An optimised versus standard dosing regimen of vancomycin in infants with Gram-positive sepsis (NeoVanc): a multi-centre randomised, open-label, phase IIb, non-inferiority trial

Authors: Louise F Hill*, Michelle N Clements*, Mark A Turner, Daniele Donà, Irja Lutsar, Evelyne Jacqz-Aigrain, Paul T Heath, Emmanuel Roilides, Louise Rawcliffe, Clara Alonso-Diaz, Eugenio Baraldi, Andrea Dotta, Mari-Liis Ilmoja, Ajit Mahaveer, Tuuli Metsvaht, George Mitsiakos, Vassiliki Papaevangelou, Kosmas Sarafidis, A Sarah Walker, Michael Sharland, on behalf of the NeoVanc Consortium†

Paediatric Infectious Diseases Research Group, Institute for Infection and Immunity, St George's, University of London, London, UK (LF Hill MBChB, Prof PT Heath FRCPCH, Prof M Sharland MD)
Medical Research Council Clinical Trials Units, University College London, London, UK (MN Clements PhD, Prof AS Walker PhD)
Institute of Translational Medicine, University of Liverpool, Liverpool, UK (Prof MA Turner PhD)
Division of Pediatric Infectious Diseases, Department of Women's and Children's Health, University of Padova, Padova, Italy (Daniele Donà PhD)
Fondazione Penta – ONLUS, Padua, Italy (Daniele Donà PhD)
University of Tartu, Tartu, Estonia (Prof I Lutsar PhD)
Department of Pediatric Pharmacology and Pharmacogenetics, Hôpital Robert Debré, Paris, France (Prof E Jacqz-Aigrain PhD)
3rd Department of Pediatrics, Aristotle University, Thessaloniki, Greece (Prof E Roilides PhD)
Therakind Ltd., London, UK (L Rawcliffe MBA)
Hospital 12 de Octubre, Madrid, Spain (C Alonso-Diaz PhD)
Azienda Ospedale-Universita' di Padova, Fondazione Istituto di Ricerca Pediatrica, Padova, Italy (Prof E Baraldi MD)
Ospedale Pediatrico Bambino Gesu', Rome, Italy (A Dotta MD)
Tallinn Children's Hospital, Tallinn, Estonia (M-L Ilmoja MD)
St Mary's Hospital, Manchester, UK (A Mahaveer MRCPCH, MD)
Tartu University Hospital, Tartu, Estonia (Prof T Metsvaht PhD)
Papageorgiou Hospital, Thessaloniki, Greece (Prof G Mitsiakos PhD)
General University Hospital, Attikon, Chaïdári, Greece (Prof V Papaevangelou PhD)
Hippokration Hospital, Thessaloniki, Greece (Prof K Sarafidis PhD)

* Louise F Hill and Michelle N Clements have contributed equally and are co-first authors

† See appendix pp 1–2 for full list of investigators included in the NeoVanc Consortium

Correspondence to:
Dr Louise F Hill, Paediatric Infectious Diseases Research Group, Institute for Infection and Immunity, St George's, University of London, Jenner Wing, Level 2, Room 2.216F, Mail Point J2C, Cranmer Terrace, London SW17 0RE, United Kingdom
lhill@sgul.ac.uk

Summary

Background Vancomycin is the most widely used antibiotic for neonatal Gram-positive sepsis, but clinical outcome data of dosing strategies are lacking. The NeoVanc programme comprised extensive pre-clinical studies to inform an optimised vancomycin dosing randomised controlled trial (RCT). The primary objective was to compare the efficacy of an optimised regimen to a standard regimen in infants with late onset sepsis, known or suspected to be caused by Gram-positive microorganisms.

Methods NeoVanc was an open-label, parallel, phase IIb, non-inferiority RCT comparing efficacy and toxicity of an "optimised" regimen of vancomycin to a "standard" regimen in infants ≤90 days. Infants with ≥3 clinical/laboratory sepsis criteria or confirmed Gram-positive sepsis with ≥1 clinical/laboratory criterion were enrolled from 22 neonatal intensive care units in 5 European countries. Randomisation was 1:1 to the optimised regimen (25mg/kg loading dose followed by 5±1 days of 15 mg/kg q12h or q8h dependent on postmenstrual age (PMA)) or standard regimen (no loading dose; a 10±2 day course at 15 mg/kg q24h, q12h, or q8h). The primary endpoint was successful outcome at end of vancomycin therapy (EVT) and no clinically/microbiologically significant relapse/new infection requiring anti-staphylococcal antibiotics within 10 days of EVT. Non-inferiority margin was –10%. Secondary endpoints included abnormal hearing screening. Recruitment stopped at 242 (120 optimised arm; 122 standard arm) infants; it was not possible to reach the sample size of 300 within remaining trial timelines. Trial registration: ClinicalTrials.gov (NCT02790996).

Findings 64/90 (71%) infants in the optimised and 73/92 (79%) in the standard arm (per-protocol analysis) had a successful primary outcome; non-inferiority was not confirmed (adjusted risk difference –7% 95% CI –15% to +2%). Incomplete resolution of clinical/laboratory signs after 5±1 days of vancomycin therapy was the main factor contributing to failure in the optimised arm. Hearing in the ITT population was abnormal in 25/84 (30%) infants in the optimised arm and 12/79 (15%) in the standard arm (adjusted risk ratio: 1·72; 95% CI (1·0–2·9).

Interpretation In the largest neonatal vancomycin efficacy trial yet conducted, no clear clinical impact of shorter duration was demonstrated. The use of the optimised regimen cannot be recommended as a potential hearing safety signal was identified; long-term follow-up will be conducted. These results emphasise the importance of robust clinical safety assessments of novel antibiotic dosing regimens in neonates.

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Background

Neonatal sepsis is a major public health concern, with ~3 million cases/year globally.[1] Coagulase negative staphylococci (CoNS) are skin and gut commensals and the most commonly isolated organisms in late onset sepsis (LOS) in high income countries,[2] particularly in association with central lines. Although overall CoNS LOS mortality rates are low,[3] CoNS sepsis is associated with neurodevelopmental sequelae.[4] CoNS are often multi-drug resistant[5] and the emergence of vancomycin heteroresistant organisms globally is concerning; these organisms are increasingly reported in neonates.[6,7]

Vancomycin is the most widely used antibiotic for Gram-positive LOS.[8] Neonatal vancomycin dosing and durations vary markedly,[9] leading to different drug exposures.[10] Robust infant pharmacokinetic (PK), safety and clinical efficacy data, for different dosing strategies, are lacking.[11] The NeoVanc project addressed this gap.

Pre-clinical components of the NeoVanc Programme included hollow-fibre infection (HFI) and rabbit models and a population PK meta-analysis (Supplementary Figure 1, appendix p 4). This work and a clinical bridging study determined that frequent dosing facilitated bacterial kill and led to quicker reduction in C-reactive protein whilst continuous infusions appeared to select for vancomycin heteroresistance.[12] The neonatal PK model suggested standard dosing regimens had low vancomycin target attainment and supported the use of a loading dose, to shorten the time to achieving therapeutic levels when combined with more frequent dosing in infants <29 weeks postmenstrual age (PMA).[13] Both the NICU bridging study and PK model indicated the need for more frequent dosing in infants <29 weeks PMA, where it can take days to achieve therapeutic levels. A vancomycin loading dose is routine in adults and has been used in neonates in association with continuous infusions[14], however, it is novel within the context of intermittent dosing. The subsequent optimised dosing regimen for the NeoVanc RCT was a short course (5±1 days) of vancomycin with a loading dose and more frequent dosing in infants <29 weeks PMA compared to a standard of care regimen of 10±2 days. Shorter vancomycin durations are supported by retrospective analyses.[15] A non-inferiority design was selected as shorter treatment duration was not expected to lead to higher efficacy than longer treatment duration but result in potential secondary benefits, including reduced rates of antimicrobial resistance and toxicity, because of lower overall vancomycin exposure.

Potential toxicity of vancomycin includes nephrotoxicity and ototoxicity. Neonatal vancomycin safety studies have historically been underpowered and relied upon retrospective analyses of routinely collected data.[16] Robust, pre-clinical neonatal vancomycin ototoxicity models are lacking.[16]

The NeoVanc RCT aimed to use a loading dose of vancomycin to provide faster target attainment with a new, shorter optimised regimen, thus reducing overall vancomycin exposure without affecting clinical efficacy or increasing toxicity when compared to the standard dosing regimen in infants with LOS known or suspected to be caused by Gram-positive microorganisms. The overall aim was to test whether the optimised regimen, which included a loading dose, was non-inferior to the standard regimen.

Methods

Study design

NeoVanc was an open-label, multi-centre, Phase IIb, randomised, active control, parallel group, non-inferiority trial recruiting participants across 22 NICUs in 5 European countries – Greece, Italy, Estonia, Spain and the United Kingdom. All were tertiary NICUs prescribing vancomycin routinely and selected to ensure representation of variation in neonatal intensive care practice across Europe.

NeoVanc was approved by the London–West London & GTAC Research Ethics Committee (REC reference: [16]/LO/1026) on 18th July 2016. Protocol amendments are outlined in the appendix (p 3). Ethics Committee and Regulatory Body approvals were gained in each participating country/hospital. Written, informed consent was obtained from all participants' parents/guardians by trained research personnel. Consent could be obtained if <24 hours of antibiotics had been administered in the current sepsis episode. Pre-consent was also allowed provided consent was re-confirmed if the infant became unwell. The study was performed in accordance with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice guidelines, local regulations and study standard operating procedures.

Participants

The protocol has been published elsewhere.[17] Briefly, infants were eligible for inclusion if they had a postnatal age of ≥72 hours and <90 days at randomisation and had clinical sepsis or blood culture positive sepsis. Modified EMA criteria were applied to identify clinical sepsis;[18] enrolment required ≥3 clinical or laboratory criteria or a positive culture with Gram-positive bacteria from a normally sterile site and ≥1 clinical or laboratory criterion, in the 24 hours prior to randomisation. Trial inclusion and exclusion criteria and post-randomisation exclusions from efficacy analysis are detailed in Supplementary Table 1 (appendix pp 5–6). Any infant who received ≥1 dose of study vancomycin was followed-up for safety.

Randomisation, minimisation and masking

Infants were randomised in a 1:1 allocation ratio for each regimen. A secure, web-based system (ClinInfo SAS Lyon, France), was adopted for randomisation, which was controlled through an authorised username and password. Infants were recruited and randomised by trained investigators at each site. A minimisation algorithm ensured balance between arms in relation to

baseline data – NICU, PMA, and presence/absence of an umbilical catheter/central line. Local investigators and parents/guardians were not blinded to regimen allocation. The trial management group and trial data analysts were blinded to aggregate outcomes apart from statisticians who were unblinded for interim analyses and Independent Data Monitoring Committee (IDMC) meetings.

Data management

Data were collected in an electronic case report form (eCRF) managed by Consorzio per Valutazioni Biologiche e Farmacologiche (Pavia, Italy). All collected data remained strictly confidential and anonymous.

Procedures

Infants received either the standard regimen: a 10±2 day course of 15 mg/kg q24h (PMA <29 weeks), q12h (PMA 29–35 weeks) or q8h (PMA >35 weeks), or the optimised regimen: a 5±1 day course of a single loading dose of 25 mg/kg followed by a maintenance dose of 15 mg/kg q12h (PMA ≤35 weeks) or q8h (PMA >35 weeks). Vancomycin hydrochloride (supplied by Laboratorio Reig Jofre, Barcelona, Spain) was administered intravenously via 60-minute infusion. In the optimised arm, the first maintenance dose was administered 8 or 12 hours after the loading dose, dependent on PMA; infants, therefore, received 10 mg/kg plus the 15 mg/kg maintenance dose (25 mg/kg in total as a "loading dose") as their first dose compared to the first maintenance dose of 15mg/kg in the standard arm. Vancomycin durations outside the specified limits were permitted based on clinician assessment. The standard treatment regimen was based on European dosing recommendations[19], with the 10±2 day duration being chosen to best-reflect current practice across European NICUs from pre-trial surveys, as no reference information from RCTs was available. Dose adjustments were permitted through routine therapeutic drug monitoring (TDM) or renal impairment, where modifications were made based on vancomycin levels and local policy.

Study visits are specified in Supplementary Table 2 (appendix pp 7–8). Clinical and laboratory parameters were monitored in accordance with the modified EMA neonatal sepsis criteria, at Day 3, Day 5±1 and Day 10±2 (standard arm or if still receiving study vancomycin only).[18] At the end of actual vancomycin therapy (EVT), improvement in overall clinical status was assessed, as defined in the protocol. Infants fulfilling these criteria proceeded to test of cure (TOC; primary endpoint visit),

10±1 days after EVT, where clinically significant new infections, microbiological relapse and/or microbiological new infections were recorded (Supplementary Table 3, appendix pp 9–11). Relapse/new infections were assessed at a short-term follow-up (STFU) visit at 30±5 days from initiation of study vancomycin.

Hearing screening was performed between EVT and 90 days after randomisation. Otoacoustic emissions (OAE) and/or auditory brainstem responses (ABR) were permitted as per local clinical practice; abnormal hearing was defined as no clear response in one ear on OAE or ABR.

Outcomes

Given the low mortality in CoNS sepsis, the primary outcome was based on clinical recovery, defined using modified EMA guidance[18] and expert consensus, as success at the test of cure (TOC) visit (10±1 days after EVT) in the per protocol population. Primary outcome success components were: participant was alive at TOC; participant had a successful outcome at EVT; participant had not had a clinically/microbiologically significant relapse/new infection requiring treatment with vancomycin or other specific anti-staphylococcal antibiotic (flucloxacillin, oxacillin, linezolid, tedizolid, daptomycin or teicoplanin) for >24 hours. Success at EVT was defined as participant was alive, there was a significant improvement in participant's overall clinical status, microbiological resolution or presumed eradication of bacteria and no new vancomycin-susceptible pathogens were identified (Supplementary Table 3, appendix pp 9–11). Success was evaluated using a clinical algorithm (Supplementary Table 3, appendix pp 9–11) that did not rely on physician assessment of outcome.

Secondary efficacy outcomes were: success at 5±1 days from initiation of study vancomycin; success at EVT; success at end of allocated therapy (EOAT; pre-specified in the Statistical Analysis Plan (SAP)); failure at TOC visit due to clinically/microbiologically significant relapse/new infection requiring treatment with non-anti-staphylococcal ("other") antibiotics for >24 hours; and failure at STFU.

Other secondary PK and microbiology outcomes will be reported separately when laboratory results are available.

Safety and adverse event assessment

Secondary safety outcomes included: abnormal renal function at STFU (urinary output <0.7 mL/kg/hours for 24 hours and/or creatinine value ≥100 µmol/L (≥1.13 mg/dL)); abnormal hearing screening tests after EVT; adverse events (AEs) up to STFU; vancomycin-related AEs; all serious adverse events (SAEs); and vancomycin-related SAEs. All AEs and SAEs occurring between the administration of the first dose of study vancomycin and the final follow-up visit were recorded in the eCRF.

Sample size

In total, 150 infants per arm provided at least 90% power to demonstrate non-inferiority using a two-sided 95% confidence interval (i.e. type I error rate of 2.5%), assuming a success rate in both arms of 95% and a non-inferiority margin on the risk-difference scale of 10% (Wilson-score method (nQuery. Statistical Solutions Ltd., Cork, Ireland)). A 5% relapse/new infection rate was based on data from neonIN, an international neonatal infection surveillance network and the magnitude of a clinically relevant effect was obtained through consensus in the NeoVanc Consortium. There is no regulatory guidance from either the U.S. Food and Drug Administration (FDA) or the EMA on neonatal sepsis trials, although a non-inferiority margin of 10% has been recommended by the FDA for acute pneumonia RCTs where treatment is believed to be highly efficacious.[20] The 10% non-inferiority margin was based on relapse/new infection and is in-keeping with adult antibiotic RCTs.[21,22] A power sensitivity analysis, without reference to the data, was performed when it became apparent that this sample size would not be met within the project timelines. This analysis indicated there would not be an appreciable increase in power gained from the expected sample size of 100 per arm (expected power = 83% using the same parameters as the original sample size calculation) to the maximum possible sample sizes, given resource and time limitations (power = 87% for 110 per arm). An IDMC reviewed the data periodically and the trial was consequently stopped before the planned recruitment target was met.

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Statistical analysis

The intention to treat population (ITT) comprised all randomised infants except post-randomisation exclusions and where consent to use data had subsequently been withdrawn (safety analysis population). The per protocol (PP) population (efficacy analyses) additionally excluded infants randomised in error, with a loading dose not administered as randomised, or duration of

vancomycin <48 hours from initiation of study vancomycin. The primary analysis used binomial regression with an identity link to report risk difference and associated 95% CI, with a non-inferiority margin of –10%. Inference was based on adjusted estimates, where PMA (<29 weeks/29-35 weeks/>35 weeks), and presence/absence of umbilical catheters/central venous lines were fixed effects and centre was a random effect. Three separate subgroup analyses were pre-specified: PMA at randomisation (<29 weeks, 29–35 weeks, >35 weeks); birthweight (<1000 g, 1000–1500 g, >1500 g); and presence or absence of an umbilical catheter/central venous line at the onset of sepsis. Bayesian analysis, pre-specified in the SAP, was used to estimate the probability of the optimised regimen truly being superior to the standard regimen under different prior assumptions (Supplementary Table 4, appendix p 11).

Analyses of secondary outcomes used risk ratios and their 95% confidence intervals from log binomial regression models, with the same adjustment factors as the primary outcome, except AEs and SAEs which were reported as the incidence rate per 1000 child days (number of infant-days recorded as alive and in the study between randomisation and STFU) with comparison using incidence rate ratios and 95% confidence intervals to allow for the possibility of multiple events occurring in the same infant and negative binomial regression to account for overdispersion. Post-hoc imputation was carried out on rates of abnormal hearing due to missing data; imputation was done separately for each arm and factors included in the model were baseline variables of PMA stratum, birthweight stratum (as above), presence or absence of umbilical catheters/central venous lines, sex, hypoxic ischaemic encephalopathy, intraventricular haemorrhage and presence/ absence of separate known risk-factor antibiotics (amikacin, ciprofloxacin, gentamicin, linezolid, netilmicin, and teicoplanin). For all analyses, 95% confidence intervals were used with no adjustment for multiple testing. Statistical analyses used Stata version 16 (StataCorp, College Station, Texas, USA).

Independent Data Monitoring Committee

An IDMC, composed of a neonatologist, microbiologist and statistician met three times throughout the trial period to monitor progress, efficacy, safety and pharmacokinetic data according to a specific Charter and without formal stopping guidelines.

Trial registration

NeoVanc was registered on ClinicalTrials.gov (NCT02790996) on 7th April 2016 and EudraCT (2015–000203-89) on 18th July 2016.

Role of the funding source

This research was funded by the European Union Seventh Framework Programme for research, technological development and demonstration under Grant No 602041. The funder had no role in study design, data collection, analysis or interpretation or writing of the report.

Results

Between 3rd March 2017 and 29th July 2019, 242 infants were randomised at 17 sites (Figure 1). Primary outcome data in the per protocol population were available for 90 infants in the optimised arm and 92 in the standard arm.

Baseline characteristics were broadly similar across arms (Table 1 and Supplementary Table 5, appendix pp 12–13). The great majority of infants (99%) had at least three clinical or laboratory signs of neonatal sepsis at baseline. A total of 80 Gram-positive bacteria were detected at baseline in 76 infants (69% *Staphylococcus epidermidis*, 10% *Staphylococcus hominis*, 9% *Staphylococcus haemolyticus*, with the remaining 12% comprising six different species (Supplementary Table 6, appendix p 13). *S. epidermidis* was relatively more common in the standard arm (34/43 (79%)) than in the optimised arm (21/37 (57%)), with *S. hominis* being relatively more common in the optimised arm (5/37 (14%) vs 3/43 (7%)); other organisms were comparable between arms. No invasive organism exhibited vancomycin resistance by EUCAST breakpoints. 101/116 (87%) available CoNS blood culture isolates demonstrated vancomycin heteroresistance by the brain heart infusion agar method [23] (51 standard arm and 50 optimised arm).

64% of infants in the optimised arm and 88% in the standard arm received vancomycin within their randomised duration window.

Continued treatment with vancomycin or another anti-staphylococcal antibiotic, likely reflecting treatment for the original infection, lasted a median of 6 days from commencement of study

vancomycin in the optimised and 10 days in the standard arm (Figure 2; Supplementary Table 7, appendix p 14). However, the difference between treatment arms became notably less when considering the total days of exposure to STFU, both of vancomycin (median of 7 days in the optimised arm and 10 days in the standard arm) and all antibiotics (median of 12 days in optimised arm and 11 days in standard arm; Figure 2; Supplementary Table 7, appendix p 14). TDM was assessed for 46 infants (25%) in seven centres, with 50% of assessed participants having at least one dosing adjustment (Supplementary Table 8, appendix p 14); assessment rates were slightly higher in the standard arm than the optimised arm.

Efficacy

A successful primary outcome was achieved in 137/182 (75%) infants: 64/90 (71%) in the optimised and 73/92 (79%) in the standard arm (Table 2). The adjusted risk difference between arms was –7% (95%CI = (–15%, 2%) and consequently non-inferiority was not concluded based on a non-inferiority margin of –10% (see Supplementary Table 9 for analysis of ITT population, appendix p 15). The lower success rate in the optimised arm seemed to be driven by higher apparent clinical failure rates at EVT when vancomycin therapy was stopped (21% in the optimised arm, at approximately Day 5, and 10% in the standard arm, at approximately Day 10; Table 2; Figure 3; Supplementary Figure 2, appendix p 15). Of those 28 infants with clinical failure at EVT, 57% had at least three clinical signs and 79% had at least one laboratory sign. Bayesian analysis showed 79%–99% probability that the optimised arm was truly worse than the standard arm, depending on the prior used, and 4%–43% probability that the optimised arm was truly worse than the standard arm by at least the 10% non-inferiority margin (Supplementary Table 10, appendix p 16; Supplementary Figure 3, appendix p 17). There was no evidence of heterogeneity in subgroup analyses (PMA, birthweight, presence of a central line) for the primary outcome (Supplementary Table 11, appendix p 17).

Secondary efficacy outcomes are outlined for the PP population in Table 2 and for the ITT population in Supplementary Table 9, appendix p 15. Success rates at Day 5±1 were lower in the optimised arm (71%) than in the standard arm (82%), although the 95% confidence interval crossed one (adjusted risk ratio: 0·90; 95%CI = (0·78, 1·04)) and post-hoc analyses as per primary outcome did not conclude non-inferiority (adjusted risk difference: –8%; 95% CI = (–19%, +3%)). Lower rates of relapse/new infections treated with non-anti-staphylococcal (“other”) antibiotics between EVT and TOC were seen in the optimised arm (3%) than in the standard arm (17%). When the primary outcome was extended to include relapse/new infections treated with any antibiotics between EVT and TOC,

there was no evidence success rates differed between the optimised (71%) and standard (74%) arms (adjusted risk ratio 0·98; 95% CI = (0·87, 1·11)) and post-hoc analyses as per the primary outcome was marginally non-inferior (adjusted risk difference: 3%; 95% CI (–10%, +6%)).

Toxicity and Safety

Abnormal hearing screening rates were twice as high in the optimised arm (30%) compared to the standard arm (15%; adjusted risk ratio = 1·93; 95% CI (1·10–3·39), p=0·02; Table 3), although only 82% of the ITT population had hearing assessed. Eleven of the 37 infants without hearing assessed had died and 70% of the remaining individuals were from two sites. Post-hoc multiple imputation (Supplementary Table 12, appendix p 18) indicated slightly higher rates of abnormal hearing in both arms (33% optimised and 19% standard adjusted risk ratio: 1·72; 95% CI (1·0–2·9)). Additional post-hoc analyses, on infants with available hearing screening results, showed higher rates of abnormal hearing in the optimised arm across all PMA groups but with weak evidence for a greater excess risk in those with the youngest PMA (Supplementary Table 13, appendix p 19), and across both hearing tests conducted (Supplementary Table 14, appendix p 19). There was no evidence that age at hearing test differed between arms (post-hoc analyses mean: 61 days (SD 30) in optimised arm, 62 days (SD 27) in standard arm; difference 1·6 days; 95% CI (–12, 9); p=0·77). Results were unchanged when repeated on the as-treated population (receiving loading dose as randomised; Supplementary Table 15, appendix p 19). Adding cumulative dose to the unadjusted model resulted in a very small decrease in the effect size although cumulative dose itself was not statistically significant (Supplementary Table 16, appendix p 20). Rates of abnormal renal function tests at STFU were extremely low, at 2% in the optimised arm and 0% in the standard arm (Table 3). There were 6 vancomycin related AEs in the optimised arm (1 SAE) and 4 in the standard arm (2 SAEs). There was no evidence that AEs and SAEs rates, both all-cause and vancomycin-related, differed across arms (Table 3).

Mortality

Eleven infants in the ITT population died (6 optimised and 5 standard arm): 4 with necrotising enterocolitis; 2 with Gram-negative infection; 3 with respiratory pathology; 1 with pericarditis and *S. epidermidis* bloodstream infection; 1 with severe brain injury secondary to vein of Galen aneurysm and septic shock.

Discussion

Main findings

NeoVanc, an open-label RCT, aimed to validate preclinical data to establish if the duration of vancomycin treatment for Gram-positive LOS could be safely reduced to 5±1 days with more frequent dosing in infants <29 weeks PMA and the inclusion of a loading dose. We could not conclude non-inferiority on the primary outcome. Additionally, a potential safety signal was detected in relation to higher abnormal hearing screening rates in the optimised arm.

The inability to conclude non-inferiority of the optimised arm in the primary outcome was multifactorial. The intended sample size was not reached which may have led to lack of power. In hindsight a non-inferiority limit of 10% of with an anticipated relapse rate of 5% could have been considered large. However, it did not impact on inference in the study. However, Bayesian analysis showed 79%–99% probability of the optimised arm being truly worse than the standard arm, implying low power may not be the only factor. Unsuccessful outcome in the optimised arm was predominantly related to lack of clinical recovery at EVT, and not because of relapse/new infection; 21% of infants (83% of failures) were clinical failures in the optimised arm compared to 10% of infants (47% of failures) in the standard arm. Microbiological failure was very low in both arms (1%), despite a Gram-positive blood culture positivity rate of >40% at baseline. The day of EVT differed between arms and secondary efficacy analyses showed higher failure rates in the optimised arm at the end of vancomycin therapy, both when therapy was randomised to end (EOAT) and when therapy actually ended (EVT). These differences may reflect the time taken for clinical/laboratory signs to normalise in infants with significant sepsis regardless of dosing regimen; assessment of both arms at Day 10±2 (EOAT in the standard arm) may have aided in elucidating this further. The new National Institute for Health and Care Excellence neonatal sepsis guidelines recommend 7 days of antibiotic treatment in babies with culture positive LOS.[24]

Only two infants demonstrated abnormal renal function at STFU. There was no evidence that the frequency of AEs and SAEs differed between study arms. Rates of abnormal hearing were almost twice as high in the optimised arm, although the associated 95% confidence interval were relatively wide and hearing screening results were only available for 82% of the ITT population. This result could reflect a genuine safety signal but may be due to low sample sizes and chance attributable to multiple testing. There was no evidence age at the time of hearing screening differed between arms.

Multiple imputation, factoring in other risk factors for hearing loss, including aminoglycoside and furosemide exposure and low birthweight, showed a slightly reduced effect size (1·7 times), smaller confidence intervals with the lower limit of the 95% confidence interval being 1.0 and consequently the pattern of missing data may be driving some of the differences observed. The protocol definition of abnormal hearing, was stricter than that used in clinical practice[25] so failure rates may be higher, although, would expect to be distributed evenly between arms. If genuine, the higher abnormal hearing screening rates in the optimised arm could be caused by either the loading dose or more frequent administration of vancomycin in infants <29 weeks PMA. There was weak evidence of an interaction between PMA group and arm on abnormal hearing screening rates, although sample sizes were low. Cumulative exposure of vancomycin has been described as a risk factor for abnormal hearing screening at NICU discharge in VLBW babies[26] although we did not find robust evidence of this. If the ototoxicity safety signal is being driven by the loading dose, then these NeoVanc results suggest cumulative dose is unlikely to be the only risk factor, particularly as the number of days of vancomycin exposure up to STFU was similar in both arms. Risk factors are likely to be cumulative and data on hearing outcomes in septic babies are sparse. Of note, a neonatal meropenem versus standard of care RCT reported abnormal hearing screening rates of up to 29% in their population of septic infants.[27] Robust, prospective long-term hearing data are required to ascertain if failure at hearing screening translates to long-term hearing loss on diagnostic auditory assessment.[16] A NeoVanc long-term follow-up study is planned with the aim of obtaining missing data and collecting follow-up hearing data in infants who failed their hearing screening.

Previous trials

Only two neonatal vancomycin RCTs have been registered on ClinicalTrials.gov and the International Standard Randomised Controlled Trial Number trial registry in the last 20 years, emphasising the paucity of efficacy trials.[28,29] Both trials were stopped prior to recruitment of their target sample size, demonstrating the difficulty of recruiting to neonatal antibiotic trials.

Trial strengths and limitations

CoNS sepsis has historically been considered to have a less severe clinical course. However, infants recruited into NeoVanc had significant clinical sepsis; 99% had ≥3 clinical/laboratory signs with blood culture positivity rate being high. The inclusion criteria clearly identified septic infants.

Test of cure assessment in neonatal antibiotic trials is not standardised and no guidance is available on neonatal sepsis trial design from the FDA and EMA.[30] Test of cure in NeoVanc was based on days from actual end of vancomycin therapy and not days from randomisation and so was at different timepoints in the optimised compared to the standard arm. Very low rates of new infection and relapse were seen in both arms. The NeoVanc trial was a pragmatic open-label study, and this may have influenced clinician decisions, particularly if they were accustomed to giving longer antibiotic course durations. The STFU visit was 30±5 days from randomisation to ensure comparability of outcome assessment with respect to the initial presenting episode and overall antibiotic exposure was comparable between arms to this timepoint, which supports lack of evidence of a difference between the arms at this later follow-up.

The NeoVanc Programme incorporated extensive pre-clinical studies[12] including the largest ever meta-analysis evaluating the vancomycin population PK in infants.[13] This RCT also provides valuable PK, safety and efficacy information on infants <29 weeks PMA, who comprised nearly a quarter of the study population.

Next steps

Interim NeoVanc PK analysis (full analysis delayed by the COVID-19 pandemic; to be published) indicate that the newly developed PK model from the pre-clinical studies, which has been externally validated, is robust, supporting the use of pre-clinical studies to optimise antimicrobial dosing regimens. However, modelling toxicity is more problematic and can only be detected within the context of a reasonably sized RCT. The ototoxicity safety signal, potentially associated with the loading dose in this RCT, was not predicted, particularly given the previous inconclusive data relating to ototoxicity in infants and considering a loading dose is recommended in critically ill children and adults.[31] Neonates may demonstrate unique toxicity profiles, and dosing recommendations should be adopted with caution if the data are generated from adult or childhood RCTs alone. Rates of ototoxicity have not been compared between continuous and intermittent vancomycin infusion within the setting of an RCT in infants.

Recruitment to neonatal antibiotic trials is challenging and the sample size required to detect safety signals is considerably more than most of the currently recruiting new neonatal antibiotic trials.[32] An approach that balances risk and unmet need seems appropriate. For antibiotics with a low risk

of toxicity (e.g. beta-lactams) and limited clinical unmet need, PK studies alone to determine optimal dosing regimens are reasonable. For drugs with a higher toxicity potential and high unmet clinical need, NeoVanc demonstrates that, robust RCTs adequately powered to identify potential novel toxicity signals may be required. Additionally, efficacy assessment should be undertaken at the same timepoint from randomisation in each arm to allow equal time for resolution of symptoms in both arms. We would not currently recommend a 25 mg/kg loading dose of vancomycin in infants or more frequent dosing in infants <29 weeks PMA in view of the identified hearing safety signal.

Contributors

Conceptualisation and funding acquisition – MAT, IL, ER, EJ-A, PTH, MS; study design – LFH, MAT, PTH, IL, EJ-A, ER, MS; methodology – LFH, MAT, PTH, IL, EJ-A, ER, MS, MNC, LR; data curation – MNC; data analysis – MNC, LFH, ASW, MS; project administration – LFH, DD, LR; resources – LFH, DD, LR; investigation –CA-D, EB, AD, M-LI, AM, TM, GM, VP, KS; supervision – LFH, MS, MAT, DD, IL, PTH, ER, LR, CA-D, EB, AD, M-LI, AM, TM, GM, VP, KS; visualisation – LFH, MNC, MS; software and validation – MNC; writing - first draft – LFH, MNC, MS; writing – review and editing – LFH, MNC, MS, MAT, DD, IL, ER, PTH, EJ-A, LR, CA-D, EB, AD, M-LI, AM, TM, GM, VP, KS, ASW

LFH* – trial coordinator, MNC* – trial statistician, MS – chief investigator, country coordinators – MAT, IL, ER, CA-D, AD

*LFH and MNC have contributed equally and are co-first authors

LFH and MNC verified the underlying data.

All authors had access to the full data set and accept responsibility to submit for publication.

Declarations of interests

PTH is a member of the National Institute for Health and Care Excellence neonatal infection guideline development group. LR is an employee of Therakind Ltd. DD's PhD was funded by Fondazione Penta – ONLUS; the capacity and remit of his PhD was independent and unrelated to his involvement with NeoVanc.

Acknowledgments

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NeoVanc clinical trial (recruiting centres and NeoVanc Consortium members are listed in the appendix pp 1–2). We thank the Sponsor, Fondazione Penta – ONLUS (Email: info@penta-id.org), and the Independent Data Monitoring Committee (Prof John van den Anker [Chair], Jim Gray and Corine Chazallon).

Data sharing

The study protocol has previously been published in detail.[17] Sharing of data will be considered based on a detailed proposal which should include aims, methods and a statistical analysis plan. Requests will be checked for compatibility with regulatory and ethics committee requirements as well as with compatibility with the participant informed consent. Proposals should be addressed to the corresponding author at lhill@sgul.ac.uk and will be evaluated by the Sponsor.

Research in context

Evidence before this study

Neonatal sepsis is a global health priority. Coagulase negative staphylococci (CoNS) are the most commonly identified organisms in neonatal late onset sepsis (LOS) in high income countries, with very low birthweight babies experiencing the greatest associated morbidity and mortality. Vancomycin is the mainstay of treatment of CoNS LOS. Prior to the initiation of this trial, a search of PubMed, ClinicalTrials.gov and ISRCTN identified only two neonatal vancomycin randomised controlled trials (RCTs) registered in the last 20 years. These two RCTs, recruited a total of 220 babies; one was an active control trial comparing cefazolin and vancomycin and the other compared continuous and intermittent infusion with pharmacokinetic endpoints.

The NeoVanc Programme completed pre-clinical studies, which informed the optimised dosing regimen evaluated in the RCT. The NeoVanc hollow fibre infection and animal models determined that more frequent dosing may be beneficial in facilitating bacterial kill and discouraging the development of vancomycin resistance. A bridging study to the NICU clinical setting, established that more frequent dosing led to a quicker and more satisfactory reduction in C-reactive protein, particularly in infants <29 weeks postmenstrual age (PMA), and supported a shorter vancomycin course. Linder, *et al* previously found, in a retrospective study, that infants with an uncomplicated clinical course treated for CoNS sepsis with vancomycin for 5 days after the last positive blood

culture, had similar outcomes to those treated for longer durations. A novel neonatal vancomycin PK model was developed within the NeoVanc programme based on a population PK metanalysis from previously published data. It included 4894 vancomycin concentrations from 1631 neonates and supported the need for more frequent dosing in infants <29 weeks PMA and predicted an optimised regimen which included a 25 mg/kg loading dose. The use of a loading dose of 25 mg/kg in seriously unwell adults and children has been supported by the Infectious Diseases Society of America in the treatment of MRSA.

Added value of this study

NeoVanc is the first RCT to evaluate a loading dose of vancomycin in conjunction with intermittent dosing in neonatal sepsis and the largest neonatal vancomycin clinical efficacy trial ever conducted. However, NeoVanc identified an ototoxicity safety signal, potentially associated with the use of a loading dose and/or more frequent dosing in infants <29 weeks PMA, which has not been previously recognised in this population. Additionally, no clear advantage was seen for adopting a shorter 5±1 day course over a standard 10±2 day course for neonates with significant clinical sepsis. The adapted EMA neonatal sepsis criteria, utilised for inclusion to the RCT, successfully identified such infants with significant clinical sepsis, with >40% of baseline blood cultures positive in trial participants.

Implications of all the available evidence

There is no evidence for reducing vancomycin course duration to 5±1 days in truly septic infants as no benefit was identified. A vancomycin loading dose and more frequent dosing in infants <29 weeks PMA should not currently be recommended in infants because of a possible ototoxicity safety signal. Long-term neonatal vancomycin hearing analyses are in progress.

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Figure Texts

Figure 1: NeoVanc participant flow

Post-randomisation exclusions: any participant found to have Gram-negative or fungal sepsis, osteomyelitis, septic arthritis, urinary tract infection, meningitis or *Staphylococcus aureus* (methicillin-susceptible *S. aureus* or methicillin-resistant *S. aureus*) bacteraemia after randomisation was excluded from efficacy analysis

Figure 2: Continued and overall antibiotic therapy duration by study arm

Continued exposure = continuous antibiotic therapy from initiation of study vancomycin.
Total exposure = total antibiotic exposure from initiation of study vancomycin to short-term follow-up visit (30±5 days from initiation of study vancomycin).
Anti-staphylococcal antibiotic = vancomycin, flucloxacillin, oxacillin, linezolid, tedizolid, daptomycin or teicoplanin

Figure 3: Number of clinical/laboratory signs of sepsis over time

Table 1: NeoVanc participant baseline characteristics (per protocol population ([primary])

	Optimised vancomycin regimen (N = 92)	Standard vancomycin regimen (N = 93)
Participating centres: *n*	14	15
Babies per country: *n (%)*		
Greece	44 (48%)	44 (47%)
Italy	28 (30%)	24 (26%)
Estonia	5 (5%)	15 (16%)
Spain	10 (11%)	9 (10%)
United Kingdom	5 (5%)	1 (1%)
Sex male: *n (%)*	47 (51%)	53 (57%)
Postnatal age at trial entry (days): *Median (IQR)*	14 (9, 28)	14 (9, 23)
Postmenstrual age at trial entry (completed weeks): *Median (IQR)*	32.5 (29, 37)	32 (29, 37)
n (%)		
< 29	20 (22%)	23 (25%)
29 to 35	44 (48%)	43 (46%)
> 35	28 (30%)	27 (29%)
Birthweight (g): *Median (IQR)*	1155 (855, 1720)	1120 (800, 1930)
n (%)		
< 1000	39 (42%)	33 (35%)
1000 to 1500	21 (23%)	27 (29%)
> 1500	32 (35%)	33 (35%)
Weight at trial entry (g): *Median (IQR)*	1465 (945, 2145)	1300 (940, 2213)
Multiple birth: *n (%)*	19 (21%)	25 (27%)
Ethnicity: *n (%)*		
White	84 (91%)	89 (96%)
Asian	2 (2%)	0 (0.0%)
Black	3 (3%)	2 (2%)
Other	2 (2%)	0 (0.0%)
Mixed	1 (1%)	2 (2%)
Umbilical catheter/central venous line present: *n (%)*	58 (63%)	58 (62%)
Number of Gram-positive bacteria detected in baseline blood culture sample: *n (%)*		
0	54 (59%)	44 (47%)
1	31 (34%)	41 (44%)
2	3 (3%)	1 (1%)
No sample	4 (4%)	7 (8%)
Postnatal age at onset of late onset sepsis (days): *Median (IQR)*	14 (8·5, 27·5)	14 (8, 23)
Congenital malformations or underlying neonatal conditions: *n (%)*	53 (58%)	52 (56%)
Surgery performed in the last month: *n (%)*	10 (11%)	17 (18%)
Risk factors for hearing impairment: *n (%)*	20 (22%)	19 (20%)
Antibiotics given within 7 days prior to trial entry: *n (%)*	62 (67%)	73 (78%)
Total criteria at trial entry: *n (%)*		
2	1 (1%)	1 (1%)
3	20 (22%)	15 (16%)
4	30 (33%)	27 (29%)
5	19 (21%)	27 (29%)
6	8 (9%)	13 (14%)
7	9 (10%)	6 (6%)
8	3 (3%)	2 (2%)
9	2 (2%)	2 (2%)

Table 2: NeoVanc participant efficacy outcomes by study arm in per protocol population

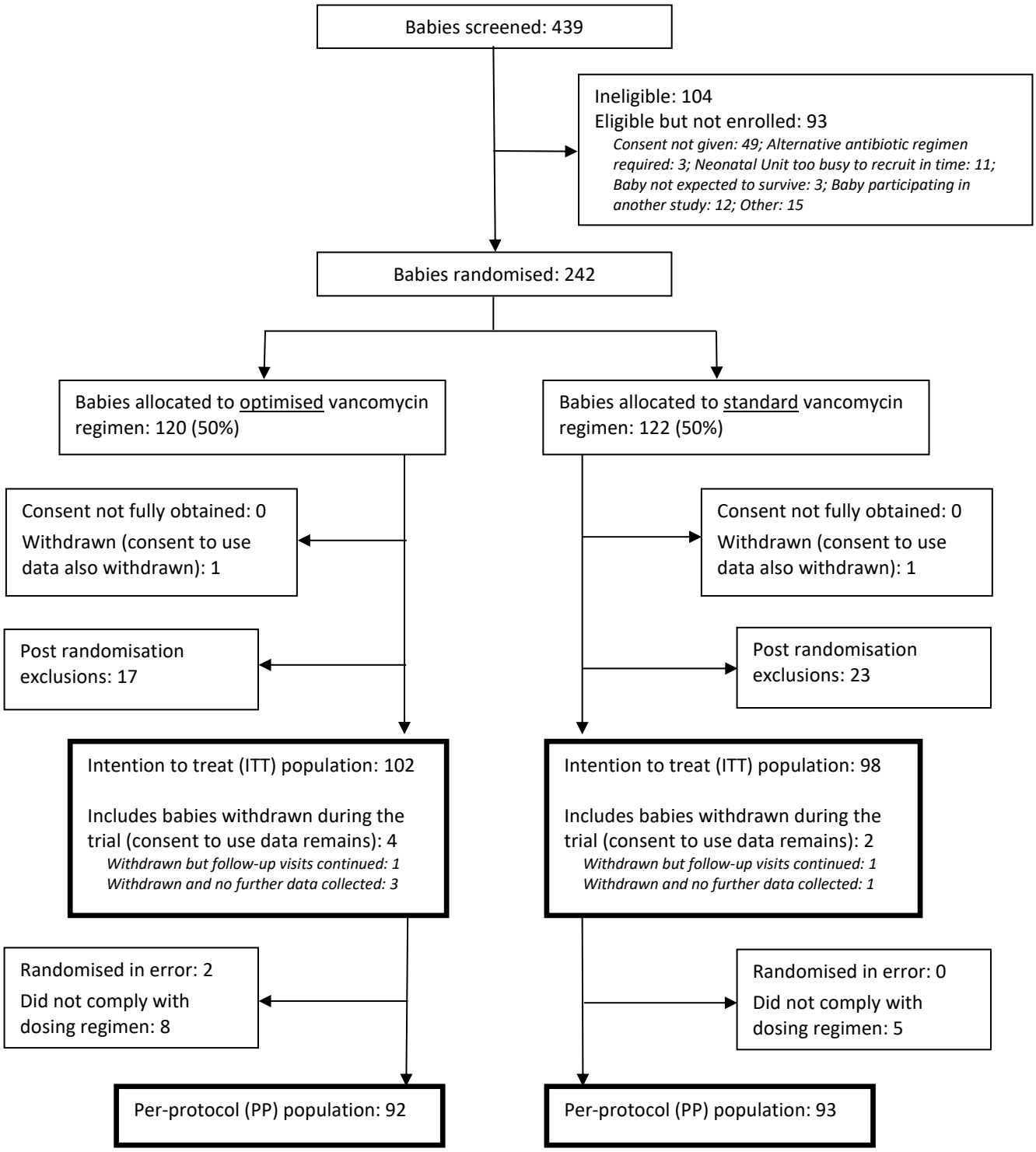
Outcome	Optimised vancomycin regimen n/N (%)	Standard vancomycin regimen n/N (%)	Adjusted risk difference (95% CI)	p-value
Success at TOC visit	64/90 (71%)	73/92 (79%)	-7% (-15%, 2%)	
Secondary outcomes			**Adjusted risk ratio (95% CI)**	
Success at 5 ± 1 days after start of allocated vancomycin therapy	65/91 (71%)	76/93 (82%)	0·90 (0·78, 1·04)	0·16
Success at end of actual vancomycin therapy	68/90 (76%)	82/92 (89%)	0·87 (0·80, 0·95)	0·001
Success at TOC visit: composite including treatment with "other" antibiotics*	64/90 (71%)	68/92 (74%)	0·98 (0·87, 1·11)	0·76
Success at STFU visit (30±5 days from initiation of study vancomycin)	56/90 (62%)	71/92 (77%)	0·81 (0·71, 0.93)	0·002
Failure between EVT & TOC caused by treatment with "other" antibiotics*	3/90 (3%)	16/92 (17%)	0·19 (0·08, 0·39)	0·001
Failure between TOC and STFU	11/90 (12%)	4/92 (4%)	2·81 (0·84, 9·38)	0·09
Success at end of allocated therapy	65/91 (71%)	83/93 (89%)	0·79 (0·69, 0·91)	0·001

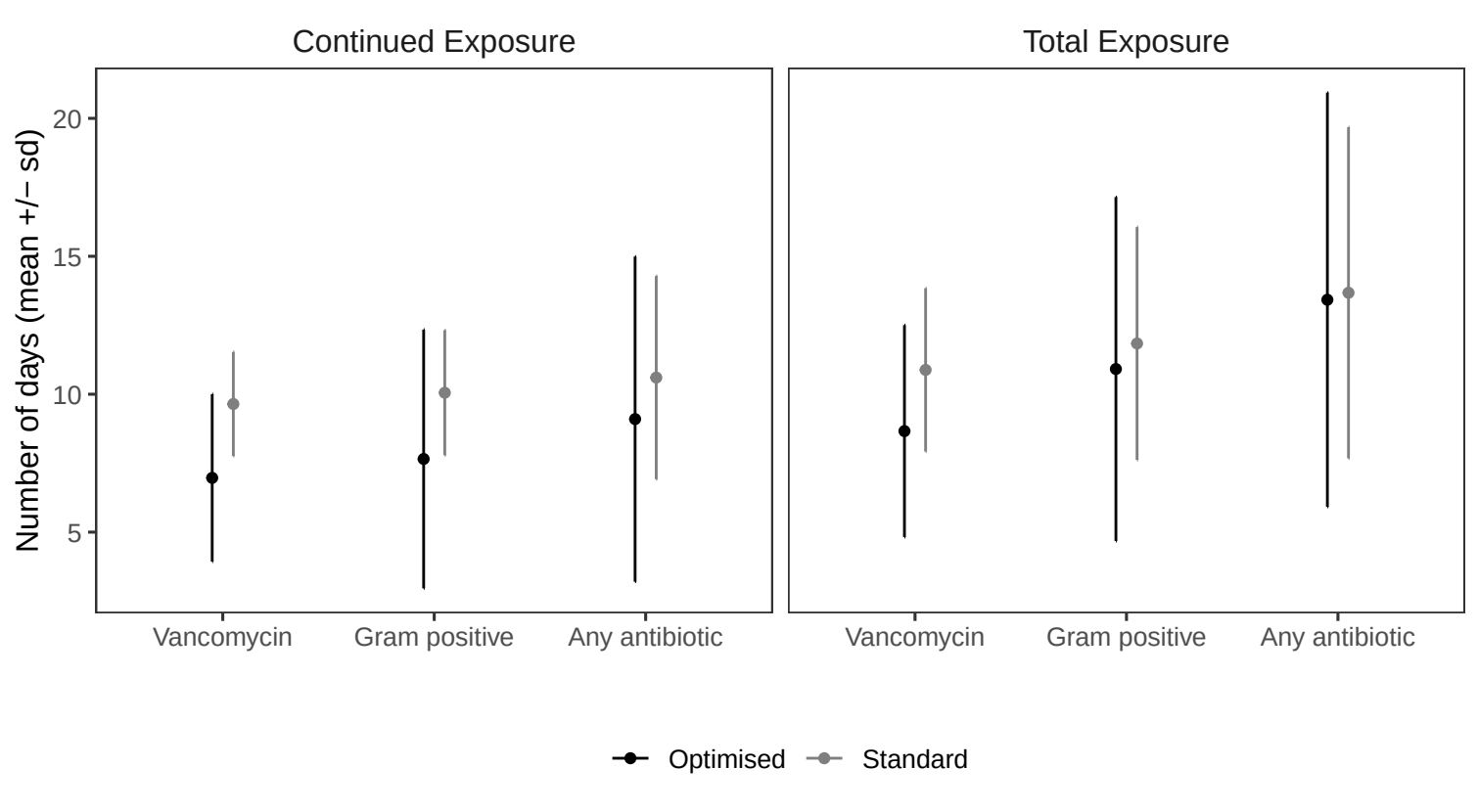
*"other" antibiotics = any antibiotic that is not vancomycin or another specific anti-staphylococcal antibiotic as specified in the protocol (flucloxacillin, oxacillin, linezolid, tedizolid, daptomycin or teicoplanin). Success at end of allocated therapy was pre-specified in SAP only. AE = Adverse Event; EVT = End of Actual Vancomycin Therapy; NA = not applicable; SAE = Serious Adverse Event; STFU =Short-term Follow-Up; TOC = Test of Cure

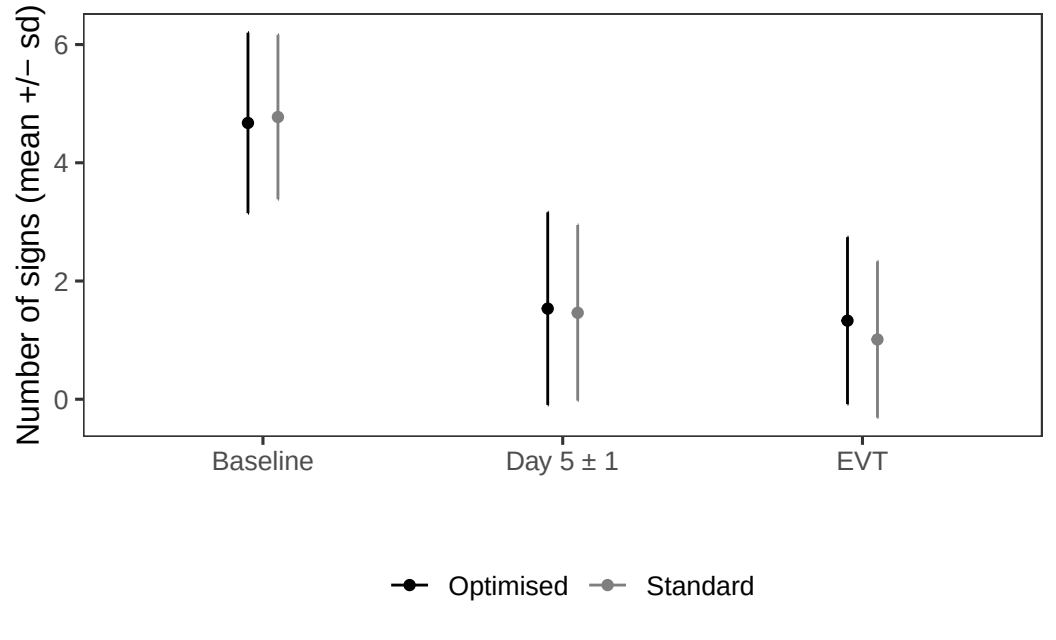
Table 3: NeoVanc participant safety outcomes by study arm in ITT population

Outcome	Optimised vancomycin regimen n/N (%)	Standard vancomycin regimen n/N (%)	Adjusted risk ratio (95% CI)	p-value
Abnormal renal function tests at the short term follow up visit:	2/84 (2%)	0/81 (0%)	0.85 (-1.7, +inf)	0.5
Abnormal hearing screening test after EVT	25/84 (30%)	12/79 (15%)	1·93 (1·10, 3·39)	0·02
Abnormal hearing screening test after imputation	33·7/102 (33%)	18·5/98 (19%)	1·72 (1·0, 2·9)	0·05
Incidence rate per 1000 child days				
Adverse events up to STFU:				
- All AE	46 (138/3012)	41(122/2956)	1·11 (0·67, 1·87)	0·41
- Vancomycin related AE	2·0 (6/3012)	1·4 (4/2956)	1·45 (0·78, 2·68)	0·24
Serious adverse events				
- All SAE	6·9 (21/3012)	9·5 (28/2956)	0·73 (0·29, 1·83)	0·5
- Vancomycin related SAE	0·33 (1/3012)	0·68 (2/2956)	0·49 (0.11, 2.15)	0·34

AE = Adverse Event; EVT = End of Actual Vancomycin Therapy; NA = not applicable; SAE = Serious Adverse Event; STFU =Short-term Follow-Up; *shows unadjusted output from Poisson regression as specified in SAP


Babies screened: 439
Ineligible: 104
Eligible but not enrolled: 93
Consent not given: 49; Alternative antibiotic regimen required: 3; Neonatal Unit too busy to recruit in time: 11; Baby not expected to survive: 3; Baby participating in another study: 12; Other: 15
Babies randomised: 242
Babies allocated to optimised vancomycin regimen: 120 (50%)
Babies allocated to standard vancomycin regimen: 122 (50%)
Consent not fully obtained: 0
Withdrawn (consent to use data also withdrawn): 1
Consent not fully obtained: 0
Withdrawn (consent to use data also withdrawn): 1
Post randomisation exclusions: 17
Post randomisation exclusions: 23
Intention to treat (ITT) population: 102
Includes babies withdrawn during the trial (consent to use data remains): 4
Withdrawn but follow-up visits continued: 1
Withdrawn and no further data collected: 3
Intention to treat (ITT) population: 98
Includes babies withdrawn during the trial (consent to use data remains): 2
Withdrawn but follow-up visits continued: 1
Withdrawn and no further data collected: 1
Randomised in error: 2
Did not comply with dosing regimen: 8
Randomised in error: 0
Did not comply with dosing regimen: 5
Per-protocol (PP) population: 92
Per-protocol (PP) population: 93


Continued Exposure
Total Exposure
Number of days (mean +/− sd)
20
15
10
5
Vancomycin
Gram positive
Any antibiotic
Vancomycin
Gram positive
Any antibiotic
Optimised
Standard


Number of signs (mean +/− sd)
6
4
2
0
Baseline
Day 5 ± 1
EVT
Optimised
Standard

Appendix

NeoVanc Consortium

Recruiting centres*

Tallinn Children's Hospital, Tallinn, Estonia (Mari-Liis Ilmoja, Maarja Hallik)

Tartu University Hospital, Tartu, Estonia (Tuuli Metsvaht, Riste Kalamees)

Aghia Sophia Children's Hospital (NICU A), Athens, Greece (Korina Karachristou, Adamantios Vontzalidis)

Aghia Sophia Children's Hospital (NICU B), Athens, Greece (Fani Anatolitou, Chryssoula Petropoulou)

Aghia Sophia Children's Hospital (NICU C), Athens, Greece (Tania Siahanidou, Eirini Nikaina)

General University Hospital, Attikon, Chaïdári, Greece (Vassiliki Papaevangelou, Pinelopi Triantafyllidou)

Hippokration Hospital, Thessaloniki, Greece (Kosmas Sarafidis, Angeliki Kontou)

Kyriakou Children's Hospital, Athens, Greece (Angeliki Nika, Kassandra Tataropoulou)

Papageorgiou Hospital, Thessaloniki, Greece (George Mitsiakos, Elias Iosifidis, Dimitra Gialamprinou)

ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy (Stefano Martinelli, Laura Ilardi)

Azienda Ospedale-Universita' di Padova, Fondazione Istituto di Ricerca Pediatrica, Padova, Italy (Eugenio Baraldi, Luca Bonadies)

Ospedale Di Venere, Bari, Italy (Antonio Del Vecchio, Caterina Franco)

Ospedale Pediatrico Bambino Gesu', Rome, Italy (Andrea Dotta, Maia De Luca)

Ospedale Sant'Anna, Turin, Italy (Paolo Manzoni, Daniele Farina)

Policlinico San Matteo, Pavia, Italy (Chryssoula Tzialla)

Università degli Studi di Palermo, Palermo, Italy (Mario Giuffrè, Vincenzo Insinga)

Hospital 12 de Octubre, Madrid, Spain (Clara Alonso-Diaz, Concepción de Alba Romero, Javier de la Cruz, Paola Catalina Morales-Betancourt)

Hospital Materno Infantil, La Paz, Madrid, Spain (Laura Sanchez Garcia, Malaika Cordeiro)

Hospital Sant Joan de Deu, Barcelona, Spain (Ana Alarcon Allen, Mar Reyné)

John Radcliffe Hospital, Oxford, UK (Charles C Roehr, Zoltan Molnar)

Royal Jubilee Maternity Hospital, Belfast, UK (Paul Moriarty)

St Mary's Hospital, Manchester, UK (Ajit Mahaveer, Nicola Booth)

*22 NICU sites were opened to recruitment; 17 sites recruited participants to the RCT

Trial oversight and coordination (NeoVanc Trial Management Group):

St George's, University of London, UK – Michael Sharland (Chief Investigator), Paul T Heath, Louise F Hill (Trial co-ordinator), Tatiana Munera Huertas, Uzma Khan

Fondazione Penta – ONLUS, Italy – Davide Bilardi, Daniele Donà

Therakind Ltd., UK – Louise Rawcliffe, Basma Bafadal, Deborah Roberts, Antonella Silvestri

MRC Clinical Trials Unit at UCL, UK – Michelle N Clements (Trial statistician)

Consorzio per Valutazioni Biologiche e Farmacologiche, Italy – Cristina Manfredi, Mariagrazia Felisi, Paola Gandini

University of Tartu, Estonia – Irja Lutsar (Country co-ordinator)

University of Liverpool, UK – Mark A Turner (Country co-ordinator)

Aristotle University, Thessaloniki, Greece – Emmanuel Roilides (Country-co-ordinator)

Servicio Madrileno de Salud, Spain – Clara Alonso-Diaz (Country-co-ordinator)

Ospedale Pediatrico Bambino Gesu', Italy – Andrea Dotta (Country-co-ordinator)

Hôpital Robert Debré, France – Evelyne Jacqz-Aigrain

Independent Data Monitoring Committee:

John van den Anker (Chair), Corine Chazallon, James Gray

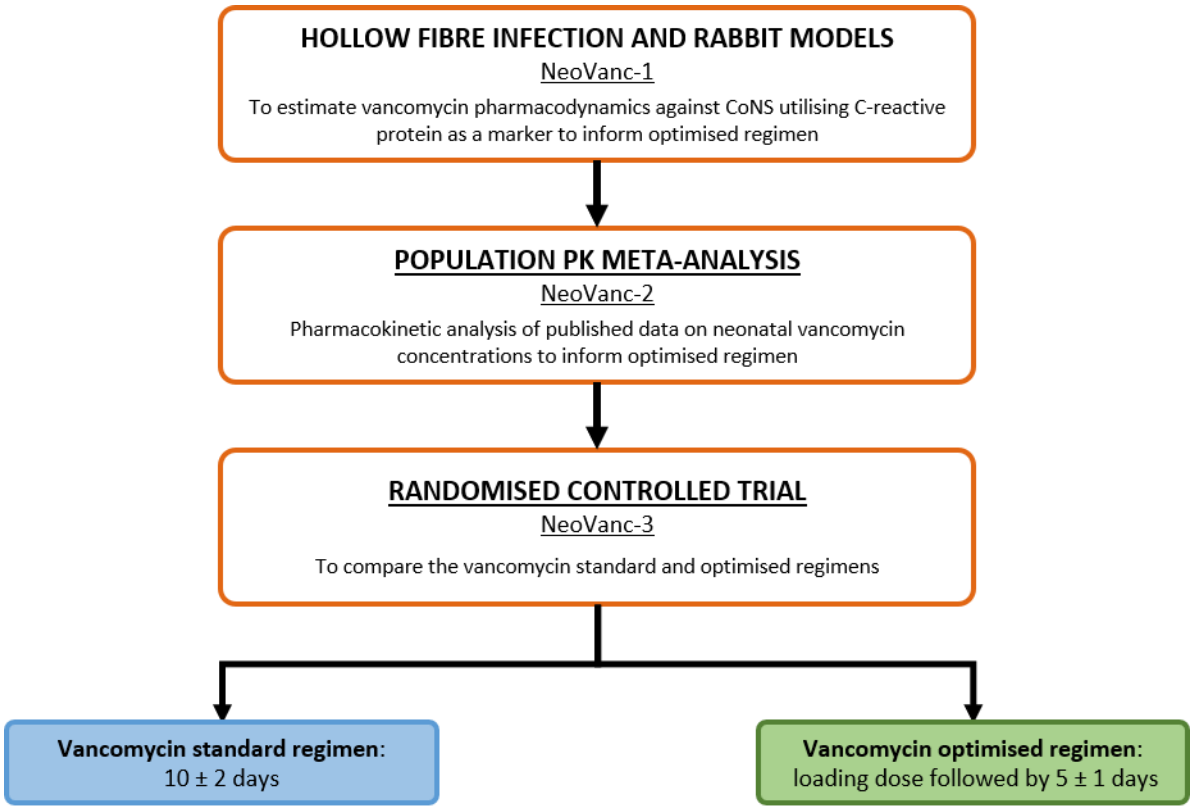
Protocol and amendments

Final protocol version 8.0

There were three substantial amendments to the protocol.

1. Protocol version 4.0 to 5.0 approved on 27/09/2016 (prior to recruitment commencement):
    a. clarification of the exclusion criteria
    b. clarification of the timeframe for starting IMP
    c. full description of the primary endpoint
    d. clarification of the secondary endpoint relating to treatment with “other” antibiotics
    e. clarification of the process of reporting AEs and SAEs including expedited reporting, addition of definition of “medical event”.
2. Protocol version 5.0 to 6.0 approved on 27/09/2016 (prior to recruitment commencement):
    a. recruitment timelines updated
    b. update of study schematic diagrams
    c. typographical error correction to inclusion criteria in relation to units for white cell count and platelet count
    d. clarification of timing of safety reporting
    e. change to ensure all blood isolates collected
3. Protocol version 6.0 to 7.0 (UK only) approved on 29/06/2018
    a. addition of follow-up sites
    b. clarification on how to manage inter-hospital transfers and discharges in relation to collecting follow-up data

A further non-substantial amendment was made from protocol version 7.0 (UK)/6.0 (other countries) to version 8.0 (final approved version) approved on 20/06/2019, which was made to update the protocol with study personnel who had changed over the course of the trial.



Supplementary Figure 1: The NeoVanc Project: how the hollow fibre infection, animal models and population pharmacokinetic meta-analysis informed the clinical trial

Supplementary Table 1: Inclusion and exclusion criteria for the NeoVanc clinical trial

Inclusion criteria

Infants are included in NeoVanc if they comply with the following criteria:

Postnatal age ≤ 90 days

AND

Postnatal age ≥ 72 hours at onset of LOS

AND

Clinical sepsis as defined by presence of any three clinical **or** laboratory criteria from the list below:

OR

Confirmed bacterial sepsis as defined by positive culture with a Gram-positive bacterium from a normally sterile site and at least one clinical **or** one laboratory criterion (at the time screening for sepsis takes place) from the list below, in the 24 hours before randomisation

Clinical criteria

- Hyper- or hypothermia
- Hypotension or impaired peripheral perfusion or mottled skin
- Apnoea or increased oxygen requirement or increased requirement for ventilatory support
- Bradycardic episodes or tachycardia
- Worsening feeding intolerance or abdominal distension,
- Lethargy or hypotonia or irritability

Laboratory criteria

- White blood cells (WBC) count < 4 or > 20 x 10^9 cells/L
- Immature to total neutrophil ratio (I/T) > 0.2
- Platelet count < 100 x 10^9/L
- C-reactive protein (CRP) > 10 mg/L
- Glucose intolerance as defined by a blood glucose value > 180 mg/dL (> 10 mmol/L) when receiving normal glucose amounts (8 – 15 g/kg/day),
- Metabolic acidosis as defined by a base excess (BE) < –10 mmol/L (< –10 mEq/L) or a blood lactate value > 2 mmol/L

Exclusion criteria

1. Administration of any systemic antibiotic regimen for more than 24 hours prior to randomisation, unless the change is driven by the apparent lack of efficacy of the original regimen
2. Treatment with vancomycin for ≥ 24 hours at any time within 7 days of randomisation
3. Known toxicity, hypersensitivity or intolerance to vancomycin
4. Known renal impairment with urinary output < 0.7 ml/kg/hour for 24 hours or a creatinine value ≥ 100 µmol/L (1.13 mg/dL)
5. Patient receiving (or planned to receive) haemofiltration, haemodialysis, peritoneal dialysis, extracorporeal membrane oxygenation (ECMO) or cardiopulmonary bypass
6. Severe congenital malformations where the infant is not expected to survive for more than 3 months
7. Patient known to have *S. aureus* (MSSA or MRSA) bacteraemia
8. Patient with osteomyelitis, septic arthritis, urinary tract infection (UTI) or meningitis
9. Patient with high suspicion of/confirmed sepsis caused by Gram-negative organisms or fungi
10. Other situations where the treating physician considers a different empiric antibiotic regimen necessary
11. Current participation in any other clinical study of an investigational medicinal product (IMP)

Post-randomisation exclusions from efficacy analysis*

Any participants found to have the following conditions following randomisation were excluded from efficacy analysis, as they would have required a longer treatment duration than the optimised arm or vancomycin would have been ineffective for the underlying condition:

1. Gram-negative or fungal sepsis
2. osteomyelitis
3. septic arthritis
4. urinary tract infection
5. meningitis
6. *Staphylococcus aureus* (methicillin-susceptible *S. aureus* or methicillin-resistant *S. aureus*) bacteraemia

*Participants who received at least one dose of study vancomycin were followed up for safety

Inclusion criteria were adapted from the European Medicines Agency “Report on the expert meeting on neonatal and paediatric sepsis”. Vol. 44. 2010
Inclusion and exclusion criteria from Hill LF (2020)

Supplementary Table 2: NeoVanc study visits & procedures

Visit Number	Visit Name	Visit Timing	Participants undertaking visit	Procedure	Laboratory	Study specific sampling	Pharmacokinetics assessment
Visit 1a	Screening & randomisation visit	Day 0	All	• Signed informed consent • Medical history • Adverse event reporting • Clinical examination	• Full blood count • Renal function measurements • Glucose/Lactate/ • Base excess • CRP • Blood culture	• Bacterial DNA PCR • Colonisation swabs • Biomarkers	
Visit 1b	Treatment initiation visit	Minimum of 24h after randomisation	All	• Adverse event reporting • Vancomycin administration	• Any laboratory tests not done at Visit 1a	• Any study specific procedures not done at Visit 1a	
Visit 2	Renal function measurement visit	Between Visits 1b and 3	All	• Adverse event reporting • Vancomycin administration	• Renal function measurements		**Infants <29 weeks PMA:** 3 pre-defined blood samples: *1st infusion*: PK1: 5 – 10 min after end of infusion PK2: 8 – 12 h from start of infusion *4th or 5th infusion* PK3: 4 to 12 h from start of infusion In addition, up to 3 scavenged samples **Babies ≥ 29 weeks PMA:** 3 to 5 scavenged PK samples
Visit 3	Early on treatment visit	72 ± 8 h after initiation of study vancomycin	All	• Clinical examination • Adverse event reporting • Vancomycin administration	• Full blood count • Renal function measurements • Glucose/Lactate/Base excess • CRP • Blood culture[a]	• Bacterial DNA PCR • Biomarkers	
Visit 4	Day 5±1/End of Allocated Therapy (Optimised arm) visit	5 ± 1 days from initiation of study vancomycin	All	• Clinical examination • Adverse event reporting • Vancomycin administration	• Full blood count • Renal function measurements • Glucose/Lactate/Base excess • CRP • Blood culture[a]	• Biomarkers	
Visit 5	Day 10±2/End of Allocated Therapy (Standard arm) visit	10 ± 2 days from initiation of study vancomycin	Any participant still receiving vancomycin	• Clinical examination • Adverse event reporting • Vancomycin administration	• Full blood count • Renal function measurements • Glucose/Lactate/Base excess • CRP	• Biomarkers	
EVT[b]	End of Actual Vancomycin Therapy (EVT) visit	End of primary course of vancomycin	Only participants whose vancomycin was stopped earlier or later than outlined in the protocol	• Clinical examination • Adverse event reporting • Vancomycin administration	• Full blood count • Renal function measurements • Glucose/Lactate/Base excess • CRP	• Biomarkers	
Visit 6	Test of Cure visit *(primary endpoint visit)*	10 ± 1 days after end of study vancomycin	All	• Clinical examination • Adverse event reporting • Assessment for relapse/new infection	• Full blood count[b] • Renal function measurements[b] • Glucose/Lactate/Base excess[b] • CRP[b]		
Visit 7	Short-term follow-up visit	30 ± 5 days from initiation of study vancomycin	All	• Clinical examination • Adverse event reporting • Assessment for relapse/new infection	• Renal function measurements	• Bacterial DNA PCR • Biomarkers	
Visit 8	Audiology follow-up visit	Up to Day 90 from initiation of study vancomycin	All	• Adverse event reporting	• Newborn hearing screening (OAE and/or ABR)		

[a] If a blood culture has been taken and is positive in the 24 h before randomisation, blood culture does not need to be repeated at Visit 1a or 1b. If blood culture is positive, further cultures should be taken at each subsequent visit until culture becomes negative up to and including the Visit 4. Blood cultures do not need to be repeated if the previous culture is negative unless clinically indicated. Blood cultures should be performed between TOC and STFU in cases of relapse/new infection

[b] Only participants whose vancomycin has been stopped earlier or later than outlined in the protocol

[c] Only if abnormal at previous visit

ABR = Auditory brainstem responses; CRP = C-reactive protein; DNA = deoxyribonucleic acid; EVT = End of actual vancomycin therapy; OAE = Otoacoustic emissions; PCR = polymerase chain reaction

Supplementary Table 3: Pre-specified rules determining outcome

Failure at TOC will be any participant who:

- died prior to TOC
- was not a success at EVT
- had a clinically significant new infection, a microbiological relapse or a microbiological new infection (as defined by the protocol)

All other scenarios will be regarded a success, however, specific outcomes will fall under secondary analyses as outlined in the protocol.

Definitions of relapse and new infection

Clinically significant (culture negative) relapse or new infection

A re-appearance of 3 or more clinical or laboratory criteria defining late onset sepsis; as defined within the protocol primary endpoint, also requiring treatment with vancomycin or other specific anti-staphylococcal antibiotics (flucloxacillin, oxacillin, linezolid, tedizolid, daptomycin and teicoplanin) for more than 24 hours within 10 days of the EVT visit.

Microbiological relapse

Clinically significant infection[¥] together with positive blood culture, from a normally sterile site, of a phenotypically similar microorganism to the baseline pathogen*; as defined within the protocol primary endpoint, also requiring treatment with vancomycin or other specific anti-staphylococcal antibiotics (flucloxacillin, oxacillin, linezolid, tedizolid, daptomycin and teicoplanin) for more than 24 hours within 10 days of EVT visit.

Microbiological new infection

Clinically significant infection[¥] together with a positive culture, from a normally sterile site, of a phenotypically different Gram-positive microorganism; as defined within the protocol primary endpoint, also requiring treatment with vancomycin or other specific anti-staphylococcal antibiotics (flucloxacillin, oxacillin, linezolid, tedizolid, daptomycin and teicoplanin) for more than 24 hours within 10 days of the EVT visit.

*These will be Gram-positive organisms (not including *Staphylococcus aureus*) as all Gram-negative organisms, fungal organisms and *S. aureus* are post-randomisation exclusions.

[¥] Only 1 clinical or laboratory criterion required to be classified as a clinically, significant infection in the presence of a positive blood culture.

Specific scenarios

<u>If a participant is alive at TOC and has had a successful outcome at EVT but there has been:</u>

Scenario for antibiotic use after end of treatment with vancomycin	**Treatment Success or Failure**
Significant use of anti-staphylococcal antibiotics targeting Gram positive bacteria for clinical or laboratory reasons	
Treatment with specific anti-staphylococcal antibiotics** for more than 24 hours, within 10 days of EVT visit, associated with appearance of 3 or more clinical or laboratory criteria associated with late onset infection and blood culture, associated with this episode, is negative.	Failure
Other use of anti-staphylococcal antibiotics targeting Gram positive bacteria for clinical or laboratory reasons**	
Treatment with specific anti-staphylococcal antibiotics** for more than 24 hours, within 10 days of EVT visit, associated with appearance of less than 3 clinical or laboratory criteria associated with late onset infection and blood culture, associated with this episode, is negative.	Success
Relapse of infection with phenotypically similar microorganism	
Treatment with specific anti-staphylococcal antibiotics** for more than 24 hours, within 10 days of EVT visit, associated with positive blood culture, from a normally sterile site, of a phenotypically similar microorganism to the baseline pathogen AND at least one clinical or laboratory criterion associated with late onset infection.	Failure
Treatment with specific anti-staphylococcal antibiotics** for more than 24 hours, within 10 days of EVT visit, associated with positive blood culture, from a normally sterile site, of a phenotypically similar microorganism to the baseline pathogen AND no clinical or laboratory criteria associated with late onset infection.	Success
New infection with Gram positive microorganism	
Treatment with specific anti-staphylococcal antibiotics** for more than 24 hours, within 10 days of EVT visit, associated with positive blood culture, from a normally sterile site, of a phenotypically different microorganism to the baseline pathogen AND at least one clinical or laboratory criterion associated with late onset infection.	Include as failure but conduct a sensitivity analysis as success
Treatment with specific anti-staphylococcal antibiotics** for more than 24 hours, within 10 days of EVT visit, associated with positive blood culture, from a normally sterile site, of a phenotypically different microorganism to the baseline pathogen AND no clinical or laboratory criteria associated with late onset infection.	Success
Other infection with Gram positive microorganism	
Treatment with anti-staphylococcal antibiotics** for more than 24 hours, within 10 days of EVT visit, associated with positive blood culture, from a normally sterile site, of a Gram positive microorganism, when there was no positive culture of a Gram positive microorganism from a normally sterile site during treatment allocation, AND at least one clinical or laboratory criterion associated with late onset infection.	Include as failure but conduct a sensitivity analysis as success
Other suspected infection	
Treatment with antibiotics (but not specific anti-staphylococcal antibiotics**) for more than 24 hours, within 10 days of EVT visit, AND 3 clinical or laboratory criteria associated with late onset infection.	Success
Other infection	
Treatment with antibiotics (but not specific anti-staphylococcal antibiotics**) for more than 24 hours, within 10 days of EVT visit, AND a positive culture (not a Gram positive microorganism) from a normally sterile site AND at least one clinical or laboratory criterion associated with late onset infection.	Success
Treatment with antibiotics (but not anti-staphylococcal antibiotics**) for more than 24 hours, within 10 days of EVT visit, AND a positive blood culture (not a Gram positive microorganism) from a normally sterile site AND no clinical or laboratory criteria associated with late onset infection	Success

Other clinical episode	
Treatment with antibiotics (but not specific anti-staphylococcal antibiotics**) for more than 24 hours, within 10 days of EVT visit, AND less than 3 clinical or laboratory criteria associated with late onset infection in the absence of a positive culture from a normally sterile site	Success

** specific anti-staphylococcal antibiotics as defined in the protocol = vancomycin, flucloxacillin, oxacillin, linezolid, tedizolid, daptomycin and teicoplanin

Supplementary Table 4: Details of Bayesian priors

Prior name	Mean	Variance	Description
non-informative	0	10000	very wide distribution
optimistic	0	0·0026	centred on zero, 2·5% of sample outside NI margin
sceptical	-0.1	0026	centred on NI margin, 2·5% of sample above 0

Three priors for the treatment effect in the primary analysis were selected and were fitted as Normal distributions with the parameters shown. The first prior was 'non-informative', with a very wide variance, and was selected to be analogous to the frequentist results. The other two priors were selected to represent opposing views on the treatment effect of the optimised regimen in comparison to the standard regimen while still acknowledging that there must be some degree of equipoise for the trial to go ahead. The 'optimistic' prior represents the anticipation of no true difference between treatments with a small probability (2.5%) that the optimistic arm is worse than the standard arm by at least 10% (the NI margin). In contrast, the sceptical prior represents the anticipation of the optimistic arm truly being worse than the standard arm by 10%, with a small probability (2.5%) that the optimistic arm is not worse than the standard arm. These contrasting optimistic and sceptical priors therefore act as a sensitivity analysis to the non-informative prior.

Supplementary Table 5: Additional baseline characteristics by study arm (per-protocol population)

	Optimised vancomycin regimen (N=92)	Standard vancomycin regimen (N=93)
Babies per centre: *n (%)*		
Papageorgiou, Thessaloniki (Greece)	26 (28%)	20 (22%)
Ospedale Universitario, Padova (Italy)	14 (15%)	11 (12%)
OPBG, Rome (Italy)	10 (11%)	9 (10%)
Hippokration, Thessaloniki (Greece)	7 (8%)	9 (10%)
12 de Octubre, Madrid (Spain)	8 (9%)	7 (8%)
Tartu University Hospital, (Estonia)	2 (2%)	9 (10%)
Tallinn Children's Hospital, (Estonia)	3 (3%)	6 (6%)
Aghia Sofia A, Athens (Greece)	3 (3%)	5 (5%)
Attikon, Athens (Greece)	4 (4%)	4 (4%)
St Mary's, Manchester (UK)	5 (5%)	1 (1%)
Ospedale Niguarda, Milan (Italy)	3 (3%)	3 (3%)
Aghia Sophia C, Athens (Greece)	4 (4%)	2 (2%)
Aglaia Kyriakou, Athens (Greece)	0 (0%)	4 (4%)
Sant Joan de Deu, Barcelona (Spain)	2 (2%)	2 (2%)
Di Venere, Bari (Italy)	0 (0%)	1 (1%)
San Matteo, Pavia (Italy)	1 (1%)	0 (0%)
Umbilical catheter/central venous line present: *n (%)*	58 (63%)	58 (62%)
Clinical criteria: *n (%)*		
1. Hyperthermia or hypothermia	33 (36%)	33 (35%)
2. Hypotension or impaired peripheral perfusion or mottled skin	50 (54%)	62 (67%)
3. Apnoea or increased oxygen requirement or increased requirement for ventilatory support	62 (67%)	60 (65%)
4. Bradycardic episodes or tachycardia	57 (62%)	56 (60%)
6. Worsening feeding intolerance or abdominal distension	41 (45%)	44 (47%)
6. Lethargy or hypotonia or irritability	37 (40%)	46 (49%)
Laboratory criteria: *n/N (%)*		
1. White blood cell (WBC) count < 4 or > 20 x 10^9 cells/L	23/89 (26%)	26/84 (31%)
2. Immature to total neutrophil ratio (I/T) > 0.2	2/6 (33%)	3/10 (30%)
3. Platelet count < 100 x 10^9/L	13/89 (15%)	5/84 (6%)
4. C-reactive protein (CRP) > 10 mg/L	71/92 (77%)	63/93 (68%)
5. Glucose intolerance as defined by a blood glucose value > 180 mg/dL (> 10 mmol/L) when receiving normal glucose amounts (8 – 15 g/kg/day)	13/92 (14%)	10/93 (11%)
6. Metabolic acidosis as defined by a base excess (BE) < –10 mmol/L (< – 10 mEq/L) or a blood lactate value > 2 mmol/L	28/84 (33%)	36/92 (39%)
Number of clinical criteria: *N (%)*		
0	1 (1%)	0 (0%)
1	13 (14%)	6 (6%)
2	14 (15%)	21 (23%)
3	29 (32%)	29 (31%)
4	24 (26%)	22 (24%)
5	10 (11%)	12 (13%)
6	1 (1%)	3 (3%)

Number of laboratory criteria: *N (%)*		
0	7 (8%)	8 (9%)
1	40 (43%)	47 (50%)
2	27 (29%)	24 (26%)
3	16 (17%)	8 (9%)
4	2 (2%)	6 (6%)

Supplementary Table 6: Gram-positive species detected at baseline by study arm

Gram positive species detected at baseline	Optimised vancomycin regimen (n=92)	Standard vancomycin regimen (n=93)
Staphylococcus epidermidis	21/37 (57%)	34/43 (79%)
Staphylococcus hominis	5/37 (14%)	3/43 (7%)
Staphylococcus haemolyticus	3/37 (8%)	4/43 (9%)
Enterococcus faecalis	2/37 (5%)	0/43 (0%)
Staphylococcus capitis	2/37 (5%)	0/43 (0%)
Staphylococcus warneri	2/37 (5%)	0/43 (0%)
Staphylococcus lugdenensis	1/37 (3%)	1/43 (2%)
Streptococcus mitis	1/37 (3%)	0/43 (0%)
Streptococcus sp.	0/37 (0%)	1/43 (2%)

Supplementary Table 7: Duration of antibiotic therapy by study arm

	Optimised vancomycin regimen	Standard vancomycin regimen
Median number of doses of study vancomycin (IQR)		
PMA < 29 weeks (10 opt / 10 std)	11·5 (10, 13), n = 20	10 (10 - 14), n= 23
PMA 29-35 weeks (10 opt / 20 std)	12 (10 - 13), n = 44	20 (17 - 21), n = 43
PMA > 35 weeks (15 opt / 30 std)	17 (13 - 18), n = 28	26 (24 - 30), n = 27
Median days of continued antibiotic treatment from start of study vancomycin (IQR)		
Vancomycin	6 (5 – 7·5)	10 (9 - 10)
Anti-staphylococcal antibiotic*	6 (5 - 8)	10 (9 - 11)
Any antibiotic	6 (5 – 11·5)	10 (9 - 11)
Median days of total antibiotic exposure to STFU (IQR)		
Vancomycin	7 (6 - 11)	10 (9 - 12)
Anti-staphylococcal antibiotic*	9 (6 - 14)	11 (10 - 12)
Any antibiotic	12 (7 - 20)	11 (10 - 15)

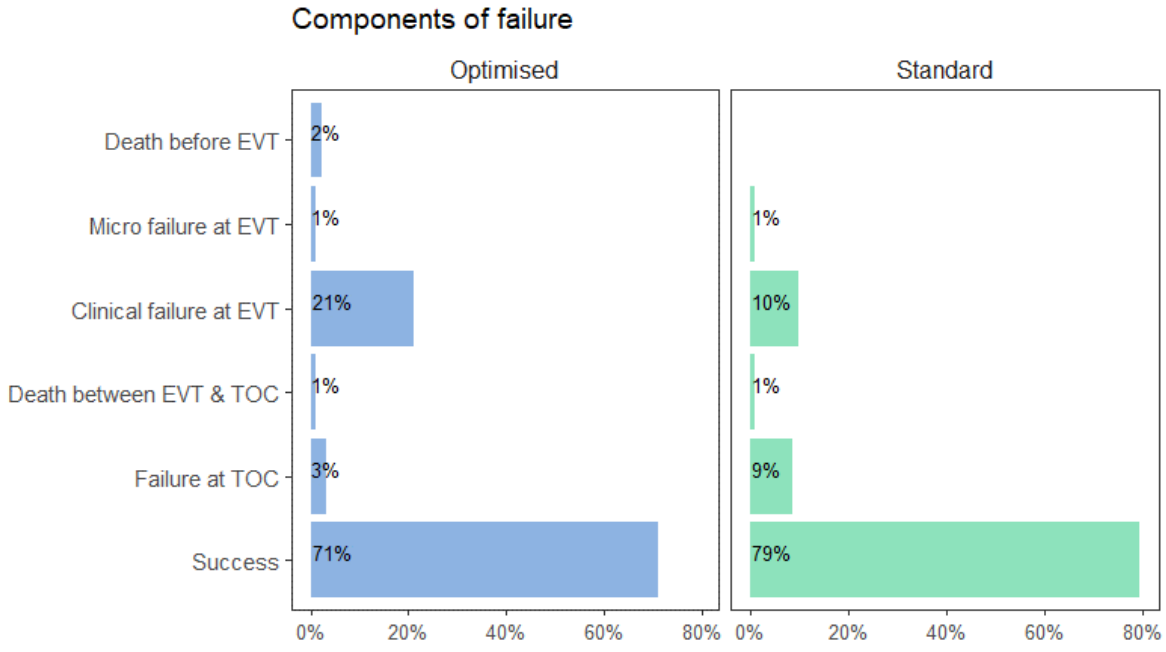
* Anti-staphylococcal antibiotics are vancomycin, flucloxacillin, oxacillin, linezolid, tedizolid, daptomycin or teicoplanin

Supplementary Table 8: Therapeutic drug monitoring by arm in the per-protocol population

	Optimised vancomycin regimen (N=92)	Standard vancomycin regimen (N=93)
Instances		
TDM assessment (N)	41	60
Dose adjustment following TDM assessment (N)	16	24
Patients		
TDM assessment (N)	20	26
Dose adjustment following TDM assessment (N)	9	14
Centres		
TDM assessment (N)	5	7
Dose adjustment following TDM assessment (N)	4	5

Supplementary Table 9: Participant outcomes by study arm in the ITT population

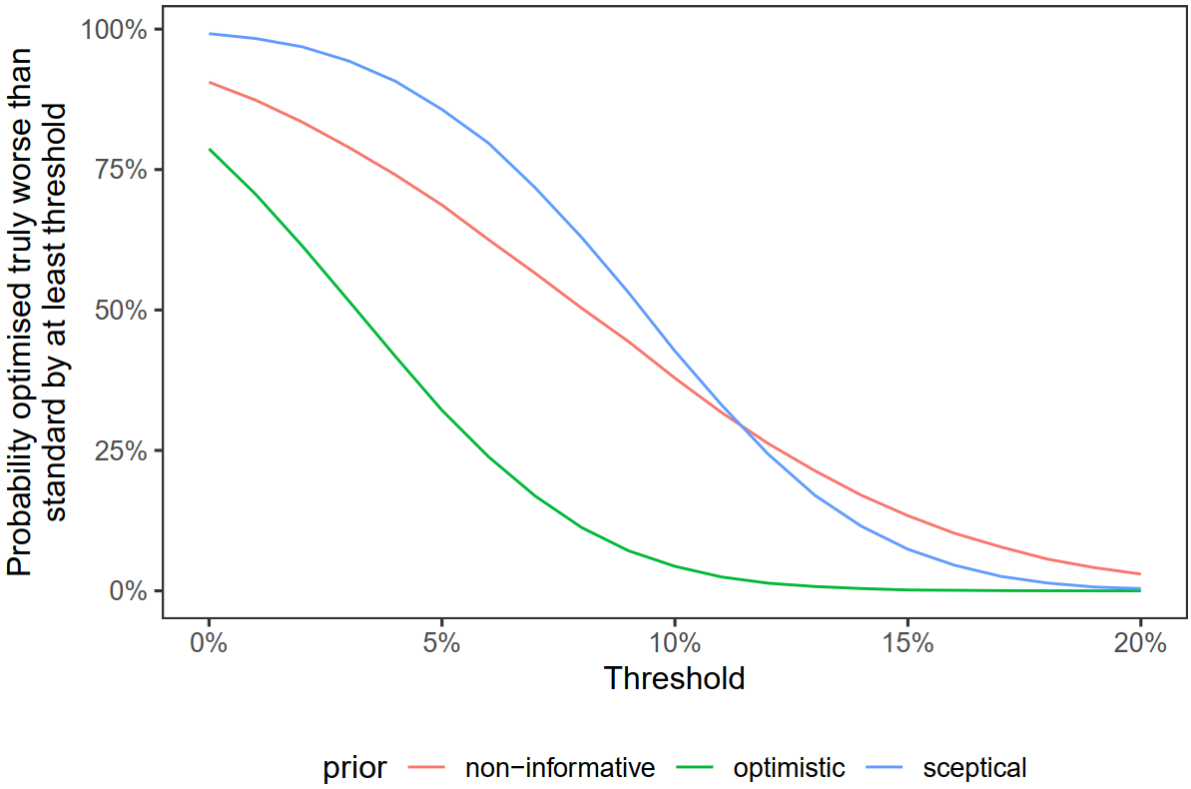
Outcome	Optimised vancomycin regimen n/N (%)	Standard vancomycin regimen n/N (%)	Adjusted risk difference (95% CI)
Success at TOC visit	68/99 (69%)	76/97 (78%)	-7% (-15%, 1%)
Secondary outcomes			**Adjusted risk ratio (95% CI)**
Success at 5 ± 1 days after start of allocated vancomycin therapy	69/100 (69%)	79/98 (81%)	0.90 (0.79, 1.03)
Success at end of actual vancomycin therapy	72/99 (73%)	85/97 (88%)	0.83 (0.73, 0.95)
Success at TOC visit: composite including treatment with "other" antibiotics*	68/99 (69%)	71/97 (73%)	0.98 (0.88, 1.09)
Success at STFU visit (30±5 days from initiation of study vancomycin)	60/99 (60%)	74/97 (76%)	0.82 (0.72, 0.93)
Failure between EVT & TOC caused by treatment with "other" antibiotics*	3/90 (3%)	16/92 (17%)	0.19(0.08, 0.39)
Failure between TOC and STFU	11/90 (12%)	4/92 (4%)	2.81 (0.84, 9.38)
Success at end of allocated therapy	69/100 (69%)	86/98 (88%)	0.78 (0.68, 0.89)



Supplementary Figure 2: Components of the primary outcome by arm

Supplementary Table 10: Bayesian analysis comparing the primary outcome by study arm

	Per-protocol (PP) population		
	Non-informative prior	**Sceptical prior**	**Optimistic prior**
Standard arm estimated success rate (95% BCI)	79% (70%, 86%)	79% (72%, 86%)	77% (69%, 84%)
Optimised arm estimated success rate (95% BCI)	71% (61%, 79%)	70% (62%, 78%)	73% (66%, 81%)
Estimated difference (95% BCI)	-8% (-21%, 4%)	-9% (-17%, -2%)	-3% (-11%, 5%)
Probability optimised arm is truly worse than standard arm by at least:			
0%	91%	99%	79%
1%	87%	98%	71%
2%	83%	97%	61%
3%	79%	94%	52%
4%	74%	91%	42%
5%	69%	86%	32%
6%	63%	80%	24%
7%	57%	72%	17%
8%	50%	63%	11%
9%	44%	53%	7%
10%	38%	43%	4%
11%	32%	33%	2%
12%	26%	24%	1%
13%	21%	17%	1%
14%	17%	12%	0%
15%	13%	7%	0%
16%	10%	5%	0%
17%	8%	3%	0%
18%	6%	1%	0%
19%	4%	1%	0%
20%	3%	0%	0%



Supplementary Figure 3: Bayesian analysis showing probability optimised arm is truly worse than standard arm by at least the threshold value, as a function of different thresholds.

Supplementary Table 11: NeoVanc participant subgroup analyses on primary outcome by study arm in per protocol population

Subgroup	Optimised vancomycin regimen (n/N (%))	Standard vancomycin regimen (n/N (%))	Adjusted risk ratio (95% CI)
Postmenstrual age (weeks):			
< 29	9/20 (45%)	17/23 (74%)	0·60 (0·38, 0·93)
29 to 35	32/43 (74%)	32/43 (74%)	0·99 (0·82, 1·20)
> 35	23/27 (85%)	24/26 (92%)	0·96 (0·77, 1·19)
			Interaction p-value = 0·13
Birthweight (g):			
< 1000	22/39 (56%)	23/33 (70%)	0·89 (0·64, 1·24)
1000 to 1500	16/21 (76%)	22/27 (81%)	0·86 (0·54, 1·15)
> 1500	26/30 (87%)	28/32 (88%)	0·97 (0·80, 1·18)
			Interaction p-value = 0·76
Umbilical catheter/central venous line present:			
No	26/33 (79%)	31/35 (89%)	0·95 (0·76, 1·19)
Yes	38/57 (67%)	42/57 (74%)	0·91 (0·72, 1·40)
			Interaction p-value = 0·80

Supplementary Table 12: Variables considered for inclusion in post-hoc multiple imputation on abnormal hearing safety outcome in ITT population

Variable	Instances observed	Included in final model
Arm	200	Yes
Sex	200	Yes
Birthweight stratum	200	Yes
Postmenstrual age stratum	200	Yes
Microtia/external ear canal atresia at baseline	1	No
Syndromes associated with hearing impairment, including Trisomy 21	3	No
Craniofacial abnormalities including cleft palate at baseline	1	No
Confirmed congenital infections, e.g. CMV, toxoplasmosis at baseline	1	No
Previous bacterial meningitis at baseline	1	No
Severe unconjugated hyperbilirubinaemia at baseline	0	No
Suspicion of or known A1555G mitochondrial mutation at baseline	0	No
Hypoxic ischaemic encephalopathy at baseline	9	Yes
Intraventricular haemorrhage at baseline	31	Yes
Family history of hearing impairment at baseline	1	No
Received amikacin	93	Yes
Received ciprofloxacin	14	Yes
Received clarithromycin	1	No
Received erythromycin	2	No
Received gentamicin	121	Yes
Received imipenem	3	No
Received levofloxacin	0	No
Received linezolid	12	Yes
Received netilmicin	30	Yes
Received teicoplanin	58	Yes
Received tobramycin	2	No
Received valganciclovir	1	No

Variables considered for inclusion in the model were demographic such as age and sex, risk factors for hearing impairment at baseline such as family history and whether or not infants had also received specific drugs with potential ototoxic effects in neonates. Some variables could not be included in the final model due to low prevalence leading to issues with perfect prediction, as shown in the final column. Multiple imputation was run to create 1,000 imputed datasets which were then analysed using the same adjusted model specified in the SAP to ensure small Monte-Carlo error rates.

Supplementary Table 13: Post-hoc subgroup analyses on abnormal hearing safety outcome in ITT population with hearing assessed

Subgroup	Optimised vancomycin regimen (n/N (%))	Standard vancomycin regimen (n/N (%))	Adjusted risk ratio (95% CI)
Post-menstrual age (weeks):			
< 29	8/19 (42%)	1/19 (5%)	7·9 (1·8, 35·1)
29 to 35	8/42 (19%)	5/38 (13%)	1·5 (0·6, 3·3)
> 35	9/23 (39%)	6/22 (27%)	1·4 (0·6, 3·2)
			Interaction p-value = 0·05

Supplementary Table 14: Post-hoc analyses of abnormal hearing safety outcome in ITT population in infants where hearing was assessed

Test	Optimised vancomycin regimen (n/N (%))	Standard vancomycin regimen (n/N (%))	Adjusted risk ratio (95% CI)
Otoacoustic Emissions	15/60 (25%)	6/55 (16%)	1·6 (0·9, 3·1)
Auditory Brainstem Response	11/46 (24%)	5/38 (13%)	1·7 (0·7, 4·3)

Note: 36 babies had hearing assessed using both methods

Supplementary Table 15: Post-hoc analyses of safety outcomes for the as-treated population

Outcome	Optimised vancomycin regimen n/N (%)	Standard vancomycin regimen n/N (%)	Adjusted risk ratio (95% CI)
Abnormal renal function tests at the short-term follow-up visit:	2/84 (2%)	0/81 (0%)	0.85 (-1.7, +inf)
Abnormal hearing screening test after EVT	25/84 (30%)	12/67 (15%)	1.96 (1.1, 3.6)
Abnormal hearing screening test after imputation	33·7/102 (33%)	18·5/98 (19%)	1·72 (1·0, 2·9)
Incidence rate per 1000 child days			
Adverse events up to STFU:			
- All AE	47 (141/3012)	42 (125/2956)	1.1 (0.64, 1.89)
- Vancomycin related AE	2.3 (7/3012)	1.4 (4/2956)	1.7 (0.89, 3.18)
Serious adverse events			
- All SAE	7.3 (22/3012)	9.8 (29/2956)	0.73 (0.29, 1.84)
- Vancomycin related SAE	0.33 (1/3012)	0.68 (2/2956)	0.49 (0.11, 2.16)

"as treated" = optimised arm – all infants receiving a loading dose; standard arm – all infants not receiving a loading dose.

Note: All except one infant in the ITT population received a loading dose as randomised. Consent was withdrawn for the infant in question after randomisation (to optimised arm) but before IMP was given. Therefore, results above are the same as Table 4.

Supplementary Table 16: Post-hoc analyses of abnormal hearing including cumulative dose of vancomycin

Parameter	Unadjusted			
	Coefficient	Lower 95% Confidence interval	Upper 95% confidence interval	p value
Model 1: No cumulative dose				
Arm: Optimised	0.67	0.06	1.29	0.032
Model 2: Linear cumulative dose				
Arm: Optimised	0.65	0.03	1.27	0.040
Cumulative dose (mg/kg)	0.0006	-0.0007	0.0018	0.373
Model 3: Fractional polynomial cumulative dose				
Arm: Optimised	0.65	0.03	1.27	0.041
Cumulative dose (mg/kg) 1	0.00000005	-0.00000001	0.00000011	0.133
Cumulative dose (mg/kg) 2	-0.00000001	-0.00000002	0.00000000	0.139
Parameter	**Adjusted**			
	Coefficient	**Lower 95% Confidence interval**	**Upper 95% confidence interval**	**p value**
Model 1: No cumulative dose				
Arm: Optimised	0.67	0.05	1.30	0.035
Model 2: Linear cumulative dose				
Arm: Optimised	0.65	0.01	1.30	0.047
Cumulative dose (mg/kg)	0.0005	-0.0008	0.0017	0.459
Model 3: Fractional polynomial cumulative dose				
Did not converge				

Individual dose data were available for vancomycin doses given as part of the trial intervention. Daily dose and number of days given were recorded for doses given before (up to 24 hours, rounded up to 24 hours of dosing) and after the trial (start and end dates were rounded up to full days of dosing). Cumulative dose was expressed as mg/kg based on the weight recorded at baseline and seven cumulative doses above 1000 mg/kg were truncated at 1000 to avoid undue influence on the model.

The table above shows the output from glm models with binomial error distribution, log link function and fixed effects of arm. Adjusted models (bottom) also include fixed effects of PMA stratum and presence/absence of central lines at baseline, and random effect of centre as per the adjusted analyses pre-specified in the SAP. Within each of adjusted/unadjusted are three models: with no adjustment for cumulative dose (as per original analyses), with cumulative dose fitted as a linear coefficient, and with cumulative dose fitted as a fractional polynomial. Results are reported as coefficients.