Synopsis – Trial AF-H-01

Title of Trial
A multicentre randomised Phase II clinical trial to demonstrate equivalent pharmacodynamic efficacy and tolerability of two updosing schedules for ALK-Flex SQ

Investigators
50 principal investigators in Germany. Signatory Investigator: Priv.-Doz. Dr. med. [redacted]

Trial Centres
50 centres in Germany

Publications
- Jung K. et al. Subcutaneous immunotherapy with a preparation with optimised allergen aluminium hydroxide ratio: a randomised parallel-group clinical trial investigating safety and tolerability. *Allergy* 2010; 65 (Suppl. 92): 4-5
- Wolf H. et al. Subcutaneous immunotherapy with a preparation with optimised allergen aluminium hydroxide ratio: Immunologic effects. *Allergy* 2010; 65 (Suppl. 92): 134

Trial Period
First subject first visit – 11 February 2009
Last subject last visit – 19 June 2009

Objectives
As stated in the protocol (Appendix I.1), the objectives of the trial were as follows:

The primary objective was:
- To demonstrate equivalence of changes from baseline in specific IgE-blocking factor against *Phleum pratense* in Group 1[1] and Group 2[1] one week after the 5th injection of ALK-Flex SQ using the equivalence limit ± 0.03

The secondary objectives were:
- To compare both groups with respect to incidences of delayed systemic adverse events (1-24 hrs) in Group 1[1] and Group 2[1] classified as "urticaria" and/or "asthma" according to EAACI
- To compare both groups with respect to incidences of early adverse events (0-60 minutes) in Group 1[1] and Group 2[1], graduated according to EAACI and classified according to MedDRA version 11.0[2]
- To compare both groups with respect to changes from baseline in specific IgG_4 against *Phleum pratense* 1 week after the 5th injection of ALK-Flex SQ
- To compare both groups with respect to changes from baseline in specific IgE against *Phleum pratense* 1 week after the 5th injection of ALK-Flex SQ

Due to safety concerns[3], the protocol was amended and the objectives were slightly changed accordingly.

The primary objective was changed to:
- To demonstrate equivalence of changes from baseline in specific IgE-blocking factor against *Phleum pratense* in Group 1 and Group 2 one week after the 7th injection of ALK-Flex SQ using the equivalence limit ± 0.03

The secondary objectives were changed to:
- To compare both groups with respect to incidences of delayed systemic adverse events (1 – 24 hrs) in Group 1 and Group 2 classified as "urticaria" and/or "asthma" according to EAACI
- To compare both groups with respect to incidences of early adverse events (0 – 60 minutes) in Group 1 and Group 2, graduated according to EAACI and classified according to MedDRA version 11.0[2]
- To compare both groups with respect to changes from baseline in specific IgG_4 against *Phleum pratense* 1 week after the 7th injection of ALK-Flex SQ
- To compare both groups with respect to changes from baseline in specific IgE against *Phleum pratense* 1 week after the 7th injection of ALK-Flex SQ

[1] Group 1 and Group 2 in the pre-amendment phase were, after an amendment was implemented (please also refer to footnote 2 below), renamed to Group A and Group B in order to separate these groups from Group 1 and Group 2 in the post-amendment phase; i.e. Group A and B was used for the pre-amendment phase and Group 1 and 2 for the post-amendment phase.

[2] MedDRA version 11.0 was intended to be used according to the protocol and protocol amendment, but MedDRA was later upgraded to version 11.1 and used for coding of adverse events in this ICTR.

[3] Evaluation of preliminary safety data revealed a number of delayed allergic reactions. As the observed level of allergic reactions was considered unacceptable by ALK, the trial was put on "temporarily hold" and modifications of the protocol with respect to the updosing schedules and the time interval between injections were implemented before continuation of the trial.

Methodology
This was a multicentre, randomised, parallel-group trial comparing the pharmacodynamic efficacy and tolerability of two updosing schedules for ALK-Flex SQ 4-grass + rye.

Number of Subjects Planned and Analysed
400 subjects were planned for inclusion; 553 subjects were screened; 473 subjects were randomised and 459 subjects were treated (14 withdrew before first injection was given). The disposition of subjects who initiated treatment pre-amendment and of subjects who initiated treatment post-amendment are shown below:

Pre-Amendment	**Group A**	**Group B**	**Total**
Full Analysis Set (FAS)	**28 (100%)**	**31 (100%)**	**59 (100%)**
Subject withdrawals	**15 (53.6%)**	**15 (48.4%)**	**30 (50.8%)**
Reason for withdrawal:			
Adverse events	11 (39.3%)	7 (22.6%)	18 (30.5%)
Withdrawal of consent	1 (3.6%)	3 (9.7%)	4 (6.8%)
Maintenance phase shortened	–	1 (3.2%)	1 (1.7%)
Other reason	3 (10.7%)	4 (12.9%)	7 (11.9%)
Subjects completed	**13 (46.4%)**	**16 (51.6%)**	**29 (49.2%)**

Post-Amendment	**Group 1**	**Group 2**	**Total**
Full Analysis Set (FAS)	**201 (100%)**	**199 (100%)**	**400 (100%)**
Subject withdrawals	**16 (8.0%)**	**26 (13.1%)**	**42 (10.5%)**
Reason for withdrawal:			
Adverse events	8 (4.0%)	15 (7.5%)	23 (5.8%)
Adverse event + withdrawal of consent	1 (0.5%	3 (1.5%)	4 (1.0%)
Withdrawal of consent	–	3 (1.5%)	3 (0.8%)
Non-compliance	1 (0.5%)	2 (1.0%)	3 (0.8%)
Maintenance phase shortened	6 (3.0%)	3 (1.5%)	9 (2.3%)
Subjects completed	**185 (92.0%)**	**173 (86.9%)**	**358 (89.5%)**

Main Selection Criteria
Subjects included in this trial had grass pollen induced allergic rhinoconjunctivitis of two years or more requiring treatment during the grass pollen season and a positive skin prick test to *Phleum pratense.* Subjects were not allowed to have FEV_1 < 70% of predicted value at screening, bronchial asthma corresponding to GINA step 3 or more, even if controlled, a history of asthma exacerbation or emergency visit or admission for asthma in the previous 12 months, and were not allowed to have been treated with immunotherapy within the previous 5 years.

Investigational Medicinal Product, Dose and Mode of Administration, Batch Number
ALK-Flex SQ 4-grass + rye; suspension for subcutaneous injection.

The IMP was provided in two strengths:

- 600 SQ-U/ml (vial A, batch no. 0000117340)
- 30,000 SQ-U/ml (vial B and C, batch no. 0000117339)

Dosing of the IMP was conducted according to the schedules listed below:

Original Dosing Schedules (Protocol dated 22 January 2009)

Injection no.	Phase	Group A		Group B	
		Dose	Time	Dose	Time
1	Updosing	300 SQ-U (0.5 ml vial A)	Day 1	300 SQ-U (0.5 ml vial A)	Day 1
2		3,000 SQ-U (0.1 ml vial B)	Day 2	3,000 SQ-U (0.1 ml vial B)	Day 3-4
3		15,000 SQ-U (0.5 ml vial B)	Day 3	15,000 SQ-U (0.5 ml vial B)	Day 7-8
4	Maintenance	15,000 SQ-U (0.5 ml vial B)	+ 14 days	15,000 SQ-U (0.5 ml vial B)	+ 14 days
5		15,000 SQ-U (0.5 ml vial C)	+ 4 weeks	15,000 SQ-U (0.5 ml vial C)	+ 4 weeks

Revised (Amended) Dosing Schedules (Protocol Amendment dated 27 February 2009)

Injection no.	Phase	Group 1		Group 2	
		Dose	Time	Dose	Time
1	Updosing	300 SQ-U (0.5 ml vial A)	Day 0	300 SQ-U (0.5 ml vial A)	Day 0
2		600 SQ-U (1 ml vial A)	Day 7	600 SQ-U (1 ml vial A)	Day 3-4
3		3,000 SQ-U (0.1 ml vial B)	Day 14	3,000 SQ-U (0.1 ml vial B)	Day 7-8
4		6,000 SQ-U (0.2 ml vial B)	Day 21	6,000 SQ-U (0.2 ml vial B)	Day 10-11
5		15,000 SQ-U (0.5 ml vial B)	Day 28	15,000 SQ-U (0.5 ml vial B)	Day 14-15
6	Maintenance	15,000 SQ-U (0.5 ml vial B or C)	+ 14 days	15,000 SQ-U (0.5 ml vial B or C)	+ 14 days
7		15,000 SQ-U (0.5 ml vial C)	+ 3-4 weeks	15,000 SQ-U (0.5 ml vial C)	+ 3-4 weeks

Reference Therapy, Dose and Mode of Administration, Batch Number
Not applicable.

Duration of Treatment
Treatment included 5 (pre-amendment) or 7 (post-amendment) injections with either 3-4 days intervals or weekly intervals.

Criteria for Evaluation –Pharmacodynamic Efficacy
Changes from baseline in specific IgE-blocking factor against *Phleum pratense* one week after the last injection of ALK-Flex SQ 4-grass + rye and changes in the specific IgG_4 and specific IgE against *Phleum pratense* one week after the last injection of ALK-Flex SQ 4-grass + rye.

Criteria for Evaluation – Safety
Adverse events, clinical safety laboratory tests, vital signs and physical examination.

Statistical Methods

The primary pharmacodynamic endpoint for the trial was changes from baseline in specific IgE-blocking factor against *Phleum pratense* at follow-up (one week after the last injection of ALK-Flex SQ 4-grass + rye). Changes from baseline in specific IgE-blocking factor against *Phleum pratense* one week after the last injection of ALK-Flex SQ 4-grass + rye was tested for equivalence of the two dosing schedules according to the principle of "confidence interval inclusion" based on Satterthwaite's modified t-test with δ=0.03 as equivalence margin.

The null hypothesis:

- H_0: $\Delta_1 \leq \Delta_2 - \delta$ or $\Delta_1 \geq \Delta_2 + \delta$

was tested against the alternative hypothesis

- H_1: $\Delta_1 > \Delta_2 - \delta$ and $\Delta_1 < \Delta_2 + \delta$

  i.e. $|\Delta_1 - \Delta_2| < \delta$

where δ was the equivalence margin; Δ_1 was the changes in Group A/1 and Δ_2 was the changes in Group B/2. For this purpose a 95% confidence interval ($CI_{lower} | CI_{upper}$) of the treatment difference $\Delta_1 - \Delta_2$ was determined. Equivalence could be derived if $-\delta < CI_{lower}$ and $CI_{upper} < \delta$.

The secondary pharmacodynamic endpoints for this trial were changes in the specific IgG_4 and specific IgE against *Phleum pratense* one week after the last injection of ALK-Flex SQ 4-grass + rye. Changes in the specific IgG_4 and specific IgE against *Phleum pratense* one week after the last injection of ALK-Flex SQ 4-grass + rye were compared between Group A/1 and Group B/2 by means of Mann-Whitney test (U-test) for the untransformed data as well as by means of Satterthwaite's t-test after logarithmic transformation.

No imputation of data was carried out in case of missing data, but all available data were used to its full extent.

No adjustment for multiplicity was required with only one primary statistical hypothesis test.

Safety endpoints were mainly analysed by descriptive statistics.

Demography of Trial Population

Demographical data for subjects who initiated treatment pre-amendment and for subjects who initiated treatment post-amendment are presented in the tables below. No major differences between dosing schedules were observed.

Demographics, Pre-amendment (FAS)

	Group A	Group B	FAS
Number of subjects	28	31	59
Age (years)			
Mean (SD)	38.4 (13.3)	36.9 (11.1)	37.6 (12.1)
Median	39.0	37.0	38.0
Minimum- maximum	19-62	19-56	19-62
Gender, N (%)			
Male	9 (32.1%)	18 (58.1%)	27 (45.8%)
Female	19 (67.9%)	13 (41.9%)	32 (54.2%)
BMI (kg/m^2)			
Mean (SD)	24.1 (3.7)	24.9 (3.1)	24.5 (3.4)
Median	23.2	24.4	24.4
Minimum- maximum	18.9-30.3	19.1-33.3	18.9-33.3
Ethnic origin, N (%)			
Caucasian	27 (96.4%)	30 (96.8%)	57 (96.6%)
Other	1 (3.6%)	1 (3.2%)	2 (3.4%)

SD: Standard deviation
N: Number of subjects

Demography of Trial Population - Continued

Demographics and Baseline Characteristics, Post-amendment (FAS)

	Group 1	Group 2	FAS
Number of subjects	201	199	400
Age (years)			
Mean (SD)	37.9 (11.1)	35.0 (12.2)	36.5 (11.7)
Median	38.0	34.0	36.0
Minimum-maximum	18-66	18-65	18-66
Gender, N (%)			
Male	97 (48.3%)	105 (52.8%)	202 (50.5%)
Female	104 (51.7%)	94 (47.2%)	198 (49.5%)
Ethnic origin, N (%)			
Caucasian	199 (99.0%)	195 (98.0%)	394 (98.5%)
Other	2 (1.0%)	4 (2.0%)	6 (1.5%)
History of rhinoconjunctivitis, N (%)			
Yes	201 (100.0%)	199 (100.0%)	400 (100.0%)
No	-	-	-
Duration of rhinoconjunctivitis (years)*			
Mean (SD)	16.8 (11.4)	15.9 (10.9)	16.4 (11.1)
Median	14.0	13.0	14.0
Minimum-maximum	2-50	2-54	2-54
History of bronchial asthma, N (%)			
Yes	65 (32.3%)	45 (22.6%)	110 (27.5%)
No	136 (67.7%)	154 (77.4%)	290 (72.5%)
Duration of bronchial asthma (years)#			
Mean (SD)	12.5 (11.2)	10.1 (8.5)	11.5 (10.2)
Median	10.0	8.0	9.0
Minimum-maximum	0-46	1-29	0-46

*: Data from one subject in Group 1 is missing
#: Data from one subject in Group 2 is missing
SD: Standard deviation
N: Number of subjects

Pharmacodynamic Results

The pharmacodynamic results are based on data from subjects who initiated treatment post-amendment. The pharmacodynamic results from the subjects who initiated treatment pre-amendment are not suitable for any conclusion due to the change in dosing schedules implemented in the protocol amendment and due to the required sample size.

Primary Pharmacodynamic Endpoint

- A statistically significant increase of IgE-blocking factor from baseline to follow-up (one week after the last injection) was observed for both dosing schedules ($p<0.0001$). The estimated mean was 0.32 for Group 1 and 0.36 for Group 2. The difference between Group 1 and Group 2 was not statistically significantly different from zero ($p=0.0590$)
- Equivalence between the two dosing schedules could not be confirmed. The 95% confidence limit for the mean difference in change from baseline between the dosing schedules was [-0.073; 0.001] and thus not fully contained within the equivalence interval of [-0.03; 0.03]

Secondary Pharmacodynamic Endpoints

- The level of IgG_4 increased from baseline to follow-up (one week after the last injection) for both dosing schedules and was statistically significant ($p<0.0001$). The geometric mean of the relevant increase was 4.8 for Group 1 and 5.6 for Group 2. The observed difference between the two dosing schedules was not statistically significant ($p=0.1068$)
- The level of IgE increased from baseline to follow-up (one week after the last injection) for both dosing schedules and was statistically significant ($p<0.0001$). The geometric mean of the relevant increase was 2.1 for Group 1 and 2.8 for Group 2. The difference between the two dosing schedules was statistically significant ($p=0.0012$)

Safety Results

- The two dosing schedules initially used in the trial caused a significant number of delayed systemic reactions and modifications of the protocol with respect to the updosing schedules and the time interval between injections were considered necessary
- Two modified dosing schedules were used for the remaining part of the trial and the following safety conclusions could be drawn:
  - The majority of the adverse events were mild or moderate in severity and occurred during the updosing period; 58% were mild and 38% moderate in Group 1 and 66% were mild and 29% moderate in Group 2. A total of 57 adverse events (5%) were considered severe – these were equally distributed between the two dosing schedules
  - 79% of the adverse events were considered related to the IMP corresponding to 76% of the adverse events reported in Group 1 and 82% of the events reported in Group 2
  - The most frequently reported adverse events (related to IMP) for both dosing schedules were events related to the injection site, the respiratory system, the skin and the eyes with injection site swelling being the most frequently reported adverse event
  - Systemic reactions occurred in 21% of the subjects in Group 1 and in 33% of subjects in Group 2. The majority of the systemic reactions occurred within 1-24 hours after injections (i.e. delayed reactions) and the most frequently reported systemic reactions were for both dosing schedules unspecific symptoms, rhinoconjunctivits and urticaria
  - 3 subjects treated according to the revised dosing schedules experienced 14 serious adverse events: 2 subjects with a total of 3 events in Group 1 (all judged to be unlikely related to IMP) and 1 subject with a total of 11 events in Group 2 (all judged to be probably related to IMP)
  - 27 subjects (7%) treated according to the revised updosing schedules withdrew due to a total of 82 adverse events: 9 subjects (4%) in Group 1 and 18 subjects (9%) in Group 2. Approximately half of the events leading to withdrawal occurred after injection with the 3,000 SQ-U dose
  - A global assessment of the tolerability of treatment showed that tolerability was judged to be good or very good by 94% of subjects in Group 1 and by 86% of subjects in Group 2. The global assessment of tolerability judged by the investigator showed a similar result
  - No safety concerns were found for clinical safety laboratory tests, FEV_1, vital signs, and physical examination

Conclusions

- Initially, two dosing schedules of ALK-Flex SQ 4-grass + rye with 3 updosing steps and injections either daily or every 3-4 days were to be investigated. These dosing schedules were shortly after initiation of the trial found not to provide an acceptable tolerability profile and the dosing schedules were changed to include 5 updosing steps and injections either weekly or every 3-4 days
- The response in the IgE-blocking factor was not demonstrated to be equivalent with respect to the pre-specified equivalence interval of ± 0.03 between the two dosing schedules with 5 updosing steps either weekly or every 3-4 days
- Both dosing schedules induced statistically significant increases in the level of IgE-blocking factor, IgG_4 and IgE. The increases were of similar sizes
- The change in dosing schedules from 3-step updosing to 5-step updosing resulted in a tolerability profile with fewer delayed systemic reactions and fewer subjects withdrew due to adverse events. The dosing schedule with 5 updosing steps with weekly injections generally caused less adverse events (local reactions, early and delayed systemic reactions as well as adverse event withdrawals) than the dosing schedule with 5 updosing steps and injections every 3-4 days
- Overall, the updosing schedule including 5 injections (300 SQ-U, 600 SQ-U, 3,000 SQ-U, 6,000 SQ-U and 15,000 SQ-U) given once weekly demonstrated an acceptable tolerability profile and did also provide a statistically significant immunological response

Date of the Report

Final, 18 August 2010

This trial was conducted in compliance with the principles of ICH Good Clinical Practice.