

Additional file 2. Description of minor protocol changes made during the conduct of the study (version date 22-Mar-2019, through version date 25-Oct-2023).

Protocol version and date	Date of Research Ethics Board (REB) approval at study coordinating centre (CHEO/Clinical Trials Ontario)	Summary of modifications¹
Protocol v: 11-Jul-2019	22-Jul-2019	1. Data collection schedule updated. 2. Flow chart added to clarify adverse event (AE) reporting. 3. Mention of completing physician oversight logs in REDCap removed.
Protocol v: 25-Jul-2019	25-Jul-2019	1. For adverse event reporting: (i) added that events will be reviewed by the sponsor-investigator before they are reviewed by the data safety monitoring board (DSMB); (ii) added that 'site study staff will complete the AE reporting form' and added 'within 24 hours of becoming aware of the SAE'; (iii) clarified meaning of 'Is the event a specified safety endpoint?'
Protocol v: 16-Dec-2019	08-Jan-2020	1. Timing of discharge questionnaire administration changed from pediatric intensive care unit (PICU) discharge to hospital discharge. Also clarified that these will only be administered if the patient is discharged after the 28-day follow-up (and if discharge questionnaires are administered within the 28-day follow-up window, they will only be administered once for both hospital discharge and Day 28). 2. Clarified that obtaining consent for participation in the biobank may occur at the same time as the consent discussion for participation in the VITdALIZE-KIDS trial, or at a separate time (e.g. follow-up visit). The option to obtain biobank consent by telephone (in the presence of an impartial witness) was also added. 3. Follow-up questionnaires (28-day and 90-day) may be sent to families by mail, if they have indicated they prefer this, or if they cannot be contacted by phone.
Protocol v: 08-Jan-2020	20-Jan-2020	1. (Editorial change)
Protocol v: 30-Nov-2020	07-Dec-2020	1. Inclusion criterion was previously an expected PICU length of stay >48 hours at the time of study enrollment (i.e. study drug administration), but was changed to allow the patient to: (1) have an

expected PICU length of stay of ≥ 48 hours at time of enrollment, OR (2) be in PICU at time of enrollment (i.e. study drug administration) and have an expected hospital length of stay of ≥ 7 days.

2. Correction of inconsistent protocol wording surrounding baseline questionnaire administration (it will be administered within 72 hours of study enrollment, *not* within 72 hours of PICU admission).
3. Removed statement indicating that the study team would keep empty syringes after drug administration (for drug accountability) due to COVID-19 precautions. This change was discussed with, and approved by, the CHEO Research Institute (RI) Quality Assurance team.
4. The following were clarified within the protocol:
 - (i) in the Prohibited Medications section, thiazide diuretics are contraindicated in conjunction with calcium administration above the daily recommended intake, and patients will be excluded if they are receiving this combination of medication;
 - (ii) it will not be considered a protocol deviation if a patient is randomized while fully eligible, but becomes NPO (nothing by mouth) and cannot receive the study drug;
 - (iii) sample volume for screening/enrollment (4-5mL) can be split over the screening and enrollment time points rather than collected in full at the time of screening;
 - (iv) it will not be considered a protocol deviation if enrollment blood and/or urine cannot be collected prior to study drug administration;
5. Agreement to participate in the VITdALIZE-KIDS Biobank can be completed via email using Docu-Sign for electronic signature.
6. If preferred by the legal guardian, the 28- and 90-day follow-ups can be completed via Zoom (password protected meeting).
7. Previously, families were given a \$25 gift card for completing both the 28- and 90-day questionnaires. This has been changed to a \$25 gift card after completion of each follow-up questionnaire (i.e. \$50 for full study completion).
8. Monitoring frequency for sites has been changed from 'every 6 months or more or less frequently as needed' to: 'every 6 months or once 12 patients have been enrolled since the previous monitoring visit, whichever occurs later, or more or less frequently as per the study monitoring plan.' This change was made as recruitment rates were lower than anticipated.
9. Monitoring of the study coordinating centre (CHEO) will be performed by the CHEO RI Quality Assurance and Regulatory Compliance program (or their representatives), rather than peer-to-peer monitoring.

Protocol v: 07-
Feb-2022

07-Mar-2022

1. Electronic signature options removed (non-validated electronic signatures cannot be used for regulatory documents)
2. Clarified that the impact of the study intervention on 25OHD status will only be evaluated in a subset of enrolled patients (all consecutively enrolled patients for whom an enrollment and post-intervention blood sample was obtained).
3. Criteria for inclusion in the biomarker and metabolomics sub-study was added to section 2.3.1 (collection of an enrollment and at least one post-intervention blood and urine sample).
4. Inclusion criterion changed from 'anticipated PICU stay ≥ 48 hours at the time of study enrollment, OR patient will be in PICU at the time of study drug administration and has an expected hospital length of stay of ≥ 7 days' to: inclusion criterion 'admitted to PICU' and exclusion criterion 'patient will be discharged from PICU before they can be enrolled (i.e. study drug administered) into the VITdALIZE-KIDS study and/or patient will be in hospital for < 3 days following enrollment.'
5. Removed inclusion criterion 'expected to require clinically indicated bloodwork in hospital > 48 hours (range: Day 2-7) after receipt of the intervention.'
6. Modification of exclusion criterion from 'treating physician refuses enteral drug administration due to gastrointestinal disorder' to: 'treating physician refuses enteral drug administration due to gastrointestinal disorder, and expects to do so for the full duration of the patient's PICU admission.'
7. Clarified that enrollment and post-intervention blood/urine samples will only be collected for consecutively enrolled patients with access to sample collection until the time that 100 patients with enrollment and post-intervention blood/urine samples have been enrolled, except at select sites; also clarified when samples will be analyzed.
8. Added the following statement: 'The duration of the recruitment period will be extended to account for recruitment delays as a result of the COVID-19 pandemic. We will re-evaluate recruitment and study duration in the summer of 2022.'
9. Added 'assuming a vitamin D deficiency rate of 60-70%' to the statement indicating an expected recruitment rate of 2 to 2.5 patients per site per month.
10. Updated protocol to reflect that questionnaires will only be administered at hospital discharge for the subset of patients with a hospital length of stay following enrollment of ≥ 14 days.
11. Removed the requirement for additional blood sampling after specific triggers (e.g. ECMO).
Note: this only applies to enrolled patients, not to rescreening of potential participants who did not initially meet vitamin D deficiency criteria but subsequently underwent interventions known to reduce 25OHD.

12. Added the following note to the description of planned interim analyses: 'At the January 2022 VITdALIZE-KIDS Steering Committee meeting, we discussed the possibility of conducting the interim analysis sooner, given the impact of the COVID-19 pandemic and a lower-than-expected vitamin D deficiency rate on recruitment. The Steering Committee (in consultation with the Chair of the VITdALIZE-KIDS Data Safety Monitoring Board), agreed to re-evaluate study progress in the summer of 2022 and make a decision at that time about modifications to the analysis schedule.
13. The title for safety endpoint 'clinically significant nephrolithiasis or nephrocalcinosis in the context of new hypercalciuria' has been updated ('in the context of new hypercalciuria' was removed, as urine samples are no longer being collected). The definition of this safety endpoint has also been modified accordingly.

Protocol v: 11-Apr-2022	13-May-2022	1. (Editorial change)
Protocol v: 16-May-2022	17-May-2022	1. (Editorial change)
Protocol v: 26-Jul-2023	12-Oct-2023	1. (Editorial changes and clarifications) 2. Study duration updated to March 2026; coincides with recruitment target changing from 2-2.5 participants/month/site to 1-2 participants/month/site, due to lower than expected VDD (~40%). 3. Cost of illness removed as tertiary outcome. 4. Clarification made to exclusion criterion (new text underlined): "<i>Patient on thiazide diuretics also receiving regular ongoing calcium supplementation above the daily recommended intake (<u>excluding calcium supplementation administered for the treatment or prevention of hypocalcemia</u>)</i>". This represents a clarification, as opposed to a change, to this criterion. 5. Follow-up questionnaires may be conducted by email or mail. 6. Clarification that IP administration should be followed by a flush of tube using local site policy, and provided additional instruction if the site does not have a local policy. This update reflects standard practice for medication administration. 7. Statement that IP dose should be calculated based on Ideal Body Weight, if clinical medication volumes are being calculated this way (standard practice). 8. Clarified that sub-analysis will be based on sex, not gender, to reflect correct terminology 9. Storage period updated from 25 to 15 years, to reflect current Health Canada requirements. 10. Protocol updated to match revised monitoring plan (i.e. sites are monitored after primary outcomes are available for the first participant enrolled; previously it was after the first two participants).

11. Added a statement to section on SAE reporting to indicate that PI will make the final decision on expectedness and causality of SAEs.
12. Added contact number for out-of-hours medical and non-medical questions from participating site QIs

Protocol v: 25- 31-Oct-2023
Oct-2023

1. Added section on participant co-enrollment with other studies.

¹Editorial changes to protocol (e.g. typo corrections, rewording) not included in this table.