Study protocol and statistical analysis plan

Measurement of breathing parameters of asthma and COPD patients with different degrees of disease severity for the computer simulation of lung deposition of aerosol drugs

Study short name: **CHOICE**

Non-interventional, observational study

Sponsor: Medisol Development Kft.

Study protocol ID: TBEP-2110/01
version: 2021 V2

February 1, 2022

Initiator and scientific supercisor: Dr. Gabriella Gálffy PhD, director-general
Workplace: County Institute of Pulmonology, Törökbálint
2045 Törökbálint, Munkácsy Mihály utca 70
Phone: +36-23-511570/121
Mobile: 06 30 7005 688
E-mail: kutatasititkarsag@torokbalintkorhaz.hu

Supervisor of numerical simulations: Dr. Árpád Farkas PhD, head of department
Workplace: Centre for Energy Research, Environmental Physics Department
1121 Budapest Konkoly-Thege Miklós út 29-33
Phone: +3613922222/3404
Mobile: +36 20 356 4255
E-mail: farkas.arpad@ek-cer.hu

Sponsor: Medisol Development Kft
1044 Budapest, Ezred utca 7. 2 ép. fszt 4
Fiscal code: 28820835-2-19
Mobile: +36 30 399 8022
E-mail: czaun.peter@gmail.com

Contact person: Dr. Árpád Farkas PhD
Mobile: +36 20 356 4255
E-mail: farkasar@gmail.com

I. Study Protocol

I.1. The purpose of the study (research objective)

Primary aim

- Computer modelling of the lung deposition of aerosol drugs based on realistic individual breathing patterns to be measured within this study.*

* given that there are currently several innovative and generic products that are available for the treatment of patients with asthma and COPD, however not all products have the necessary data for computer modelling provided by companies or retrievable in the open literature, hence we are only modelling for products that have input data on drug aerodynamic characteristics.

Secondary goals

- Examination of the correlation between the lung deposition efficiency (the dose depositing in the lungs divided by the dose metered in the inhalation device) and the patients current health status.
- Evaluation of patients' inhalation device usage and suggestions for optimal usage according to the acquired breathing breathing data and calculated lung deposition values.

I.2. Characteristics of patients to be included in the study (inclusion and exclusion criteria)

Inclusion criteria

□ diagnosed obstructive lung disease (asthma or COPD)
□ proper usage of inhalation tool after being educated
□ availability of whole body plethysmography measurement data from the last 6 monts or the possibility to perform them before the start of the study (the most important parameter is reserve volume, RV)
□ subject under outpatient/inpatient therapy
□ over 18 years of age
□ capable of acting and cooperating

Exclusion criteria

□ the patient doesn't align with any of the criteria mentioned above
□ the patient is incapable of filling out the questionnaire/questionnaires' parts that are accorded to him/her
□ the patient doesn't agree to have data collected of him/her
□ diagnosed heavy, not treated chronic illness
□ not properly carried out /not evaluable lung function (spirometry) examination

I.3. Sampling and recruiting principles

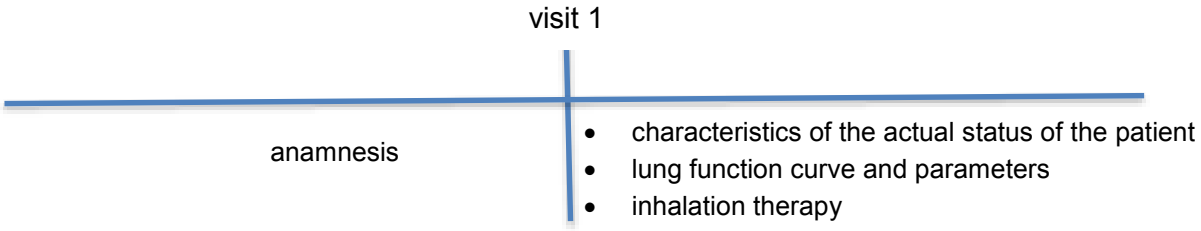
Every involved medical professional should opt for randomness in sampling. This includes the selection process but also the device order. We are involving examiners

for our test from different levels of healthcare servicing (university centre, regional institute, county hospital, panel clinic) aiming to ensure that the sampling venues (centrum related) to minimize centre specific effects.

Participation of patients is on a volunteer basis. Patient involvement in ambulant patient care occurs strictly in the time frame when the examination would take anyway place, no special visit (only for the study) will be needed. Only patients with proven records of COPD or asthma can be involved. In principle, in every centrum anyone who shows up for examination with asthma or COPD and is willing to cooperate can participate, given that it passes the inclusion criteria and no exclusion criterion applies to him/her. Children under 18 cannot be involved in the examination. There is no upper age constraint. There are no restrictions on gender or ethnicity. Minimum number of patients planned is 200.

I.4. The examination

Patient involvement will take place after the possibly emerging questions of the subject are answered and the patient is told about the whole goal of the examination and all of its details, has read and signed the leaflet and the patient-consent statement. After that the data of illnesses are obtainable according to the daily routine which are present suitably during the ambulant daily routine examination anyways.



The study not involving intervention in the care and treatment of patients consists of only one visit. Within this, as indicated on the data sheet, answers to questions in accordance with standard medical practice and individual respiratory function profile are recorded. The latter takes place only if the doctor has planned lung function measurement independently of the study.

I.5. Actions to be undertaken during the visit

The table below summarizes the data collection during the visit. The patients can only be involved and data can be registered only if these data would have anyway been obtained through a regular visit. In addition to the patient data, the centrums' data will be registered as well in the eCRF system, as complementary data helping the evaluation of the examination.

Patients' data collection	**visit 1 (patient involvement)**
Time	0^{th} day
Evaluation of the inclusion and exclusion criteria	X
Comprehensive informing of the patient	X
Signing of patient information leaflet and patient consent sheet	X
Recording of patient demographic data	X
Recording of the anamnesis	X
Recording of diagnostic	X
Recording of actual status	X
Smoking habit	X
Applied drug therapy, dose	X
Education on the use of inhalers	X
Whole body plethysmography measurement, only if it was not performed in the last 6 months (recording of RV and FVC)	X
Recording of inhalation time and breath-holding time.	X
Registration of eventual side effects or unexpected events.	X

I.6. Beginning and duration of the examination

The first patient can be involved if the Scientific Council for Health Scientific and Research Ethics Committee approves and afterwards the National Institute of Pharmacy and Food Health (OGYÉI) grants permission. In the first part of the study all the input data necessary for numerical modelling will be obtained.

Beginning date: 2022.08.01
End date: 2025.06.30

I.7. Legal background

The legal frame is provided by:

- The European Commission 520/2012/EU implementing regulation (Annex II, paragraph 38), the 726/2004/EK Regulation of the European Parliament and of the Council and the 2001/83/EK implementation of pharmacovigilance activities provided for in the Directive of the European Parliament and of the Council
- XCV. law from 2005 on medicinal products for human use and amending other laws governing the pharmaceutical market
- CLIV. Law on Health Care from 1997
- 235/2009. (X. 20.) Government regulation of medical research in humans, clinical examination of test artefacts for human use, and on the regulations of enabling the use of medical tools intended to be used for clinical examinations in humans.
- 23/2002. (V. 9.) EüM Regulation on medical research on humans.
- 15/2012. (VIII. 22.) EMMI Regulation on pharmacovigilance of medicinal products for human use.

I.8. Data protection, publishing principles, IPD share plan

The recorded personal information during the study is confidential. In all respects, the regulation of non-interventional examinations should be taken into account when collecting data (23/2002 EüM regulation), according to the year 2011. CXII. Law on the right to information self-determination, freedom of information, as well as the European Parliament and council (EU) 679/2016 General Data Protection Regulation.

The publication of the results of the study is likely to be useful information for pulmonologists, primary care workers, and also for specialists working in other fields, but its research value also manifests itself as research development potential. It can help to recognize the connections between the general condition of the patient, his drug use effectiveness, and the planning of later, prospective studies. It can also serve as a basis for more extensive studies. The head of the research shall decide on the preparation and publication of the communication on results as well as the naming of co-authors

In the case of an appropriate sample number (at least 30 patients per medicine and device) it is also possible to conduct interim analysis and publish the results of the study. Individual lung function results allow individual (personalized) modelling of drug deposition to be performed. The test can also be considered successful if statistically representative groups are not able to be formed. In this case, we perform modelling for individual measurements without aggregate results.

Individual Patient Data Share Plan

The data sharing plan contains statements on the availability of individual participant data (including data dictionaries), information on what data in particlular will be shared, what other documents will be available, when will data be available, with whom, for what type of analyses. In addition, the plan specifies the mechanism the data are be made available.

According to the plan those IPD will be available that underlie the results published in research article(s) will be shared after deidentification.

The frame of IPD sharing is defined below:

Supporting information: Study Protocol

Time frame: Between months 9 and 36 after the publication of article(s).

Access criteria: IPD will be shared with investigators whose proposed use of the data has been approved by an independent review committee identified for this purpose. IPD will be shared to achieve aims in the approved proposal. Proposal may be submitted to farkasar@gmail.com.

URL: https://data.mendeley.com

I.9. Exploitation of results

The research team (all experts included in this research) is looking forward to making the results public in the form of presentations in national and international congresses and publicizing it even if the series of measurements does not end with a positive result that can be evaluated for the endpoints. Moreover, if the results show that the deposition results based on individual breathing patterns and the aerodynamic characteristics of different drug particles differ significantly among each other, then they will be used in research aiming at the optimisation and personalization of inhaler choice and aerosol drug therapy.

I.10. Recording and reporting of side effects

The head of research shall be responsible for reporting any undesirable drug effects detected by all researchers in connection with the study or in connection with the use of an inhaled medicine in connection with the study, and for complying with the relevant regulatory requirements in the field.

Experienced side effects are reported individually by the researchers towards OGYÉI, and they shall immediately be noted to the head of research and the sponsor. The fact of side effects is also recorded on the test sheets on a mandatory basis, which can be checked by the sponsor and the head of research.

The head of research takes responsibility to report the adverse reactions, side effects and other specific cases (hereinafter referred to collectively as "medicinal product safety information") experienced during the entire period of the non-interventional observational study and during the processing of the results as follows:.

Side effect (adverse drug reaction): harmful and unwanted effects caused by medicines**.** According to the perceptive doctor, the side effect may be causal to the use of the medicine. In addition to the harmful and unwanted effects resulting from the use under the marketing authorisation during the normal administration of medicinal products, adverse unwanted effects resulting from the pharmaceutical error and use not covered by the marketing authorisation shall also be considered as a side effect, including improper use and abuse of the medicinal product.

Unexpected side effect: a side effect which is not included in the prescription for use of the medicinal product or the frequency, nature, severity or outcome does not correspond to the side effect listed in the prescription for use.

Serious side effect: a side effect that endangers life, requires or prolongs hospitalization, causes permanent or significant damage to health, disability, congenital abnormalities, birth defects or death. It is medically significant, i.e. it does not meet any of the severity criteria listed above, but without intervention it leads to the appearance of one of the criteria.

Special cases, which shall also be reported to the sponsor if there are no side effects associated with the case:

- use of medicinal products during pregnancy
- use of medicinal products during breastfeeding
- overdose (either intentionally or accidental)
- ineffectiveness
- incorrect application
- medication error
- quality defect
- drug interactions
- paternal exposure
- misuse of medicinal products
- occupational harm to medicinal products
- transmission of an infectious agent by medicinal product
- off label (out-of-indication) drug application

The above-described information on the safety of medicines, whether or not there have been any side effects in connection with the specific case, or whether the side effect is well known or unexpected, or serious or not serious, shall be forwarded to the head of research and sponsor within 24 hours of the date on which the person concerned became aware. Study participants can also report side effects to the National Institute of Pharmacy through the contact details provided on the Institute's official website.

To avoid unnecessary administration and duplication, it is important that researchers report side effects only on one reporting route. The reports received by the head of research and sponsor will be forwarded/notified to the competent authorities in accordance with the legal requirements.

I.11. Authorisation

The document describing the study (with all its annexes, patient information, patient consent statement) is to be approved by the National Institute of Pharmacy and Nutrition (OGYÉI). The research plan is evaluated from an ethical point of view by the Scientific and Research Ethics Committee of the Medical Research Council (ETT TUKEB).

Bibliography

1. Global Initiative for asthma (GINA) - https://ginasthma.org
2. Global Initiative for Chronic Obstructive Lung Disease (GOLD) - https://goldcopd.org
3. Korányi Évkönyv - https://szakmai.koranyi.hu/bulletin/ -

4. Borgström L, Derom E, Stahl E, Wahlin-Boll E, Pauwels R. The inhalation device influences lung deposition and bronchodilating effect of terbutaline. Am J Respir Crit Care Med 153:1636–1640, 1996.

5. Borghardt JM, Kloft C, Sharma A. Inhaled Therapy in Respiratory Disease: The Complex Interplay of Pulmonary Kinetic Processes. *Can Respir J*. 2018;2018:2732017. Published 2018 Jun 19. doi:10.1155/2018/2732017 6. Busse WW, Brazinsky S, Jacobson K, Stricker W, Schmitt K, Vanden Burgt J, et al. Efficacy response of inhaled beclomethasone dipropionate in asthma is proportional to dose and is improved by formulation with a new propellant. J Allergy Clin Immunol 104: 1215–1222, 1999. 7. Buttini F, Brambilla G, Copelli D, Sisti V, Balducci AG, Bettini R, Pasquali I. Effect of Flow Rate on In Vitro Aerodynamic Performance of NEXThaler(®) in Comparison with Diskus(®) and Turbohaler(®) Dry Powder Inhalers. *Journal of Aerosol Science and Pulmonary Drug Delivery* 29: 167-178, 2015.

8. Corradi M, Chrystyn H, Cosio BG, Pirozynski M, Loukides S, Louis R, Spinola M, Usmani OS. NEXThaler, an innovative dry powder inhaler delivering an extrafine dry powder combination of beclometasone and formoterol to treat large and small airways in asthma. *Expert Opinion in Drug Delivery* 11: 1497-1506, 2014.

9. deBoer AH, Gjaltema D, Hagedoorn P, Frijlink HW. Can 'extrafine' dry powder aerosols improve lung deposition? *European Journal of Pharmaceutics and Biopharmaceutics* 96: 143-151, 2015.

10. Grant AC, Walker R, Hamilton M, Garrill K. The ELLIPTA Dry Powder Inhaler: Design, Functionality, In Vitro Dosing Performance and Critical Task Compliance by Patients and Caregivers. *J Aerosol Med Pulm Drug Deliv* 28: 474-485, 2015. 11. Hamilton M, Leggett R, Pang C, Charles S, Gillett B, Prime D. In Vitro Dosing Performance of the ELLIPTA Dry Powder Inhaler Using Asthma and COPD Patient Inhalation Profiles Replicated with the Electronic Lung (eLungTM). *J Aerosol Med Pulm Drug Deliv* 28: 498-506, 2015.
12. Horváth, Alpár ; Balásházy, Imre ; Sárkány, Zoltán ; Tomisa, Gábor ; Bártfai, Zoltán ; Farkas, Árpád Optimized and individualised inhalation therapy targeted MEDICINA THORACALIS 69 : 2 pp. 76-86. , 11 p. (2016)

13. Horváth A, Farkas Á, Szipőcs A, Tomisa G, Szalai Zs, Gálffy G. Numerical simulation of the effect of inhalation parameters, gender, age, and disease severity on the lung dose of drugs emitted by Turbuhaler®, Breezhaler® and Genuair® in COPD patients. European Journal of Pharmaceutical Sciences 154: 105508, 2020.

14. Horváth A, Balásházy I, Tomisa G, Farkas Á. Significance of breath-hold time in dry powder drug therapy pf COPD patients. European Journal of Pharmaceutical Sciences 104: 145-149, 2017.
15. Jókay Á, Farkas Á, Füri P, Horváth A, Tomisa G, Balásházy I. Computer modelling of airway deposition of Foster® NEXThaler®and Seretide® Diskus®dry powder combination drugs. European Journal of Pharmaceutical Sciences 88: 210-218, 2016. 16. Lewis D. A priori design of metered dose inhalers. *Respiratory*

Drug Delivery Asia 187-197, 2016.

17. Mariotti, F., Sergio, F., Acerbi, D., Meyer, T., Herpich, C. Lung deposition of extrafine dry powder fixed combination beclomethasone dipropionate plus formoterolfumarate via the NEXT DPI® in healthy subjects, asthmatic and COPD
patients. *Eur. Respir J.* 38, 830, 2011.

18. Saeed H, Salem HF, Rabea H, Abdelrahim MEA. Effect of Human Error, Inhalation Flow, and Inhalation Volume on Dose Delivery from Ellipta® Dry-Powder Inhaler. *J Pharma Inno* 14: 239–244, 2019.

19. Zanker D, Ehlich H, Sommerer K, Mariotti F, Riolo D, Cocconi D, Lutero E. Development of a robust technique of radiolabelling beclometasone dipropionate (BDP) with 99mTechnetium in a BDP/Formoterol dry powder inhaler. Poster: Drug Delivery to the Lungs. In: DDL Conferences. Edinburgh, 2011.

II. Statistical analysis plan

The data shall be processed and evaluated using statistical methods adopted in the case of tests not involving intervention.

Single-variable statistical methods: frequency (distribution), relative frequency, average, standard deviation, median, lower and upper quartiles. Multivariate regression analysis is used to analyse primary and secondary endpoints, if necessary.

The statistical analysis plan contains two parts, that is, the description of methods and tools for the processing of measured breathing parameters, and the description of methods and tools for the processing of the calculated deposited doses.

II.1. Processing of the measured breathing data

The analysis of the dependence of PIFdev for each selected device on patient demographic data (age, gender), anthropomorphic characteristics (height, weight), disease condition (expiratory volume at the end of the first second of forced exhalation: FEV1, forced vital capacity: FVC, Tiffeneau index: FEV1/FVC, GOLD grades) and baseline spirometric data characterizing the inhalation (peak inspiratory flow: PIF, inspiratory vital capacity: IVC, inhalation time: tin) is performed by an in-depth statistical evaluation of the interrelationships.

Cross-correlation analysis of the above parameters (except gender, and GOLD grades) in connection with each device is performed.
Correlations between different parameters are expressed by *Pearson coefficients*.
Predictors of PIFdev are looked for by stepwise *multiple regression analysis* using a backward elimination technique.
Mean PIFdev values of different patient subgroups upon gender, age, body mass index (BMI) and disease stages (GOLD grades) are analysed by conducting *two-sample t-tests*. Two age groups are formed. The cut-off age is 65 years. By the same token, two groups are formed upon BMI by using a cut-off value of 25 kg/m2. Two groups are analysed also based on disease severity.
All statistical analyses are performed by the application of *OriginPro 2018* (version b9.5.0.193, OriginLab Corporation, Northampton, Massachusetts, USA) and *Minitab®* (version 18.1, Minitab, Inc., State College, Pennsylvania, USA) software packages. A value of $p < 0.05$ is considered significant throughout the study.

II.1. Processing of the computed lung deposition data

An analysis of the correlation of the computed lung doses with the breathing parameters characterizing the inhalation through the selected devices (Q, tb-h, IV, tin, PIFdev) is performed. The strength of the correlation is expressed in terms of *Pearson coefficients* and any correlation is considered significant when $p<0.05$.

In addition, the correlation of lung deposition with baseline spirometric breathing parameters expressing the breathing status of the patient (expiratory volume at the end of the first second of forced exhalation: FEV1, forced vital capacity: FVC, Tiffeneau index: FEV1/FVC, peak inhalation flow: PIF, peak expiratory flow PEF, inspiratory vital capacity: IVC) is studied. (PIF denotes the peak inhalation flow measured by standard spirometry, while PIFdev is the peak inhalation flow measured while inhaling through the device, PIFdev<PIF).

Mean values of lung doses characteristic of different age, gender and GOLD groups are analysed conducting *two-sample t-tests*. Two age groups are formed. The cutoff age is 65 years. Two groups are analysed also upon the classification of patients based on symptoms and exacerbation history.

All statistical analyses are performed by the application of *OriginPro 2018* (version b9.5.0.193, OriginLab Corporation, Northampton, Massachusetts, USA).