

2. Synopsis

Study Title: A Phase IIb, Multicentre, Randomised, Double-Blind, Placebo-Controlled, Crossover Study to Evaluate the Efficacy and Safety of Dirocaftor/Posenacaftor/Nesolicaftor in Subjects with Cystic Fibrosis Aged 18 Years or Older (CHOICES)
Study Number: HIT-CF-001
Study Phase: IIb
Study Drug: Combination of dirocaftor, posenacaftor, and nesolicaftor (DIR/POS/NES), and matching placebo.
Study Sponsor: University Medical Center Utrecht Department of Paediatric Respiratory Diseases Heidelberglaan 100 3584 CX Utrecht The Netherlands
Study Centres: This study was conducted at 15 centres throughout Europe. The coordinating investigator was C.K. van der Ent.
Publications: A total of 2 abstracts based on the study were published: Destoop M, Bierlaagh MC, Michel S, van der Ent CK. Subject selection plan for CHOICES clinical trial aiming to assess efficacy of new CFTR modulators and to validate the intestinal organoid model. Journal of Cystic Fibrosis 2024; 23S1: S19. Cipriani A, Beekman J, van der Ent K, Charitou P. Safety assessment of the FAIR therapeutics CFTR modulators nesolicaftor, posenacaftor and dirocaftor in CF patients. Journal of Cystic Fibrosis 2025; 24: S62.
Study period: 27 May 2024 to 20 June 2025
Study objectives: The primary objective was: • to evaluate the efficacy of DIR/POS/NES in terms of pulmonary function after 8 weeks compared to placebo in ○ cystic fibrosis (CF) patients with rare cystic fibrosis transmembrane conductance regulator (CFTR) mutations and a high organoid response to DIR/POS/NES ○ CF patients with rare CFTR mutations not pre-selected based on their organoid response.

Secondary objectives were:

- to evaluate the efficacy in terms of sweat chloride concentration, weight, and respiratory symptom severity after 8 weeks of DIR/POS/NES compared to placebo in
 - CF patients with rare CFTR mutations and a high organoid response to DIR/POS/NES
 - CF patients with rare CFTR mutations not pre-selected based on organoid response.

In addition, the study aimed to evaluate the safety and tolerability of DIR/POS/NES in CF patients with rare CFTR mutations, as well as the pharmacokinetics (PK) of DIR/POS/NES and their respective metabolites, when relevant.

Exploratory objectives were:

- to evaluate a possible difference in efficacy of DIR/POS/NES between
 - CF patients with rare CFTR mutations and a high organoid response to DIR/POS/NES and
 - CF patients with rare CFTR mutations not pre-selected based on organoid response
- to evaluate the correlation between participants' measured organoid responses and individual outcomes on efficacy variables.

Methodology:

CHOICES was a randomised, double-blind, placebo-controlled, crossover trial conducted at multiple sites in Europe. Participants had a diagnosis of CF and had completed organoid screening in the HIT-CF organoid study (NTR7520). There were 2 cohorts in the study:

- Pre-selected cohort: planned to consist of 26 participants with the highest organoid response to DIR/POS/NES in the HIT-CF organoid study.
- Randomly-selected cohort: planned to consist of 26 participants without such pre-selection.

The cohort of participants without pre-selection was determined before the selection of the participants with high organoid responses. This allowed the cohort of randomly-selected participants to include participants with a high organoid response.

Eligible participants were randomised at a 1:1 randomisation ratio into 1 of 2 treatment sequences: either DIR/POS/NES once daily for 8 weeks followed by placebo once daily for 8 weeks, or placebo once daily for 8 weeks followed by DIR/POS/NES once daily for 8 weeks. Treatment periods were separated by an 8-week wash-out period.

Before starting the second treatment period, disease stability was assessed by phone to ensure that the participant could start the treatment period. If the participant's health status had changed over the washout period, the washout period could be extended.

Throughout the study, participants visited the study site and were contacted by phone at pre-determined time points to assess safety, efficacy, and PK.

Number of participants (planned and analysed):

A total of 52 participants were planned, 44 were screened, 41 were randomised, and 35 completed the study. The target sample size in the pre-selected cohort was not reached. Due to pre-defined selection criteria, only patients meeting the high-responder criteria were eligible for inclusion in the pre-selected cohort. Within this cohort, patients were ranked from highest to lowest response, and invitations to participate were sent starting with the highest responders. If a patient was ineligible or declined participation, the next patient on the ranked

list was approached. Over time, the pool of patients meeting the high-responder criteria was exhausted, and it was decided not to include additional patients.

Diagnosis and main criteria for inclusion:

Main inclusion criteria:

- Male or female, at least 18 years of age
- Having completed the HIT-CF organoid study
- Having a confirmed diagnosis of CF

Main exclusion criteria:

- Having at least 1 of the following CFTR-mutations: F508del, G551D, G1244E, G1349D, G178R, G551S, S1251N, S1255P, S549N, S549R, R117H, A455E, 3849+10kbC>T
- Having a combination of any 2 of the following mutations: any nonsense mutation, 1717-1G>A, 621+1G>T, 3120+1G>A, 1898+1G->A, CFTRdele2,3, and 2183AA->G

Test product, dose, and mode of administration:

The test products were 300 mg/day DIR, 600 mg/day POS, and 10 mg/day NES. DIR/POS/NES was given as oral capsules.

Duration of treatment:

Participants were treated for 8 weeks with DIR/POS/NES and for 8 weeks with placebo.

Reference therapy, dose, and mode of administration:

Placebo was given as identical oral capsules.

Criteria for evaluation:

The primary endpoint was the average of the percentage predicted forced expiratory volume in 1 s (ppFEV₁) measurements taken after 4, 6, and 8 weeks of treatment.

The secondary endpoints were:

- The average of the sweat chloride measurements taken after 4, 6, and 8 weeks of treatment.
- The average of the body weight measurements taken after 4, 6, and 8 weeks of treatment.
- The average of the cystic fibrosis questionnaire revised (CFQ-R) respiratory domain measurements taken after 4, 6, and 8 weeks of treatment.
- Safety and tolerability assessments based on adverse events (AEs), clinical laboratory tests, physical examinations, electrocardiography (ECG), and vital signs.
- PK parameter estimates and metabolites of DIR/POS/NES derived from plasma.

Exploratory endpoints were based on the combined data from the 2 cohorts:

- Comparison of the treatment effect on efficacy endpoints between CF patients with rare CFTR mutations and a high organoid response to DIR/POS/NES, and a random sample of CF patients with rare CFTR mutations.
- Correlation between organoid response and individual clinical efficacy endpoints.

Statistical methods:

Analysis of primary endpoint:

For each cohort, the treatment effect was estimated using a linear regression model with the average of the 3 post-baseline measurements per period for ppFEV₁ as the dependent

variable, and the treatment indicator, the period indicator, the fixed participant effect, and the period baseline value as independent variables. The treatment effect estimate was presented with the 2-sided 95% confidence interval (CI) and a p-value; α was 0.05.

The primary analysis was based on the intention to treat principle, i.e., including all randomised patients according to their treatment allocation. Data were used regardless of e.g., treatment adherence or use of rescue medication. Reasons for treatment non-adherence and treatment discontinuation were documented.

Missing data were handled using multiple imputation strategies.

Analysis of secondary and exploratory endpoints:

For sweat chloride assessments, CFQ-R, and body weight, the analysis for each cohort was analogous to the analysis of the primary outcome: based on the mean of the values at the 4, 6, and 8 week visits. Furthermore, for these outcomes, as well as for the primary endpoint, the analysis was performed using the measurements at 4, 6, and 8 weeks separately (i.e., without averaging).

Listings and summary tables were provided for the assessment of safety and tolerability for AEs, clinical laboratory tests, physical examinations, ECG, and vital signs.

To compare the treatment effect on efficacy endpoints (ppFEV₁, sweat chloride concentrations, CFQ-R, and body weight) between the 2 cohorts, all treatment effect estimates were tabulated and graphically presented with 95% CIs.

The correlation of individual clinical efficacy endpoints with organoid response was assessed via scatter plots showing the change scores (the difference between the mean of the post-baseline scores and the baseline score) in the period of receiving active treatment and the organoid response. Spearman and Pearson correlation coefficients (with 95% CIs) were determined. These correlation analyses were performed on data of each of the 2 cohorts separately and on the combined data.

Any PK parameters were planned to be assessed using population PK models, followed by a PK-pharmacodynamic model to assess the relationship between plasma exposure and efficacy and safety.

Results:

Efficacy:

Except for the main analysis of the primary endpoint, all secondary and exploratory analyses were considered explorative and were not adjusted for Type 1 error multiplicity, and should therefore be interpreted with caution.

Pre-selected cohort:

In the pre-selected cohort, the primary efficacy endpoint, which was the average ppFEV₁ of the 3 post-baseline measurements, did not show a statistically significant effect of DIR/POS/NES treatment. Nonetheless, ppFEV₁ seemed to suggest a favourable effect of DIR/POS/NES treatment. Statistical significance was reached for ppFEV₁ at the Week 4 assessment, but not at the Week 6 and 8 assessments.

Similar to the primary efficacy endpoint, none of the secondary efficacy endpoints showed a statistically significant effect of DIR/POS/NES treatment. Nonetheless, the average sweat chloride concentration of the 3 post-baseline measurements and the average CFQ-R respiratory domain scores of the 3 post-baseline measurements seemed to suggest a favourable effect of DIR/POS/NES treatment. In contrast, the average weight of the 3 post-

baseline measurements did not show any indication for a favourable effect of DIR/POS/NES treatment.

Randomly-selected cohort:

In the randomly-selected cohort, the primary efficacy endpoint, which was the average of ppFEV₁ of the 3 post-baseline measurements, did not show a statistically significant effect of DIR/POS/NES treatment.

Similarly, none of the secondary efficacy endpoints showed a statistically significant effect of DIR/POS/NES treatment. Only the sweat chloride concentration of the 3 post-baseline measurements seemed to suggest a favourable effect of DIR/POS/NES treatment, but effect sizes were smaller than in the pre-selected cohort.

Pharmacokinetics:

Pharmacokinetic data have not been analysed.

Safety:

DIR/POS/NES treatment was generally safe and well tolerated. Serious adverse events other than infective pulmonary exacerbations of CF were only observed during treatment with placebo.

Photosensitivity reactions occurred in more than a third of the participants during DIR/POS/NES treatment, whereas they did not occur during placebo treatment. Other (possibly) related AEs that appeared to be more prevalent during DIR/POS/NES treatment than during placebo treatment were sunburn, rash, increased hepatic enzyme, and diarrhoea. Events of decreased glomerular filtration rate also occurred more often during DIR/POS/NES treatment than during placebo treatment, but the investigators did not consider these events (possibly) related to study drug. None of the (possibly) drug-related AEs were serious.

The incidence of infective pulmonary exacerbation of CF was lower during DIR/POS/NES treatment than during placebo treatment in the pre-selected participants.

Conclusions:

In CF patients with rare CFTR mutations, DIR/POS/NES treatment was generally safe and well tolerated. The most prominent adverse events related to DIR/POS/NES treatment were photosensitivity reactions.

Analyses of the efficacy endpoints did not show any statistically significant benefit of DIR/POS/NES treatment in CF patients with rare CFTR mutations. However, in the cohort of participants who were pre-selected based on their organoid response to DIR/POS/NES exposure, there appeared to be a possible benefit from DIR/POS/NES treatment on ppFEV₁, sweat chloride concentrations, and CFQ-R respiratory domain scores.

Date of Clinical Study Report:

09Feb2026