Fel d 1–derived peptide antigen desensitization shows a persistent treatment effect 1 year after the start of dosing: A randomized, placebo-controlled study

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Background: Allergic rhinoconjunctivitis is an increasingly common source of morbidity, with sensitivity to cats accounting for 10% to 15% of disease burden. Allergy to cats is also a major risk factor for the development of asthma.

Objectives: We sought to probe the persistence of the treatment effect of a novel Fel d 1–derived peptide antigen desensitization (Cat-PAD) 1 year after the start of treatment in subjects with cat allergy–induced rhinoconjunctivitis after standardized allergen challenge.

Methods: In a randomized, double-blind, placebo-controlled, parallel-group clinical trial, subjects attended an environmental exposure chamber in which they were exposed to cat allergen before and after treatment with 2 different regimens of Cat-PAD over a 3-month period. Clinical efficacy was assessed as a change in total rhinoconjunctivitis symptom scores 18 to 22 weeks and 50 to 54 weeks after the start of treatment.

Results: Treatment with Cat-PAD showed greater efficacy with 4 administrations of a 6-nmol dose 4 weeks apart than with 8 administrations of a 3-nmol dose 2 weeks apart. The treatment effect of 6 nmol persisted 1 year after the start of treatment and was significantly different from that of 3 nmol ($P = .0342$) and placebo ($P = .0104$). The treatment effect was apparent on both nasal and ocular symptoms at 1 year.

Conclusions: A short course of Cat-PAD improves the ocular and nasal components of rhinoconjunctivitis symptoms in subjects with cat allergy, with the treatment effect persisting 1 year after the start of treatment. (J Allergy Clin Immunol 2013;131:103-9.)

Key words: *Allergic rhinitis, allergic rhinoconjunctivitis, cat allergy, environmental exposure chamber, Fel d 1 peptide immunotherapy, T-cell epitope, immune tolerance, persistence*

Allergic rhinitis is a common disorder, with prevalence ranging from approximately 10% to 40% in adults and up to 40% in children.[1] The reduction in quality of life and the economic effect of allergic rhinitis are substantial.[2] Of a population of 310 million persons in the United States, approximately 60 million have allergies,[3] and 17% of the population have positive skin prick test responses to cat.[4] Furthermore, sensitization to cat allergens is a potent risk factor for the development of asthma; almost 30% of allergic asthma in the United States is attributable to cat sensitization.[5]

The morbidity associated with allergic diseases disproportionately affects socioeconomically disadvantaged populations, particularly children.[6] Fel d 1 is detectable in many public places at levels capable of sensitizing or exacerbating symptoms in susceptible subjects, including children in schools.[6]

Peptide immunotherapy uses synthetic peptides consisting of T-cell epitopes derived from major allergens and autoantigens to induce antigen-specific tolerance. Delivery of T-cell epitopes intradermally is thought to lead to the induction of T cells with a regulatory phenotype, which results in downregulation of the response to antigen.[7] The identification of a set of T-cell epitopes derived from Fel d 1 (Cat-peptide antigen desensitization [Cat-PAD]) has been described previously.[8]

Evaluation of the efficacy of therapeutic interventions for allergic rhinitis frequently relies on measurement of symptoms with or without medication use in clinical trials in which symptoms are elicited through natural exposure to allergen. Although this methodology is believed to reflect "real-life" exposure, it is confounded by marked variability in levels of allergen exposure. The use of an environmental exposure chamber (EEC) allows for clinical trial subjects to be exposed to aeroallergens in a highly controlled manner and allows the use of pre-established allergen levels known to induce symptoms at the moderate-to-severe level.[9,10] In a previous unrelated pilot EEC study we demonstrated that treatment with a 3-nmol dose of Cat-PAD with 4 or 8 injections over a 12- or 14-week period resulted in a greater reduction in mean total rhinoconjunctivitis symptom scores (TRSSs) than placebo, with a mean treatment difference of 2.9 units between 1 and 3 hours on days 2 and 4 of consecutive 3-hour EEC challenges 17 to 21 weeks after the start of treatment.[11]

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Abbreviations used
AE: Adverse event
EEC: Environmental exposure chamber
PAD: Peptide antigen desensitization
PTC: Posttreatment challenge
TEAE: Treatment-emergent adverse events
TNSS: Total nasal symptom score
TOSS: Total ocular symptom score
TRSS: Total rhinoconjunctivitis symptom score

The purpose of the present study was to evaluate whether efficacy could be further improved by administering the 6-nmol dose of Cat-PAD (also known as ToleroMune Cat [Circassia Limited, Oxford, United Kingdom]) 4 times over a 12-week period (4×6 nmol) and to compare this regimen with 8×3 nmol 2 weeks apart (8×3 nmol) and placebo. The study was originally designed with change in TRSSs measured 18 to 22 weeks after the start of treatment. The data presented here summarize the results at 18 to 22 weeks and present a follow-up study in which subjects returned to the EEC 1 year after the start of dosing and approximately 9 months after the last dosing visit, without any further retreatment.

METHODS

Subjects

The study received prior ethical approval from Institutional Review Board Services (Aurora, Ontario, Canada) and from Health Canada's Biologics and Genetic Therapies Directorate (the Canadian federal authority that regulates biological drugs). The study was registered at www.clinicaltrials.gov (identifiers: NCT01033344 and NCT01272323). Subjects were male or female (18-65 years of age), had a history of rhinoconjunctivitis with or without Global Initiative for Asthma step 1 asthma (http://www.ginasthma.com/) on exposure to cats for at least 1 year, provided written informed consent, and were able to comply with study requirements. Subjects with persistent asthma or subjects using inhaled corticosteroids or leukotriene modifiers to manage their asthma symptoms were excluded. Further details are provided in the Methods section in this article's Online Repository at www.jacionline.org.

Clinical study design

This was a randomized, double-blind, placebo-controlled, parallel-group study evaluating the safety and efficacy of intradermal injections of 2 dosing regimens of Cat-PAD performed at the Cetero Research EEC in Mississauga, Ontario, Canada. Subjects attended a screening visit and then a baseline challenge of 4 consecutive days of 3 hours in the EEC, which occurred before the first administration of study medication (see Fig E1 in this article's Online Repository at www.jacionline.org). Two hundred two subjects were randomized to one of 3 regimens (8×3 nmol 2 weeks apart, 4×6 nmol 4 weeks apart with infill placebo to maintain blinding, or $8 \times$ placebo). Table E1 in this article's Online Repository at www.jacionline.org provides further details of the dosing regimens and treatment administration. After dosing, subjects returned to the EEC for a further 4 consecutive days of 3-hour allergen exposures 18 to 22 weeks after the start of treatment. Eighty-nine subjects were re-enrolled in a follow-up study and returned to the EEC for a further 4 consecutive days of 3-hour allergen exposures at 50 to 54 weeks. Subjects and study staff remained blind to treatment. A new principal investigator was appointed to maintain blinding, and subjects were issued with new subject numbers to ensure blinding of the Cetero, Adiga, and Circassia staff directly involved in the study. The EEC is designed with clean-room technology using 100% fresh high-efficiency particulate air filtering. Cat allergen was dispersed into the chamber by an aerosol generator to achieve a consistent mean airborne level of 48.3 ± 2.09 ng of Fel d 1/m^3 using a fully validated method (Cetero Research). Each participant was subjected to challenge at a consistent time throughout the study (eg, in the morning or afternoon) to minimize the effect of circadian variations.

Study medication

Cat-PAD is an equimolar mixture of 7 peptides, the sequences of which (CPAVKRDVDLFLT, EQVAQYKALPVVLENA, KALPVVLENARILNCV, RILKNCVDAKMTEEDKE, KENALSLLDKIYTSPL, TAMKKIQDCY VENGLI, and SRVLDGLVMTTISSSK) are derived from Fel d 1, as previously described.[8] Study medication was supplied as a room temperature–stable lyophilisate of the 7 peptides or placebo and was reconstituted with sterile diluent or water for injection by an unblinded pharmacist before dosing. When reconstituted, the resulting solutions contained the 7 peptides at 50 or 100 nmol/mL or placebo. The reconstituted placebo product comprised the vehicle used to formulate the peptides. All subjects received a 60-μL intradermal injection volume. The 3-nmol dose was equivalent to approximately 37.5 μg of the 7 peptides.

Primary efficacy measurement

The primary efficacy measurement was based on the TRSS. At all EEC visits, subjects recorded symptoms in a diary just before entering the chamber and at 30-minute intervals thereafter. Symptoms were divided into nasal (running nose, sneezing, blocked nose, and itchy nose) and ocular (itchy eyes, watery eyes, red eyes, and sore eyes) symptoms. For each symptom, the subject rated the severity as follows: 0, absent; 1, mild and barely noticeable; 2, moderate and annoying/bothersome; and 3, severe and very annoying/very bothersome. The TRSS was calculated by summing the nasal and ocular symptom scores at each time point in the EEC. Subjects were required to have a TRSS of at least 10 of 24 and a total nasal symptom score (TNSS) of at least 6 of 12 on at least 1 diary card on days 3 and 4 of baseline challenge. The protocol-specified primary end point was the mean change in TRSSs at 50 to 54 weeks from 1 hour onward on days 2 to 4 of the posttreatment challenge (PTC) compared with baseline values for nonasthmatic subjects. The primary end point at 50 to 54 weeks was also analyzed in the total study population (pooled asthmatic and nonasthmatic subjects).

Secondary efficacy measurements

Secondary end points included the mean change from baseline values in scores for ocular and nasal symptoms at time points after 1 hour on days 2 to 4 of PTC in the treatment groups compared with the placebo group and the mean change in TRSSs and nasal and ocular symptoms at all time points on all days.

An exploratory analysis of mean change from baseline concentrations of cat-specific IgE at follow-up compared with that in the placebo group was also performed.

Safety measurements

Safety parameters included adverse events (AEs), physical examination results, vital signs, clinical laboratory test results (hematology, blood biochemistry, and urinalysis), spirometric results (FEV_1), visual analog scale scores of breathlessness and nasal symptoms, and local reactions at the injection site.

AEs

At each visit, the investigator determined whether AEs had occurred by asking nonleading questions. AE reporting began from obtaining signed informed consent forms and ended after the follow-up visit.

Statistical analysis

A comparison of each Cat-PAD dose with placebo was made by using an analysis of covariance model with treatment as a factor and baseline measurements as covariates. Statistical significance was accepted at a P value of less than .05.

RESULTS

Study participants

Two hundred two subjects received at least 1 dose of study medication and were included in the safety population. One hundred seventy-four subjects completed at least 1 PTC visit to the EEC and were included in the intention-to-treat analysis at 18 to 22 weeks (170 subjects with EEC data on days 2-4 of the PTC were analyzed). Missing data were not replaced. All subjects were invited to participate in a 1-year follow-up study. Ninety agreed, and after 1 screening failure, 89 subjects were enrolled in the blinded 1-year follow-up study detailed here and attended at least 1 EEC visit 1 year after the start of Cat-PAD. Demographic details (see Table E2 in this article's Online Repository at www.jacionline.org), disposition of subjects, and numbers analyzed (see Fig E2 in this article's Online Repository at www.jacionline.org) are presented in this article's Online Repository.

Primary efficacy variable

A summary of the change from baseline in mean TRSSs for the nonasthmatic subjects participating in the 1-year follow-up study after the start of treatment can be found in Table I; data for the same subjects at the 18- to 22-week visit to the EEC are also shown. For the nonasthmatic population, the results demonstrated a decrease in mean TRSSs from baseline (ie, a greater decrease in symptom severity) for 6 nmol (−6.778) when compared with both 3 nmol (−3.893) and placebo (−2.908). Median values showed the same trend. The change in TRSSs observed with 6 nmol was significantly different than the changes observed with 3 nmol ($P = .0342$) and placebo ($P = .0104$).

The treatment effect at 18 to 22 weeks in subjects who attended the 1-year follow-up study showed a greater mean change from baseline TRSSs (ie, a greater decrease in symptom severity) for both 6 nmol (−5.406) and 3 nmol (−5.136) when compared with placebo (−2.786). Median values showed the same trend. Comparison of the 50- to 54-week visit versus the 18- to 22-week visit showed the treatment effect (difference between active and placebo treatment) might have increased for 6 nmol. The treatment effects seen at 18 to 22 weeks in subjects who participated in the 1-year follow-up study were similar to those seen at 18 to 22 weeks in the full study population (mean change in TRSS: 6 nmol = −5.556, 3 nmol = −4.870, and placebo = −3.524). This demonstrates the subjects who participated in the 1-year follow-up study were representative of the main study population.

Fig 1, *A*, shows mean TRSSs at each time point on each day for the EEC visits at baseline challenge. A modest priming effect of sequential allergen exposure was observed, with an approximate peak TRSS of 14 on day 1 and increasing to 16 on day 4. A brisker response to allergen exposure was also observed with time. On day 1, a 1-hour exposure elicited a TRSS of approximately 9, whereas on day 4, a score of greater than 12 was achieved. Fig 1, *B*, shows mean TRSSs at each time point on each day for the EEC visits at 18 to 22 weeks, and Fig 1, *C*, shows mean TRSSs at each time point on each day for the EEC visits at 50 to 54 weeks. It is noteworthy that on day 4 of the EEC challenge, TRSSs decreased from a plateau of approximately 16 at the end of the baseline challenge to less than 7 during the 1-year follow-up visit for subjects receiving 6 nmol of Cat-PAD.

Fig 2 shows the mean difference in TRSSs between the baseline challenge and the 1-year follow-up visit for 6 nmol and placebo. The 6-nmol regimen shows a mean treatment effect of approximately 5 TRSS units more than placebo at the end of day 4 during the 1-year follow-up visit.

TABLE I. Change from baseline values in TRSSs at 1 to 3 hours on days 2 to 4 after PTC at 18 to 22 and 50 to 54 weeks (primary efficacy analysis nonasthmatic population)

Population	No.	Mean	SD	Median
TRSS change from baseline to PTC at 18-22 wk				
Placebo	29	−2.786	5.283	−3.067
8 × 3 nmol	23	−5.136	5.137	−4.067
4 × 6 nmol	21	−5.406	5.795	−5.733
TRSS change from baseline to PTC at 50-54 wk				
Placebo	29	−2.908	5.558	−3.267
8 × 3 nmol	23	−3.893	5.559	−3.733
4 × 6 nmol	21	−6.778	5.711	−6.800

	TRSS difference between active and placebo groups at 50-54 wk			
	LS means	LS means	95% CI	*P* value
Placebo	−2.997	0	0	NA
8 × 3 nmol	−3.509	−0.512	−3.539 to 2.515	.7369
4 × 6 nmol	−7.074	−4.077	−7.165 to −0.989	.0104
TRSS difference between active treatments				
4 × 6 nmol vs 8 × 3 nmol		−3.565	−6.856 to −0.274	.0342

Least squares means, 95% CIs, and *P* values are based on an analysis of covariance, with change from baseline as the dependent variable, treatment as the fixed effect, and baseline as the covariate. *P* values are from a 2-sided test at the 5% level.
LS means, Least squares means; *NA*, not applicable.

Secondary efficacy variables

Analysis of changes in TRSSs at 1 year for the pooled asthmatic and nonasthmatic subjects (total population) was performed for sensitivity testing (Table II). The results showed a larger decrease in mean change from baseline TRSSs (ie, a greater decrease in symptom severity) for 6 nmol (−6.353) when compared with 3 nmol (−3.636) and placebo (−2.488). Median values showed the same trend. The change in TRSSs observed with 6 nmol was significantly different from that observed with 3 nmol ($P = .0311$) and placebo ($P = .0057$). The small number of asthmatic subjects did not permit a separate statistical analysis for the effect of peptide immunotherapy in this category of patients.

A summary of the changes in TNSSs and total ocular symptom scores (TOSSs) for the nonasthmatic population at 50 to 54 weeks can be found in Tables III and IV, respectively. The results showed a larger decrease in mean change from baseline TNSSs (ie, a greater decrease in symptom severity) for 6 nmol (−3.435) when compared with both 3 nmol (−2.177) and placebo (−1.625). Median values showed the same trend. The change in TNSSs observed with 6 nmol was significantly different from that seen with placebo ($P = .0200$). The results also showed a larger decrease in mean change from baseline TOSSs (ie, a greater decrease in symptom severity) for 6 nmol (−3.343) when compared with both 3 nmol (−1.716) and placebo (−1.283). Median values showed the same trend. The change in TOSSs observed with 6 nmol was significantly different from that observed with 3 nmol ($P = .0292$) and placebo ($P = .0121$).

An exploratory analysis of changes in cat-specific IgE levels demonstrated no obvious or significant changes after the 1-year

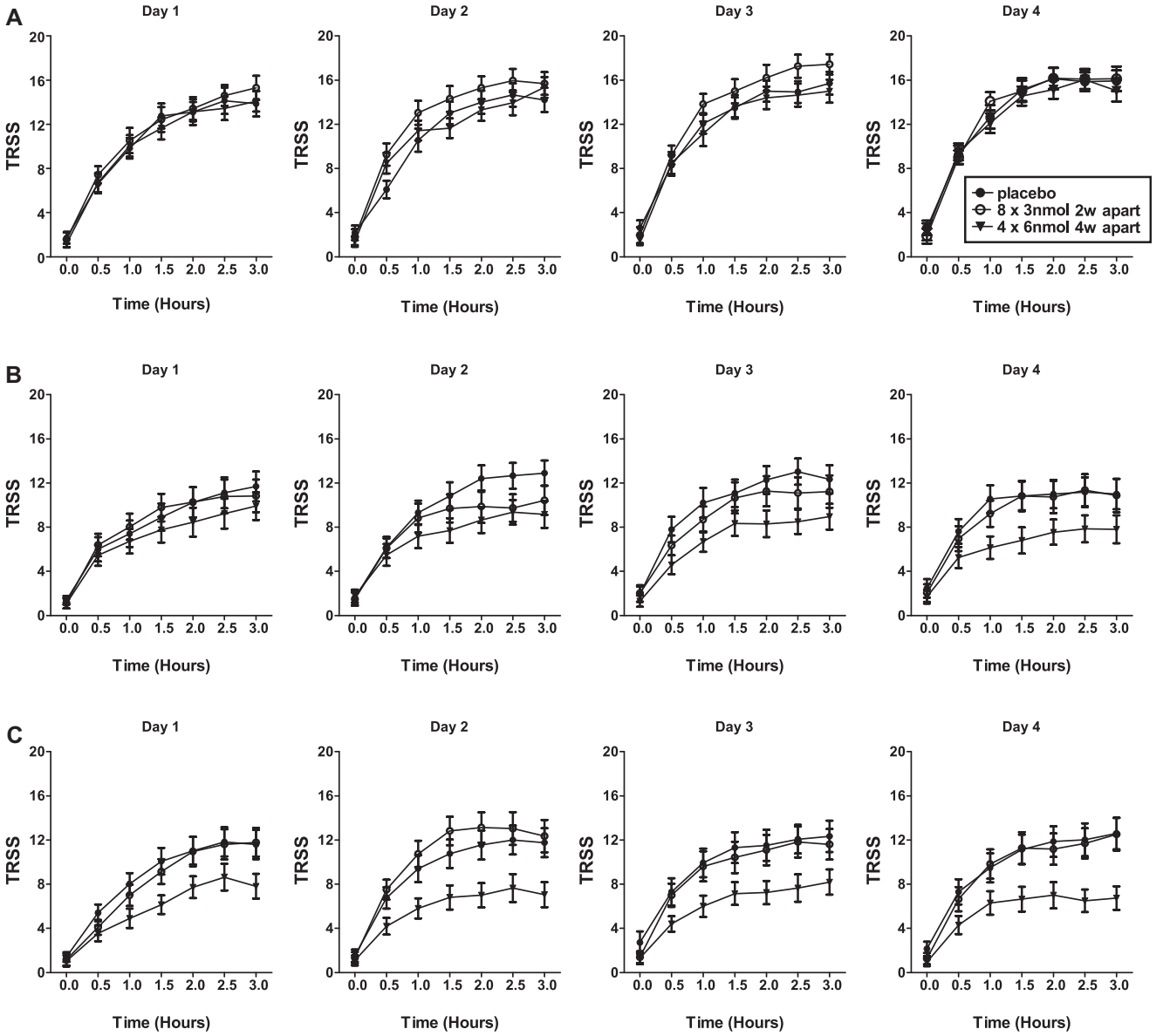

FIG 1. TRSSs (means ± SEMs) at each 30-minute time point (3 hours per day) in the chamber over 4 consecutive days: score at baseline **(A)**, score at 18 to 22 weeks after the start of treatment **(B)**, and score at challenge 50 to 54 weeks after the start of treatment **(C)**.

follow-up visit for any of the treatment regimens when compared with the baseline EEC visit or the visit to the EEC at 18 to 22 weeks (see Table E3 in this article's Online Repository at www.jacionline.org).

Safety

Table V summarizes treatment-emergent adverse events (TEAEs) by treatment arm in the safety population (all subjects receiving ≥1 dose) for the initial study. Table E4 in this article's Online Repository at www.jacionline.org summarizes the TEAE frequency by using the Medical Dictionary for Regulatory Activities System Organ Class and treatment group. There were no deaths reported during this study. There was 1 serious AE, a skin laceration, in a subject in the placebo group, which was judged to be of moderate severity and not related to the study drug. There were no serious AEs in the asthmatic population relating to their asthma.

A total of 267 TEAEs were reported: 84 TEAEs were reported by 44 different subjects after receiving placebo, 103 TEAEs were reported by 48 different subjects after receiving 3 nmol, and 80 TEAEs were reported by 36 different subjects after receiving 6 nmol. There was no evidence that the higher-dose regimen led to more TEAEs.

The majority of recorded TEAEs were mild in severity, with no TEAEs rated as severe. Six subjects did not complete the study because of a TEAE (placebo: 1 subject with back pain; Cat-PAD 3 nmol: 1 subject with arthralgia and pain in extremities and 2 subjects with bronchospasm; and Cat-PAD 6 nmol: 1 subject with hypersensitivity and 1 subject with presyncope and convulsion). The majority of TEAEs, including 5 of the 6 AEs leading to withdrawal, were assessed as unrelated to the study drug. The

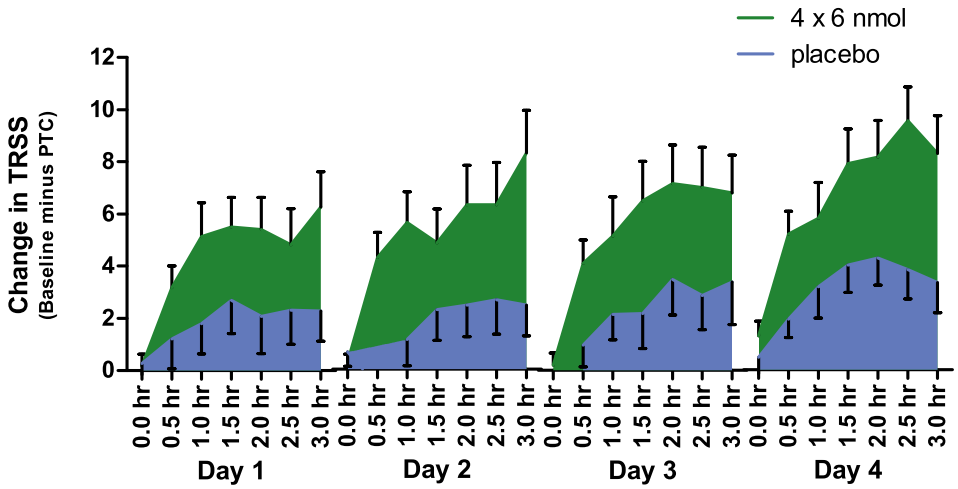

FIG 2. Difference in TRSSs (means ± SEMs) at each 30-minute time point (3 hours per day) in the chamber over 4 consecutive days: score at baseline challenge minus score at PTC 50 to 54 weeks after start of treatment.

TABLE II. Secondary efficacy analysis: change from baseline in TRSSs at 1 to 3 hours on days 2 to 4 after PTC at 18 to 22 and 50 to 54 weeks (pooled asthmatic and nonasthmatic population)

Population	No.	Mean	SD	Median
TRSS change from baseline to PTC at 18-22 wk				
Placebo	36	−3.096	4.984	−3.600
8 × 3 nmol	28	−4.610	5.136	−4.100
4 × 6 nmol	24	−4.992	5.679	−5.200
TRSS change from baseline to PTC at 50-54 wk				
Placebo	36	−2.488	5.385	−3.200
8 × 3 nmol	28	−3.636	5.136	−3.300
4 × 6 nmol	24	−6.353	5.748	−6.033

	TRSS difference between active and placebo groups at 50-54 wk			
	LS means	LS means	95% CI	*P* value
Placebo	−2.629	0	0	NA
8 × 3 nmol	−3.322	−0.693	−3.313 to 1.926	.600
4 × 6 nmol	−6.507	−3.878	−6.598 to −1.158	.0057
TRSS difference between active treatments				
4 × 6 nmol vs 8 × 3 nmol		−3.185	−6.074 to −0.296	.0311

Least squares means, 95% CIs, and *P* values are based on an analysis of covariance, with change from baseline as the dependent variable, treatment as the fixed effect, and baseline as the covariate. *P* values are from a 2-sided test at the 5% level.
LS means, Least square means; *NA*, not applicable.

TABLE III. Secondary efficacy analysis: change from baseline in TNSSs at 1 to 3 hours on days 2 to 4 after PTC at 18 to 22 and 50 to 54 weeks (nonasthmatic population)

Population	No.	Mean	SD	Median
TNSS change from baseline to PTC at 18-22 wk				
Placebo	29	−1.515	2.811	−1.067
8 × 3 nmol	23	−2.452	2.404	−2.200
4 × 6 nmol	21	−2.838	3.015	−2.800
TNSS change from baseline to PTC at 50-54 wk				
Placebo	29	−1.625	2.953	−1.400
8 × 3 nmol	23	−2.177	2.758	−2.000
4 × 6 nmol	21	−3.435	3.047	−2.667

	TNSS difference between active and placebo groups at 50-54 wk			
	LS means	LS means	95% CI	*P* value
Placebo	−1.649	0	0	NA
8 × 3 nmol	−1.988	−0.339	−1.945 to 1.268	.6753
4 × 6 nmol	−3.609	−1.960	−3.601 to −0.319	.0200
TNSS difference between active treatments				
4 × 6 nmol vs 8 × 3 nmol		−1.621	−3.380 to 0.138	.0702

Least squares means, 95% CIs, and *P* values are based on an analysis of covariance, with change from baseline as the dependent variable, treatment as the fixed effect, and baseline as the covariate. *P* values are from a 2-sided test at the 5% level.
LS means, Least squares means; *NA*, not applicable.

hypersensitivity in a subject receiving 6 nmol of Cat-PAD was assessed as related to the study drug.

There were no clinically significant findings related to any clinical laboratory evaluation. No reductions in FEV_1 of greater than 30% (prospectively defined cutoff) were observed in any treatment group at any treatment visit. The majority of injection-site inspections were assessed as normal, and no abnormal assessment was considered clinically significant. Visual analog scale scores of breathlessness and nasal symptoms remained low in the 1 hour after dosing for the active treatment regimens and placebo on all dosing days.

Analysis of the respiratory system TEAEs shows no evidence of any safety signal. Three subjects receiving 6 nmol experienced an episode of dyspnea, bronchospasm, or asthma, whereas 14 subjects receiving 3 nmol and 11 subjects receiving placebo reported such an episode. The majority of these events occurred during the PTC in the EEC at 18 to 22 weeks.

DISCUSSION

We have previously identified 7 synthetic peptides that contain the major T-cell epitopes from the cat allergen Fel d 1.[8] The proliferative and cytokine responses to these 7 peptides when administered together were equivalent to those observed with whole cat dander extract. Unlike whole cat dander extract, however, the 7 peptides did not induce histamine release in blood basophils.

A previous proof-of-concept study conducted in the EEC demonstrated that either 4 or 8 administrations of 3 nmol

TABLE IV. Secondary efficacy analysis: change from baseline in TOSSs at 1 to 3 hours on days 2 to 4 after PTC at 18 to 22 and 50 to 54 weeks (nonasthmatic population)

Population	No.	Mean	SD	Median
TOSS change from baseline to PTC at 18-22 wk				
Placebo	29	−1.271	3.000	−1.200
8 × 3 nmol	23	−2.684	3.052	−1.867
4 × 6 nmol	21	−2.568	3.123	−1.867
TOSS change from baseline to PTC at 50-54 wk				
Placebo	29	−1.283	2.923	−1.267
8 × 3 nmol	23	−1.716	3.169	−1.467
4 × 6 nmol	21	−3.343	3.048	−3.000

	TOSS difference between active and placebo groups at 50-54 wk			
	LS means	LS means	95% CI	*P* value
Placebo	−1.353	0	0	NA
8 × 3 nmol	−1.531	−0.179	−1.767 to 1.409	.8231
4 × 6 nmol	−3.448	−2.096	−3.718 to −0.473	.0121
TOSS difference between active treatments				
4 × 6 nmol vs 8 × 3 nmol		−1.115	−2.464 to 0.235	.0292

Least squares means, 95% CIs, and *P* values are based on an analysis of covariance, with change from baseline as the dependent variable, treatment as the fixed effect, and baseline as the covariate. *P* values are from a 2-sided test at the 5% level.
LS means, Least squares means; *NA*, not applicable.

TABLE V. TEAEs in the safety population (subjects receiving ≥1 dose of Cat-PAD or placebo)

	Placebo (N = 69)		8 × 3 nmol (N = 67)		4 × 6 nmol (N = 66)	
TEAEs	No. (%)	E	No. (%)	E	No. (%)	E
All TEAEs	44 (63.8%)	84	48 (71.6%)	103	36 (54.5%)	80
Serious TEAEs	1 (1.4%)	1	0 (0.0%)	0	0 (0.0%)	0
TEAEs leading to withdrawal	1 (1.4%)	1	3 (4.5%)	4	2 (3.0%)	4
TEAEs considered related to study drug by principal investigator	13 (18.8%)	18	18 (26.9%)	25	14 (21.2%)	20

E, Total number of TEAEs; *N*, number of subjects exposed; *No.*, number of subjects exposed who experienced 1 or more TEAEs; %, percentage based on N.

of Cat-PAD over a 12- or 14-week period resulted in greater changes in TRSSs than placebo 17 to 21 weeks after the start of treatment.[11] One of the purposes of this study was to evaluate the persistence of clinical efficacy 1 year after the start of treatment with 4 administrations of 6 nmol of Cat-PAD over a 12-week period or with 8 administrations of 3 nmol of Cat-PAD over a 14-week period compared with placebo.

The use of precisely standardized pretreatment and posttreatment allergen challenges in an EEC allowed the accurate determination of efficacy, including the onset of action, magnitude, and duration of the treatment effect. The mean level of Fel d 1 in the EEC in the present study of 48 ng/m^3 was within the range of 10 to 200 ng/m^3 reported for airborne Fel d 1 levels in homes with cats.[12] Previously, studies of allergen immunotherapy have not systematically evaluated the effect of dose and dosing regimen on the persistence of a treatment effect after withdrawal of therapy. In the current study the strongest treatment effect in the 1-year follow-up was observed in the group treated with 6 nmol of Cat-PAD. This group showed statistically significant differences in mean TRSSs versus treatment with placebo and 3 nmol of Cat-PAD, as well as the component scores, TNSS and TOSS, versus placebo.

As exemplified in Fig 2, there is a substantial reduction in mean TRSSs in the nonasthmatic population between subjects who received 6 nmol of Cat-PAD and those who received placebo. At time points from 1 hour onward on days 2 to 4 in the EEC, the mean difference between placebo and 6 nmol was 3.9 units on the TRSS scale.

Although the same cumulative antigen dose (24 nmol) was administered with both regimens and a treatment effect was seen at 18 to 22 weeks for both, the reduction in rhinoconjunctivitis symptoms persisted to a much greater extent at the 1-year follow-up for the 6-nmol dose compared with the 3-nmol dose. We speculate that a threshold immunologic event was triggered with the 6-nmol dose, resulting in the maintained effect. Further studies are needed to evaluate the long-term immunomodulatory changes arising as a result of peptide immunotherapy, as well as the immunologic effect of the different dosing regimens, to understand the nature of this threshold immunologic event.

Previous studies have demonstrated reduced allergen-specific proliferation and T_H2 cytokine responses in PBMCs after peptide immunotherapy.[13,14] In contrast, IL-10 production was increased and, in a murine model of peptide immunotherapy for cat allergy, played a central role in immunologic tolerance to the allergen.[15] Peptide treatment was associated with upregulation of CD5, a molecule involved in T-cell activation and division, perhaps limiting T-cell expansion.[16] It has also been demonstrated that the tolerogenic environment created by treatment with a limited number of peptides can result in limited local tolerance to other T-cell epitopes within the same allergen.[15]

The TRSS used in this study has been used as a subjective outcome measure in numerous clinical trials of pharmacotherapy and allergen immunotherapy.[17-19] The observed change of 3.9 TRSS units in the current study represents a substantial improvement over current approaches because these approaches have been evaluated in a similar system (ie, in studies using TRSS outcomes with an EEC design). For example, studies of similar design reported TRSS changes of approximately 2 units for adjuvanted subcutaneous immunotherapy[20] and daily sublingual grass immunotherapy,[21] whereas a single 180-mg dose of the antihistamine fexofenadine achieved a mean difference in TRSSs of 1.3.[22] Moreover, the change in TRSSs reported here was observed after just 4 administrations of Cat-PAD and persisted 9 months after treatment cessation.

In conclusion, the optimal dose and dosing regimen of Cat-PAD has been identified as 4 administrations of a 6-nmol dose 4 weeks apart, and a persistent improvement in rhinoconjunctivitis symptoms lasting at least 1 year after the start of treatment has been demonstrated.

Clinical implications: Short-course (4 administrations) immunotherapy with T-cell epitopes significantly improves clinical symptoms of cat allergen–induced rhinoconjunctivitis 1 year after the start of treatment.

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METHODS

Clinical characteristics of subjects in the clinical study

Male or female subjects aged 18 to 65 years were required to have a 1-year documented history of cat allergen–induced rhinoconjunctivitis and a positive skin prick test response to cat allergen with a wheal diameter at least 3 mm larger than that produced by the negative control. Female subjects of childbearing potential were required to practice an acceptable form of contraception. Subjects must have achieved minimum qualifying symptom scores on at least 1 symptom diary card during EEC exposure on the third and fourth days during the baseline challenge. Minimum qualifying symptom scores were defined as a TRSS of at least 10 of a possible 24 and a TNSS of at least 6 of a possible 12, and the subject must have been willing and able to comply with the study requirements.

Subjects were excluded from the study if they had persistent asthma falling under the Global Initiative for Asthma classifications "partly controlled" and "uncontrolled," an FEV_1 of less than 70% of normal value, or a history of anaphylaxis to cat allergen or received allergen immunotherapy in the last 12 months or cat dander immunotherapy ever. Subjects with seasonal allergic rhinoconjunctivitis who could not complete the clinical study outside the relevant pollen season, who had significant allergy to other animal dander that could not be avoided during the study period, or who were unable to tolerate the baseline challenge were also excluded. Furthermore, subjects were not permitted to use corticosteroids, cromones, antihistamines other than loratadine, leukotriene inhibitors, anticholinergic agents, α-adrenergic agonists, tricyclic antidepressants, monoamine oxidase inhibitors, β-blockers, α-adrenoceptor blockers, tranquilizers, or psychoactive drugs during the study. Additional exclusion criteria included subjects for whom administration of epinephrine was contraindicated (eg, subjects with acute or chronic symptomatic coronary heart disease or severe hypertension) and subjects being treated with β-blockers. Additionally, subjects with symptoms of a clinically relevant illness within 6 weeks before the screening visit and subjects with clinically relevant abnormalities detected on physical examination vital signs, or laboratory values outside the normal ranges were excluded. Female subjects who were pregnant, lactating, or planning a pregnancy during the study were also excluded. Finally, subjects were also excluded if they had a significant history of alcohol or drug abuse or a history of immunopathologic diseases; had previously been randomized into this study or had received Cat-PAD previously; had a history of severe drug allergy, severe angioedema, or an anaphylactic reaction to food; had received treatment with an investigational drug within 6 months before study screening; were unable to communicate or understand the requirements of the study; had any significant disease or disorder that, in the opinion of the investigator, might either have put the subject at risk because of participation in the study or influenced the results of the study or the subject's ability to participate in the study; or had a known allergy to thioglycerol.

Randomization

In the first part of the study, in which the treatments were administered, subjects were randomly assigned to Cat-PAD (8 × 3 nmol), Cat-PAD (4 × 6 nmol), or placebo in a 1:1:1 ratio after the baseline challenge in the EEC. Randomization was performed by using a computer-generated algorithm. Subjects were randomized in blocks of 6. Randomization was stratified according to whether the subjects had regular exposure to a cat and by diagnosis of asthma. Cetero Research generated the random allocation sequence. Blinded Cetero Research staff enrolled participants and assigned participants to treatments according to the randomization schedule. In the 1-year follow-up that constitutes the main part of this article, all subjects from the initial study were contacted, and 89 agreed to participate. No further treatment was administered.

Study medication administration

Treatments were administered by means of intradermal injection into the flexor surface of the left forearm every 2 weeks (±2 days) for 14 weeks (Table E1). The 8 × 3-nmol regimen consisted of 8 injections of 3 nmol, with each injection separated by 2 weeks. The 4 × 6-nmol regimen consisted of 4 injections of 6 nmol, with each injection separated by 4 weeks. Placebo injections were inserted into the dosing schedule for the 4 × 6-nmol regimen to maintain blinding. The placebo arm consisted of 8 injections of placebo, with each injection separated by 2 weeks. Previous studies have shown both Cat-PAD and placebo lead to a mild erythema and wheal response after intradermal injection. Consequently, no additional actions were considered necessary to maintain blinding after product administration.

Study medication manufacture

The peptides were synthesized by Bachem (Bubendorf, Switzerland), according to current Good Manufacturing Practice. The lyophilized Cat-PAD product was formulated, filled, and finished by Patheon (Monza, Italy) and the lyophilized placebo was manufactured by Aptuit (Glasgow, United Kingdom), also according to current Good Manufacturing Practice. The materials were tested at Patheon and Gen-Probe (Livingston, United Kingdom) and released in accordance with the European Union (Directive 2001/20/EC) and Canadian (Food and Drug Act, Section C.05.010) regulations after labeling and packaging at Aptuit (Bathgate, United Kingdom).

Recruitment

For the initial study, the first subject was screened on December 7, 2009, and the last subject completed on October 6, 2010. For the 1-year follow-up, the first subject was screened on February 7, 2011, and the last subject completed on April 28, 2011. In both cases the trial ended after successful completion of the last subject.

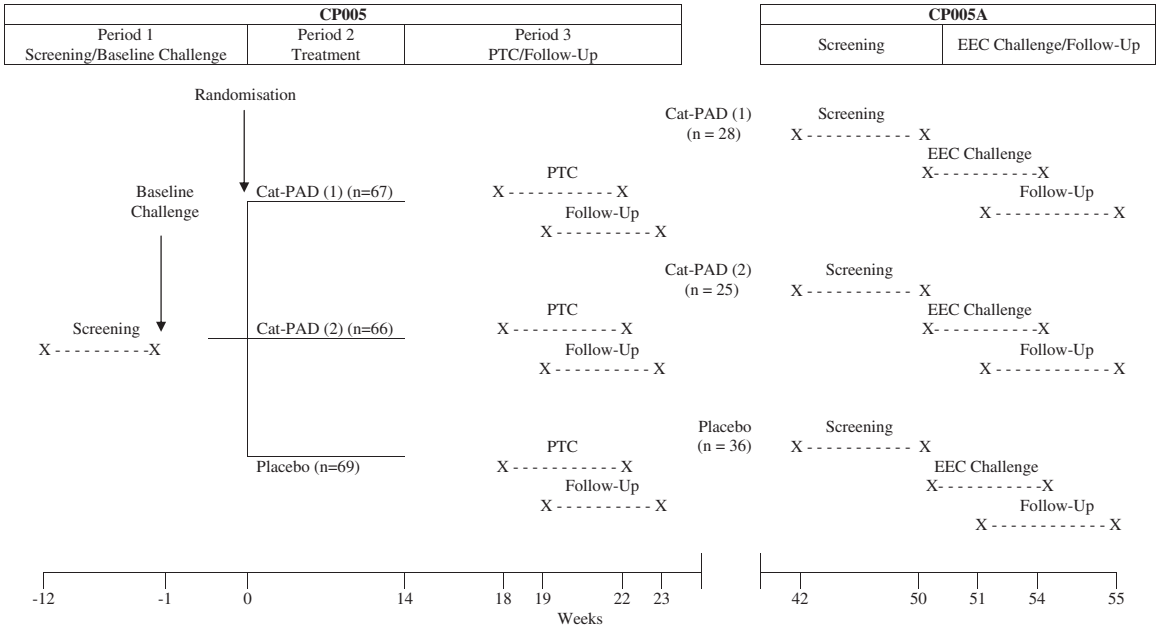

FIG E1. Overall study design. The baseline challenge and PTC both consisted of 3 hours in the EEC exposed to cat allergen on 4 consecutive days. Two dosing regimens of Cat-PAD were compared with placebo: *Cat-PAD (1)*, 8 × 3 nmol administered 2 weeks apart; *Cat-PAD (2)*, 4 × 6 nmol administered 4 weeks apart.

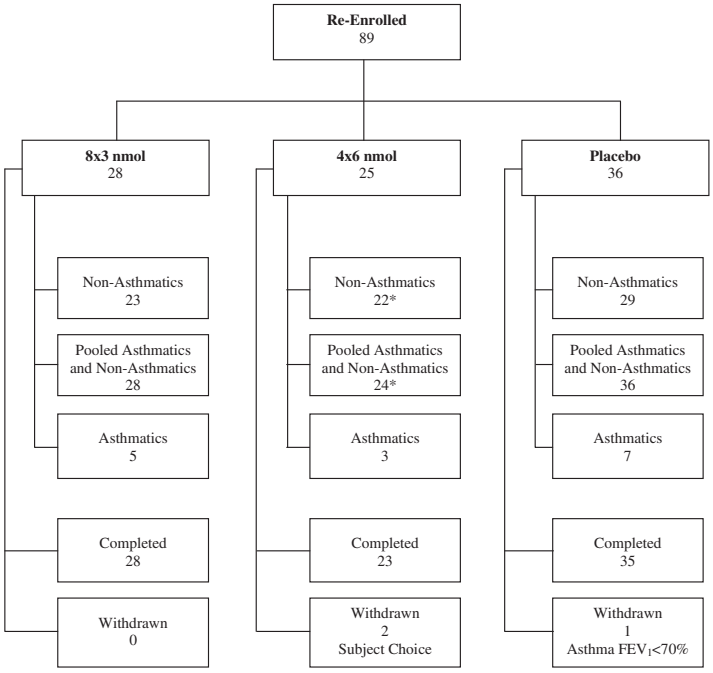

FIG E2. Disposition of subjects and populations for analysis. *One subject in the 4 × 6-nmol regimen included in the nonasthmatic population withdrew after PTC at weeks 50 to 54 on day 1 and therefore had no data for the analysis, which used data from days 2 to 4.

TABLE E1. Treatment regimens and treatment schedule for Cat-PAD

Visit	3A	3B	3C	3D	3E	3F	3G	3H
Week	0	2	4	6	8	10	12	14
Regimen								
8 × 3 nmol	X	X	X	X	X	X	X	X
4 × 6 nmol	X	P	X	P	X	P	X	P
Placebo	P	P	P	P	P	P	P	P

P, Placebo administration; *X*, Cat-PAD administration.

TABLE E2. Demographic data of subjects participating in the 1-year follow-up study

Characteristic	Placebo (n = 36)	8 × 3 nmol (n = 28)	4 × 6 nmol (n = 25)
Race, no. (%)			
American Indian/Alaska Native	0 (0.0)	0 (0.0)	1 (4.0)
Asian	2 (5.6)	1 (3.6)	2 (8.0)
Black/African American	4 (11.1)	4 (14.3)	0 (0.0)
Mixed	3 (8.3)	0 (0.0)	2 (8.0)
White	27 (75.0)	23 (82.1)	20 (80.0)
Ethnicity, no. (%)			
Hispanic or Latino	5 (13.9)	1 (3.6)	5 (20.0)
Not Hispanic or Latino	31 (86.1)	27 (96.4)	20 (80.0)
Sex, no. (%)			
Male	21 (58.3)	9 (32.1)	15 (60.0)
Female	15 (41.7)	19 (67.9)	10 (40.0)
Age (y)			
Mean ± SD	38.0 ± 11.2	39.5 ± 11.6	35.9 ± 9.1
Range	19-62	21-65	22-56

TABLE E3. Exploratory efficacy analysis: change from baseline in cat-specific IgE levels (kIU/L) after PTC at 18 to 22 and 50 to 54 weeks

	Treatment group		
Parameter	**Placebo**	**8 × 3 nmol**	**4 × 6 nmol**
No. of subjects	29	23	22
Mean ± SD cat-specific IgE level at screening visit	6.651 ± 8.602	22.048 ± 39.303	11.951 ± 19.955
Median cat-specific IgE at screening visit	3.050	3.960	4.080
Mean ± SD cat-specific IgE after EEC challenge at 18-22 wk	9.180 ± 11.958	21.564 ± 32.919	12.122 ± 17.569
Median cat-specific IgE level after EEC challenge at 18-22 wk	3.810	6.910	6.640
Mean ± SD cat-specific IgE level after EEC challenge at 50-54 wk	8.234 ± 10.771	18.546 ± 26.986	10.555 ± 17.960
Median cat-specific IgE level after EEC challenge at 50-54 wk	3.500	6.850	4.565

No significant changes were found for any of the treatment regimens.

TABLE E4. TEAE frequency by System Organ Class and treatment group after administration of Cat-PAD or placebo

	Treatment group		
System Organ Class Preferred Term	**Placebo (n = 69)**	**8 × 3 nmol (n = 67)**	**4 × 6 nmol (n = 66)**
Subject with ≥1 AE	44 (63.8%)	48 (71.6%)	36 (54.5%)
Nervous system disorders	23 (33.3%)	15 (22.4%)	15 (22.7%)
Respiratory, thoracic, and mediastinal disorders	20 (29.0%)	18 (26.9%)	13 (19.7%)
Infections and infestations	9 (13.0%)	16 (23.9%)	12 (18.2%)
Skin and subcutaneous tissue disorders	4 (5.8%)	7 (10.4%)	2 (3.0%)
Immune system disorders	2 (2.9%)	4 (6.0%)	5 (7.6%)
Injury, poisoning, and procedural complications	4 (5.8%)	3 (4.5%)	4 (6.1%)
Musculoskeletal and connective tissue disorders	2 (2.9%)	4 (6.0%)	5 (7.6%)
Eye disorders	2 (2.9%)	5 (7.5%)	0 (0.0%)
Gastrointestinal disorders	3 (4.3%)	5 (7.5%)	2 (3.0%)
General disorders and administration site conditions	1 (1.4%)	2 (3.0%)	1 (1.5%)
Pregnancy, puerperium, and perinatal conditions	1 (1.4%)	0 (0.0%)	2 (3.0%)
Psychiatric disorders	0 (0.0%)	1 (1.5%)	0 (0.0%)
Renal and urinary disorders	0 (0.0%)	0 (0.0%)	1 (1.5%)
Reproductive system and breast disorders	0 (0.0%)	0 (0.0%)	1 (1.5%)
Vascular disorders	0 (0.0%)	1 (1.5%)	1 (1.5%)
Ear and labyrinth disorders	0 (0.0%)	1 (1.5%)	0 (0.0%)

Data are shown for 202 subjects randomized to treatment and cover the period to the close-out visit occurring 3 to 10 days after the EEC visit at 18 to 22 weeks. Counts reflect numbers of subjects reporting 1 or more AEs that map to the Medical Dictionary for Regulatory Activities System Organ Class/Preferred Term. At each level of summarization (System Organ Class or Preferred Term), subjects reporting more than 1 AE are counted only once.
n, Number of subjects exposed; %, percentage based on n.