Biostatistics Research Group,

Population Health Sciences Institute, Newcastle University

Stage 2 - Statistical Analysis Plan

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This current version of the SAP and all preceding versions will be stored in the Statistical Section of the Trial Master File held by the IHS Biostatistics Research Group.

This statistical analysis plan (SAP) provides a framework and guidelines for the statistical analysis and reporting of the *MCDS-Therapy* trial. **This SAP applies to Stage 2 of the *MCDS-Therapy* trial only.**

The SAP applies to a clean and validated dataset. Detailed information on data collection tools, data validation, consistency and accuracy checks and data storage and archiving can be found in the current version of the Data Management Plan (version 3.0 [02/12/2021]).

Any deviations from the methods outlined in this SAP will be documented in the statistical end of trial report. Example Tables, Figures and Listings are for illustrative purposes only and are subject to change.

This SAP, along with all other documents relating to the analysis of this trial, will be stored in the 'Statistical Section' of the Trial Master File (TMF) held and maintained by the PHSI Biostatistics Research Group. The final signed SAP will also be stored in section 16 of the main TMF (16. Statistics / 16.1 Final signed Statistical Analysis Plan).

Revision history

Version	Date	Changes made	Justification for change	Timing of change
1.0	08/02/2024	First version	NA	NA

Abbreviations

ABBREVIATION	DEFINITION
ADR	Adverse Drug Reaction
AE	Adverse Event
AR	Adverse Reaction
CNS	Central Nervous System
CBZ	Carbamazepine
CDMS	Clinical data management system
CI	Chief Investigator
COL10A1	Collagen Type X Alpha 1 Chain - Schmid Metaphyseal Chondrodysplasia
CRF	Case Report Form
CRL	Crown-rump length
CTA	Clinical Trial Authorisation
CTCAE	Common Terminology Criteria for Adverse Events
CTIMP	Clinical Trial of an Investigational Medicinal Product
DLT	Dose Limiting Toxicity
eCRF	Electronic Case Report Form
EMA	European Medicines Agency
EQ-5D-Y	EuroQoL 5 Dimension Youth Questionnaire
ER	Endoplasmic Reticulum
EudraCT	European Clinical Trials Database
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GP	General Practitioner
HRQOL	Health Related Quality of Life
HRA	Health Research Authority
HTA	Human Tissue Authority
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of technical requirements for
IDMC	Independent Data Monitoring Committee
IMP	Investigational Medicinal Product
ISF	Investigator Site File
ISRCTN	International Standard Randomised Controlled Trials Number
LPLV	Last Patient Last Visit
MCDS	Metaphyseal chondrodysplasia type Schmid
MedRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare products Regulatory Agency
MTD	Maximum Tolerated Dose
NCTU	Newcastle Clinical Trials Unit
NHS	National Health Service
NIMP	Non-Investigational Medicinal Product
NUTH	The Newcastle upon Tyne Hospitals NHS Foundation Trust
PEDSQL	Paediatric Quality of Life Inventory Pain coping inventory/questionnaire
PI	Principal Investigator
PIC	Participant Identification Centre
PIS	Participant Information Sheet

QA	Quality Assurance
QC	Quality Control
QP	Qualified Person
R&D	Research & Development
REC	Research Ethics Committee
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SDV	Source Data Verification
SOP	Standard Operating Procedure
SmPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
TSC	Trial Steering Committee
TMF	Trial Master File

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1 Introduction

1.1 Background and rationale

The aim of the MCDS therapy trial is to evaluate the effect of carbamazepine (CBZ) on children with a diagnosis of Metaphyseal chondrodysplasia type Schmid (MCDS) with confirmed COL10A1 pathogenic mutation. There is currently no specific treatment for patients with MCDS, and patient care is based only on the management of symptoms.

The trial is based on the results of preclinical studies which support the efficacy of CBZ on cells and in mice with a COL10A1 mutation, both at a molecular level on the pathogenic mechanism reducing endoplasmic reticulum (ER) stress, and on growth and bone alignment in mice.

CBZ is a well-established drug, which has been widely marketed throughout Europe since the 1960s and is routinely used in paediatric care for the treatment of epilepsy and neuropathic pain. It has a well-known safety profile. Based on clinical experience of the MCDS patient population there is no clinical reason to expect a different safety profile of CBZ on patients with MCDS compared to patients of similar ages treated with CBZ for epilepsy. Its effects on patients with MCDS have never been investigated.

This trial includes children with MCDS; to evaluate the effect of CBZ on growth and bone alignment it is necessary to evaluate this on patients who have not reached bone maturity.

MCDS is a very rare disease for which there are no existing natural history studies. Baseline data will be collected in the first year of the trial for all patients before starting administration of CBZ to allow comparison of pre and post treatment characteristics in each individual.

The trial is divided into an initial dose titration and tolerability stage (Stage 1 – now completed) followed by a treatment stage (Stage 2). The efficacy of CBZ in the treatment of children with MCDS will be evaluated in full at the end of Stage 2 based on growth velocity, bone alignment, quality of life and pain.

1.2 Stage 1 summary

The primary objective of Stage 1 was to assess the safety and tolerability of CBZ in the treatment of children with MCDS who are ambulant but have not yet reached skeletal maturity (open epiphyses) and to determine an appropriate dose of CBZ to inform the treatment of children in Stage 2.

A cohort of eleven patients were evaluated through a review of safety and tolerability. Data presented to the DMC included the number and reason for any DLTs, laboratory safety assessments, abnormal physical examinations, pain scores and adverse events. Initially, the Stage 1 safety review [report dated 9/8/2021] was presented to the IDMC when six patients in Stage 1 reached their maximum tolerated dose or 20mg/kg. A subsequent report was presented to the DMC [25/10/2021] once the last patient on CBZ in Stage 1 reached the 6-month time point.

Following these reviews, the IDMC recommended continuation of the trial according to the current version of the protocol *(*Version 6.0 dated 30 June 2021) with no changes and agreed that the IMP dose regimen to be implemented in Stage 2 of the study should be 20 mg/kg.

1.3 Stage 2 Objectives

1.3.1 Primary objective

To evaluate efficacy of CBZ for the treatment of children with MCDS who are ambulant but have not yet reached skeletal maturity (open epiphyses) and to determine if the level of efficacy warrants a subsequent formal development programme for CBZ in this indication.

1.3.2 Secondary objectives

To evaluate the effect of CBZ on height, bone conformation, pain, and health related quality of life in children with MCDS over 12 months.

2 Study Methods

2.1 Study setting and patient population

Stage 1 of MCDS-Therapy was conducted at two centres within the UK: The Newcastle upon Tyne Hospitals NHS Foundation Trust Newcastle upon Tyne UK (NuTH) and Guys and St. Thomas' Hospital London UK (GSTFT).

Stage 2 includes sites across the UK, and self-sponsored trial sites across Europe and Australia.

The following sites were expected to recruit to Stage 2:

I. The Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne UK (NuTH)
II. Guy's and St. Thomas' Hospital, London UK (GSTFT)
III. Rizzoli Orthopaedic Institute, Bologna Italy (IOR)
IV. University of Freiburg- Freiburg Germany (UKL-FR)
V. Assistance Publique – Hôpitaux de Paris – Paris France (AP-HP)
VI. Murdoch Children Research Institute, Melbourne Australia (MCRI)
VII. University of Antwerp, Antwerp Belgium (UZA)

As the dosing regimen for Stage 2 remains the same as for Stage 1 (20 mg/kg), Stage 1 participants will be included in Stage 2 analysis.

2.2 Eligibility criteria

For a full list of inclusion and exclusion criteria see the protocol, version 9.0 (16th October 2023).

2.2.1 Inclusion criteria

Participants will be those with a proven diagnosis of MCDS; a pathogenic mutation in the gene encoding the Collagen Type X Alpha 1 Chain - Schmid Metaphyseal Chondrodysplasia (COL10A1) protein and ambulant at the time of consent/assent, with open epiphyses. Female patients of childbearing potential must have a negative pregnancy test at baseline and agree to regular pregnancy testing during the trial; sexually active female patients of childbearing potential are required to practice true abstinence.

2.2.2 Exclusion criteria

Patients will be excluded if they: have reached skeletal maturity; are known to have atrioventricular block; have had a prior adverse reaction to CBZ or a similar drug; have a history of bone marrow suppression/depression; have evidence of chronic hepatic or renal impairment; have acute intermittent porphyria; have received a monoamine oxidase inhibitor within 14 days of commencing therapy; or have abnormal blood screening results at the time of treatment initiation. Patients of Han Chinese, Thai and

other Asian origins who carry the HLA-B*1502 allele are excluded, as are patients who have a planned surgery or planned osteotomy.

2.3 Randomisation and blinding

There is no randomisation or blinding in this trial.

2.4 Definition of outcome measures

2.4.1.1 Primary objectives/endpoints

	Objective	Outcome
Primary	To evaluate efficacy of CBZ for the treatment of children with MCDS who are ambulant but have not yet reached skeletal maturity (open epiphyses)	Alteration from baseline in growth velocity over 12 months
	To determine if the level of efficacy warrants a subsequent formal development programme for CBZ in this indication.	Growth velocity follow-up data at 12 months post treatment initiation

Participants undergo an observational phase (12-18 months for Stage 1 participants; 6 months for Stage 2 participants) prior to starting treatment. Measurements taken include data on height and weight. The same measurements are taken at various time points during the treatment period, allowing an assessment of growth velocity and any change which might be due to treatment.

2.4.1.2 Secondary objectives/endpoints

	Objective	Outcome
Secondary	To evaluate the effect of CBZ on height in children with MCDS over 12 months	Alteration from baseline in height percentile over 12 months
	To evaluate the effect of CBZ on bone conformation in children with MCDS over 12 months	Alteration from baseline in long bone alignment and configuration over 12 months as measured by X-ray analysis
	To evaluate the effect of CBZ on pain in children with MCDS over 12 months	Alteration from baseline in pain perception over 12 months as measured by: • PEDSQL Pain Coping Inventory • PEDSQL Pain Questionnaire

	To evaluate the effect of CBZ on health related quality of life of children with MCDS over 12 months	Alteration from baseline in HRQOL scores over 12 months as measured by: • Paediatric Quality of Life Inventory (PedsQL) • EQ-5D-Y

2.4.1.2.1 Alteration from baseline in height percentile over 12 months

Total height (length if age <2 years); sitting height (age $\geqslant$ 5 years) and crown-rump length (age <5 years) are recorded at each baseline and treatment visit.

Height for age and sex, ***zscores*** are calculated using the ZANTHRO function in STATA; the "British 1990 Growth Reference" charts are used as the standard.

Vidmar, S. I., Cole, T. J., & Pan, H. (2013). Standardizing Anthropometric Measures in Children and Adolescents with Functions for Egen: Update. The Stata Journal, 13(2), 366–378. https://doi.org/10.1177/1536867X1301300211

2.4.1.2.2 Alteration from baseline in long bone alignment and configuration over 12 months as measured by X-ray analysis.

Using X-ray images of the hip and knees, bone alignment will be evaluated based on the angle formed between the head and neck of the femur and its shaft (Mikulicz angle), the femoral neck-shaft angle (NSA). To ensure consistency, the NSA will be assessed centrally by at least two independent radiologists to ensure consistency across sites. This will be measured according to the standards described in Keats TE Atlas of Roentenographic measurement Mosby 1990 p.289 p.320.

Outcome measures are:

A. Bone age SD – age related zscore (taken from the left wrist x-ray)

B. Tanner and Whitehouse bone age (TW3)??

C. Femoral neck-shaft angle (NSA) on left and right legs

D. Deviation from midline at knee

2.4.1.2.3 Pain perception

Alteration from baseline in pain perception over 12 months is measured by:

- PedsQL Pain Coping Inventory
- PedsQL Pain Questionnaire

PEDSQL Pain Coping Inventory and PEDSQL Pain Questionnaire - to measure pain perception and pain coping strategies. (Varni, J.W., Waldron, S.A., Gragg, R.A., Rapoff, M.A., Bernstein, B.H., Lindsley, C.B., & Newcomb, M.D. (1996). Development of the Waldron/Varni Pediatric Pain Coping Inventory. Pain, 67, 141-150.

https://www.pedsql.org/about_pedsql.html

Separate versions of the PedsQL for completion by the subject (for children aged 5 and over) and for the parent(s)/ legal guardian(s) acting as a proxy for the child are used. When the child is capable of self-completing an age-appropriate version, as assessed by parental and clinician judgement, they will do so. However, if the child is deemed incapable, the proxy version is used alone. It is recommended where possible, the same parent/caregiver should complete the questionnaires at all visits. Thus, two questionnaires, one from the child and one from the parent/guardian, could be available for analysis at each time point.

2.4.1.2.3.1 PedsQL Pediatric Pain Coping Inventory

Composed of 41 questions comprising 5 dimensions:

1. Cognitive Self-Instruction
2. Problem-Solving
3. Distraction
4. Seeks Social Support
5. Catastrophizing / Helplessness

A 3-point Likert scale from 0 (Never) to 2 (Often) is used to score each question. Total score is calculated as the sum of all question scores divided by the number of questions answered. If more than 50% of the questions in the scale are missing, the score should not be computed. If 50% or more of the questions are completed: the mean of the completed questions is imputed for those that are missing.

Outcome is the total score which will vary between 0 and 2 and will be treated as a continuous variable.

2.4.1.2.3.2 PedsQL Pediatric Pain Questionnaire

The Pediatric Pain Questionnaire is composed of 3 items. Questions on "*how you feel now" and "worst pain you had this week"* scored on a VAS and converted to a score of 0 (no pain) to 100 (Severe pain). The third item refers to the localisation of pain and is not scored.

2.4.1.2.4 PedsQL™ Generic Core Scales Version 4.0 Short Form (SF15)

Alteration from baseline in HRQOL scores over 12 months as measured by Paediatric Quality of Life Inventory (PedsQL).

Composed of 15 questions comprising 4 dimensions:

1. Physical functioning
2. Emotional functioning
3. Social functioning
4. School functioning

A 5-point Likert scale from 0 (Never) to 4 (Almost always) is used to score each question except for the self-reported version completed by young children (ages 5-7) which uses a 3-point scales: 0 (Not at all), 2 (Sometimes) and 4 (A lot). Questions are reversed scored and linearly transformed to a 0-100 scale as follows: 0=100, 1=75, 2=50, 3=25, 4=0.

Score by Dimensions: If more than 50% of the questions in the scale are missing, the scale scores should not be computed. Mean score is calculated as the sum of the question scores divided by the number of questions answered.

Psychosocial Health Summary Score = Sum of the question scores divided by the number of questions answered in the Emotional, Social, and School Functioning Scales.

Physical Health Summary Score = Sum of the question scores divided by the number of questions answered in the Physical Functioning Scale.

If more than 50% of the questions in the scale are missing, the score should not be computed. If 50% or more of the questions are completed: the mean of the completed questions is imputed for those that are missing.

2.5 Study assessments

***Baseline observation* and *Treatment* visits have a ± 14-day allowable visit window.**

Any AEs reported during trial visits, telephone calls or that come to the attention of the trial team by other means, are recorded in the patient's medical notes and the eCRF, within the trial MACRO database.

History of surgical interventions to manage MCDS symptoms are recorded at the screening visit. In addition, any incidence of surgical intervention to manage MCDS-related symptoms during the trial is recorded on the AEs crf.

Blood samples for laboratory safety assessments are taken at each *Baseline observation* visit and 6 monthly during the treatment phase. Bone biochemistry (tests comprise of total calcium and/or adjusted calcium*, 25-OHD [or total Vitamin D, depending on local reporting parameters], Phosphate and PTH) and X-rays are recorded at the same visits

Physical examinations are performed at each visit.

Pain questionnaires (PEDSQL Pain Coping Inventory and PEDSQL Pain Questionnaire) are administered at *Baseline observation* and *Treatment* visits as for AE reporting.

The PedsQL™ Generic Core Scales Version 4.0 Short Form (SF15) is used to capture participant QoL and is administered at Baseline observation and Dose titration visits.

To aid with study participant retention due to COVID 19, the 12-month visit window can be up to 12 months plus a further 8 weeks.

2.5.1 MCDS-Therapy participant visit schedule (modified from Protocol version)

	Stage 1: Dose titration participant			***Stage 2: Treatment phase participants***		
Stage of trial	**Visit**	**Timepoint**		**Visit**	**Timepoint**	
Consent/screening and eligibility	Consent Screening visit 1*			Consent Screening visit		
	Screening visit 2*	Up to 28 days post consent		Screening visit 2*	Up to 28 days post consent	
Baseline observation phase	Visit 1*	0	Months since **Baseline observation**	Visit 1*	0	Months since **Baseline observation**
	Visit 2	6**		Visit 2	6	
	Visit 3*	12***				
Dose titration phase	Visit 1.1*	0	Weeks since start of **Dose titration phase**			
	Visit 1.2	2				
	Dose evaluation telephone calls	1 -11				
	Visit 1.3	3	Months since start of **Dose titration phase**			
	Visit 1.4	6				
	Visit 1.5	9				
	Visit 1.6	12				
Treatment phase	Visit 2.1*	12*		Visit 2.1*	0	Months since start of **Treatment phase**
	Visit 2.2	15		Visit 2.2	3	
	Visit 2.3	18		Visit 2.3	6	
	Visit 2.4	21		Visit 2.4	9	
	Visit 2.5	24		Visit 2.5[֍]	12	
Optional extended treatment phase	Visit 2.6 [Δ]	27				
	Visit 2.7 [Δ]	30				
	Visit 2.8 [Δ]	33				
	Visit 2.9 [Δ ֍]	36				

*Visits which may coincide with the preceding or proceeding visit: - Screening visit 1 and 2/Visit 1 baseline observation; Visit 3 baseline observation/ visit 1.1 dose titration; Visit 3 baseline observation/Visit 2.1 treatment phase

**6 month visit may be missed for participants who have experienced delays to their visit schedule due to COVID-19 halt to the trial

***12 month visit may be rescheduled to 18 month visit for participants who have missed the 12 month visit due to COVID-19

[Δ] This is an optional visit subject to an additional informed consent

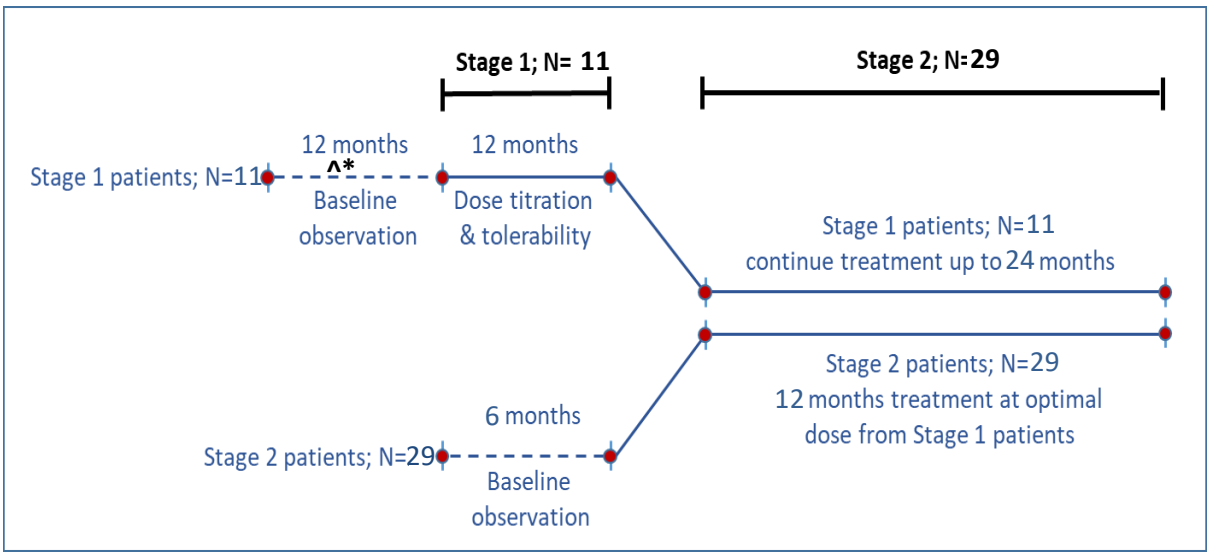
[֍] Visit 2.5 in Stage 2 and visit 2.9 in Stage 1 must occur no later than 31st January 2024.

2.6 Sample size and power

The sample size was chosen on the basis of the feasible number of patients that could be recruited to the trial in the specified time period. No statistical criteria were used to choose the sample size.

The trial was initially designed to have a cohort of 12 participants in Stage 1, the dose determination stage, and a cohort of 28 participants in the treatment stage, Stage 2. However due to the COVID-19 pandemic delays, this has been amended to Stage 1; N=11 and Stage 2; N=29. The overall sample size remains the same (N=40)

2.7 Overview of MCDS-Therapy Clinical Trial (from Protocol)



*The 12 month baseline observational visit may be rescheduled to a 18 month visit for participants who have missed the 12 month visit due to COVID-19 (Stage 1 UK participants only).

^ To aid with study participant retention due to COVID 19, with discussion with the CI, the 12 month baseline observational visit window can be up to 12 months and 8 weeks. This will be assessed on a case by case basis through discussion with the site PI[a] with approval from the CI and the NCTU team notified.

Eligible Stage 1 participants may continue treatment for further 12 months (up to 36 months) subject to a separate informed consent at visit 2.5.

Last Patient Last Visits in Stage 1 and Stage 2 must be completed by 31st January 2024.

3 Study Population

3.1.1 Screening, eligibility, and recruitment

Screening data, number assessed for eligibility, number not meeting eligibility criteria (with reasons), number declining participation (with reasons) and any other reasons for not entering the study will be reported.

3.1.2 Protocol deviations

Protocol deviations including missed safety assessments; incorrect dose escalations; missed study assessments; participants not using IMP as prescribed; and lack of compliance will be reported.

Example Table: Line listing of protocol deviations

Trial ID	Deviation type	Major/minor	Details
	Ineligible/Consent/Treatment not given as per-protocol/Withdrawal from treatment by investigator/Withdrawal from treatment due to participant choice/Use of prohibited concomitant medication/Study procedures/Visit schedule		

3.1.3 Follow-Up

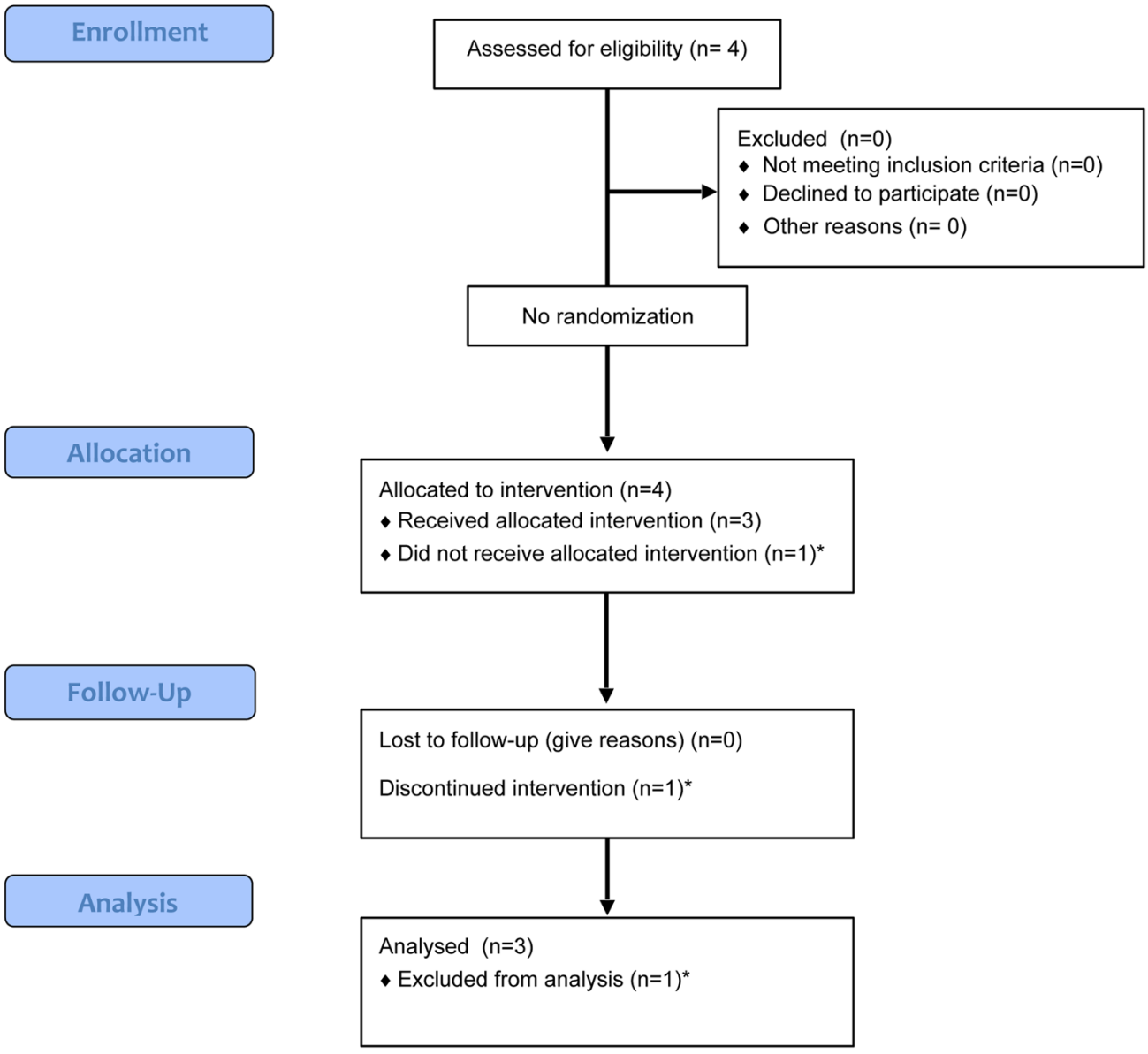
Participants have the right to withdraw from the trial at any time without having to give a reason. If a trial participant withdraws from the trial all data collected to the point of withdrawal will be retained. Consent/assent will be sought to allow this.

The number of patients withdrawing from trial specific follow-up visits, the number withdrawing from any follow-up data to be collected and the number lost to follow up, with timing of withdrawal will be reported.

3.1.4 Participant flow through trial

Patient flow through the trial will be presented using a CONSORT diagram (example below). Information will be provided on numbers and reasons (where appropriate) for: screened patients not being eligible; eligible patients not being consented; patients found to be ineligible after consent; patients not evaluable for the primary endpoints; withdrawal from follow-up; withdrawal of consent and all protocol violations.

Example Figure: CONSORT flow diagram



3.1.5 Baseline characteristics

Demographic, clinical and surgical characteristics recorded at the first visit during the baseline observation period will be summarised. Categorical data will be presented as frequencies and percentages, continuous data will be presented as number of patients, mean, median, standard deviation, minimum, maximum and range.

Demographic characteristics: age, sex, height (or length if <2 years old), sitting height in children >=5 years old, crown-rump length (CRL) in children <5 years old.

Clinical characteristics: age at onset of first symptoms, pain at diagnosis of MCDS (yes/no), current frequency of pain, joints affected with pain, surgical interventions to manage MCDS symptoms (yes/no), bone age, head/neck of femur angle.

Example Table: Patient demographics at baseline

	n=X	
Sex Male Female		
Age (years) Median Mean		
Median BMI		
Blood pressure (mmHg) Systolic Diastolic		

3.1.6 Treatment received

Stage 2 participants will receive Tegretol® for a 12 month period. Dosage will be calculated according to the participant's weight. As the trial is recruiting paediatric participants, participants will be weighed every 3 months as part of the physical examination and dose adjusted accordingly to ensure the appropriate dose is given to the participants as they grow. For Stage 2, participants CBZ will be titrated to a dose of 20mg/kg .

Participants will be initiated on a daily CBZ dose of 5mg/kg/day, divided into two separate doses. If the current dose is tolerated following seven days of treatment, the daily dose will be increased by 5mg/kg/day for a further seven days. The dose level will be increased every seven days until a maximum dose of 20mg/kg/day or MTD is achieved.

At weekly intervals and until they reach a stable dose, participants will receive a dose evaluation telephone call to determine if the current dose has been tolerated. Participants are asked to provide information regarding drug side effects, AEs, changes to concomitant medication, drug compliance and details of missed doses; a Diary is provided in which to record this information between calls/visits. All dosage decisions, IMP side effects, AEs and any changes to concomitant medication noted during the dose evaluation calls are recorded in the appropriate eCRF within the trial MACRO database.

If the participant reports side effects/AEs of significant concern to the clinician which are suspected to be related to the IMP, they are invited for further laboratory safety blood assessments and review. If the

participant reports severe side effects which prevent adherence to the trial protocol or in the opinion of the clinical investigator deem the participant not suitable to continue participation, then CBZ will be permanently discontinued, and the patient withdrawn from the trial. Participants who do not tolerate dosing will be kept on a lower dosing or dosing reduced.

For each participant, the following will be reported:

1. Initial prescribed daily dose (mg/kg) of CBZ and form (tablet/suspension).

2. Daily Dose (mg/kg) and prescribed daily dose (mg or ml, depending on CBZ form) will be shown graphically over the treatment period.

3. Compliance will be reported as the number of missed CBZ doses reported at each visit or dose evaluation phone call together with the percentage of the prescribed daily dose taken.

4. MTD

4 Statistical considerations

4.1 Timing of analyses

The *Stage 1 safety review* was conducted to formally agree the IMP dose regimen to be implemented in Stage 2 of the study, report to DMC dated 25/10/2021. The final analysis of Stage 2 will take place after data cleaning following the end of trial – this is defined as the date of last data collection for the last participant and is expected to be spring 2024.

4.2 Analysis populations

There will be two analysis populations:

1. Participants who have completed the baseline observation period and have received at least one dose of CBZ.

2. Participants who have completed their baseline observation period and have been prescribed, on average, at least 15 mg/kg of CBZ over their first 12 -months of treatment, i.e. a dose which is at least 75% of the dose recommended from Stage 1. Note that some participants will have longer than 12 months of treatment.

Adverse events will be reported for all participants who start their baseline period.

4.3 Primary analysis

The primary objective of Stage 2 is to evaluate efficacy of CBZ for the treatment of children with MCDS who are ambulant but have not yet reached skeletal maturity and to determine if the level of efficacy warrants a subsequent formal development programme for CBZ in this indication.

4.3.1 Statistical model for growth

For subject *i* (*i = 1,..,N*), let τ_{ij} be a treatment indicator taking the value 0 for measurements observed during the baseline period and 1 for measurements observed during the treatment period; treatment for individual *i* starts at time T_i.

Let y_{ij} denote a continuous response variable (e.g., height), observed at measurement times t_{ij} (j = 1,..,n_i), the model for which is:

$$y_{ij} = \beta_1 + \beta_2 t_{ij} + \beta_3 \tau_{ij}(t_{ij} - T_i) + \varepsilon_{ij},$$

where β_1, β_2 and $\beta_3$are regression parameters to be estimated using Generalised Estimating Equations (GEE). The "working" correlation matrix is given by,

$$Corr(\varepsilon_{ij}, \varepsilon_{ij'}) = \begin{cases} 1 & if\ \ j = j' \\ \rho & otherwise \end{cases}$$

This is sometimes referred to as an exchangeable covariance model. The first observation on each individual, y_{i1}, takes place at time t_{i1}=0.

- During the baseline period, $y_{ij} = \beta_1 + \beta_2 t_{ij} + \varepsilon_{ij.}$
- At the start of the treatment period, $y_{ij} = \beta_1 + \beta_2 T_i + \varepsilon_{ij}$.
- During the treatment period, $y_{ij} = (\beta_1 - \beta_3 T_i) + (\beta_2 + \beta_3) t_{ij} + \varepsilon_{ij.}$

The parameter β_3 represents the change in slope between the baseline and treatment periods, i.e., the change in growth velocity.

4.3.2 Analysis

All patients who have completed the baseline observation period and have received at least one dose of CBZ will be included in the primary analysis.

Height measurements will be displayed graphically by individual indicating measurements taken during baseline and treatment periods.

Height measurement data will be analysed using the STATA commend ***xtgee*** to estimate the parameters of the regression model in 4.3.1. The parameter β_3 representing the change in growth velocity will be reported together with 95% confidence interval. The Huber/White/sandwich estimator of variance will be used to obtain confidence intervals [in STATA: vce(robust)].

If fitting the growth model in section 4.3.1 is problematic, for example, lack of convergence, then a simplified analysis will be considered.

For subject *i* (*i = 1,..,N*), let v_{ik} denote growth velocity (a continuous response variable) measured during the baseline period (*k*=1) and the treatment period (*k*=2). Growth velocity during the baseline period for each participant will be estimated using the first available and last available measurement during the baseline period; the growth velocity during the treatment period will be estimated in a similar manner.

The model for growth velocity is:

$$v_{ik} = \beta_1 + \beta_{2i} + \beta_3 P_k + \varepsilon_{ik},$$

where $\beta_1, \beta_{2i}\ and\ \beta_3$ are regression parameters to be estimated using simple linear regression. P_k takes the value 0 for baseline period velocities (*k=1*) and 1 for treatment period velocities (*k=2*). As for the previous GEE model, the parameter β_3 represents the change in growth velocity between treatment and baseline periods. The parameter β_3 representing the change in growth velocity will be reported together with 95% confidence interval.

4.3.3 Secondary analysis of primary outcome

4.3.3.1 Surgical interventions

Surgical interventions are recorded throughout the baseline and treatment periods for all participants. Following completion of the trial a review will be conducted by the CI to assess whether any of the surgical interventions could have the potential to affect growth. In the event that some interventions are thought to have potentially affected growth, the primary analysis will be repeated using an extension to the primary analysis model which allows the growth rate to change following surgery.

Let δ_{ij} be an indicator variable taking the value 0 for measurements observed before time T_i^* and 1 for measurements observed after time T_i^*, where T_i^* denotes the time of the surgical intervention for participant *i.*

The model used to adjust for surgical interventions is then given by:

$$y_{ij} = \beta_1 + \beta_2 t_{ij} + \beta_3 \tau_{ij}(t_{ij} - T_i) + \beta_4 \delta_{ij}(t_{ij} - T_i^*) + \varepsilon_{ij}$$

Before the surgical intervention the model remains unchanged from the model used in the primary analysis, however after the surgical intervention, the model becomes

$$y_{ij} = (\beta_1 - \beta_4 T_i^*) + (\beta_2 + \beta_4) t_{ij} + \beta_3 \tau_{ij}(t_{ij} - T_i) + \varepsilon_{ij}$$

and so allows for a change in growth rate from the point of surgery onwards. The change in growth rate is estimated by the parameter β_4.

4.3.3.2 25-OH Vitamin D

25-OH vitamin D levels might predict secondary effects on bone health and could be considered a potential confounding variable. Bone biochemistry bloods (25-OHD vitamin D [nmol/L]) are recorded 6 monthly throughout the trial. 25-OH vitamin D levels will be included as a time-varying covariate in the primary analysis model to adjust for any effects of vitamin D level. A last observation carried forward approach will be taken to impute vitamin D levels when missing.

Let z_{ij} be the standardised 25-OH vitamin D level for participant *i* at time t_{ij} (j = 1,..,n_i),

i.e. $z_{ij} = (x_{ij} - \bar{x})/\sigma_x^2$, where x_{ij} is the observed 25-OH vitamin D level, and $\bar{x}$ and σ_x^2 are the sample mean and variance of all 25-OH vitamin D observations across all participants.

The model used to adjust for the effect of vitamin D is given by:

$$y_{ij} = \beta_1 + \beta_2 t_{ij} + \beta_3 \tau_{ij}(t_{ij} - T_i) + \beta_4 z_{ij} t_{ij} + \varepsilon_{ij}.$$

This can be written as:

$$y_{ij} = \beta_1 + (\beta_2 + \beta_4 z_{ij}) t_{ij} + \beta_3 \tau_{ij}(t_{ij} - T_i) + \varepsilon_{ij},$$

which makes clear how the time varying covariate, 25-OH vitamin D, affects the rate of growth.

4.3.4 Sensitivity analysis of primary outcome

The primary analysis will be repeated including only those participants who have completed their baseline observation period and have been prescribed, on average, at least 15 mg/kg of CBZ over their first 12 - months of treatment, i.e. a dose which is at least 75% of the dose recommended from Stage 1.

Note that some participants will have longer than 12 months of treatment. Dosing details during these extended periods of treatment will not be considered when determining if a participant is included in this sensitivity analysis. However, if a participant is included, then the participant's growth data for this extended period of treatment will be included in the analysis.

4.4 Analysis of secondary outcomes

To evaluate the effect of CBZ on height, bone conformation, pain, and health related quality of life in children with MCDS over 12 months.

4.4.1 Descriptive and graphical analysis

Outcome measures:

1) Height, for age and sex, *zscore*
2) Bone age SD – age related zscore (taken from the left wrist x-ray)
3) Tanner and Whitehouse bone age (TW3)
4) Femoral neck-shaft angle (NSA) on left and right legs
5) Deviation from midline at knee
6) PedsQL Pain Coping Inventory total score by participant (both parent and child completed if available) ***[Average score of 41 questions – 0, 1 or 2 for each question]***
7) Pediatric Pain Questionnaire: VAS scores for the questions *"how you feel now" and "worst pain you had this week"* (both parent and child completed if available) converted to a score of 0 (no pain) to 100 (Severe pain)
8) Paediatric Quality of Life Inventory (PedsQL) total score
   [*Likert scale used to score each of 15 questions transformed to a 0-100 scale as follows: 0=100, 1=75, 2=50, 3=25, 4=0*]
9) PedsQL Psychosocial Health sub-domain score
10) PedsQL Physical Health sub-domain score

will be plotted by individual for each available visit, indicating visits which took place during the baseline observation period and during the treatment phase.

4.4.2 Statistical modelling

A more general version of the statistical model used for the analysis of the primary outcome will be used to assess changes in secondary outcome measures possibly related to treatment. The model additionally allows for a possible step change in response at the start of the treatment period.

Defining τ_{ij}, t_{ij} and T_i in the same manner as used for the primary outcome analysis, let y_{ij} denote a continuous response variable, the model for which is:

$$y_{ij} = \beta_1 + \beta_2 t_{ij} + \beta_3 \tau_{ij}(t_{ij} - T_i) + \beta_4 \tau_{ij} + \varepsilon_{ij},$$

where $\beta_1, \beta_2, \beta_3$ and β_4 are regression parameters to be estimated using Generalised Estimating Equations (GEE). The "working" correlation matrix is the same as used for the primary analysis.

- During the baseline period, $y_{ij} = \beta_1 + \beta_2 t_{ij} + \varepsilon_{ij.}$
- At the start of the treatment period, $y_{ij} = \beta_1 + \beta_2 T_i + \beta_4 + \varepsilon_{ij}.$
- During the treatment period, $y_{ij} = (\beta_1 - \beta_3 T_i + \beta_4) + (\beta_2 + \beta_3) t_{ij} + \varepsilon_{ij.}$

The step change in response at the start of the treatment period is modelled by the parameter β_4; the parameter β_3 represents the increase in slope during the treatment period compared to the baseline period as previously.

Height *zscore,* PedsQL Pain Coping Inventory total score, Pediatric Pain Questionnaire VAS score, Paediatric Quality of Life Inventory (PedsQL) total score, PedsQL Psychosocial Health sub-domain score and PedsQL Physical Health sub-domain score will be analysed using the STATA commend ***xtgee*** to estimate the parameters of the regression model above. The parameter β_3 and β_4 will be reported together with 95% confidence interval. The Huber/White/sandwich estimator of variance will be used to obtain confidence intervals [in STATA: vce(robust)].

4.4.3 Additional secondary analyses

Use of surgical interventions to manage the condition will be captured on the eCRF over the whole follow up period. These will be reported descriptively as the type and proportion of participants experiencing an event per three month period from baseline until the end of trial follow up.

5 Safety

Laboratory safety assessments outside the appropriate local normal reference range will be reported at a participant level. Abnormal physical examinations will be reported at a participant level together with any further details provided.

All AEs occurring from the point of consent to the end of trial participation are recorded in the trial MACRO database eCRF. AEs are assessed for their relationship to treatment and coded as: Unrelated, Unlikely, Possible, Probable, Definitely, or Not assessable. Severity is graded according to the Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0, Published: November 27, 2017.

Grading is based on the following general guideline:

- **Grade 1 Mild**; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- **Grade 2 Moderate**; minimal, local, or non-invasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL).
- **Grade 3 Severe** or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
- **Grade 4 Life-threatening** consequences; urgent intervention indicated.
- **Grade 5 Death** related to AE.

AEs are coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 22.0. The Clinical Data Management System (CDMS) auto-codes the majority of AEs to the latest version of the MedDRA dictionary. If this is not possible, AEs are coded from the MedDRA dictionary by the CI.

SARs occurring from the first participant's dose of IMP to the last participant's end of follow-up are recorded on the trial MACRO database eCRF. Following this defined active monitoring period for SARs, investigators are still required to report any SARs or SUSARs which are brought to their attention.

For each SAE the following information is collected:

1. Full details in medical terms and case description
2. Event duration (start and end dates, if applicable)
3. Action taken
4. Outcome
5. Seriousness criteria
6. Causality in the opinion of the investigator
7. Whether the event is considered expected or unexpected in accordance with the approved RSI if a causal relationship is suspected.

5.1.1 Adverse events

The number of adverse events and adverse reactions (those possibly, probably or definitely related to treatment) and the worst grade AE and AR per participant will be summarised descriptively.

Example table: Number of events per participant

	Mean (SD)	Median (Q1, Q3)	Min, Max
All adverse events			
Adverse reactions (possibly, probably, definitely)			

Example table: Worst grade reported per participant (number, %)

	None	Mild	Moderate	Severe
All adverse events				
Adverse reactions (possibly, probably, definitely)				

The number of participants reporting each type of adverse event (by MedDRA System Organ Class) will be tabulated.

Example table: Number and percentage of participants affected by each adverse event, reported by System Organ Class

Event term	Total		Mild		Moderate		Severe	
	N	%	N	%	N	%	N	%
Blood and lymphatic system disorders								
Cardiac disorders								
Endocrine disorders								
Eye disorders								
Gastrointestinal disorders								
General disorders and administration site conditions								
Immune system disorders								
Infections and infestations								
Injury, poisoning and procedural complications								
Investigations								
Metabolism and nutrition disorders								
…..								
…..								

The number and percentage of patients experiencing at least one high grade event (grade 3/4/5).

A line listing of all AEs will be reported, by participant, including severity, seriousness and relationship to study treatment.

5.1.2 Serious adverse events

A chronological listing of serious adverse events (SAEs) will be presented. The number of SAEs and the number of patients reporting at least one SAE will be reported.

Patient ID	Description	MedDRA Preferred Term	Severity	Relationship to treatment	Onset Date	Date of resolution	Duration	Seriousness criteria	Action taken in relation to SAE	Outcome

6 Statistical Software

Data will be output directly from MACRO into a STATA format by the NCTU at time-points agreed by the TMG. Statistical analyses will be carried out by the Trial Statistician at the PHSI BRG using Stata version 16. All programs and output will be stored in the School Statistics folder on the PHSI server.

7 Appendix: CTCAE grading relevant to MCDS-Therapy IMP DLTs

MedDRA Code	MedDRA SOC	CTCAE Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Definition	
10002272	Blood and lymphatic system disorders	Anaemia	Haemoglobin (Hgb) <LLN - 10.0 g/dL; <LLN - 6.2 mmol/L; <LLN - 100 g/L	Hgb <10.0 - 8.0 g/dL; <6.2 - 4.9 mmol/L; <100 - 80g/L	Hgb <8.0 g/dL; <4.9 mmol/L; <80 g/L; transfusion indicated	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by a reduction in the amount of haemoglobin in 100 ml of blood. Signs and symptoms of anaemia may include pallor of the skin and mucous membranes, shortness of breath, palpitations of the heart, soft systolic murmurs, lethargy, and fatigability.	
1019491	Blood and lymphatic system disorders	Haemolysis	Laboratory evidence of haemolysis only (e.g., direct antiglobulin test; DAT; Coombs'; schistocytes; decreased haptoglobin)	Evidence of hemolysis and >=2 g decrease in haemoglobin	Transfusion or medical intervention indicated (e.g., steroids)	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by laboratory test results that indicate widespread erythrocyte cell membrane destruction.	
10001551	Investigations	Alanine aminotransferase increased	>ULN - 3.0 x ULN if baseline was normal;	>3.0 - 5.0 x ULN if baseline was normal; >3.0 - 5.0	>5.0 - 20.0 x ULN if baseline was	>20.0 x ULN if baseline was normal; >20.0 x	-	A finding based on laboratory test results that indicate an	Also consider Hepatobiliary

			1.5 - 3.0 x baseline if baseline was abnormal	x baseline if baseline was abnormal	normal; >5.0 - 20.0 x baseline if baseline was abnormal	baseline if baseline was abnormal		increase in the level of alanine aminotransferase (ALT or SGPT) in the blood specimen.	disorders: Hepatic failure
10001675	Investigations	Alkaline phosphatase increased	>ULN - 2.5 x ULN if baseline was normal; 2.0 - 2.5 x baseline if baseline was abnormal	>2.5 - 5.0 x ULN if baseline was normal; >2.5 - 5.0 x baseline if baseline was abnormal	>5.0 - 20.0 x ULN if baseline was normal; >5.0 - 20.0 x baseline if baseline was abnormal	>20.0 x ULN if baseline was normal; >20.0 x baseline if baseline was abnormal	-	A finding based on laboratory test results that indicate an increase in the level of alkaline phosphatase in a blood specimen.	
10005364	Investigations	Blood bilirubin increased	>ULN - 1.5 x ULN if baseline was normal; > 1.0 - 1.5 x baseline if baseline was abnormal	>1.5 - 3.0 x ULN if baseline was normal; >1.5 - 3.0 x baseline if baseline was abnormal	>3.0 - 10.0 x ULN if baseline was normal; >3.0 - 10.0 x baseline if baseline was abnormal	>10.0 x ULN if baseline was normal; >10.0 x baseline if baseline was abnormal	-	A finding based on laboratory test results that indicate an abnormally high level of bilirubin in the blood. Excess bilirubin is associated with jaundice.	Also consider Hepatobiliary disorders: Hepatic failure
10011368	Investigations	Creatinine increased	>ULN - 1.5 x ULN	>1.5 - 3.0 x baseline; >1.5 - 3.0 x ULN	>3.0 x baseline; >3.0 - 6.0 x ULN	>6.0 x ULN	-	A finding based on laboratory test results that indicate increased levels of creatinine in a biological specimen.	Also consider Renal and urinary disorders: Acute kidney injury
10064848	Renal and urinary disorders	Chronic kidney disease	eGFR (estimated Glomerular Filtration Rate) or CrCl (creatinine clearance) <LLN - 60 ml/min/1.73 m2 or proteinuria 2+ present; urine protein/creatinine >0.5	eGFR or CrCl 59 - 30 ml/min/1.73 m2	eGFR or CrCl 29 - 15 ml/min/1.73 m2	eGFR or CrCl <15 ml/min/1.73 m2; dialysis or renal transplant indicated	Death	A disorder characterized by gradual and usually permanent loss of kidney function resulting in renal failure.	