Hydroxychloroquine in pediatric ILD
START randomized controlled in parallel-group, then switch placebo to active drug,
and
STOP randomized controlled in parallel-group
to evaluate the efficacy and safety of hydroxychloroquine (HCQ)

HCQ in pediatric ILD

Statistical Analysis Plan

Version 1.1, 07.12.2016

X

1/10/20

Prof. Dr. med. M. Griese, Sponsor

Date, Signature

Klinikum der Universität München
Kinderklinik im Dr. v. Haunerschen Kinderspital
Christiane Herzog Ambulanz
Behandlungszentrum für
Cystische Fibrose (Mukoviszidose)
Lindwurmstr. 4, D-80337 München
Tel: 089/5160-7877 Fax -7879

Christian Ruckes, Biometrician

Date, Signature

Unterschrift fehlend, nachgeholt am 1/10/20 DS

Contents

1 Background

1.1 Study Objectives

The primary objective of the trial is to investigate the following:
***START* HCQ block**
To evaluate the efficacy of HCQ after 28 days of treatment in chILD compared to placebo.
***STOP* HCQ block**
To evaluate the efficacy of HCQ after 84 days of treatment in chILD compared to placebo.

Secondary objectives of the study are:
***START* HCQ block**

- To evaluate the efficacy of HCQ after 56 days of treatment in chILD compared to 28 days.
- To evaluate the safety of HCQ after 28 and 56 days of treatment in chILD.
- To evaluate blood levels of HCQ after 28 and 56 days of treatment in chILD.

***STOP* HCQ block**

- To evaluate the efficacy of chronic (> 3 months) HCQ treatment in chILD compared to placebo. This analysis differs from the primary analysis, only if there is a substantial HCQ therapy before study begin.
- To evaluate the safety of HCQ after > 3 months of treatment in chILD.
- To evaluate blood levels of HCQ before and after treatment in chILD.

1.2 Study Design

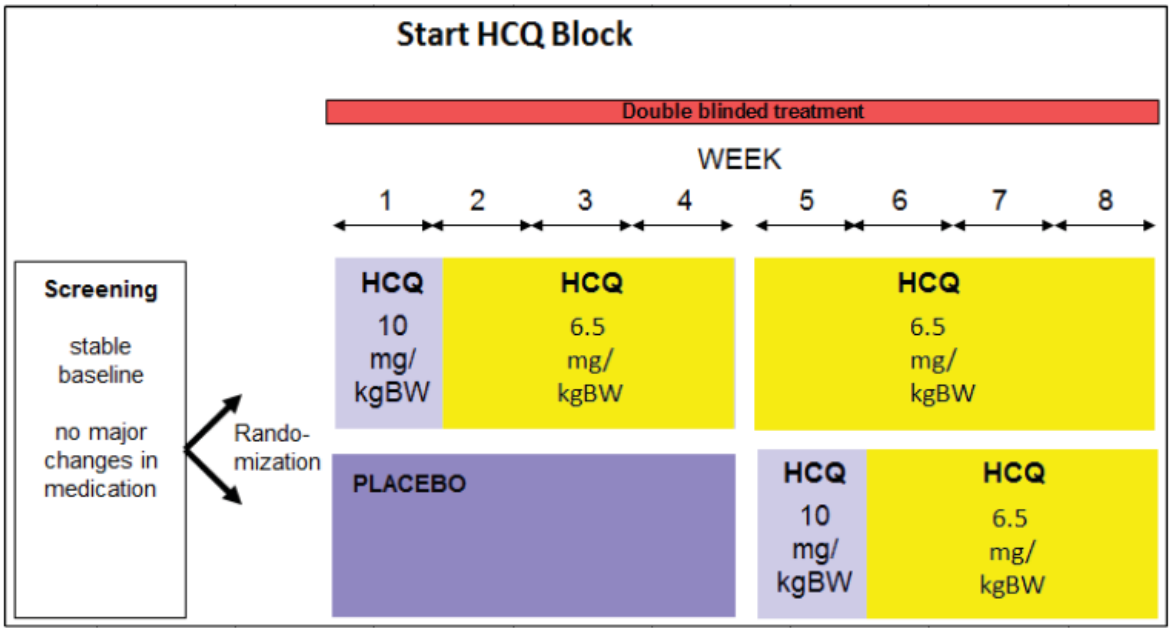
This study is an explorative, prospective, randomized, double-blind, placebo controlled investigation of hydroxychloroquine (HCQ) in pediatric ILD. The treatments are organized in START and STOP blocks, which can be initiated in sequence, as needed by the subjects. Each patient can participate in each block, but only once in each block.

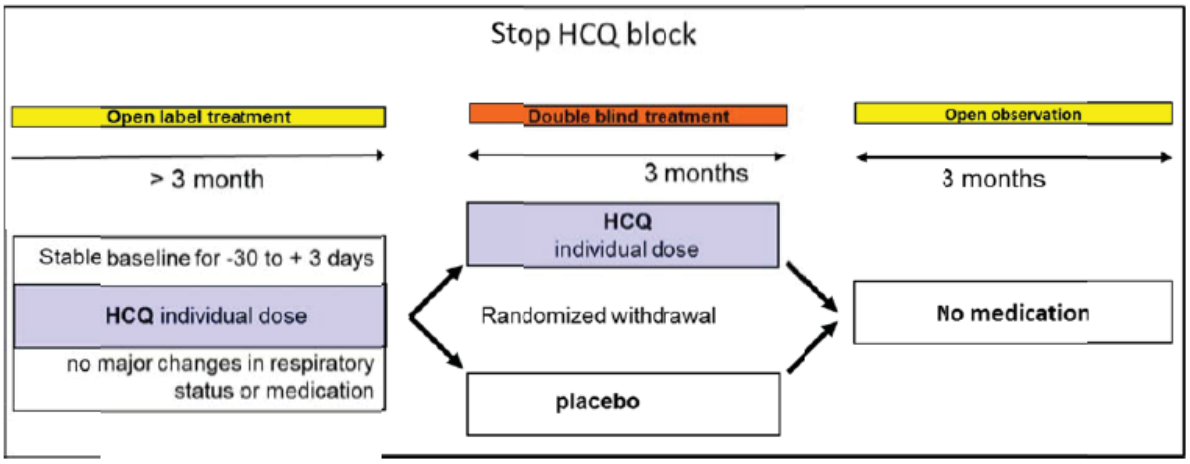
In the START block subjects are randomized to parallel-groups, then the placebo group is switched to active drug.

In the STOP block, subjects on HCQ are randomized into parallel-groups treated with placebo or HCQ to investigate the withdrawal of HCQ for assessment of its efficacy.

It is planned to assess >100 subjects for eligibility and to allocate 80 subjects. An equal distribution between START and STOP blocks is expected.

Study flow chart





Trial schedule START HCQ Block

Visit	Screening evaluations and Visit 1	Visit 2	No Visit	Visit 3	No Visit	Visit 4
Action	„Start of baseline“	“End of baseline” + Day 1 of treatment	Every 7 days at home + web/tele feed-back	End of 1st Tx period	Every 7 days at home + web/tele feed-back	“End of trial”
Trial day	-28 to -3	1		28 (-4, +7)		56 (-4, +7)
Demographics (e.g. sex, age, race)	X					
Patient information, informed consent	X					
Previous and concomitant diseases	X	X		X		X
Previous and concomitant treatments	X	X		X		X
Inclusion/exclusion criteria	X					
Pregnancy test [k]	X			X		X
Randomization [a]	X					
Physical examination	X	X		X		X
Vital signs (BP, pulse, temp, cardiotoxicity, hypoglycaemia)	X	X		X		X
ECG	X*			X		X
Ophthalmologic review[j]	X*					
Lab [c] (GOT/GPT/Crea/BUN/CK/GGT/LDH/BB/DIFF drug level /save EDTA, blood glucose)	X*			X		X
pO_2, pCO_2 (capillary)	X	X***		X***		X***
Primary outcome [e] O_2-sat, in room air	X	X	-	X	-	X
Secondary outcomes						
Respiratory rate, in room air	X	X	X	X	X	X
Retractions	X	X	X	X	X	X
Coughing	X	X	X	X	X	X
Oxygen demand	X	X	X	X	X	X
Chest x-ray scoring for chILD	X*			X****		
Pulmonary hypertension (Echo)	X*					
Quality-of-life [i]	X*					X
Health Economics [h]	X*					X
Weight for height [b]	X*					X
Adverse events		X	X	X	X	X
Additionally **in children > 5 years**						
FEV1 % predicted (recorded in L) [d]	X	X		X		X
FVC % predicted (recorded in L) [d]	X	X		X		X
6 minute walking distance (meter) [g]	X	X		X		X
O_2-saturation before and after 6MWT	X	X		X		X
Borg scale [f]	X	X		X		X
Additionally **in ventilated** patients						
Oxygenation index (OI)	X	X	X	X	X	X
Duration (h) of mechanical ventilation	X	X	X	X	X	X
NO (%), ECLS (VV/VA/ECLA/Flow/time since insertion)	X	X	X	X	X	X

X* Tests must be done before first drug dosing
X** Test can be done according to the opinion of the treating physician
X*** Test can be done facultative, but should be done, if initial pCO2 has been elevated
X**** Chest x-ray is recommended if clinically indicated e.g. to answer the question if the disease is stable

Trial schedule STOP HCQ Block

Visit	Visit 1 + Screening	Visit 2	No Visit	Visit 3	No Visit	Visit 4	No Visit	Visit 5	Visit 6
Action	„Begin of baseline“	“End of baseline” + Day 1 of withdrawal treatment	2 weeks of withdrawal, at home + web/tele feed-back	4 weeks of withdrawal treatment	6 weeks of withdrawal , at home + web/tele feed-back	8 weeks of withdrawal treatment	10 weeks of withdrawal , at home + web/tele feed-back	“End of trial” after 12 weeks of withdrawal treatment	Follow up after 12 weeks of open observation
Trial day	-30 to -10	1 (-1, +8)	14 (-7,+7)	28 (-7, +7)	42 (-7, +7)	56 (-7, +7)	70 (-7, +7)	84 (-7, +7)	168 (-7, +7)
Demographics (e.g. sex, age, race)	X								
Patient information, informed consent	X								
Previous and concomitant diseases	X	X		X		X		X	X
Previous and concomitant treatments	X	X		X		X		X	X
Inclusion/exclusion criteria	X								
Pregnancy test [k]	X			X		X		X	
Randomization [a]	X								
Physical examination	X	X		X		X		X	X
Vital signs (BP, pulse, temp, cardiotoxicity, hypoglycaemia)	X	X		X		X		X	X
ECG	X*			X		X		X	
Ophthalmologic review[j]	X*								
Lab [c] (GOT/GPT/Crea/BUN/CK/GGT/LDH/ BB/DIFF, blood glucose, drug level (EDTA blood)	X*			X		X		X	X (only EDTA for drug level)
pO_2, pCO_2 (capillary)	X	X**		X**		X**		X**	X***
Primary outcome [e] O_2-sat, in room air	X	X	-	X	-	X	-	X	X
Secondary outcomes									
Respiratory rate, in room air	X	X	X	X	X	X	X	X	X
Retractions	X	X	X	X	X	X	X	X	X
Coughing	X	X	X	X	X	X	X	X	X
Oxygen demand	X	X	X	X	X	X	X	X	X
Chest x-ray scoring for chILD	X*							X***	
Pulmonary hypertension (Echo)	X*								
Quality-of-life [l]	X*							X	X
Health Economics [h]	X*							X	X
Weight for height [b]	X*							X	X
Adverse events	X	X	X	X	X	X	X	X	X
Additionally **in children > 5 years**									
FEV1 predicted (recorded in L) [d]	X	X		X		X		X	X
FVC predicted (recorded in L) [d]	X	X		X		X		X	X
6 minute walking distance (meter) [g]	X	X						X	X
O_2-saturation before and after 6MWT [g]	X	X						X	X
Borg scale [f]	X	X						X	X
Additionally **in ventilated** patients									
Oxygenation index (OI)	X	X	X	X	X	X	X	X	X
Duration (h) of mechanical ventilation	X	X	X	X	X	X	X	X	X
NO (%), ECLS (VV/VA/ECLA)Flow/time since insertion	X	X	X	X	X	X	X	X	X

X* Tests must be done before first drug dosing
X** Test can be done facultative, but should be done, if initial pCO2 has been elevated
X*** Chest x-ray is recommended if clinically indicated e.g. to answer the question if the disease is stable

a Randomization must occur after all inclusion and exclusion criteria are met directly after consent to the study is given and at least 1 week before first dose of HCQ or withdrawal treatment. This is necessary to allow for preparation and shipping of the study medication.

c Blood sample should include: Blood count with differential, GOT, GPT, gGT, Creatinine, LDH, BUN, Creatine kinase, drug level, 3 ml EDTA-blood

d Spirometry will be performed pre-bronchodilator in children age ≥ 5 years. SOP will be provided.

e Measurement of O_2-Saturation, respiratory rate and O_2 flow (if necessary) in awake patient at rest:
If not on O_2: after 5 min at rest, room air, then measure twice over 1 min. each, at least 1 min. apart
If on O_2:
- BEFORE withdrawal (after 5 min at rest with steady state O_2 supplement, then measure over 1 minute)
- Then withdraw O_2 to obtain steady state O_2-Sat. in room air (at least 30 seconds without change). Then measure twice over 1 min. each, at least 1min. apart. If falls below SpO_2<80% place back in oxygen and note as <80%.

f Borg-Scale: assessment of dyspnea (0 – 10: 0 = no dyspnea at all, 5 = severe dyspnea, 10 = extremely severe dyspnea)

g Standard exercise testing (6-minute-walk test): SOP will be provided

h Health economics assessment with the use of a special questionnaire which has to be filled out by the parents

l Quality of Life assessment with the use of the questionnaire PedsQL for different age groups

j For ophthalmological review please see Chapter 5.1

k Pregnancy test only has to be performed in girls of childbearing age and only if sexual relations are known or probable. It is at the discretion and responsibility of the attending physician to decide, whether a pregnancy test is necessary or not.

2 Analysis Populations

2.1 Definitions

All subjects with signed informed consent are considered as **enrolled subjects**, even if they did not receive any trial treatment.

All randomised subjects will be included in the **Intention-to-treat (ITT)** populations. This population is the primary analysis population. Within ITT population analyses subjects will be assigned to the treatment to which they were randomised. For the combined analysis the ITT Population consists of all patients that are at least in the START or the STOP block.

The **safety** population comprises all subjects who received at least one dose of trial treatment. In analyses of the safety population subjects will be assigned to the treatment which they actually received.

The analysis populations will be defined prior to unblinding of the study.

2.2 Scope

The primary population for the analyses of efficacy are the ITT Populations (randomised patients) for each block. For the combined analysis the ITT Population consists of all patients that are at least in the START or the STOP block.

Safety parameters will be analysed based on the safety population for the assessment of safety.

2.3 Major Protocol Violations

No per protocol analysis will be done.

3 Study Centres

Recruitment and treatment of subjects is expected to be performed in > 100 trial centres.

4 Analysis Variables

4.1 Demographics and Baseline Characteristics

Demographics

- Birth date (not analysed)
- Gender
- Age (calculated)
- Nationality

Baseline Characteristics

- Concomitant diseases (in the year before screening / current)
- **Physical examination (without finding yes/no, several body systems normal/abnormal)*
- **Vital signs (height (cm), weight (kg), heart rate (b/min), blood pressure systolic (mmHg), blood pressure diastolic (mmHg), BMI (calculated), temperature, cardiotoxicity, hypoglycaemia)*
- **O2-Saturation (not done / method, O2-Saturation % on room air, O2-Saturation % and O2-flow (l/min) on oxygen via nasal cannula or face mask)*

O2-Saturation – air challenge (not done for 5 minutes, O2-Saturation %, unable to tolerate RA challenge time to saturation of 80%)
O2-Saturation – on high-flow nasal cannula (O2-Satuation %, Flow air, Flow O2)
O2-Saturation – on non-invasive ventilation (O2-Saturation %, PEEP, PIP, Respiratory rate not done)
O2-Saturation – on invasive ventilation (O2-Saturation %, PEEP, PIP, Respiratory rate not done)
O2- saturation - on HFO (O2-saturation %, frequency FHFO, MAP, Amplitude VTHFO)
O2-saturation – on ECMO (O2-saturation %)

***Standard exercise testing (6min-walk not done/not completed/completed, reason for not done) – distance, oxygen saturation, heart rate, Borg-scale (0=nothing at all, …, 10=very, very severe (max.) at rest and immediately after exercise), if on oxygen: oxygen supplement and flow rate, if test was discontinued: reason and discontinued after xy seconds)*

**Laboratory parameters (value, value normal/abnormal)*

Pregnancy test (male/female without childbearing potential yes/no, lactation period yes/no, pregnant yes/no, test performed yes/no, if test not done: reason)

ECG

Ophthalmological review (Visual acuity and reading acuity, Central visual field, using an Amsler Chart (preferably red on black) or automated perimetry, Slit lamp examination of the cornea, Stereoscopic slit lamp examination of the retina)

pO2, pcO2 (capillary)

**respiratory rate (b/min)*
**retractions*
**coughing*
**oxygen demand*

Chest x-ray scoring for chILD

Pulmonary hypertension (echo)

**measured at subsequent visits as well. Additionally in ventilated patients: Oxygenation index (OI), Duration (h) of mechanical ventilation, NO(%), ECLS (VV/VA/ECLA/Flow/time since insertion)*
*** measured at subsequent visits as well. Additionally in children >5years: FEV1% predicted (recorded in L), FVC% predicted (Recorded in L), 6 minute walking distance (meter), O2 sat before and after 6MWT, Borg scale*

4.2 Primary Variable

START HCQ block and STOP HCQ block

The primary variable is the response rate measured by the oxygen saturation in room air.

START HCQ block

Relative change from trial day 1, i.e. Visit 2, through day 28, i.e. Visit 3, change from active compound compared to change from placebo

STOP HCQ block

Relative change from trial day 1, i.e. Visit 2, through day 84, i.e. Visit 5: change from active compound compared to change from placebo

A) Non-ventilated patients in room air or on O2-supplement

Oxygen saturation in room air

Assessment:

1) If not on O2:

(after 5 min at rest room air, then measure twice over 1 minute each, at least 1 min apart)
** O2 Sat (mean value)*

** Respiratory rate (mean value)*

2) If on O2: BEFORE withdrawal:

(after 5 min at rest with steady state O2 supplement, then measured over 1 minute)

** O2 Flow*

** O2 Sat (mean value)*

** Respiratory rate*

Then withdraw O2:

Steady state O2 Sat in air obtained. Steady state minimum SpO2 should be at least 30 seconds without change. Then measured twice over 1 minute each, at least 1 min apart).

If falls to SpO2<80% place back in oxygen and note as <80%.

** O2 Sat (mean value)*

** Respiratory rate (mean value)*

B) If on high-flow nasal prongs:

O2-flow and air-flow

C) Ventilated patients:

Oxygenation index (OI)

OI = (FiO2* MPAW)/PaO2

with

FiO2 = Fraction of inspired oxygen

MPAW = Mean airway pressure (= ((frequency * inspiration time)/60) * ((inspiratory pressure (PIP) – PEEP) + PEEP)))

PaO2 = Partial pressure of oxygen in arterial blood

4.2.1 Efficacy

The following variables will be investigated:

- Oxygen saturation (O2-sat, in room air) (only absolute, as relative already primary outcome) assessed at every visit (not web/tele feedback)
- Respiratory rate (RR, in room air) (relative and absolute) assessed at every visit and every web/tele feedback

- Retractions (yes/no) assessed at every visit and every web/tele feedback
- Coughing last 24 h (yes/no) assessed at every visit and every web/tele feedback
- Oxygen requirement assessed at every visit and every web/tele feedback

- pO2, pCO2 (capillary, in room air)
- Chest x-ray scoring for chILD assessed at screening and visit 3 (START HCQ Block) and visit 5 (STOP HCQ Block)
- Overall survival
- If > 5y old
  - FEV1 % predicted (recorded in L)
  - FVC % predicted (recorded in L)
  - reference values according to the GLI 2012 lung function regression equations
  - 6 minutes walking distance (meter)
  - O2-saturation before and after 6MWT
  - Borg scale
- If ventilated:
  - Duration (h) of mechanical ventilation
  - NO (%), ECMO yes/no, if yes VA/VV, double lumen vs. single lumen, Flow-rate.

4.2.2 Safety/Tolerability

Adverse events, clinical laboratory values (GOT, Creatinine, gGT, blood count, differential, LDH, BNP, steady state drug level), ECG, ophthalmologic review
Adverse events will be documented during the study at each visit (exemption: START HCQ between Visits 1 and 2. However, occurring AEs between those visits are not treatment emergent).
Laboratory data will be assessed at screening, visit 3 and visit 4 in the START HCQ Block and at screening, visit 3, 4 and visit 5 and EDTA additionally at visit 6 in the STOP HCQ Block.
Vital signs (respiratory rate, height, weight, BMI, heart rate, blood pressure (systolic and diastolic), temperature, hypoglycaemia (yes/no), appropriate observation of blood glucose levels (yes/no), cardiotoxicity (yes/no), appropriate ECG (yes/no)) will be assessed at screening, and every following visit (not web/tele feedback).
A physical examination will be done at the same visits.
Symptoms and clinical course of treatment will be documented at the same time points as the physical examination is performed and at telephone contact visit.

4.2.3 Quality of Life (Peds QL)

Not applicable.

4.2.4 Health Economics

Not applicable.

5 Treatment of Missing Values and Outliers

5.1 Missing Values

No substitution of missing values is planned.

5.2 Outliers

There will be no methods employed for detecting outliers.

6 Statistical Analyses

The default summary statistics for quantitative variables will be the number of observations (n), mean, standard deviation (SD), median, first quartile (Q1), third quartile (Q3), minimum and maximum for those patients with data available.
For qualitative variables, the number (n) and percentage (%) of patients with non-missing data per category will be the default summary presentation. If appropriate, the number of missing values will be displayed as a "Missing" category.
A value will be considered missing if a patient attends the visit (visit date is available) but no value is documented.
All variables will be displayed by treatment group and with a total column.
Listings of all AE data available will be provided only.

6.1 Patient Disposition

The following patient disposition variables will be presented by numbers and percentages:

- Informed consent given to study participation / study treatment
- Inclusion and exclusion criteria

- Size of analysis populations (enrolled, ITT, safety)
- Study completion according to protocol
- Reasons for premature discontinuation of study treatment
- Type of major protocol violation

Protocol deviations which are documented in the CRF will be listed additionally.

6.2 Demographics and Baseline Characteristics

All parameters (except birth date) mentioned in chapter 4.1 will be summarised descriptively. Appropriate exploratory p-values of t-test (unequal variances) or chisquare test to test on differences between treatment groups will be shown.

6.3 Prior and Concomitant Diseases

Concomitant diseases will be coded by MedDRA (Medical Dictionary for Regulatory Activities) terminology and presented by number and percentages within preferred term and system organ class (current version).

6.4 Prior and Concomitant Medication/Therapies

Concomitant medication/therapies and pain medication will be coded using the current WHO drug dictionary version and will be presented within ATC-2 and ATC-4 level.

Patients with at least one concomitant medication/therapy will be displayed by number and percentages.

6.5 Extent of Exposure / Compliance

The number of capsules used since last visit will be summed up for each patient to a total of capsules used. The following compliance calculation will be tabulated:
100 * ((total number of used capsules) / number of days between randomisation and study end)

The compliance will be analysed by descriptive methods.

6.6 Primary Analysis

The variable assessing the primary outcome is the response rate measured by the oxygen saturation in room air.

START HCQ block
Relative change from trial day 1 (Visit 2) to day 28 (Visit 3) and day 56 (Visit 4) and from day 28 to day 56 change from active compound compared to change from placebo
STOP HCQ block
Relative change from trial day 1, i.e. Visit 2, through day 84, i.e. Visit 5: change from active compound compared to change from placebo

Both blocks separately
a) A difference between the changes in placebo and active phases is considered significant as described in the table below.

Patient's condition	**Measured parameter**	**Definition of significant change or responder**
Stable in room air or on	SpO2 in room air	Increase ≥ 5%

O2-supplement		
On high-flow nasal cannula	O2-flow, air-flow	Decrease ≥ 20% or support not needed any more
Ventilated	Oxygenation index	Decrease ≥ 20% or support not needed any more
On ECMO		Decrease ≥ 20% or support not needed any more

The analysis will be done first by discarding the information on the patient's condition. Descriptive analyses will be done overall and for all patients conditions separately.

b) Patients are defined as responder as described in the table above. The numbers of responders are counted and compared by Fisher's exact test as well as logistic regression models with factors for treatment group and age group.
The following SAS code can be used:

```
PROC LOGISTIC DATA=indata;
        CLASS treatment age_gr;
        MODEL responder_YN = treatment age_group treatment*age_group;
RUN;
```

Both blocks together
Not applicable. There were too few patients in both blocks.

6.7 Secondary Analyses

All analysis of secondary endpoint will be interpreted purely exploratory. For all hypotheses two-sided exploratory p-values will be provided. Descriptive statistics will be displayed for all.

6.7.1 Efficacy

For both, START and STOP HCQ blocks absolute and relative change under the active compound from trial day 1, i.e. Visit 2, to START block: day 28, day 56 STOP block: day 84 each, will be compared between treatment groups .
The following variables will be investigated:

- Oxygen saturation (O2-sat, in room air) (only absolute, as relative already primary outcome)
- Respiratory rate (RR, in room air) (relative and absolute)

- Retractions (yes/no)
- Coughing last 24 h (yes/no)
- Oxygen requirement (yes/no)

- pO2, pCO2 (capillary, in room air)
- Chest x-ray scoring for chILD
- Overall survival (time from randomization until death; if no death observed, time will be censored at time point of last (follow-up-)visit): a Kaplan-Meier plot will be provided; if possible, the median overall survival and corresponding 95%-confidence interval will be calculated
- Cumulative amounts of steroid equivalents per time period
- If > 5y old
    - FEV1 % predicted (recorded in L)

- FVC % predicted (recorded in L)
- reference values according to the GLI 2012 lung function regression equations
- 6 minutes walking distance (meter)
- O2-saturation before and after 6MWT

- If ventilated:
  - Duration (h) of mechanical ventilation
  - NO (%), ECMO yes/no, if yes VA/VV, double lumen vs. single lumen, Flow-rate.

Additionally, FEV_1, FVC, FVC predicted, 6 min walking distance will be displayed by Box-Plots and All-in-one plots over each time point.

6.7.2 Safety/Tolerability

6.7.2.1 Adverse Events

Only treatment emergent adverse events (AEs) will be analysed. These are adverse events that occurred after taking study medication. In particular this means only the following events are considered as treatment emergent:

START BLOCK: Events starting at visit 2 or later

STOP BLOCK: Events starting at visit 1 or later

In case of missing start data, adverse events are considered as treatment emergent.

Frequencies of subjects experiencing at least one AE will be displayed by body system and preferred term according to MedDRA terminology. Detailed information collected for each AE will include: A description of the event, duration, whether the AE was serious, intensity, relationship to trial drug, action taken, and clinical outcome.

Summary tables will present the number of subjects observed with AEs and corresponding percentages. Additional subcategories will be based on seriousness of event, event intensity and relationship to trial treatment.

An overview table will be produced showing the number of patients with
- any AEs
- any SAEs
- any deaths
- any severe AEs
- any related AEs
- any related severe AEs
- any related serious AEs
- any fatal related AEs
- any AEs leading to premature study discontinuation

A subject listing of all AEs will be prepared.

6.7.2.2 Laboratory Parameters

Listings will be prepared for each laboratory measure and will be structured to permit review of the data per subject as they progress on treatment.

Summary tables will be prepared to examine the changes of laboratory measures over time by descriptive statistics and exploratory p-values.

6.7.2.3 Vital Signs

Absolute values and changes from baseline of the vital signs will be analysed descriptively and by exploratory p-values.

6.7.2.4 Others

Not applicable.

6.7.3 Quality of Life (Peds QL)

Each item will be reversed scored and linearly transformed to a 0-100 scale as follows: 0=100, 1=75, 2=50, 3=75 and 4=0 (higher scores indicate better quality of life).
If more than 50% of the items in a scale are missing, the scale score should not be computed.
Mean scores are defined as sum of the items over the number of items answered.
The psychosocial health summary score is the sum of the items over the number of items answered in the emotional, social and school functioning scales.
The total score is the sum of all items over the number of items answered on all the scales.
Descriptive statistics and exploratory p-values (t-tests with unequal variances) for absolute values and changes to baseline will be tabulated.

6.7.4 Health economics

The analysis will not be done by the IZKS.

6.8 Subgroup Analyses

Not applicable.

6.9 Interim Analysis

No interim analysis is planned.

7 Software

All analyses will be performed using the Statistical Analysis Software (SAS), Version 9.4.