A Randomized, Double-Blind, Placebo-Controlled, Dose-finding Study to Evaluate the Efficacy and Safety of Aerosolized Moli1901 in Adolescents (12 Years of Age or Older) and Adults with Cystic Fibrosis

Clinical Study Report

Study Code

Moli1901-010B

Sponsor

AOP Orphan Pharmaceuticals AG

Information has been removed according to **EU GDPR**

Investigator

[redacted]

Clinical Study Report according to ICH E3 and ICH E9

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1. TITLE PAGE

A Randomized, Double-Blind, Placebo-Controlled, Dose-finding Study to Evaluate the Efficacy and Safety of Aerosolized Moli1901 in Adolescents (12 Years of Age or Older) and Adults with Cystic Fibrosis

Title:	A Randomized, Double-Blind, Placebo-Controlled, Dose-finding Study to Evaluate the Efficacy and Safety of Aerosolized Moli1901 in Adolescents (12 Years of Age or Older) and Adults with Cystic Fibrosis
Name of test medication:	Moli1901
Indication studied:	Cystic fibrosis in Adolescents (12 Years of Age or Older) and Adults with Cystic Fibrosis
Design:	Randomized, double blind, placebo-controlled dose finding study with 3 different dosage regimes of Moli1901
Name of the sponsor:	AOP Orphan Pharmaceuticals AG
Protocol:	Protocol Moli1901-010B final V 1.1, Dec. 13th, 2006 including Amendments 1-2-3-4-5 and non-significant Amendment A Nov. 4th, 2008
Phase of development:	Phase II dose finding study
Studied period:	Observation for 8 weeks (double blind treatment phase) with follow-up 4 weeks later
Coordinating investigator:	[redacted]
Name of sponsor signatory:	Dr. Rudolf Widmann, CEO
Date of report:	24.10.2012

This study was performed according to the principles of the current edition of the Declaration of Helsinki, according to the German drug law (AMG), and according to Good Clinical Practice (GCP), including the archiving of essentials documents.

2. SYNOPSIS

Name of the Sponsor/Company: AOP Orphan Pharmaceuticals AG **Name of the finished product:** Moli 1901 **Name of the active ingredient:** Polycyclic peptide of 19 amino acids, obtained by fermentation of *Streptomyces cinnamoneous*	
Title of the Study:	A Randomized, Double-Blind, Placebo-Controlled, Dose-finding Study to Evaluate the Efficacy and Safety of Aerosolized Moli1901 in Adolescents (12 Years of Age or Older) and Adults with Cystic Fibrosis
Protocol Number:	Moli1901-010B
Investigator:	[redacted]
Studied period:	Observation for 8 weeks (double blind treatment phase) with follow-up 4 weeks later. First Patient in : October 16th, 2007 Last Patient out: July 15th, 2009
Phase of development:	Phase II dose finding study
Objectives:	This is a dose-finding study for the drug Moli1901 in the exploratory phase IIb. The study objective is to establish minimum effective dose (MED), optimal dose, and maximum safe dose (MSD). Additionally, the tolerability of Moli1901 shall be investigated.
Methodology:	Study Moli1901-010B was performed as a multi center, parallel group, placebo controlled, double-blind, phase IIb efficacy and safety evaluation of three different dosage schedules of aerosolized Moli1901 in adolescents (12 years of age or older), and adults. The study started with a screening period (visit 1) followed by an 8 weeks double blind comparative treatment period (visit 2-7). Thereafter, subjects were observed for additional 4 weeks without treatment.
Number of patients planned:	The study was planned with a sample size of 40 patients per group resulting in 160 patients in total.
Number of patients analyzed:	Safety Population: 161 Patients ITT Population : 157 Patients PP Population: 136 Patients
Diagnosis and main criteria for inclusion:	Indication studied is cystic fibrosis in adolescents (12 years of age or older) and adults.

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	Main inclusion criteria were: 1. Male or female subjects of 12 years of age or older 2. Body mass index equal or above -2 SDS (Standard Deviation Score-According to CDC standards) for 12-19 year old patients and ≥18.5 kg/m^2 for adults 20 years old and older 3. Confirmed diagnosis of cystic fibrosis: Positive sweat chloride value ≥ 60 mEq/l by quantitative pilocarpine iontophoresis (on at least 2 occasions, if no genotype was determined) *and/or* Genotype with two identifiable mutations consistent with CF, accompanied by one or more clinical features consistent with the CF phenotype. Measurement of nasal potential difference could be considered as an additional diagnostic tool, if results of sweat chloride test and genotype test were not unambiguous. Nasal potential difference test could not replace any of the above tests. 4. Screening FEV_1 between 50% and 100% of predicted. 5. Oxygen saturation level measured by pulse oximetry (SpO_2) > 90 % on room air.
Test product, reference product, dose and mode of administration, batch number:	Test product: Moli 1901adminsitered per inhalation Dosage regimen: Patients were allocated to one of the four treatment arms with single inhalations once daily of either Moli1901 or placebo according to the following dosage schedule for 8 weeks: **1.** 2,5 mg/day (0.5 mg/ml; 5 ml) Moli1901, daily **2.** 2,5 mg/day (0.5 mg/ml; 5 ml) Moli1901, every other day **3.** 2,5 mg/day (0.5 mg/ml; 5 ml) Moli1901, twice a week **4.** Placebo Batch numbers are for Moli: 061108, 070420, 070413, 080930. Batch numbers are for Placebo: 061031, 070504, 070620, 071101, 080926.
Duration of treatment:	Treatment duration was 8 weeks.

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CRITERIA FOR EVALUATION	
Efficacy:	**Primary Efficacy Criterion (Confirmatory Evaluation)** • Change in the percentage of the predicted FEV_1 (forced expiratory volume in first second) value **Co-Primary Criterion (Supportive Evaluation)** • The ensemble of two subject reported clinical scores, which are physical and respiratory dimensions of the self-report, disease-specific quality of life test, the cystic fibrosis questionnaire in the revised version. **Secondary Efficacy Criteria** • Change in the percentage of the predicted FVC (forced vital capacity) • Change in the percentage of the predicted FEF_{25-75} (forced expiratory flow 25–75% of FVC) • Change in the absolute values of FEV_1 (forced expiratory volume in first second) • Number of pulmonary exacerbations • Number of hospitalization days due to pulmonary exacerbation • Duration of therapy needed during study participation to treat bronchial obstruction, infection or inflammation (i.e. antibiotics, mucolytics, anti-inflammatory drugs). • Time to first pulmonary exacerbation • Change in oxygen saturation as measured with pulse oximetry • Quality of life questionnaire (cystic fibrosis questionnaire-revised)
Safety:	• Complete physical examination • Lung auscultation • Vital signs • ECG • Spirometry (FEV_1, FVC, and FEF_{25-75}) • Clinical laboratory tests (chemistry, hematology) • Adverse events • Concomitant medications • Change in microbiologic markers

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	• Pulmonary exacerbations
Statistical methods:	The definition of the optimal target dose is based on the objective measurement of FEV % predicted. The analysis of the co-primary criterion is expected to give supportive information for a second level of the burden of disease. This means that the analysis of the primary criterion is performed with the full alpha level and the results may be regarded as confirmatory. The co-primary criterion is also analyzed with full alpha, but the results should be interpreted as supportive. There are 4 treatment groups: placebo and three dose groups with the active substance to be studied. Confirmatory testing for determining the minimum effective dose will be done using two-group comparisons with the placebo group in a pre-specified order: • Starting with the high dose group • Followed by the intermediate dose group • And then followed by the low dose group. All three group comparisons are performed with full level alpha, all with full control of the experimentwise level alpha as long as the order of testing is adhered to and tests are confirmatory proof if the preliminary test has given a significant result. (See for instance, Bauer, et al, [1998], Hothorn and Hauschke [2000], Hauschke and Hothorn [2003], Bretz, Hothorn, and Hsu [2003]). The level of significance is defined as 0.025, one-sided. The primary analysis is the sequence of three tests for FEV_1 % predicted, expressed as change from baseline. With the Mann-Whitney measure of superiority as an effect size measure, the hypothesis setup for the one-sided test for superiority may be formulated as follows: H0: $MW_{TP} \leq 0.5$ HA: $MW_{TP} > 0.5$ (T = test and P = placebo, MW = Mann-Whitney Estimator The minimum effective dose (MED) is defined as the smallest dose that shows clinically relevant and statistically significant effect (Ruberg [1995]). P-values are calculated to determine statistical

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	significance. For the determination of relevance, the lower bound of the confidence interval is calculated with the Wilcoxon-Mann-Whitney test. Well-known benchmarks for relevance are (Colditz et al, 1985): MW = 0.50 equality MW = 0.56 small superiority MW = 0.64 medium-sized, relevant superiority MW = 0.71 large superiority Because a rapid and continuing effect is expected of the test drug, the series of observations over all points in time during the treatment period will be tested with one powerful global directional test using the multivariate generalization of the Wilcoxon-Mann-Whitney test as proposed by Wei and Lachin (Wei and Lachin [1984], Thall and Lachin [1988], Lachin [1992], Rosenberger and Lachin [1995]). All tests will be performed one-sided with alpha = 0.025.
SUMMARY – CONCLUSIONS **Efficacy results:**	The confirmatory evaluation was based on the criterion FEV_1 (percent predicted) to be analysed in the ITT population. A combined test including all follow-up visits of the treatment phase was performed (Wei Lachin procedure). For the comparison Moli HD versus placebo the combined result was MW = 0.4924 (CI-LB = 0.3897; P = 0.5573) expressing no difference between Moli HD and placebo in the study patients. Thus, the study cannot provide confirmatory proof of superiority of Moli HD. The comparison Moli ID versus placebo showed a combined result of MW = 0.5036 (CI-LB = 0.4086; P = 0.4704), and for the Moli LD group a combined result of MW = 0.4980 (CI-LB = 0.4087; P = 0.5171) was seen. In these groups there also is no indication for superiority of the Moli treatment. As a consequence, no optimal dose could be determined. The finding in the PP population does not differ substantially from the result of the ITT analysis. In all evaluations, very similar results were found for the criterion FEV_1 expressed in absolute values.

Name of the Sponsor/Company: AOP Orphan Pharmaceuticals AG **Name of the finished product:** Moli 1901 **Name of the active ingredient:** Polycyclic peptide of 19 amino acids, obtained by fermentation of *Streptomyces cinnamoneous*	From the CFQ-R, the respiratory scale and the physical scale were chosen as co-primary criteria, as these scales may be regarded as the clinical counterpart of lung function. In this study the results for these domains were as follows: For physical and respiratory scale, the combined result including all follow-up visits was MW = 0.5051 (CI-LB = 0.4270; P = 0.4488) for the comparison moli HD versus placebo. The comparison for Moli ID versus placebo resulted in MW = 0.5134 (CI-LB = 0.4354; P = 0.3684) and the finding for Moli LD versus placebo was MW = 0.5468 (CI-LB = 0.4693; P = 0.1183). Neither for the individual results of the physical scale nor for the individual results of the respiratory scale at any of the follow-up visits a clear indication for superiority of any of the active treatment groups could be found. More favourable results are found when considering the sum score of all CFQ-R scales: At all visits, the Mann-Whitney estimators indicate superiority of the Moli HD group. The effects vary from very small (visit 7: MW = 0.5295; CI-LB = 0.3981) to medium-sized (visit 5: MW = 0.6435; CI-LB = 0.5112). The combined result still shows a small superiority (MW = 0.5890; CI-LB = 0.4794) and misses statistical significance in a descriptive sense only by a small margin. Also for the Moli ID group, the Mann-Whitney estimators indicate superiority at all visits. The effects are small and vary from MW = 0.5495 (CI-LB = 0.4218) at visit 6 to MW = 0.5711 (CI-LB = 0.4419) at visit 4. The combined result also shows a small superiority (MW = 0.5557; CI-LB = 0.4516). For the Moli LD group, the Mann-Whitney estimators indicate superiority at all visits but visit 5 (MW = 0.4721; CI-LB = 0.3407 describing a very small inferiority). At the other visits the effects vary from MW = 0.5226 (CI-LB = 0.4218) at visit 7 to MW = 0.6462 (CI-LB = 0.4419) at visit 4. The combined result also shows a small superiority (MW = 0.5479; CI-LB = 0.4421). Additional criteria related to lung function were FVC (prc pred), FEF_{25-75} (prc pred) and blood oxygen saturation as measured by pulse oximetry.

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	The results of the global test for FVC (prc pred) are: For Moli HD and Moli ID the combined tests show small inferiority to placebo (Moli HD: MW = 0.4184, CI-LB = 0.3080, P = 0.9265 / Moli ID: MW = 0.4290, CI-LB = 0.3313, P = 0.9229). This inferiority also goes true for all individual visits, at some of them there is an even more than small inferiority to placebo. The low dose group constantly shows practically no difference compared to placebo, the global test results in MW = 0.5353 (CI-LB = 0.4437; P = 0.2252). The findings for Moli HD and Moli ID allow the conclusion that almost with certainty Moli will not be able to improve FVC (prc pred) within 8 weeks (P-values are much larger than 0.5!). In contrast, it should be discussed if there is a possibility that Moli HD and Moli ID exert a negative effect on FVC that does no longer occur when the dose is reduced to the level of the Moli LD group. With regard to FEF_{25-75}, none of the result of the combined tests shows hints for superiority. The same goes true for the results at the individual visits. There is no indication for inferiority of any of the active treatment groups. Considering pulse oximetry there is some indication for a small superiority of the verum groups with Mann Whitney estimators for the combined result within the range of very small superiority (Moli ID: MW = 0.5290, CI-LB = 0.4325, P = 0.2778) to small superiority (MW = 0.5855, CI-LB 0.4857, P = 0.0465). The tests for the individual visits show similar results. There is no clear evidence for differences between the different doses. Pulmonary exacerbations were evaluated under 3 different aspects: Time to 1st pulmonary exacerbation, patients with pulmonary exacerbation including the number of exacerbations as score, and patients with at least 1 pulmonary exacerbation. None of the analyses indicated a superiority of one of the active treatment groups. It should be noted that pulmonary exacerbation is a very poorly defined criterion. The symptoms stated on the adverse event reports defined as pulmonary exacerbation indicate that investigators may have differing points of view on what to consider as a pulmonary exacerbation, also with regard to both, symptoms and severity of symptoms. These circumstances preclude the detection of treatment differences unless unusually large. Also the evaluation of new antibiotic treatment and new anti-

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inflammatory treatment showed no indication for superiority of any of the active treatment groups. As with pulmonary exacerbation, also this criterion is not well defined. For example, oral ibuprofen and inhaled corticosteroid both fulfil the criterion anti-inflammatory treatment and are included in the cumulation of treatment days although both treatments are barely comparable. Again, these circumstances preclude the detection of treatment differences unless unusually large. Additionally, the number of such events was quite small.

There is some indication, albeit weak, that a treatment effect might be more pronounced in youths. At week 8, the WMW tests resulted in small to medium superiorities for all active treatment groups with regard to the criterion FEV_1 (prc pred). One may argue that this should at least be partly attributed to a small deterioration in the placebo group (which probably is caused by chance). Yet, very similar results are found for FEV_1 (absolute) and for this criterion the placebo groups shows practically no deterioration.

For the Moli HD group the WMW test based on the change in FEV_1 (absolute values, week 8 versus baseline) resulted in MW = 0.6050, CI-LB = 0.4265. There is also a tendency towards improvement in the Moli ID group (MW = 0.6270, CI-LB = 0.4513) and in the Moli LD group (MW = 0.5698, CI-LB = 0.3890). Yet, there is no indication for a correlation between age and treatment effect based on FEV1 (prc pred), with r = – 0.04. Additional tests with inclusion of youths only were performed calculating the combined results of the criteria FEV_1 (prc pred), FEV_1 (absolute), CFQ-R respiratory scale and CFQ-R physical scale. The global test including all 4 criteria results in small superiorities for Moli HD (MW = 05539; CI-LB = 0.4242) and Moli ID (MW = 0.5764; CI-LB = 0.4578). For Moli LD a very small superiority was found (MW = 0.5445; CI-LB = 0.4084).

Other aspects speaking in favour of including youths only are: The study will have the more power to detect a positive result the less inhomogeneities among study patients occur (note that also the age adjusted results were slightly better than the unadjusted results, see 15.4.3.1 on page 188). This statistical rule speaks in favour of deciding for one age group only. From the clinical point of view it may well be that the treatment effect is the better the earlier treatment starts. All these aspects speak in favour of a study including youths only, even if this cannot be corroborated beyond doubt by the results

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	of the study at hand. There is weak indication that it might also be advisable to exclude patients with FEV1 > 85 %.
Safety results:	The evaluation of adverse events that were considered to be related to treatment (causality categories "possible" to "certain") showed strong indication that patients treated with any of the active medications are more likely to suffer adverse events than patients in the placebo group. Findings that were reported to occur more often in the active treatment groups than in the placebo group describe conditions that are quite typical in cystic fibrosis patients. Considering these events as possible untoward treatment effects would lead to the hypothesis that the active treatments aggravate the condition instead of having a beneficial effect or no effect at all. Very high sample sizes would be required to clarify this based on adverse event analyses. It cannot be excluded for certain that Moli HD group might suffer more often from headache than patients in the other active treatment groups or in the placebo group. The vast majority of the events were described to be of mild to moderate nature. The evaluation of serious and of alerting adverse events provided no indication for group differences. Noteworthy findings in laboratory evaluation were: The sign tests for haemoglobin showed a descriptively significant tendency towards decrease in the Moli HD group at week 4 (increases: 8; no change: 2; decreases: 22; P = 0.0161) and at week 8 (increases: 8; no change: 3; decreases: 21; P = 0.0241). The median percentage changes were small (week 4: Moli HD - 2.0 %; placebo 0.4 % / week 8: Moli HD -1.6, placebo -0.7). With regard to potassium, the sign tests showed a descriptively significant tendency towards decrease (increases: 7; no change: 3; decreases: 22; P = 0.0081) in the Moli HD group at week 8. A similar result was found for the Moli LD group (increases: 8; no change: 4;

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	decreases: 26; P = 0.0029) at the same time. In the Moli LD group, there was also a significant result of the sign test at the follow-up (increases: 9; no change: 3; decreases: 26; P = 0.0060). Note that there were no relevant changes in the Moli ID group. Larger studies are needed to gain more reliable safety information. The evaluations of ECG, lung auscultation, lung function and pulse oximetry showed no suspicious findings.
Conclusion	In summary, some of the evaluations provide hints for efficacy of Moli. Yet, most of these positive effects observed in the study patients are small and it is not possible to separate them clearly from chance. This goes true for all evaluations, as they all are subject to the problem of the small sample size. At the same time, the study does also not provide enough indication for ineffectiveness of Moli, as absence of evidence must not be interpreted as evidence of absence. Further recommendations for future studies are: The observation period should be extended, as it seems as if at least there is no rapid onset of a treatment effect. Assuming that the benefit will increase in the course of time it will also be the easier to detect treatment differences the longer the patients are treated. There is some indication that for a proof of concept study or a dose finding study it might be helpful to include patients between 12 and 17 years of age only, and to exclude patients with FEV_1(prc pred) > 85 %. Pulmonary exacerbation is not a suitable criterion unless better defined. Cumulative days of treatment will not be suitable to detect group differences unless precisely defined and observed in long-term studies of at least ½ to 1 year duration.