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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

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Appendix

Optimised versus standard dosing of vancomycin in infants with Gram-positive sepsis (NeoVanc): a multicentre, randomised, open-label, phase 2b, non-inferiority trial

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NeoVanc Consortium

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

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Independent Data Monitoring Committee:

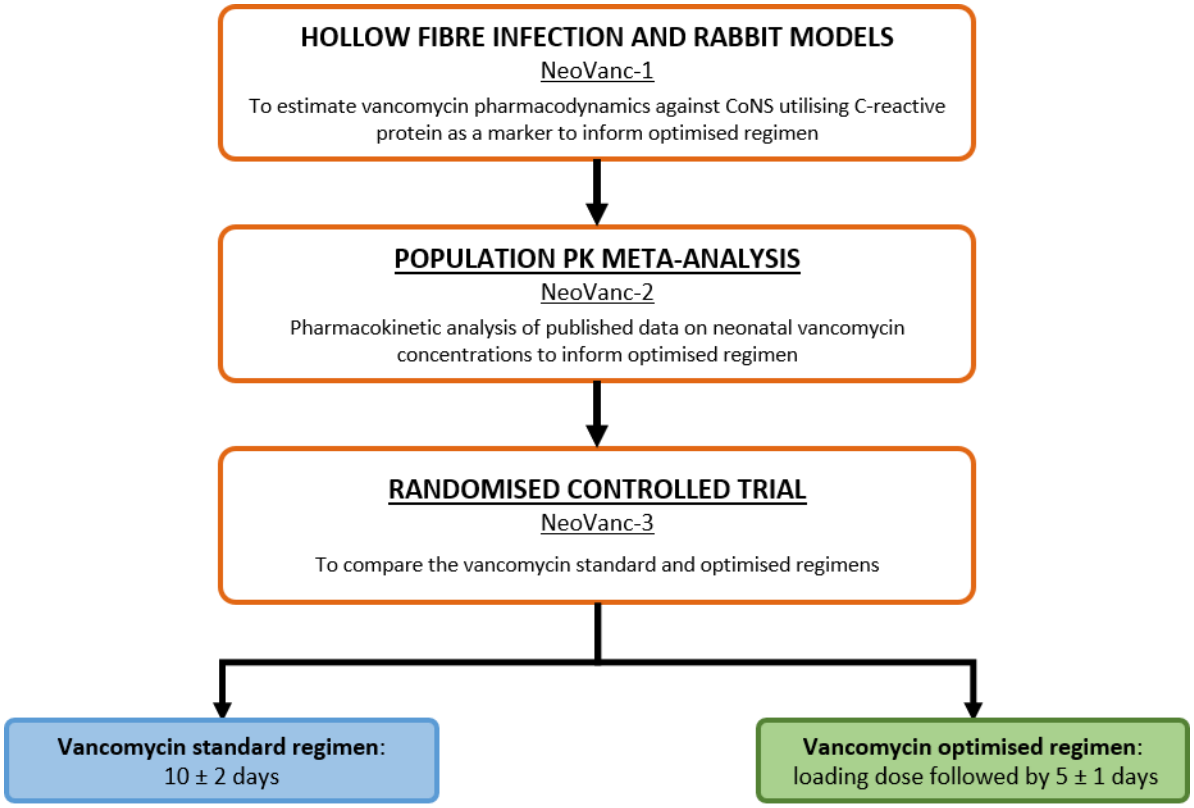
- John van den Anker (Chair)
- Corine Chazallon
- James Gray

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.[1]

Inclusion and exclusion criteria from Hill LF (2020).[2]

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

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

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)

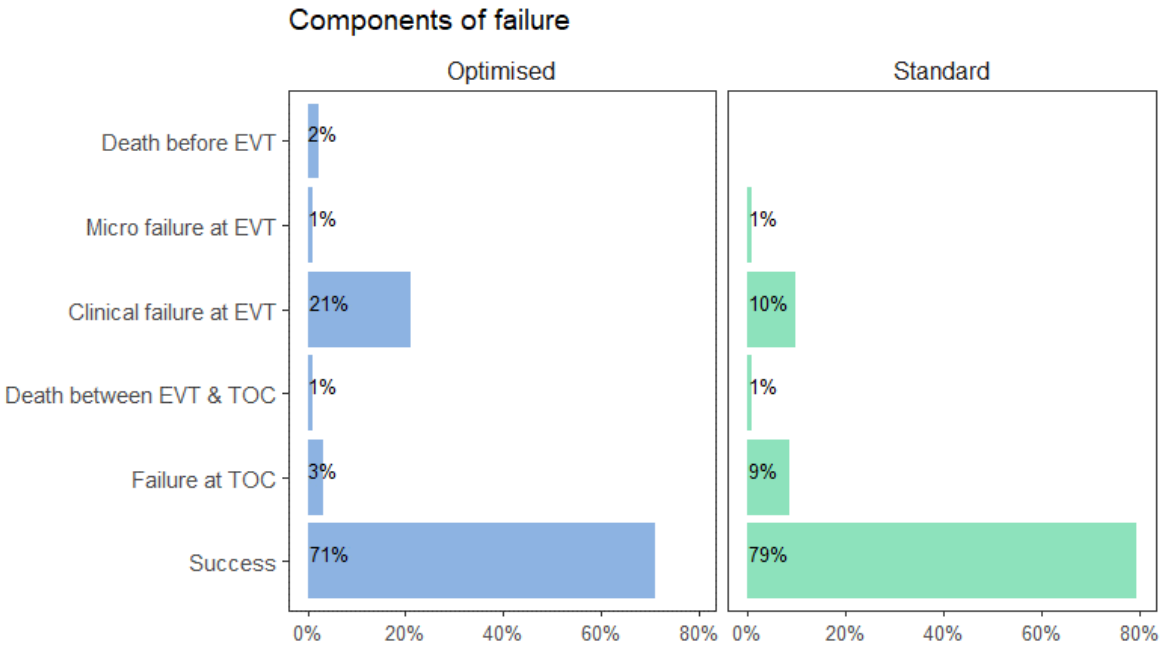
* Anti-staphylococcal antibiotics are vancomycin, flucloxacillin, oxacillin, linezolid, tedizolid, daptomycin or teicoplanin

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

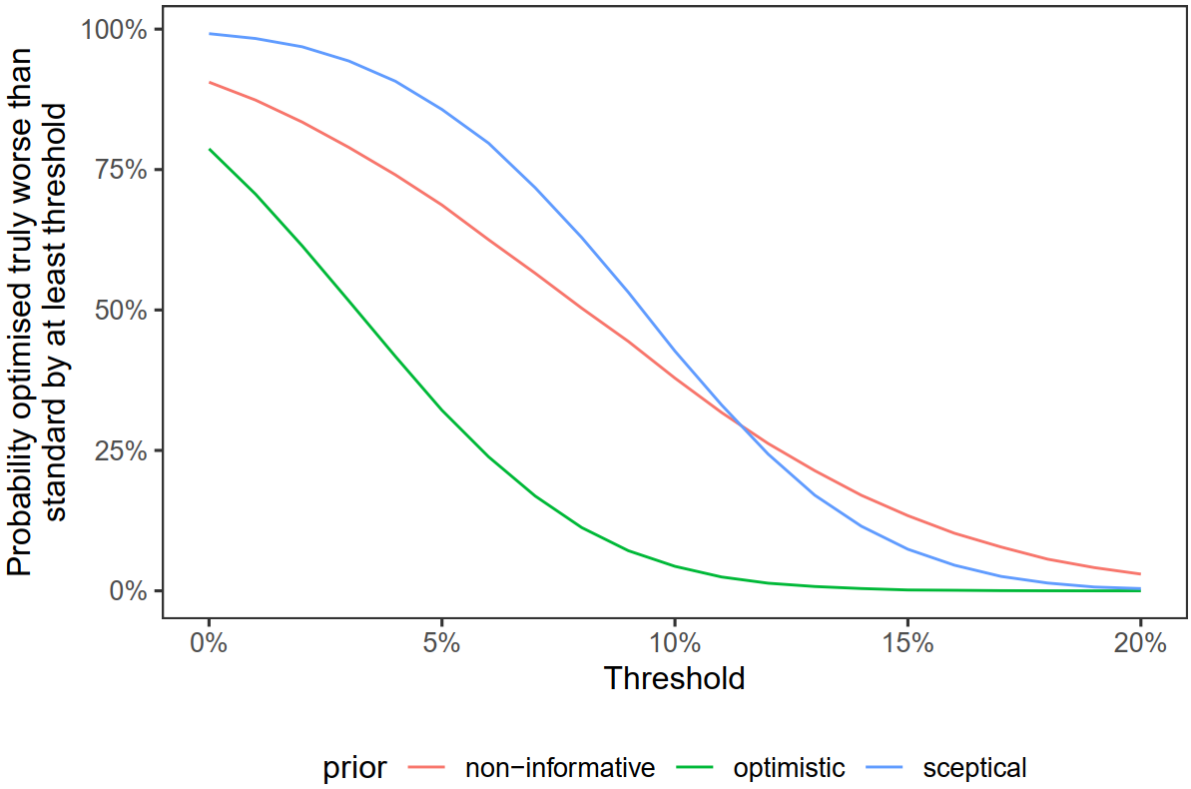
Outcome	Optimised vancomycin regimen n/N (%)	Standard vancomycin regimen n/N (%)	Adjusted risk difference (95% CI)
Success at TOC visit	68/99 (69%)	76/97 (78%)	-7% (-15%, 1%)
Secondary outcomes			**Adjusted risk ratio (95% CI)**
Success at 5 ± 1 days after start of allocated vancomycin therapy	69/100 (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.

References

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