Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Afamelanotide for Erythropoietic Protoporphyria: Two Placebo-Controlled Trials

TABLE OF CONTENTS

1. TABLE OF CONTENTS 1
2. COLLABORATORS: 2
3. SUPPLEMENTARY METHODS: 3
   A. Quality of life questionnaires (EPP-QoL) 3
   B. Effect of afamelanotide and placebo on erythrocyte free protoporphyrin IX levels 3
   C. Photoprovocation studies 3
4. TABLE S1: EPP-QoL: Questions, answer options and scoring points. 5
5. TABLE S2: Effect of afamelanotide and placebo administration on erythrocyte free protoporphyrin IX levels over the study period in the EU trial.* 7
6. TABLE S3. Photoprovocation results in the US trial: Changes from baseline in minimum symptom dose* (MSD) in J/cm² on the hand and back. 8
7. REFERENCES 9

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SUPPLEMENTARY METHODS:

A. Quality of life questionnaires (EPP-QoL)

EPP has a major impact on the quality of life of the patients. In interviews described by Rufener (1) and Wahlin et al (2) patients report a lack of understanding by others about their suffering, and a feeling of solitude and psychosocial isolation. The photosensitivity severely limits their social and work activities. Most patients have learnt to avoid exposure to light by staying indoors, wearing protective hats, gloves and clothing, and covering windows at home and in automobiles with light-filtering films. Attempts have been made to use health related quality of life questionnaires to compare the impact of EPP with that of other skin diseases, and as an instrument to detect the effects of novel treatments. Three studies have been performed using the dermatology-specific QoL DLQI questionnaire (Dermatology Life Quality Index) in EPP cohorts (3,4,5). The DLQI comprises 10 questions, and was developed as an instrument to measure quality of life in dermatological conditions. In a review article, the DLQI was found to be the most frequently used instrument in studies of randomized controlled trials in dermatology (6). It is short, simple, and easy to administer and does not require any external assistance. However, recently there have been some concerns regarding its unidimensionality and the varying responses depending on age, gender, culture etc (7,8). A further issue is the underrepresentation of the emotional aspects of the dermatological patients' lives in the DLQI. (9). As in preliminary studies the DLQI was found not to be sensitive to change in EPP patients, a new, EPP disease-specific quality of life questionnaire (EPPQoL) was developed according to standard procedures (10) Following the validation step for the severity questions, this resulted in a 12 item questionnaire which reflect quality of life in two domains: general well being (questions 1 and 11), and severity of impact of disease on Interpersonal Relations, Occupational Activity, and Leisure and Recreational Activity (questions 2-10, 12). The questions and possible responses are given in supplementary table 1.

B. Effect of afamelanotide and placebo on erythrocyte free protoporphyrin IX levels

In the EU trial protocol, erythrocyte free protoporphyrin IX levels were measured at each visit, to determine whether afamelanotide might cause changes in protoporphyrin IX concentrations. As this was not the case, and as similar lack of change had been observed in the phase II study performed in the US, measurements of free protoporphyrin IX levels were not included in the protocol of the present US study.

C. Photoprovocation studies

Photoprovocation testing was performed in subgroups in both EU and US trial, using purpose built apparatus and exposing a small area of the skin on the dorsum of the hand and on the lower back to a calibrated broad-spectrum light source.

Photoprovocation testing using a standardized and calibrated broad spectrum light source to irradiate a small area of the dorsum of the hand or the lower back from a fixed distance. The exposure time to first prodromal symptoms such as tingling or burning in the exposed areas was registered, and if no

symptoms occurred, irradiation was stopped after the maximal intensity was achieved. The photoprovocation tests were performed in subsets of patients in Düsseldorf and Newport in the EU study and in New York in the US study. In the EU study the maximum light dose was 200 J/cm^2 and a high proportion of subjects were able to tolerate the maximum irradiation dose without experiencing any prodromal symptoms. As a result, the median for minimum symptom dose was found to be the maximum applied dose. This weakened the analysis and is likely to have resulted in smaller, non-significant differences between the treatment groups (results not shown.)

In the US study, the maximum irradiation dose was increased to 300 J/cm^2, in which a 300 watt xenon arc lamp and a filter system for wavelengths 400 to 650 nm (Newport Corporation/Oriel Instruments, Model 6285, Irvine, CA) was used. Areas of ~ 33 mm in diameter were irradiated on the dorsum of the hand and lower back on days 0, 30, 60, 90 and 120 at a range of doses up to a maximum of 300 J/cm^2. Exposure time to the subject's first prodromal symptom (e.g., burning, tingling) and the light source energy output were used to calculate the "Minimum Symptom Dose" (MSD) in J/cm^2 (MSD 400-650nm = [output value 400-650nm (unit: mW/cm2) x time (sec)] / 1000.

TABLE S1: EPP-QoL: Questions, answer options and scoring points.

	QUESTION	OPTIONS	POINTS
1	Over the last two months, how has your well-being been affected by EPP? I have been:	Much better Better Same Worse	3 2 1 0
2	Over the last two months, how much has EPP influenced the choice of the clothes you wear on a sunny day?	Very much A lot A little Not at all	0 1 2 3
3	Over the last two months, how often did you feel you were at risk of developing EPP symptoms?	Very often Often A little Not at all	0 1 2 3
4	Over the last two months, how much has EPP affected any social or leisure activities on a sunny day?	Very much A lot A little Not at all	0 1 2 3
5	Over the last two months, how much has EPP influenced your need to plan before leaving your house?	Very much A lot A little Not at all	0 1 2 3
6	Over the last two months, has EPP limited your ability to undertake activities in a spontaneous manner?	Very much A lot A little Not at all	0 1 2 3
7	Over the last two months, how much has EPP interfered with your going shopping or looking after your home (indoors and outdoors) or garden on a sunny day?	Very much A lot A little Not at all	0 1 2 3
8	Over the last two months, how much has EPP prevented you from attending outdoor social activities with family and friends?	Very much A lot A little Not at all	0 1 2 3
9	Over the last two months, how much has EPP limited your amount of outdoor activities?	Very much A lot A little Not at all	0 1 2 3
10	Over the last two months, how often did you experience typical EPP skin complaints?	Very much A lot A little Not at all	0 1 2 3
11	Over the last two months, how much has your quality of life improved?	Very much A lot A little Not at all	3 2 1 0
12	Over the last two months, how much has EPP influenced your method of transportation or seating preference during transportation?	Very much A lot A little Not at all	0 1 2 3

*Different response option labels are included in this questionnaire, but each item includes 4 response options. Items are scored on a scale of 0 to 3, or 3 to 0 depending on the polarity of the question. A negative statement is therefore scored in the opposite direction to a positive statement. The scoring pattern for each item is shown in supplementary table 1. The item responses are transformed onto a

0-100 scale. Domain scores are calculated based upon the responses to the items within each domain only (general well-being and severity), or for the total. The calculation of the scores is as follows:
The raw score (RS) is first calculated as being the mean (average) of the component items. For the 2 subscales and total score, the score is calculated using

Score = {raw score/range} x 100

Each score is transformed to a 0 to 100 range. A high score for a domain represents high levels of satisfaction or high quality of life. The table in the manuscript provides the changes in total score.

TABLE S2: Effect of afamelanotide and placebo administration on erythrocyte free protoporphyrin IX levels over the study period in the EU trial.*

	Afamelanotide		***Placebo***	
Erythrocyte free protoporphyrin IX levels in µmol/L				
Day	n		n	
0	38	34.6 (5.3, 150)	36	30.6 (14.9, 274)
60	38	34.0 (6.5, 180)	35	33.6 (13.5, 303)
120	36	36.9 (10.2, 230)	35	35.2 (14.4, 253)
180	35	35.3 (9.2, 120)	35	36.9 (13.5, 239)
240	33	36.6 (9.3, 140)	35	32.0 (13.9, 257)
270	38	35.4 (6.3, 190)	35	32.1 (11.9, 263)

*Data are presented as median (min,max). The erythrocyte protoporphyrin levels did not change significantly from baseline at the 5 subsequent visits, nor was a seasonal variation observed. The upper limit of normal is 1.5 µmol/L erythrocytes.

TABLE S3. Photoprovocation results in the US trial: Changes from baseline in minimum symptom dose* (MSD) in J/cm² on the hand and back.

Dorsum of Hand	*Afamelanotide*		*Placebo*		*Afamelanotide*	*Placebo*
		Median (range)			**Mean ± SD**	
	n		**n**			
Baseline: Day 0, prior to dose 1	10	48.9 (2.3, 172)	10	21.0 (1.1, 200)	61.8 ± 53.1	60.6 ± 75.5
Change at Day 30 (mid-dose 1)	10	109 (6.4, 191)	10	25.6 (-42.7, 289)	105 ± 64.0	84.6 ± 114
Change at Day 60 (prior to dose 2)	10	128 (-62.8, 298)	9	68.3 (-1.5, 157)	128 ± 143	65.4 ± 53.0
Change at Day 90 (mid-dose 2)	10	208 (41.6, 298)†	8	56.2 (-51.3, 289)†	204 ± 82.0	67.5 ± 104
Change at Day 120 (prior to dose 3)	10	162 (22.9, 291)‡	9	30.0 (-54.3, 289)‡	160 ± 97.0	59.1 ± 103
Lower Back	*Afamelanotide*		*Placebo*		*Afamelanotide*	*Placebo*
		Median (range)			**Mean ± SD**	
	n		**n**			
Baseline: Day 0, prior to dose 1	11	32.0 (2.1, 157)	10	24.1 (3.7,200)	40.1 (43.2)	72.2 (81.4)
Change at Day 30 (mid-dose 1)	11	137 (9.1, 185)	10	44.8 (-104, 294)	104 (71.8)	70.4 (117)
Change at Day 60 (prior to dose 2)	11	50.7 (-56.4, 285)	9	4.3 (-133, 124)	78.9 (112)	-2.9 (85.9)
Change at Day 90 (mid-dose 2)	11	227 (96.0, 298)ʃ	8	-2.4 (-33.3, 124)ʃ	197 (75.3)	12.3 (56.2)
Change at Day 120 (prior to dose 3)	11	82.5 (10.0, 271)¶	9	12.1 (-87.4,124)¶	112 (101)	15.3 (61.4)

*MSD calculated using the irradiation output (mW/cm^2) and time (sec) to first symptoms using the following formula:
MSD 400-650nm = [output value 400-650nm (unit: mW/cm^2) x time to first symptoms (sec)] / 1000.
†p=0.01; ‡p=0.045; ʃp<0.001; ¶p=0.03

REFERENCES

1. Rufener EA. Erythropoietic protoporphyria: a study of its psychosocial aspects. Br J Dermatol 1987; 116: 703–8
2. Wahlin S, Floderus Y, Stål P, Harper P. Erythropoietic protoporphyria in Sweden: demographic, clinical, biochemical and genetic characteristics. J Intern Med. 2011 Mar;269(3):278-88.
3. Holme SA, Anstey AV, Finlay AY, Elder GH, Badminton MN. Erythropoietic protoporphyria in the U.K.: clinical features and effect on quality of life. Br J Dermatol. 2006;155:574-81.
4. Jong CT, Finlay AY, Pearse AD, Kerr AC, Ferguson J, Benton EC, Hawk JL, Sarkany RP, McMullen E, Rhodes LE, Farr PM, Anstey AV. The quality of life of 790 patients with photodermatoses. Br J Dermatol. 2008;159:192-7.
5. Spelt JM, de Rooij FW, Wilson JH, Zandbergen AA. Vitamin D deficiency in patients with erythropoietic protoporphyria. J Inherit Metab Dis. 2010 Dec;33:Suppl 3:S1-4.
6. Basra MK, Fenech R, Gatt RM, Salek MS, Finlay AY. The Dermatology Life Quality Index 1994-2007: a comprehensive review of validation data and clinical results. Br J Dermatol. 2008 Nov;159(5):997-1035.
7. Both H, Essink-Bot ME, Busschbach J, Nijsten T. Critical review of generic and dermatology-specific health-related quality of life instruments. J Invest Dermatol 2007; 127:2726–39.
8. de Korte J, Mombers FMC, Sprangers MAG, Bos JD. The suitabilityof quality-of-life questionnaires for psoriasis research. Arch Dermatol 2002; 138:1221–7
9. Kent G, Al-Abadie M. Factors affecting responses on Dermatology Life Quality Index items among vitiligo sufferers. Clin Exp Dermatol 1996; 21:330–3.
10. FDA. Guidance for Industry : Patient-Reported Outcome Measures: Use in Medical Product Development to Support Labeling Claims. 2009;1-39 http://www.fda.gov/downloads/Drugs/Guidances/UCM193282.pdf