SUPPLEMENTARY MATERIAL

Shortened up-dosing with 7 injections of subcutaneous allergy immunotherapy (Alutard SQ) is safe and well tolerated

Methods

Trial design

The trial design is displayed in Supplementary figure 1. It was conducted in accordance with the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH)-Guidelines for Good Clinical Practice and the Declaration of Helsinki. The protocol was approved by relevant competent and ethical bodies in Germany and Spain. All subjects gave their written informed consent prior to their inclusion in the trial.

Subjects with a positive skin prick test (wheal diameter ≥ 3 mm) at screening and a documented positive specific IgE response (≥ CAP class 2 or equivalent, ≥ 0.70 kU/L) determined in the previous 2 years of their allergy history were eligible. Subjects with asthma who were at risk of exacerbation and with inadequate symptom control according to the Global Initiative for Asthma (GINA) recommendations [1], and subjects with a forced expiratory volume in 1 second (FEV_1) < 70% of predicted value in adults, and < 80% in adolescents were excluded. Subjects were also excluded if they were currently treated with any AIT. Further exclusion criteria were: previous treatment with AIT to an allergen related to the allergens included in the trial within the past 5 years, history of anaphylaxis with cardiorespiratory symptoms, recurrent generalised urticaria during the last 2 years, history of angioedema (drug induced or hereditary), clinically relevant chronic disease of ≥ 3 months duration, systemic disease affecting the immune system, immunosuppressive treatment within 3 months prior to screening, treatment with tricyclic antidepressants, catechol-amine-o-methyltransferase or mono-amine-oxidase inhibitors, antidepressant or antipsychotic medication with antihistaminic effect, monoclonal anti-IgE antibody treatment or ß-blockers as contraindications specified in the product label of Alutard SQ.

In subjects with multiple allergies the most relevant allergy according to clinical evaluation was treated.

Up-dosing of AIT was performed either by an 11-injection schedule (Alutard SQ 6-grasses and rye) or a 7-injection schedule (Alutard SQ 6-grasses and rye, birch, house dust mites,

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ALK, Denmark) [2-4]. Medication was supplied in 3 vials containing 5 ml with concentrations of 1,000; 10,000 and 100,000 SQ-U/ml (Supplementary figure 2).
Two maintenance injections were applied 14 and 42 days after reaching the maximum dose in all groups (approximate duration of treatment: 16 weeks (grass-11) and 12 weeks (grass-7, tree-7, HDM-7).
Secondary endpoints were number of local reactions, number of adverse events (AEs) leading to discontinuation, number of systemic allergic reactions, number of early (< 30 minutes post injection) and delayed systemic reactions (> 30 minutes), number of serious AEs and change in observations in physical examination from screening to last planned assessment, and in vital signs from pre-injection to 60 minutes post-injection, and the percentage of injections with a decrease in peak expiratory flow (PEF) values > 20% from pre-injection to 60 minutes post-injection.

Sample size estimation

No formal sample size calculation was done. The planned sample size of 320 subjects followed empirical considerations. In order to evaluate the safety profiles of the 7-injection up-dosing schedule, the sample size was based on a need to detect a sufficient number of AEs to be able to evaluate if there was any significant difference. Based on the number of subjects with treatment-related adverse events (TRAEs) seen in previous trials with Alutard SQ (around 50% of subjects in the active treatment group experienced at least one TRAE) it was expected that with 320 subjects (80 subjects in each treatment group) a sufficient number of events in the 7-injection up-dosing-schedule would be detected to be able to evaluate whether the safety and tolerability of the 7-injection up-dosing schedule of the respective allergen was comparable to the 11-injection up-dosing schedule with Alutard SQ 6-grasses and rye. Because safety and tolerability of SCIT appears to be similar in adults and adolescents [5], a subgroup of 25% adolescents (20 subjects in each treatment arm) was planned to be included.

Statistical and safety analyses

All statistical analyses were descriptive. The evaluation whether the safety profile of the 7-injection up-dosing schedule was acceptable was based on a comparison to the 11-injection up-dosing schedule for the grass pollen allergen extract. No adjustment for multiplicity was done, since no hypothesis testing was performed. For statistical analyses, SAS version 9.4 (SAS Institute, Cary, North Carolina) was used.
AEs were coded according to version 20.1 of the Medical dictionary for Drug Regulatory Activities (MedDRA). The standardised MedDRA query (SMQ) ‘anaphylactic reaction’ and

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the MedDRA preferred term (PT) 'hypersensitivity' were used to identify systemic reactions among all AEs reported. The method was based on a 2 step algorithm identifying systemic allergic reactions and potential systemic allergic reactions as defined by the SMQ. First, a narrow term search was conducted using the PTs that alone indicate a systemic allergic reaction. Second, a search was conducted using the SMQ which defines groups of PTs that when co-reported (more than one symptom from 2 or more organ systems reported at the same time) could indicate an anaphylactic reaction.

Results

Patients

A total of 357 subjects were screened, 341 were allocated to treatment and 340 started treatment. Most common reason for screening failures (N=16) was lack of willingness or ability to comply with the trial protocol. The flow of patients through the trial (CONSORT diagram) is displayed in Supplementary figure 3.

The baseline characteristics of subjects are shown in Supplementary table 1.

AEs and TRAEs in adolescent and adult subgroups

All AEs and TRAEs in the 4 treatment groups in adolescents and adult subgroups are displayed in Supplementary table 2.

TRAEs (primary endpoint)

The number of TRAEs and the proportion of subjects are displayed in Supplementary figure 4 A and B).

Local reactions

A total of 1935 local administration site reactions were reported in 261 (77%) subjects. No major difference in the proportion of subjects experiencing local AEs was observed between the treatment groups (grass-11: 74%, grass-7: 78%, tree-7: 74%, HDM-7: 82%). The majority (> 99%) of these reactions were assessed as treatment-related. Most local reactions were mild (93%) or moderate (7%) in severity, did not lead to treatment change (95%) and resolved (> 99%). This pattern applied to all treatment groups. None of the events were assessed as serious. One subject in the grass-7 group and 2 in the HDM-7 group discontinued the trial due to treatment-related local reactions. The most common local reactions were injection site swelling, injection site pruritus and injection site erythema together accounting for 91% of all local administration site reactions (Supplementary figure 5).

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Systemic allergic reactions

Systemic allergic reactions defined by narrow search in MedDRA SMQ 'anaphylactic reaction' and potential anaphylactic reactions defined by co-reported AEs are displayed in Supplementary Table 3. It cannot be ruled out that these co-occurring AEs were in fact true systemic reactions, however, none of the AEs were assessed as severe or serious and all subjects continued treatment.

Serious AEs and serious TRAEs (case narratives)

The following cases were serious: An adult subject in the grass-11 group experienced a life threatening anaphylactic shock, 5 minutes after injection 13 (final injection; dose 100,000 SQ-U), with symptoms of palmar itching, shortness of breath, redness of conjunctivae and face, facial pallor and hypotension (69/38 mm Hg). The subject was treated with antihistamine (po and iv), corticosteroid (iv), fluid (NaCl), adrenaline (im and iv) and beta-2-agonist (inh) and was considered recovered after 20 minutes.

Other serious TRAEs in the grass-11 group were delayed (after > 30 minutes) anaphylactic reactions in 1 adolescent (dose: 300 SQ-U; symptoms: generalised urticarial exanthema at upper body, cough, conjunctival injection) and 1 adult subject (dose: 80,000 SQ-U; symptoms: urticaria, cough, local swelling, generalised exanthema, mild dyspnea, unusual/tingling sensation in the mouth), and an immediate reaction of hypersensitivity in 1 adult subject (dose: 100,000 SQ-U; symptoms: conjunctival erythema, pruritus, rhinitis, abdominal pain, nausea, difficulty in breathing, dizziness). In the grass-7 group 1 adult subject experienced a delayed reaction of hypersensitivity (dose: 10,000 SQ-U; symptoms: generalised urticaria/pruritus, dyspnea/cough).

All subjects recovered after treatment with antihistamines and corticosteroids, salbutamol or adrenaline (applied after the immediate reactions in 3 subjects).

In one adult subject with a medical history of arterial hypertension in the grass-7 group a hypertensive crisis 7 days after the injection was not assessed as treatment-related.

Other endpoints

No clinically relevant differences with regards to vital signs, physical examination and PEF-measurements were identified between the treatment groups.

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1. GINA Executive Committee. Global Initiative for Asthma; Global Strategy for Asthma Management and Prevention. National Heart, Lung and Blood Institute; National Institute of Health, Bethesda, Maryland, USA; 2017.
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3. Summary of product characteristics [SmPC] Alutard SQ® trees. ALK; 2016.
4. Summary of product characteristics [SmPC] Alutard SQ® house dust mites. ALK; 2016.
5. Larenas-Linnemann DE, Pietropaolo-Cienfuegos DR, Calderon MA. Evidence of effect of subcutaneous immunotherapy in children: complete and updated review from 2006 onward. Ann Allergy Asthma Immunol 2011;107:407-416.

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Supplementary Tables and Figures

Supplementary Table 1. Baseline characteristics

	Grass-11	**Grass-7**	**Tree-7**	**HDM-7**
Subjects, n	85	86	87	83
Male, n (%)	43 (51)	42 (49)	43 (49)	40 (48)
Female, n (%)	42 (49)	44 (51)	44 (51)	43 (52)
Age, median (range)	29 (12-64)	30 (12-63)	31 (12-64)	26 (12-58)
Adolescents, n (%)	22 (26)	23 (27)	21 (24)	23 (28)
Adults, n (%)	63 (74)	63 (73)	66 (76)	60 (72)
Rhinitis and/or conjunctivitis, n (%)	85 (100)	86 (100)	87 (100)	83 (100)
Allergic asthma	15 (18)	16 (19)	26 (30)	18 (22)
Years with rhinitis and/or conjunctivitis, mean (±SD)	13.5 (±10.4)	12.6 (±11.0)	12.3 (±8.2)	10.6 (±7.6)
SPT positive to, n (%):				
Grass *(Phleum pratense)*	85 (100)	86 (100)	45 (52)	38 (46)
Birch *(Betula verrucosa)*	40 (47)	31 (36)	87 (100)	38 (46)
Hazel *(Corylus avellana)*	27 (32)	27 (31)	74 (85)	25 (30)
Alder *(Alnus glutinosa)*	27 (32)	25 (29)	75 (86)	28 (34)
HDM *(D. pteronyssinus)*	31 (36)	24 (28)	36 (41)	82 (99)
HDM *(D. farinae)*	28 (33)	23 (27)	31 (36)	83 (100)
IgE class to grass, tree or HDM, n (%):				
2-3	48 (56)	50 (58)	41 (47)	45 (54)
4-6	37 (44)	36 (42)	46 (53)	38 (46)

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Supplementary Table 2. All AEs and TRAEs in the four treatment groups in adolescents, adults and all subjects

	Grass-11		Grass-7		Tree-7		HDM-7	
	adolescents (N=22) n (%), e	**adults (N=63)** n (%), e	**adolescents (N=23)** n (%), e	**adults (N=62)** n (%), e	**adolescents (N=21)** n (%), e	**adults (N=66)** n (%), e	**adolescents (N=23)** n (%), e	**adults (N=60)** n (%), e
All AEs	21 (95), 150	53 (84), 709	22 (96), 119	57 (92), 610	19 (90), 121	56 (85), 490	21 (91), 131	53 (88), 425
TRAEs	19 (86), 107	46 (73), 604	18 (78), 87	50 (81), 474	15 (71), 80	51 (77), 364	19 (83), 109	51 (85), 337
Severity, mild	18 (82), 92	45 (71), 565	15 (65), 76	49 (79), 412	15 (71), 78	51 (77), 320	19 (83), 105	50 (83), 301
moderate	6 (27), 12	13 (21), 34	5 (22), 7	18 (29), 52	2 (10), 2	14 (21), 44	3 (13), 4	13 (22), 35
severe	2 (9), 3	5 (8), 5	1 (4), 4	5 (8), 10	-	-	-	1 (2), 1
Serious TRAEs	1 (5), 1	3 (5), 3	-	1 (2), 1	-	-	-	-
Dose not changed	18 (82), 96	45 (71), 570	17 (74), 77	47 (76), 416	15 (71), 80	50 (76), 351	19 (83), 97	50 (83), 319
Dose reduced	6 (27), 10	14 (22), 34	3 (13), 6	15 (24), 43	-	5 (8), 11	4 (17), 12	8 (13), 14
Treatment interrupted	-	-	1 (4), 4	-	-	1 (2), 2	-	-
Treatment withdrawn	1 (5), 1	-	-	7 (11), 15	-	-	-	3 (5), 4
Event leading to discontinuation	1 (5), 1	-	-	7 (11), 15	-	-	-	3 (5), 4
Treated by medication	9 (41), 28	21 (33), 67	9 (39), 20	22 (35), 78	3 (14), 5	15 (23), 58	7 (30), 12	21 (35), 64
Immediate onset (<30 minutes)	4 (18), 11	19 (30), 88	5 (22), 9	21 (34), 97	3 (14), 6	14 (21), 46	4 (17), 8	16 (27), 48
Delayed onset (>30 minutes)	19 (86), 96	45 (71), 516	18 (78), 78	50 (81), 377	15 (71), 74	50 (76), 318	18 (78), 101	49 (82), 289

AE=adverse event, TRAE= treatment-related adverse event

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Supplementary Table 3. Systemic treatment-related allergic reactions defined by narrow search in MedDRA SMQ 'anaphylactic reaction'[†] and potential anaphylactic reactions defined by co-reported AEs

Preferred term	*Injection/dose*	*Immediate/delayed*[‡]	*Severity*	*Serious*	*Change of treatment*	*Outcome*
Systemic TRAEs defined by narrow search in MedDRA SMQ 'anaphylactic reaction'						
Grass-11						
Anaphylactic reaction	300 SQ-U	Delayed	Severe	Yes	Withdrawn	Recovered
Anaphylactic reaction	80,000 SQ-U	Delayed	Severe	Yes	Dose reduced	Recovered
Anaphylactic shock	100,000 SQ-U	Immediate	Severe	Yes	Dose not changed	Recovered
Hypersensitivity	100,000 SQ-U	Immediate	Severe	Yes	Dose not changed	Recovered
Grass-7[§]						
Anaphylactic reaction	30,000 SQ-U	Delayed	Severe	No	Withdrawn	Recovered
Hypersensitivity	10,000 SQ-U	Delayed	Severe	Yes	Withdrawn	Recovered
Hypersensitivity	10,000 SQ-U	Delayed	Moderate	No	Dose reduced	Recovered
Treatment-related potential systemic allergic reactions defined by co-occurring MedDRA SMQs anaphylactic reaction'						
Grass-11						
Urticaria Dyspnoea	60,000 SQ-U	Delayed	Moderate Mild	No	Dose not changed	Recovered
Grass-7						
Sneezing Erythema	30,000 SQ-U	Delayed	Mild Moderate	No	Dose reduced	Recovered
Eyelid oedema Cough	10,000 SQ-U	Delayed	Moderate Moderate	No	Dose not changed	Recovered
Tree-7						
Sneezing Pruritus	5,000 SQ-U	Delayed	Moderate Mild	No	Dose not changed	Recovered

[†]and Preferred Term 'hypersensitivity'

[‡]Immediate=treatment-related and <30 minutes after injection, delayed=treatment-related and >30 minutes after injection

[§]In addition, one subject in the grass-7 group experienced 6 re-occurring events of hypersensitivity. On different dates, the subject experienced AEs all described as "allergy" assessed as mild in severity and unlikely treatment-related, treatment was continued

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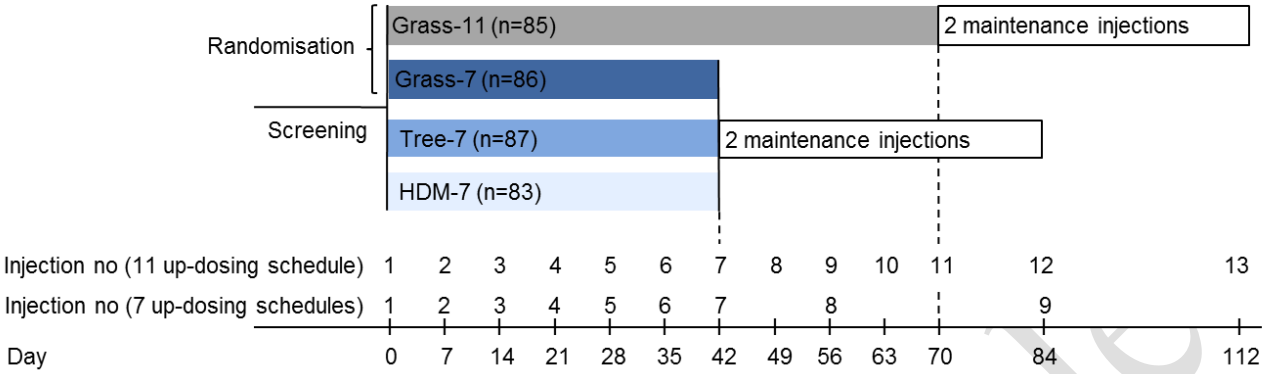
Supplementary Figure 1. Study diagram



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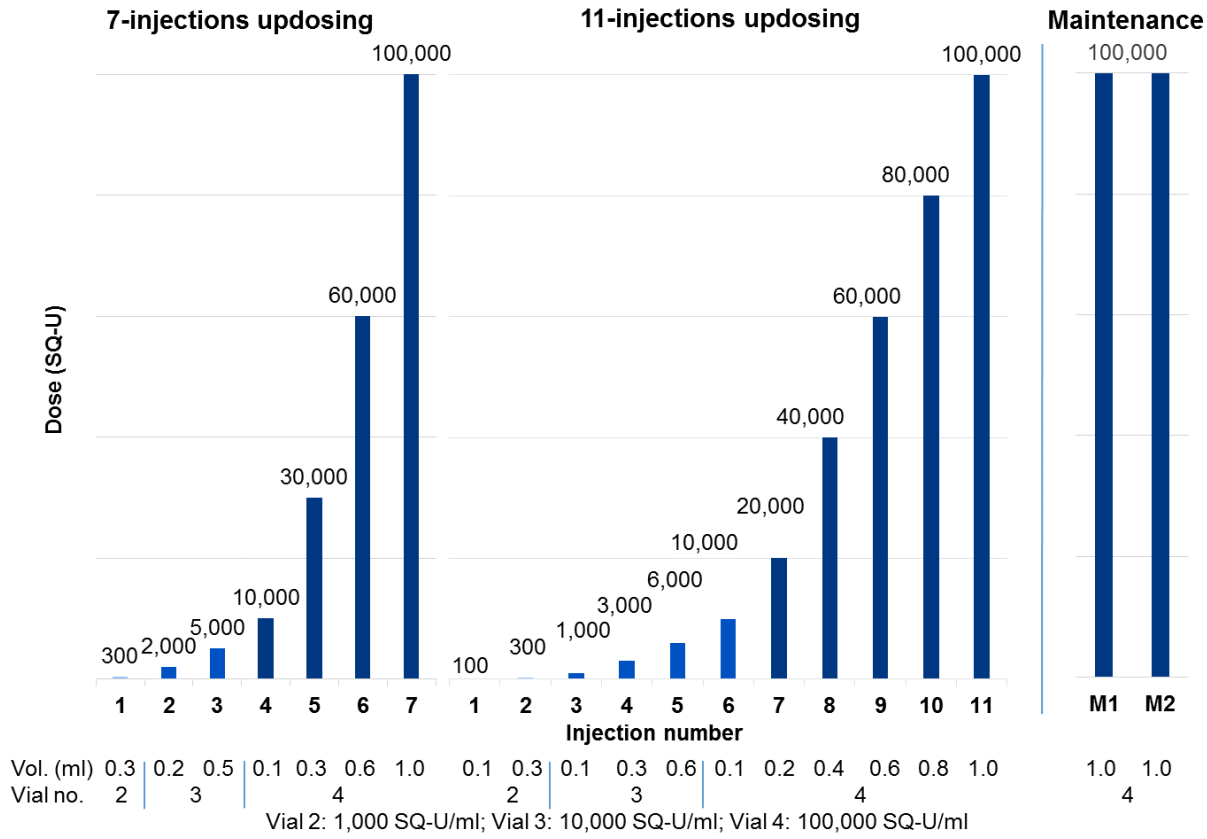
Supplementary Figure 2. Dosage schedules, volumes injected and vial concentrations.



Subjects were up-dosed by a 7-injection or an 11-injection schedule followed by 2 maintenance doses. Doses were applied from vials with increasing concentrations (vial 2, 3, 4) by injecting the equivalent volume from the vial.

(Vol. = volume; M1, M2=number of maintenance injection).

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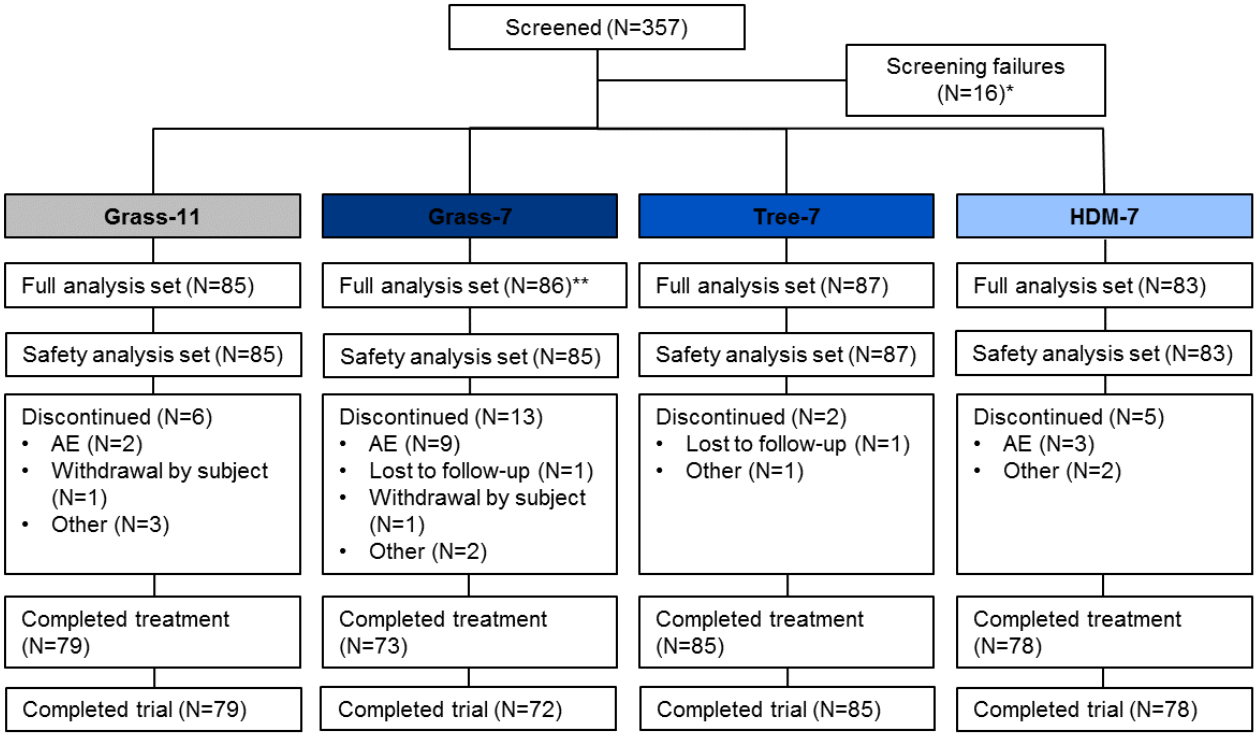
Supplementary Figure 3. Flow of subjects through the study (CONSORT-diagram).



*The most common reason was lack of willingness or ability to comply with protocol

**1 subject was randomised but discontinued before first administration of AIT

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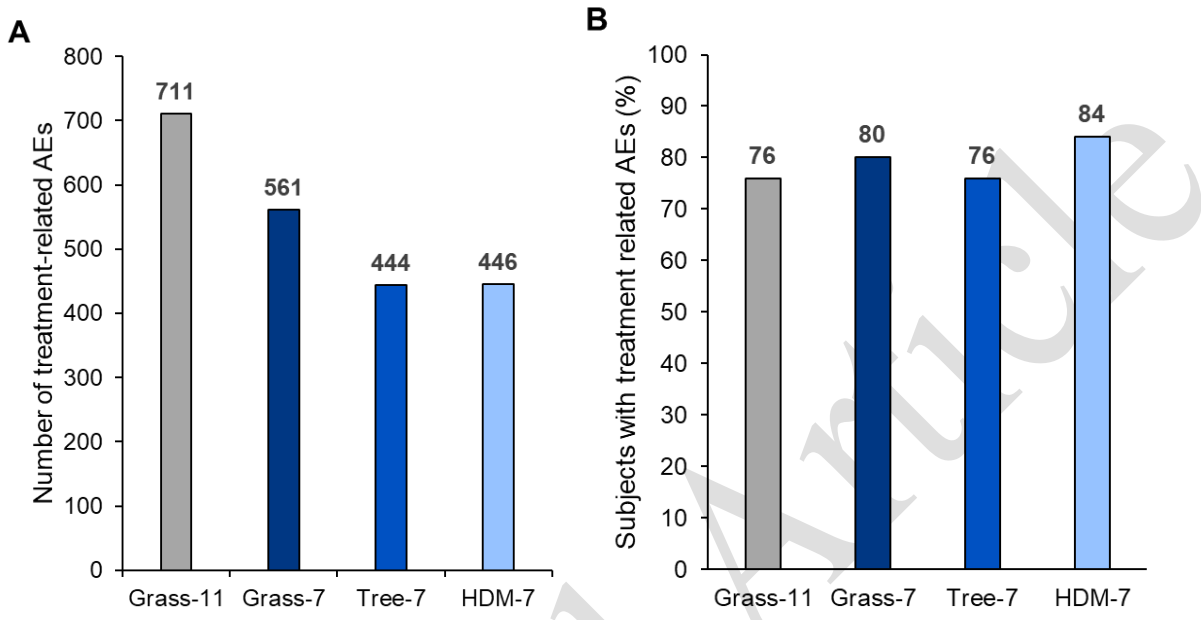
Supplementary Figure 4. Number of treatment related adverse events **(A)** and proportion of subjects with TRAEs **(B)** in the 11-injection up-dosing schedule with grass (grass-11) and the 7-injection up-dosing schedules with grass (grass-7), birch (tree-7) and house dust mite (HDM-7).



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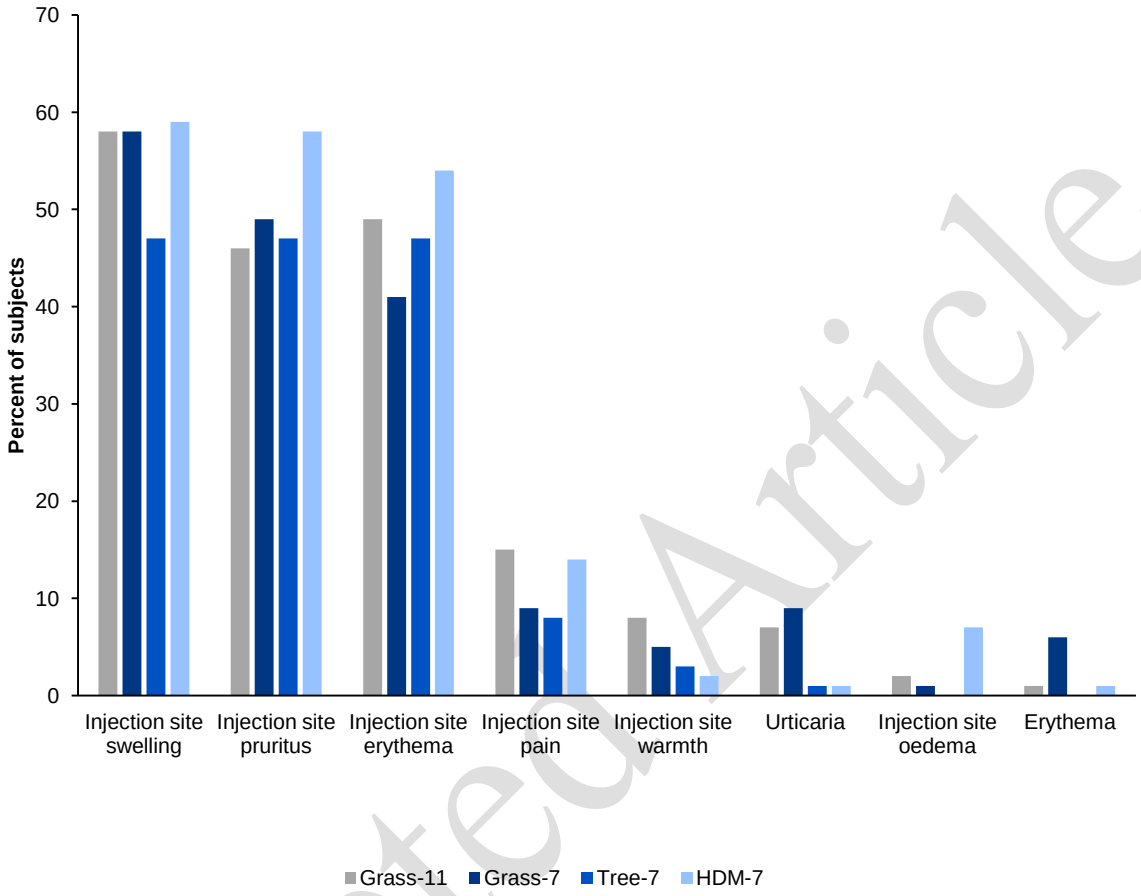
Supplementary Figure 5. Most frequently reported treatment-related adverse events (TRAEs); (≥5% of subjects in any group).



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