full analysis set). Number (%) of subjects

	CT327 0.05% n=40	CT327 0.1% n=40	CT327 0.5% n=40	CT327 Overall n=120	Vehicle n=40
Total treatment emergent adverse events*	13 (33)	17 (43)	22 (55)	52 (53)	21 (53)
Nasopharyngitis	1 (3)	0	3 (8)	4 (3)	1 (3)
Headache	2 (5)	2 (5)	1 (3)	5 (4)	0
Diarrhoea	0	1 (3)	2 (5)	3 (3)	1 (3)
Psoriasis	0	0	2 (5)	2 (2)	1 (3)
Upper respiratory tract infection	0	0	0	0	2 (5)
Pruritus	4 (10)	2 (5)	3 (7.5)	9 (7.5)	6 (15)
Back pain	0	2 (5)	0	2 (2)	0
Fatigue	2 (5)	0	0	2 (2)	0
Cough	2 (5)	0	0	2 (2)	0

\*Counting is by subject, not event.