Centre for Trials Research
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AZTEC
Azithromycin Therapy for Chronic Lung Disease

AZTEC: Azithromycin Therapy for Chronic Lung Disease of Prematurity

A randomised, placebo-controlled trial of azithromycin for the prevention of chronic lung disease of prematurity in preterm infants

VERSION 3.0 20/06/2019

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SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the relevant trial regulations, GCP guidelines, and CTR's SOPs.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the trial publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the trial will be given; and that any discrepancies from the trial as planned in this protocol will be explained.

Trial Sponsor: **Name: Chris Shaw**	**Signature**	**Date** 25/6/2019
Director: MIKE HARLING **Name: ~~Kerry Hood~~**	**Signature**	**Date** 5.7.19
Chief Investigator: **Name: Sailesh Kotecha**	**Signature**	**Date 20/06/2019**

General Information This protocol describes the AZTEC clinical trial and provides information about the procedures for entering participants into the trial. The protocol should not be used as a guide, or as an aide-memoire for the treatment of other participants. Every care has been taken in drafting this protocol; however, corrections or amendments may be necessary. These will be circulated to the known Investigators in the trial. Problems relating to the trial should be referred, in the first instance, to CTR.

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Trial Co-ordination:

The AZTEC trial is being coordinated by the Centre for Trials Research (CTR) Cardiff University, a United Kingdom Clinical Research Collaboration (UKCRC) registered trials unit.

This protocol has been developed by the AZTEC Trial Management Group (TMG)

For **all queries** please contact the AZTEC team through the main trial email address. Any clinical queries will be directed through the Trial Manager to either the Chief Investigator or a Co-Investigators.

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Randomisations:

Randomisation
To randomise a participant, visit the AZTEC randomisation site https://ctu1.phc.ox.ac.uk/*AZTEC*
In the event online randomisation is not available, contact the CTR (during office hours) on 029 2068 7990 and AZTEC@Cardiff.ac.uk
Clinical queries
AZTEC@Cardiff.ac.uk
All clinical queries will be directed to the most appropriate clinical person.
SAE reporting
Where the adverse event meets one of the serious categories, an SAE form should be completed by the responsible clinician and submitted to the CTR PV & S team within 24 hours of becoming aware of the event (See section 16 for more details).
CTR-Safety@Cardiff.ac.uk
SAE Fax number:
0203 0432 376

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Glossary

AE	Adverse Event
AR	Adverse Reaction
BPD	Bronchopulmonary Dysplasia
CI	Chief Investigator
CLD	Chronic Lung Disease of Prematurity
CPAP	Continuous Positive Airway Pressure
CRF	Case Report Form
ETA	Endotracheal Aspirates
FIO_2	Fraction of Inspired Oxygen
GMP	Good Manufacturing Practice
IDMC	Independent Data & Safety Monitoring Committee
IMP	Investigational Medicinal Product
i.v.	Intravenous
kg	Kilograms
mcg	Micrograms
MHRA	Medicines and Healthcare products Regulatory Agency
mg	Milligram
ml	Millilitre
NHS	National Health Service
NPA	Nasopharyngeal aspirates
PAS	Pediatric Academic Society
PI	Principal Investigator
PK	Pharmacokinetic
PMA	Postmenstrual age
QP	Qualified Person
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TM	Trial Manager
TMG	Trial Management Group
TPN	Total Parenteral Nutrition
TSC	Trial Steering Committee

Amendment History

The following amendments and/or administrative changes have been made to this protocol since the implementation of the first approved version.

Amendment No.	Protocol version no.	Date issued	Summary of changes made since previous version
Amendment 1 (Substantial)	2.0	20/02/2019	- Porting of protocol to CTR template - Section 1.1 flow diagram updated to reflect that placebo is sterile water (rather than saline) - Section 3.2 Removal of reference to 2-year follow up - Section 3.4 Removal of reference to 2-year follow-up - Section 9.3 updated to remove requirement for temperature monitoring of the IMP - Section 9.3 updated to clarify that acceptable diluents are those as listed in the SmPC. - Section 11 updated to clarify data collected at 36 weeks PMA (11.10) and at discharge (11.11) - Section 13.3 updated to reflect that unblinding will be conducted online rather than use of envelopes - Section 14.1.1 updated in line with CTR policy- no interim analysis planned - Section 16 updated to reflect that follow-up post-hospital discharge will be undertaken as an independent study, subject to funding. - Section 18: End of trial definition updated to reflect CTR policy - Throughout: Minor grammatical and typographic corrections
Amendment 3	3.0	20/06/2019	- Synopsis: Change secondary objectives and outcome list to bulleted list. Addition of CLD severity as secondary outcome. Expression of dosage in mL/kg - 1.1 flow diagram: Express dosages as mL/kg - 3.2 Secondary objectives: Change to bulleted list. Update to include severity of CLD

			- 3.4 Secondary outcome measures: Update to include severity of CLD - 9.4 Treatment prescribing and preparation: Inclusion of y-site compatibility. Inclusion of wording on re-establishing IV access - 14.1 Analysis Plan: Reference to clustering effect of multiple births added - Reference list updated

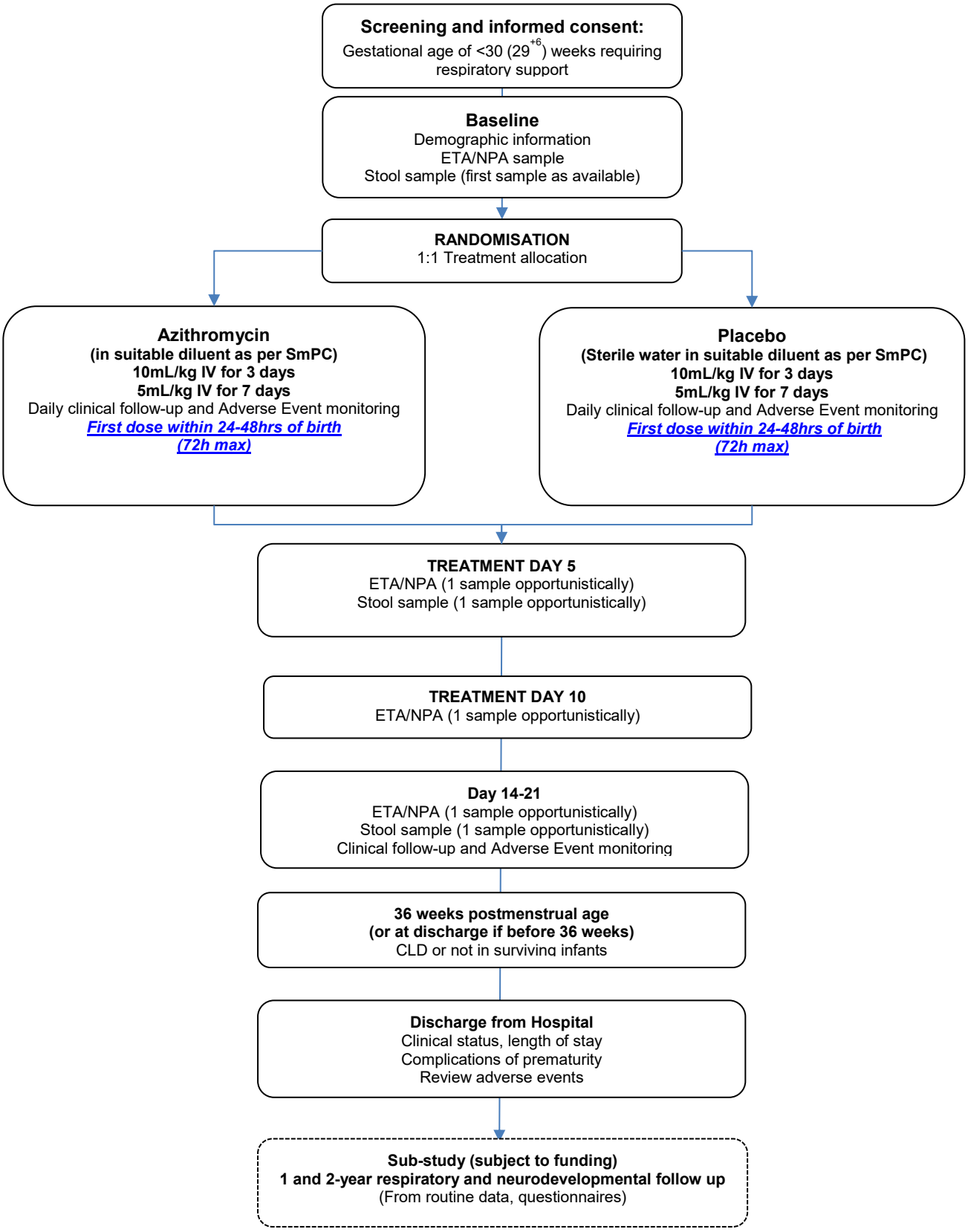
Synopsis

Short title	Azithromycin Therapy for Chronic Lung Disease of Prematurity
Acronym	AZTEC
Clinical phase	III
Funder and ref.	NIHR HTA 16/111/106
Trial design	Double blind, randomised placebo-controlled
Trial participants	Preterm neonates, <30 weeks' gestational age
Planned sample size	796
Planned number of sites	approx. 25
Inclusion criteria	1) Gestational age ≤29w+6d (including infants born as one of a multiple birth) 2) Neonates who have had respiratory support for at least 2 continuous hours duration during the first 72 hours of life (intubated, or by non-invasive mechanical ventilation including continuous positive airway pressure and high flow nasal cannula or a combination thereof) 3) Presence of an indwelling intravenous line for drug administration 4) Written informed consent within 72 hours of birth 5) Anticipating administration of first dose within 72 hours at the latest (within 24 hours of life for inborn and 48 hours for outborn infants) 6) Reasonable to expect completion of 10 days of trial treatment whilst resident at the recruiting site 7) Inborn, or born at site within the recruiting site's neonatal network where follow up will be possible 8) In the opinion of the PI, reasonable prospect of survival pas the first 72 hours of life
Exclusion criteria	1) Exposure to another systemic macrolide antibiotic (not maternal) 2) Presence of major surgical or congenital abnormalities (not including patent ductus arteriosus or patent foramen ovale) 3) Contraindication of azithromycin as specified in the summary of characteristics of the product 4) Participation in other interventional trial that precludes participation in AZTEC
Treatment duration	10 days
Follow-up duration	Until discharge from hospital
Planned trial period	30 months recruitment
Primary objective	To determine the effectiveness of azithromycin in increasing survival without physiologically defined CLD (moderate/severe) when compared to placebo
Secondary objectives	• To determine the effect of azithromycin on mortality rate (at 36 weeks' postmenstrual age) • To determine the effectiveness of azithromycin in reducing duration of positive pressure respiratory support (i.e. conventional mechanical ventilation/HFOV, continuous positive airway pressure, high flow nasal cannula) • To determine the safety and tolerability of azithromycin

	• To determine if azithromycin alters resistance to macrolides amongst S*treptococcus* and *Staphylococcus* spp. microbes isolated from respiratory and stool samples • To investigate if colonisation with *Ureaplasma* spp. Prior to randomisation modifies the treatment effect of azithromycin compared to placebo
Primary outcomes	CLD or death at 36 weeks' postmenstrual age
Secondary outcomes	• Mortality • Severity of Chronic Lung disease • Number of days of respiratory support • Development of complications of prematurity • Serious adverse events/reactions • Resistance to macrolides among microbes isolated from respiratory and stool samples
Sub-studies	Subject to funding, an independent sub-study will be performed (with separate REC and relevant approvals) to determine if azithromycin alters neurodevelopmental and respiratory outcomes in infancy at 1 and 2 years of age
Investigational medicinal products	Azithromycin or Placebo
Form	Lyophilised powder for solution for infusion
Dose	10mL/kg daily for 3 days; 5mL/kg for 7 days (10 days total)
Route	Intravenous

1 Trial summary & schema

1.1 Participant flow diagram



1.2 Trial lay summary

Premature births account for a tenth of all world-wide births. Many premature babies especially those who are born extremely prematurely will not survive. Many extremely premature babies who survive will develop the disease called Chronic Lung Disease of Prematurity (CLD), which is also often called bronchopulmonary dysplasia or BPD. CLD develops due to delivery of babies with underdeveloped lungs and also because of the treatment used which includes breathing machines (mechanical ventilators) and oxygen therapy which these babies need for survival. CLD is most commonly defined as needing oxygen at 36 weeks "corrected" gestation. Although most babies will come off oxygen prior to discharge, some go home on oxygen placing enormous burden on the parents. Babies with CLD also have many hospital admissions and chest infections in childhood.

We have noted that inflammation (like redness or soreness) of the lungs is often seen in babies who develop CLD. In addition, we and others have shown that the germ called Ureaplasma is more prevalent in the lungs of babies who develop CLD. Because the germ is acquired from the mother's birth canal or from the womb, some doctors think that it is a simple 'bystander' not causing disease, but others believe that it is actively causing harm resulting in CLD. We have shown that babies who have Ureaplasma have lung inflammation; we have also reviewed all the articles published and showed that if the premature babies' lungs have Ureaplasma, they have much greater chances of developing CLD than those who do not have Ureaplasma in their lungs. Studies have used antibiotic of the group called macrolides to treat the Ureaplasma, with the most recent studies using azithromycin. A recent report collated the data from 3 studies showing that rates of CLD may improve but total numbers studied are small. Therefore, most researchers say that a large trial using azithromycin is required to see if azithromycin can really improve CLD rates).

Azithromycin is very attractive as it has two important actions. Firstly, it decreases inflammation; secondly, it is an antibiotic against Ureaplasma. We, therefore, plan to study if a ten-day course of intravenous azithromycin improves survival without CLD in premature babies born at less than 30 weeks in gestation. During the treatment period, we shall collect lung fluid samples from babies via their breathing tube or from their nose/back of the mouth during routine care by the nurse and nappy stool samples. The samples will be used to: a) see if lung Ureaplasma is removed by azithromycin; and b) if common germs found in the gut and lungs develop antibiotic resistance. We believe that studying antibiotic resistance is important as azithromycin will be widely used if the study shows that azithromycin improves rates of CLD.

2 Background

Advances in neonatal care have improved the survival of extremely preterm infants especially in those born at 30 weeks' gestation or less. However, morbidity resulting from lung injury in the newborn has been observed in survivors. Respiratory distress syndrome (RDS) is the prototypical lung condition of preterm infants characterised by inadequate surfactant production by the immature lungs. This results in respiratory failure necessitating active support in the form of mechanical ventilation or increased oxygen concentrations. Partly due to such interventions, lung inflammation peaking at 7 – 10 days of age is observed especially in infants who progress to develop CLD. Whilst the lung injury will resolve in most infants, 30% of infants born at <30 weeks' gestational age develop CLD (most frequently defined as supplemental oxygen dependency at 36 weeks' postmenstrual age). Some will be discharged on home oxygen, with additional associated family, societal and health costs. Any interventions decreasing rates of CLD will have not only economic and health benefits but also practical and emotional benefits for both the babies and their families.

Besides factors that are known but not easily modified (prematurity, mechanical ventilation, oxygen therapy, male sex and patent ductus arteriosus), microbial colonisation is strongly associated with the development of CLD (Beeton 2011) and is amenable to therapeutic manipulation. A frequent finding in babies who develop CLD is colonisation of the preterm lung with the microbe *Ureaplasma* spp- a (class Mollicutes) vaginal microbial inhabitant of 50 to 85% of pregnant women. As such, the perinatal transmission from those colonized mothers occurs in 22 to 55 % of full term infants and in 60 % of preterms (Sanchez 1987; Dinsmoor 1989; Abele-Horn, 1997; Jobe, 2001). Although this transmission can be seen as a normal and physiological colonization process, however the presence of *Ureaplasma* spp. *in utero* is associated with increased risks of preterm labour and delivery, higher risks of foetal and maternal inflammation (Figure 1),

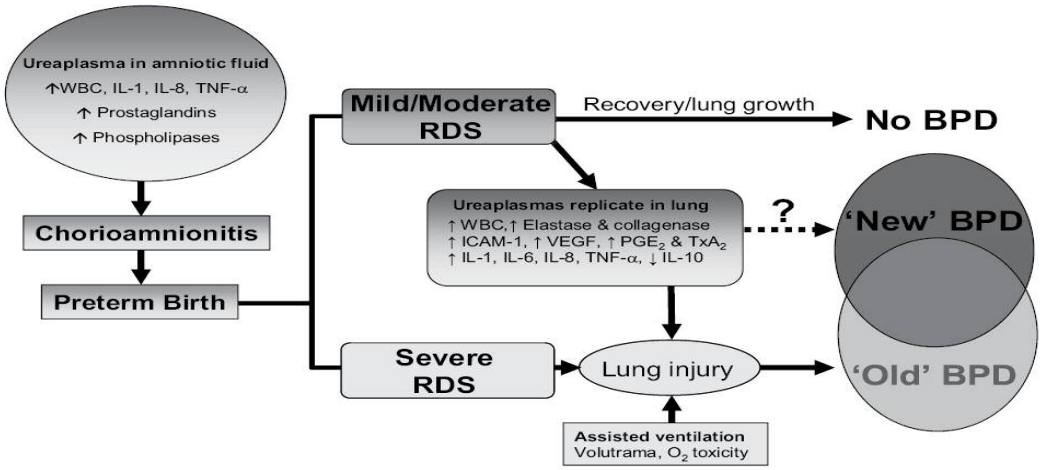
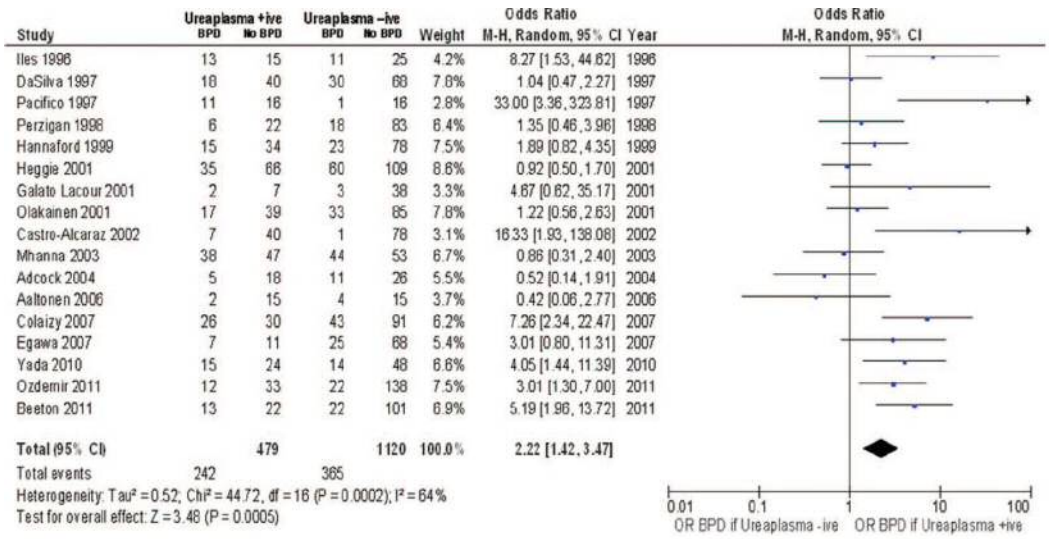


Figure 1 Schematic presentation of the pathophysiology of Ureaplasma spp. infection and the consequence on neonates. (RDS – respiratory distress syndrome; WBC – white blood cells; IL-interleukin; TNF- tumour necrosis factor; VEGF – vascular endothelial growth factor; ICAM – intercellular adhesion molecule; PG – prostaglandin; Tx thromboxane) (Reproduced from Waites 2009)

The most recent meta-analysis by Lowe *et al* noted the association between the presence of pulmonary *Ureaplasma* spp. and the development of CLD both defined as supplemental oxygen dependency at 28 days of age (OR 3.04 95% CI 2.41, 3.83) and at 36 weeks' postmenstrual age (OR 2.22 95% CI 1.42, 3.47) (Figure 2a). Since previous suggestions were that *Ureaplasma* spp. association was with gestational age rather than with respiratory illness, the authors performed meta-regression to show that the association between *Ureaplasma* spp. colonisation and development of CLD was not affected by gestational age of the baby (Figure 2b).



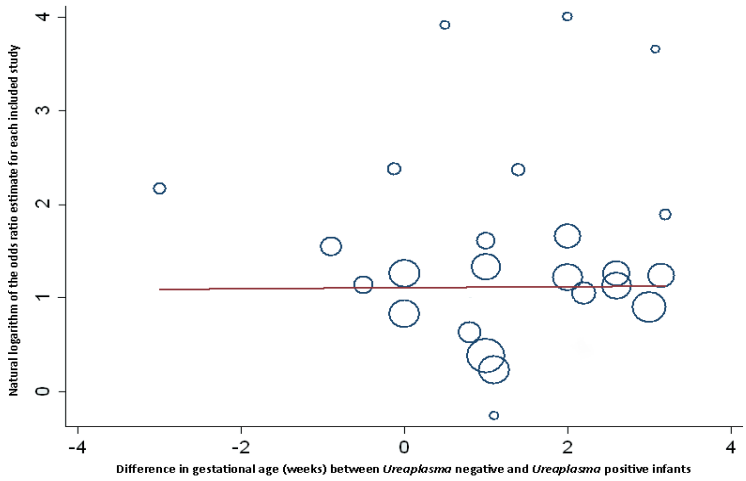


Figure 2 a) Forest plot of association between Ureaplasma spp. colonisation on development of CLD, and b) meta-regression showing association between Ureaplasma spp. colonization and CLD28, controlling for difference in gestational age between the colonized and non-colonized groups (p=0.96).

Most importantly, almost all previous papers called for an adequately powered randomised trial to definitively confirm or refute the causative role of *Ureaplasma* spp. in the development of CLD by assessing its rates after treatment with a macrolide, the most effective treatment for this age group.

2.1 Rationale for current trial/Justification of Treatment Options

There is evidence of unmet therapeutic need in the area of chronic lung disease of prematurity. Due to the advances in care, preterm births are increasing in absolute numbers and as a proportion of all births. According to NHS data for England in 2006, 2000 births (0.3% of all births) were 23 to 25+6 weeks' gestation. Similar numbers are reported by other Western European countries.

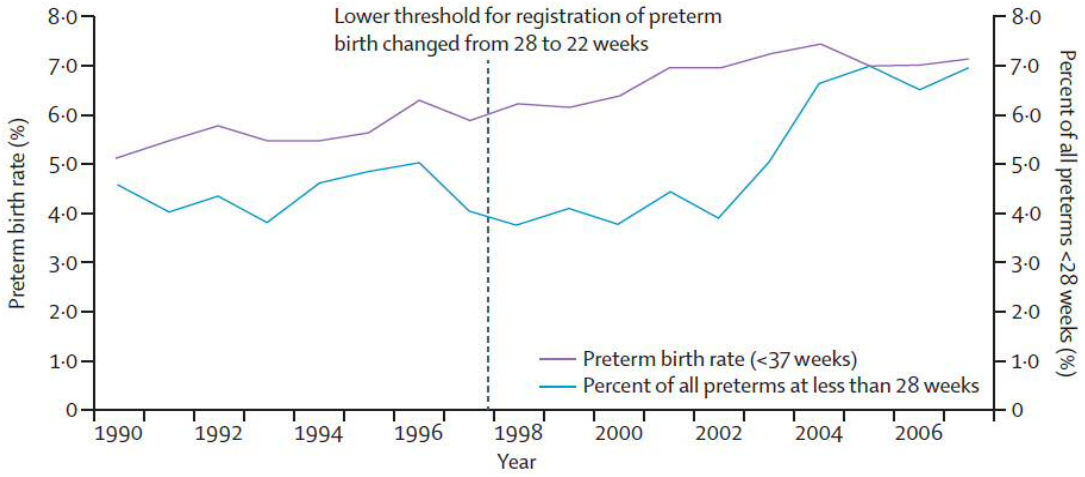


Figure 3 Variation in preterm birth rate and proportion of preterm births at less than 28 weeks with a reduction in the lower threshold for registration of preterm births from 28 to 22 weeks' gestation in Denmark Analysis of 1 191 000 livebirths 1990 to 2007 Data source: National Board of Health. (Blencowe, 2012)

However, these improvements are associated with a high mortality rate in the neonatal period (25% to 30% in premature babies). In addition, neurodevelopmental sequelae (cerebral palsy) and other morbidities, including CLD, are possible outcomes. Reducing rates of CLD would shorten hospital stay and reduce burden on families after discharge.

Initial clinical trials targeting *Ureaplasma* spp. in preterm neonates to prevent CLD used erythromycin, a macrolide antibiotic previously commonly used to treat *Ureaplasma* spp. The Cochrane review by Mabanta et al included two studies examining reduction in CLD by treating *Ureaplasma* spp (Mabanta 2013). Erythromycin eradicated colonisation in 12/14 (86%) but did not alter duration of supplemental oxygen. Importantly, neither trial noted adverse effects for 7–10-day courses of treatment. Since these two studies differed markedly in their design, the results were not combined in the Cochrane meta-analyses (Mabanta 2003). A larger trial (n=250) used the macrolide clarithromycin, 10 mg/kg twice daily for 10 days, in low birthweight infants with confirmed *Ureaplasma* spp. infection in the first 3 days of life, compared to placebo (Ozdemir 2011). The results noted a decreased rate of CLD (15.9% vs 36.4% p<0.01) when compared to placebo, but suggested the need for additional randomised studies to establish the routine use of macrolides in preterm infants including in those who were not colonised with *Ureaplasma* spp. Taken together, the current literature demonstrates the potential effectiveness of macrolides to prevent CLD but requires sufficiently powered studies before it can be introduced into routine neonatal practice.

Azithromycin has a better safety profile than erythromycin thus is a better choice of macrolide to evaluate its anti-infective action against *Ureaplasma* spp. and its potent anti-inflammatory actions in preterm babies at risk of developing CLD. It has become established as the macrolide of choice in several inflammatory/infective respiratory diseases including cystic fibrosis and chronic obstructive pulmonary disease (COPD) (Wolter 2002, Van Bambeke 2001, Blasi 2005). It is actively concentrated in alveolar macrophages thus is attractive to treat pulmonary conditions (Legrand 2014, Hand 2001). If *Ureaplasma* spp. colonisation is on the causal pathway to the development of CLD, then azithromycin should reduce the incidence of CLD in combination with its anti-inflammatory effects.

The studies by Ballard and colleagues (Ballard 2007, 2011) provide proof of concept that azithromycin may affect surrogate outcomes, for the anti-inflammatory effects of azithromycin in preterm neonates and demonstrates the tolerability of azithromycin in the target population (Turner 2011).The recent meta-analysis by Nair (Nair 2014) and colleagues supports our hypothesis: when pooling the three

available studies (Ballard 2007, Ballard 2011 and Gharehbaghi 2012), azithromycin demonstrated a significant reduction in CLD, and also the composite outcome CLD/Death (irrespective of *Ureaplasma* spp. status): respective risk ratios of 0.83 and 0.86. Preliminary Phase IIb data presented by Professor Rose Viscardi at the Pediatric Academic Society (PAS) meeting in May 2017 showed that a three-day course of 20 mg/kg per day of azithromycin eradicated *Ureaplasma* spp. in preterm-born subjects who had *Ureaplasma* spp.-positive tracheal aspirates at baseline (N=44) (Viscardi 2017). This provides further evidence of the effectiveness of azithromycin in treating *Ureaplasma* spp. infection.

As survival increases, and rates of CLD increase, addressing this problem is increasingly important. Studies of corticosteroids, for example inhaled budesonide (Bassler 2015) demonstrated a lower incidence of CLD in extremely preterm infants but this benefit may have been achieved at the expense of increased mortality. Prophylactic low-dose hydrocortisone (Baud 2016) was also noted to increase rates of survival without CLD with no differences in neurodevelopmental outcomes reported between the active and placebo groups at two years of age (Baud 2017). However, concerns persist that corticosteroids may have side effects and other modes of prevention are required.

An adequately powered randomised controlled trial relevant to UK neonatal care is needed to address the role of azithromycin in the development of CLD in preterm neonates: the current proposal has resulted after the substantial support shown by our two surveys of neonatologists in the UK (Maxwell 2009) and in Europe (Pansieri 2014). Implementation, if the trial were a success, would be relatively easy: all eligible neonates already have vascular access in place for total parenteral nutrition (TPN) that is usually required until 9 or 10 days of age. An internal audit of inborn Cardiff babies showed that 100% of all babies at 23-25 weeks' and 80% of all 26-28 weeks' gestation had an intravenous (i.v.) line in situ at 10 days of age, (figures which are likely to be higher if only those with respiratory illness are recruited), and so the expectation is that no additional i.v. access will be necessary.

It will be important to use a physiological approach to the diagnosis of CLD which involves dividing babies into two groups: those with clear-cut CLD and those with borderline CLD. Babies with borderline CLD are subject to a short period during which they are monitored closely without supplemental oxygen in order to determine whether they truly require supplementary oxygen. Identification of CLD using a physiological test, such as an air challenge, is more precise and has a stronger relationship to neurodevelopmental outcomes (Ehrenkranz 2005) than a clinical definition based solely on describing the amount of oxygen being administered at one time point. In this proposed therapeutic confirmatory trial CLD will be diagnosed with a physiological test at 36 weeks post-conception age (Quine 2006).

2.2 Rationale for azithromycin dosage and duration

Azithromycin plasma concentration needs to be within 1-4 µg/ml to achieve minimum inhibitory concentration required to inhibit the growth of 90% of organisms (MIC90) against *Ureaplasma* spp. (Merchan 2015) although recent data from the UK suggest a concentration of only 0.25 µg/ml may be sufficient for MIC50 and MIC90 for *Ureaplasma* spp. (Beeton 2016). Previous studies have used azithromycin doses of 10 mg/Kg in preterm babies (Ballard 2011, Gharehbaghi 2012). In addition, we have previously conducted extensive calculations in the TINN2 project using data obtained by SK from pharmacokinetic (PK) studies performed by Professor Rose Viscardi (Hassan 2011, Merchan 2015) to establish the optimal dosage to use (Figure 1). A variety of simulations were performed using 1000 trials per combination of dosage and duration (10mg/kg vs. 20mg/kg; 3 vs. 10 days' duration; with vs. without loading dose) to establish that a dosage of 10 mg/ml was sufficient to achieve plasma concentrations of 1-4 µg/ml (Table 1).

TABLE 3 Comparison of MIC_{50} and MIC_{90} values for various antibiotics between *U. parvum* and *U. urealyticum*

Antibiotic	Total no. of *Ureaplasma* drug-resistant samples	*U. parvum* (μg/ml)		*U. urealyticum* (μg/ml)		*P* value[a]
		MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	
Tetracycline	3	0.25	0.5	0.5	2	<0.001
Ciprofloxacin	2	1	2	2	4	<0.001
Erythromycin	0	1	2	2	4	<0.003
Azithromycin	0	0.25	0.25	0.25	0.25	NS
Chloramphenicol	0	2	4	2	4	NS
Gentamicin	130	32	64	64	128	<0.01

Table 1: MIC50 and MIC90 against both Ureaplasma spp. urealyticum and parvum was 0.25 μg/ml. Please also note antibiotic resistance was zero against azithromycin in 130 Ureaplasma spp. positive samples including 95 samples from preterm infants (Beeton 2016).

However, new data from Viscardi Phase IIb PK studies presented at the PAS in May 2017 provides new evidence from actual eligible preterm babies. Any dosing regimen will need to balance the activity against *Ureaplasma* spp. and against the pulmonary inflammation that occurs after preterm birth peaking between 7-10 days of age. In light of the data from Viscardi demonstrating effective eradication of *Ureaplasma* spp. using 20mg/kg/d for 3 days (Viscardi 2017), we propose a dosing regimen of 20 mg/kg/d for 3 days, followed by 10 mg/kg/d for 7 days to ensure both maximal *Ureaplasma* spp. eradication and appropriate anti-inflammatory effects over the 7-10 days period. PK simulations showed that this regimen would establish a concentration (1-4mcg/ml) effective against *Ureaplasma* spp. by 24 hours after the first dose, with 20% of infants having a trough concentration >1mcg/mL on day 1 and 92% by day 3 and peak concentration between 1 and 4 mcg/ml.

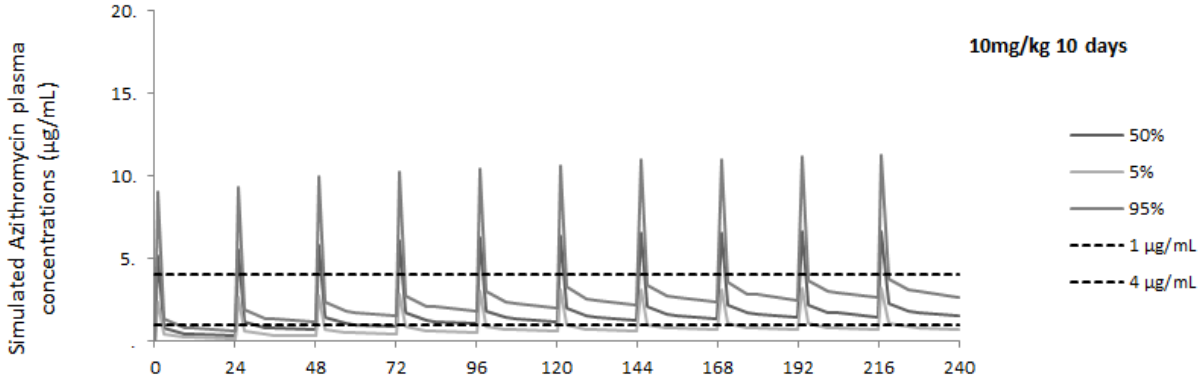


Figure 4: PK dose modelling simulation using 10mg/kg daily, for 10 days showing that the dose between 1 – 4 μg/ml is achieved.

2.3 Risks and Benefits

The meta-analysis by Nair summarises the potential benefit of prophylactic azithromycin therapy in preterm infants with a RR for the combined outcome of CLD/Death of 0.86 for the three published azithromycin studies. However, clearly the effects require replication in a large-scale randomised controlled trial.

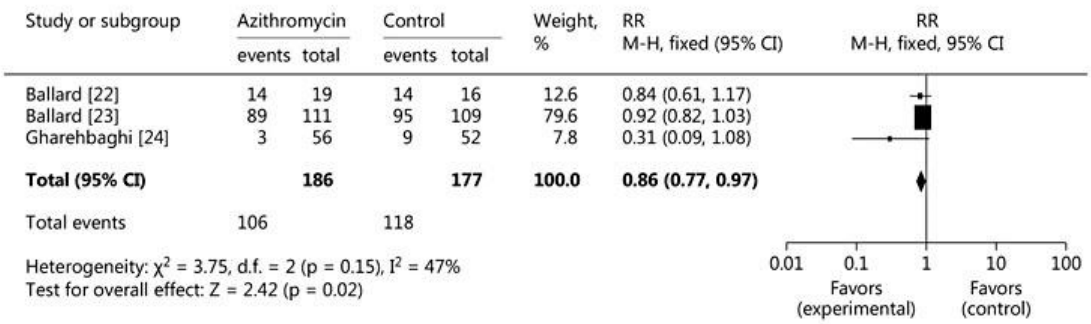


Figure 5: Forest plot for effect of prophylactic azithromycin on BPD/death from (From Nair 2014)

The Ballard studies did not report any significant adverse effects of the use of azithromycin for up to 6 weeks. This lack of adverse effects in the neonatal population is supported by the recent systematic review (Smith 2015) which reported few events. There were no cardiovascular events identified in contrast to the recent reports in at-risk adults (Ray 2012), which had several limitations including lack of randomisation, quality of data collection (death certificates only) and definition of participant population (out participants only). Due to the immaturity of the gut in preterm infants ≤32 weeks' gestation (Jadcherla 2002), azithromycin is unlikely to affect intestinal function and has low risk of developing infantile hypertrophic pyloric stenosis, which has occasionally been associated with macrolide administration (Eberly 2015). Azithromycin is not known to interact with other drugs. Despite the low potential for these adverse outcomes, we shall carefully monitor the incidence of such reactions in our study.

Classification	Adverse event	Number	Risk of AE per 1000 neonates	95% CI
Gastrointestinal	Vomiting	12	37	21 to 64
	Feeding intolerance/poor feeding	10	31	16 to 57
	NEC	8	25	12 to 49
	Abdominal tenderness	6	19	8 to 41
	Diarrhoea	4	12	5 to 33
	Other gastrointestinal symptoms	13	40	24 to 68
	Total gastrointestinal AE	53	163	128 to 209
Respiratory	BPD	96	296	251 to 350
	Respiratory distress	1	3	4 to 22
	Total respiratory AE	97	299	253 to 354
CNS	At least grade 3 IVH	29	90	63 to 127
	Abnormal hearing	22	68	45 to 102
	PVL	15	46	28 to 76
	Others	4	12	5 to 33
		74	228	187 to 279
Hepatobiliary	Elevated transaminase	16	49	31 to 80
Cardiovascular	PDA	20	62	40 to 94
Metabolic	Hyperkalaemia	2	6	2 to 25
Others	Sepsis	11	34	19 to 61
	Other infections	96	296	251 to 350
	Allergy	2	6	2 to 25
Total		371		

AEs, adverse event; BPD, bronchopulmonary dysplasia; RCT, randomised controlled trials.

Table 2: Classification and risk of adverse events from RCT and observational studies (n=324). Reproduced under CC-BY-NC license from Smith 2015

There are no other risks involved in participating in the trial. With the exception of Investigational Medicinal Product (IMP) administration and microbiology sample collection (stool and

tracheal/nasopharyngeal aspirates), no additional procedures will be involved over and above standard clinical care.

3 Trial objectives/endpoints and outcome measures

3.1 Primary objectives

To determine the effectiveness of azithromycin in increasing survival without physiologically defined CLD (moderate/severe) when compared to placebo.

3.2 Secondary objectives

- To determine the effect of azithromycin on CLD severity and mortality rate (at 36 weeks' postmenstrual age).
- To determine the effectiveness of azithromycin in reducing duration of positive pressure respiratory support (i.e. conventional mechanical ventilation/HFOV, continuous positive airway pressure, high flow nasal cannula, number of days of oxygen dependency).
- To determine the safety and tolerability of azithromycin.
- To determine if azithromycin alters resistance to macrolides amongst S*treptococcus* and *Staphylococcus* spp microbes isolated from respiratory and stool samples.
- To investigate if colonisation with *Ureaplasma* spp. prior to randomisation modifies the treatment effect of azithromycin compared to placebo.

3.3 Primary outcomes measure(s)

Objectives	**Outcome Measures**	**Timepoint(s) of evaluation of this outcome measure (if applicable)**
To determine the effectiveness of azithromycin in increasing survival without physiologically defined CLD (moderate/severe) when compared to placebo	**Survival without physiologically defined CLD** - Death - Physiologically defined CLD at 36 weeks postmenstrual age	At or before 36 weeks postmenstrual age

3.4 Secondary outcomes measure(s)

Objectives	Outcome Measures	Timepoint(s) of evaluation of this outcome measure (if applicable)
To determine the effect of azithromycin on CLD Severity and mortality rate (at 36 weeks' postmenstrual age)	**Mortality rate** **CLD severity (mild, moderate/severe)**	At or before 36 weeks postmenstrual age
To determine the effectiveness of azithromycin in reducing duration of positive pressure respiratory support	**Number of days of respiratory support required**: - conventional mechanical ventilation/HFOV - continuous positive airway pressure - high flow nasal cannula - number of days of oxygen dependency	All assessed from birth to 36 weeks postmenstrual age or discharge home or death (whichever occurs earlier)
To determine the safety and tolerability of azithromycin	**Development of complications of prematurity** - Nosocomial infection (line-sepsis, meningitis, pneumonia); confirmed microbiologically or antibiotic treatment for 5 days or more (not including trial treatment). - Severe intraventricular haemorrhage (grade III/IV); - Necrotising enterocolitis (Bell stage II and above); - Treatment for retinopathy of prematurity; - Treatment for patent ductus arteriosus;	All assessed from birth to 36 weeks postmenstrual age or discharge home or death (whichever occurs earlier)

	- Liver and renal function **Serious adverse events/reactions**	
To determine if azithromycin alters resistance to macrolides amongst S*treptococcus* and *Staphylococcus spp* microbes isolated from respiratory and stool samples	Resistance to macrolides among microbes isolated from respiratory and stool samples	Baseline and day 14-21

4 Trial design and setting

The trial is designed as a multi-centre, double blind, randomised, placebo-controlled trial of azithromycin for the prevention of chronic lung disease of prematurity in preterm infants. We plan to enrol 796 infants <30 weeks' gestational age over a 30 month recruitment period from approximately 25 level III neonatal units in the UK. Trial treatment (azithromycin or placebo) will be daily for 10 days, with follow up until 36 weeks postmenstrual age and at discharge from hospital. Serial respiratory fluid and stool samples will be collected until approximately 21 days of life.

5 Site and Investigator selection

This trial will be carried out at participating sites within the UK. All sites who are interested in participating in the trial will be required to complete a registration form to confirm that they have adequate resources and experience to conduct the trial.

Before any Site can begin recruitment a Principal Investigator (PI) at each site must be identified. The following documents must be in place and copies sent to the AZTEC Trial email account (see contact details on page 4):

- Confirmation of Capacity and Capability from the NHS organisation's R&D Department, following submission of the local information pack
- Favourable opinion of Research Ethic Committee (REC)
- A signed Clinical Trial Agreement
- Current Curriculum Vitae (CV) and GCP training certificate of the PI
- Completed Site Delegation Log and Roles and Responsibilities document
- Full contact details for all host care organisation personnel involved, indicating preferred contact
- Returned copy of the Self-Evident Correction Log signed by the PI.

Upon receipt of all the above documents, the Trial Manager (TM) will send written confirmation to the PI/lead Research Nurse detailing that the centre is now ready to recruit participants into the trial. This letter/email must be filed in each site's Site File. Along with the written confirmation, the site

should receive their trial drug supplies and a trial pack holding all the documents required to recruit into the Trial.

Occasionally during the trial, amendments may be made to the trial documentation listed above. CTR will issue the site with the latest version of the documents as soon as they become available. It is the responsibility of the CTR to ensure that they obtain local R&D approval for the new documents.

Site initiation will be by site visit, regional launch meeting, or by teleconference if attendance of key personnel is unfeasible.

6 Participant selection

Participants are eligible for the trial if they meet all of the following inclusion criteria and none of the exclusion criteria apply. All queries about participant eligibility should be directed to the TM before randomisation.

6.1 Inclusion criteria

a) Gestational age ≤29w^{+6d} (including infants born as one of a multiple birth)

b) Neonates who have had respiratory support for at least 2 continuous hours duration during the first 72 hours of life (intubated, or by non-invasive mechanical ventilation including continuous positive airway pressure and high flow nasal cannula or a combination thereof)

c) Presence of an indwelling intravenous line for drug administration

d) Written informed consent within 72 hours of birth by parent(s)/guardian(s)

e) Anticipating administration of first dose within 72 hours at the latest (within 24 hours of life for inborn and 48 hours for outborn infants)

f) Reasonable to expect completion of 10 days of trial treatment whilst resident at the recruiting site

g) Inborn, or born at site within the recruiting site's neonatal network where follow up will be possible

h) In the opinion of the PI, reasonable prospect of survival past the first 72 hours of life

6.2 Exclusion criteria

a) Exposure to another systemic macrolide antibiotic (not maternal)

b) Presence of major surgical or congenital abnormalities (not including patent ductus arteriosus or patent foramen ovale)

c) Contraindication of azithromycin as specified in the summary of characteristics of the product

d) Participation in other interventional trial that precludes participation in AZTEC

6.3 Co-enrolment Guidelines

To avoid potentially confounding issues, ideally participants should not be recruited into other interventional trials during their participation in AZTEC.

Where recruitment into another trial is considered to be appropriate and without having any detrimental effect on the AZTEC trial this must first be discussed with the TM who will contact the Chief Investigator (CI).

7 Recruitment and screening

7.1 Participant identification

Screening will be performed by local clinical care team who will review new admissions to the NICU on a daily basis against the eligibility criteria. Parent(s)/legal guardian(s) of a potentially eligible infant will then be approached by a member of the research team to consider participation. The decision to confirm eligibility and enter the infant in to the trial will be made by a clinician.

7.2 Screening logs

A screening log of all ineligible and eligible but not consented/not approached will be kept at each site so that any biases from differential recruitment will be detected. The screening log should be sent to AZTEC@Cardiff.ac.uk every month (see section 22) for further detail on data monitoring/quality assurance).

7.3 Recruitment rates

A total of 796 participants will be recruited at an expected rate of 38 per month, once all centres are open to recruitment.

7.4 Informed consent

This trial is exploring an antibiotic drug in a critically ill population requiring prompt interventions. Due to its very nature, parent(s)/legal guardian(s) are required to be informed about the trial and decide regarding entry to facilitate the start of treatment within 72 hours of the child's birth.

These constraints will be explained to parent(s)/guardian(s) and reassurance provided that if they do not feel they can decide within this time window then the ongoing treatment of their baby will not be affected. Discussions will be adapted to meet the information needs of parent(s)/guardian(s) in order that they feel that they are sufficiently informed to make the decision.

Upon reviewing the informed consent documents, the investigator will explain the research trial to the parent(s)/guardian(s). This information will emphasise that participation in the trial is voluntary and that the parent(s)/guardian(s) may withdraw the participant from the trial at any time and for any reason. All parent(s)/guardian(s) will be given opportunity to ask any questions that may arise, and

time to consider the information prior to agreeing to participate. A full explanation of the treatment options, including the conventional and generally accepted methods of treatment, will be given. A contact point where further information about the trial may be obtained will be provided. The rights and welfare of the infant participants will be protected by emphasising that the quality of medical care will not be adversely affected if the parent(s)/guardian(s) declines to consent to the infant's participation in this trial, or subsequently withdraws them from either further protocol treatment or trial follow-up.

Consent for an infant's participation in the AZTEC trial must be provided by those who have parental responsibility for the infant. Although consent is only needed from one person with parental responsibility, it is recommended that all persons with parental responsibility be involved in the discussions and agree to the infant's participation. The participant's written informed consent must be obtained using the trial Consent Form, which follows the Parent Information Sheet. The parent(s)/guardian(s) should be given sufficient time after the initial invitation to participate before being asked to sign the Consent Form. Informed consent must be obtained prior to the parent(s)/guardian(s) undergoing procedures that are specifically for the purposes of the trial. Consent may be taken by a delegated member of the trial team who, in the opinion of the local PI, be experienced at imparting important information and with experience in obtaining informed consent in the NICU environment.

Please note, only when written informed consent has been obtained from the parent(s)/guardian(s) and the infant has been randomised/enrolled into the trial can they be considered a trial participant.

Parent(s)/guardian(s) should always be asked to sign a consent form. One copy should be given to the Parent(s)/guardian(s), but the original copy should be kept in the investigator site file and a further copy should be kept with participant's hospital notes. A copy will also be sent to CTR.

The right of the parent(s)/guardian(s) to refuse permission for their infant to participate in the trial without giving reasons must be respected. After the infant has entered the trial, the investigator must remain free to give alternative treatment to that specified in the protocol, at any stage, if he/she feels it to be in the best interest of the infant. However, the reason for doing so should be recorded and the infant will remain within the trial for the purpose of follow up and data analysis according to the treatment option to which he/she has been allocated. Similarly, the parent(s)/guardian(s) must remain free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment. If the parent(s)/guardian(s) are willing to provide a reason this should be recorded to inform ongoing trial decisions.

7.5 **Randomisation**

The randomisation code list will be generated by a statistician (who is not involved with the AZTEC trial) at the CTR. Randomisation lists will be generated (1:1 ratio) using block randomisation with random variable block length. Treatment allocation of azithromycin or placebo will be blinded such that the allocation will not be known by clinicians, the baby's family or the trial outcome assessors.

Randomisation will be managed via a secure web-based randomisation facility administered by the CTR. Babies of multiple births will be randomised individually. If necessary, the code may be broken for a single baby at the request of the site PI or clinician in charge of the baby. See Section 13.3 for the procedure for unblinding treatment allocation.

All assessments for enrolment must be performed before randomisation and before administration of trial treatment. The infant may only be randomised once full eligibility has been confirmed by a

medical doctor and written informed consent has been obtained. Infants should be randomised within sufficient time to start treatment within 24 hours of life for inborn and 48 hours for outborn infants (72 hours at the latest).

At randomisation, designated Day 1, an authorised member of the site research team will access the web randomisation system. After confirming that the participant qualifies for randomisation, the randomisation system will assign both a study ID and a pack ID number. The Pack ID links the participant to a unique treatment pack of the trial drug to be allocated. These packs will be stored on the neonatal unit. An automated email confirming the randomisation will be sent to the site team and a copy of the email will also be sent to the local site pharmacy team and to the CTR AZTEC trial team.

In the event the online randomisation system is unavailable at site, then the local investigator may contact the CTR (during office hours). Randomisation may be performed by CTR staff on request of the local investigator.

8 Participant transfer, withdrawal & lost to follow-up

8.1 Participant transfers

Infants may be transferred to another neonatal unit (i.e. to a level II or I neonatal unit) when clinically appropriate, or for surgical procedures. In the event a transfer occurs to another recruiting site during the 10-day trial treatment period, arrangements will be made for continuation of the trial intervention in addition to sample and data collection. In the event of transfer to a designated 'continuing care' site, sampling and data collection only will continue but the participant will be withdrawn from the intervention. Data collection will continue to ensure inclusion in the intention-to-treat analysis. If transfer is not to a recruiting or a designated continuing care site, authorisation will be urgently sought by the TM, but this may not always be granted.

Detailed guidance on the transfer process and associated procedures will be provided to sites. Consideration should be given by the PI as to whether follow-up will be possible, particularly if the infant is not in-born and/or has been transferred from outside the normal geographical coverage of a recruiting site.

8.2 Withdrawal

Parent(s)/guardian(s) have the right to withdraw consent for their infant's participation in any aspect of the trial at any time. The infants care will not be affected at any time by declining to participate or withdrawing from the trial.

If a parent(s)/guardian(s) initially consents but subsequently withdraws from the trial, clear distinction must be made as to what aspect of the trial the family is withdrawing from. These aspects could be:

1. Withdrawal of Trial Treatment
2. Withdrawal from Samples
3. Withdrawal from follow-up assessments
4. Withdrawal of Consent to all of the above

The withdrawal of parent(s)/guardian(s) consent shall not affect the trial activities already carried out and the use of data/samples collected prior to withdrawal. The use of the data/samples collected prior to withdrawal of consent is based on informed consent before its withdrawal.

Furthermore, it is important to collect safety data ongoing at the time of withdrawal, especially if the participant withdraws because of a safety event. There is specific guidance on this contained in the Parent Information Sheet but briefly:

If a parent(s)/guardian(s) wishes to stop taking part in the trial completely, the infant will need to be seen one last time for the applicable assessment and tests. If the infant is suffering a serious reaction to the trial treatment when they decide to stop, you will need to continue to collect information about them for as long as the reaction lasts.

An infant may be withdrawn from trial treatment for the following reasons:

- Intolerance to trial medication
- Withdrawal of consent for treatment by the parent(s)/guardian(s)
- Any alteration in the infant's condition which justifies the discontinuation of the treatment in the Investigator's opinion
- Non-compliance

A withdrawal form should be completed on the eCRF by the researcher/clinician based on information provided by the participant. Any queries relating to potential withdrawal of a participant should be forwarded to AZTEC trial email address.

8.3 Lost to follow up

All efforts will be made to identify possible sites of transfer and have appropriate approvals in place to obtain follow-up data. Follow-up data (e.g. discharge summary) may be obtained through linkage via National Neonatal databases. Explicit consent for database linkage will be sought during the informed consent process.

9 Trial intervention

9.1 Trial Intervention

AZTEC is a placebo-controlled trial. The active IMP and the placebo are clear solutions with identical transparent appearance after reconstitution with a diluent. Participants will be randomised to receive either azithromycin or placebo daily for a period of 10 days.

9.2 Treatment(s)

Active IMP for AZTEC trial is: azithromycin 500mg (Zedbac™), Aspire pharma Ltd, UK.
The azithromycin pharmaceutical form is a lyophilized powder for solution for infusion in a 10mL vial under vacuum, equivalent to 500 mg of azithromycin for intravenous administration (524mg of azithromycin dihydrate = 500mg azithromycin base, citric acid, sodium hydroxide).

Placebo: The placebo will be an empty sterile 10 mL vial under vacuum.

The allocated vials will be prepared for administration in accordance with the Zedbac™ SmPC, i.e. the vial will be injected with 4.8 ml of water for injection. The reconstituted solution prepared in this way contains:

- **Active IMP:** 100 mg azithromycin per ml.
- **Placebo:** Water for injection only

9.3 Treatment packaging, supply and storage

- Packaging

Azithromycin powder for solution for infusion and placebo will be packaged in identical 10 ml vials with the same cap, same stopper and same vial.
Each vial of active IMP and placebo will be blinded with a tamper-evident custom cardboard to ensure contents are not visible during the reconstitution process.

Figure 6: Example of tamper-evident blinding device

Treatment units will be packaged into individual "Treatment Packs". Each participant's Treatment Pack will contain 12 units of 10 ml vials of azithromycin or placebo.

- **Labelling**

Labelling will comply with Annex 13 of GMP (including provision for labelling of small packaging units).

- **Distribution & Supply**

The IMP and placebo will be distributed to the sites after technical and regulatory release has been confirmed by the sponsor, and the site has completed the CTR initiation procedure. The IMP and placebo will be distributed via specialised courier to the address of the pharmacy provided by the PI of each site.

The site pharmacist will issue treatment packs (azithromycin or placebo) to the neonatal unit as defined by the online pack management system where they will be stored in a lockable, access-controlled storage unit (e.g. medicine cabinet). This will facilitate out-of-hours recruitment. An accountability trail of issue and return of medication to the pharmacy will be maintained using the online pack management system and on the logs provided to each site. Separate guidance on use of the pack management system will also be provided.

- **Storage & Stability**

The supplied products will be stored under the responsibility of the local pharmacist according to the manufacturer's guidelines

On receipt of the IMP shipment at each of the individual trial sites, the responsible pharmacist will ensure treatment packs are stored in an appropriate secure location accessible only to authorised

personnel. In accordance with the SmPC, there are no particular temperature-related storage requirements for the treatment packs.

- **Expiry date and resupply management**

Initial supply and resupply of treatment packs to sites will be arranged via the Trial Manager at the CTR.

9.4 Treatment prescribing and preparation

The trial treatment should be prescribed on the participant drug chart/notes by competent member of the trial team (as delegated by the PI). The prescription should clearly state the azithromycin dose and "or placebo", method of delivery and frequency for administration.

Reconstitution will be as described in the Zedbac™ azithromycin 500mg SPC:

4.8mL of sterile water for injection will be added via non-automated syringe to the vial and **and the vial left to stand for at least 1 minute.**

The required proportion of the resultant 100mg/mL solution will be added to a volume of diluent (as per options in the SmPC) to yield a final dose concentration for administration of 20mg/kg, or 10mg/kg respectively. Detailed guidance will be provided to sites regarding the preparation of the trial intervention.

The solution should be inspected visually for particulate matter when drawn from the vial prior to further dilution. If particulate matter is evident in reconstituted fluids, the solution should be discarded.

Syringes are prepared for each dose as required by drawing the appropriate volume based on the infant's weight to achieve a daily dose of 20 mg/kg (days 1-3) or 10mg/kg (days 4-10), equivalent to fluid volumes of 10mL/kg (days 1-3) and 5mL/kg (days 4-10). The volume should be sufficient to account for priming of the i.v. line.

Trial treatment should be infused via an existing i.v. line (umbilical venous catheter, long line, peripheral line). Data on Y-site compatibility regarding other products which may be co-administered will be followed (DynaMed 2018). The infusion should be given over a period of at least 60 minutes. Each administration will be logged on the CRF.

Wherever possible, 10 days of treatment should be completed. If a clinical decision is taken to re-establish IV access, taking in to account the condition of the infant, a missed dose should be given within 12 hours of the scheduled time.

Detailed guidance on the preparation, dosage and administration of the IMP will be provided to trial sites.

9.5 Dose modification for toxicity

No deviations from the prescribed dosages are permitted. If in the event a dose is omitted or unable to be administered in full this will be recorded in the eCRF and next dose will be given at next dose timepoint.

9.6 Management of overdose

Potential or confirmed overdoses should be immediately reported to the CTRC for assessment under the advice of the CI. A dosing error form should be submitted within 24 hours of the site becoming aware of the event.

9.7 Prohibited medications and interaction with other drugs

All concomitant medications clinically indicated are permitted, except those for whom azithromycin is known to be contraindicated (according to the SmPC).

9.8 Accountability procedures

An accountability check will be performed at the end of the treatment period to reconcile doses given with vials remaining in the treatment pack. This will be recorded on the IMP reconciliation form. More details will be provided in a separate guidance document.

9.9 Compliance

Compliance will be monitored using the eCRF. A compliance update, indicating the number of missed doses, and reasons, will be included in the monthly monitoring report.

10 Sample Management

The figure below outlines the flow of sample collection and processing. Sites will be provided with the required materials for sample collection, labelling and shipping to the Child Health laboratory at Cardiff University.

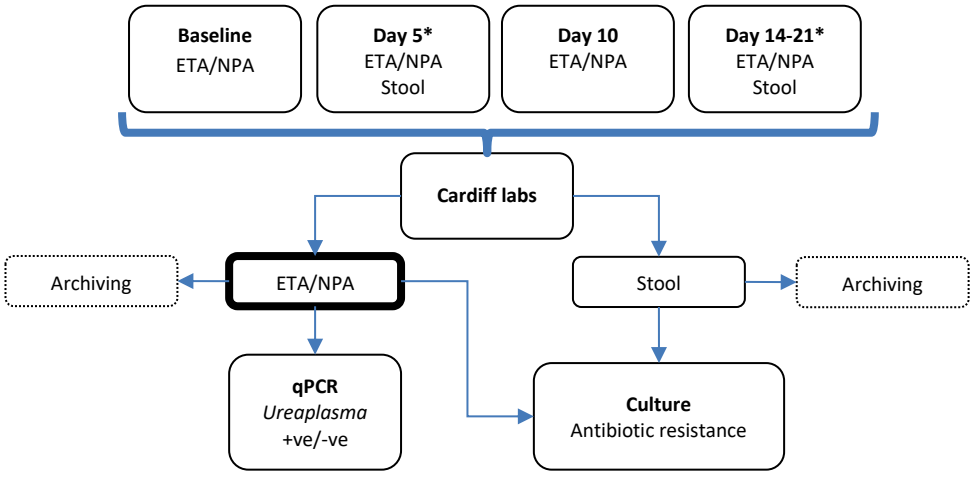


Figure 7: Schematic of AZTEC biological sample analysis * Day 4-6 is acceptable for the day 5 samples. Day 8-12 is acceptable for the day 10 sample. Day 14-21- one sample of each type during this period.

Endotracheal/nasopharyngeal aspirates

Endotracheal aspirates (ETA) (if intubated) or nasopharyngeal aspirates (NPA) (if not intubated) will be collected opportunistically at timepoints as specified above and sent to the central laboratory at Cardiff University for analysis of presence of *Ureaplasma* spp.

Detailed information will be provided to sites on the collection, storage and transport of these samples.

Stool sample

Stool samples will be collected opportunistically at the timepoints specified above and sent to the central laboratory at Cardiff University for culture. Positive cultures will undergo antibiotic resistance testing on any identified microbes.

Detailed information will be provided to sites on the collection, storage and transport of this sample.

11 Trial procedures

11.1 Baseline

Within 24 hours of birth (inborn) or 48 hours of birth (out-born) (in either case, 72 hours at the latest):

- Screening to assess eligibility of baby for entry into the trial according to inclusion/exclusion criteria
- Confirmation of eligibility (by medically qualified doctor)
- Informed consent
- Collect baseline neonatal and maternal demographic details (data collection as per eCRF)
- Collect ETA for Ureaplasma spp. colonisation status, if intubated; or NPA sample, if not intubated
- Stool sample (opportunistically, first sample when available)

11.2 Day 1

- Randomisation to treatment arm (azithromycin or placebo)
- Azithromycin 20mg/kg or placebo, once daily intravenously
- Clinical follow up and pharmacovigilance monitoring (new events and review those ongoing)

11.3 Day 2-3

- Azithromycin 20mg/kg or placebo, once daily intravenously
- Clinical follow up and pharmacovigilance monitoring (new events and review those ongoing)

11.4 Day 4

- Azithromycin 10mg/kg or placebo, once daily intravenously
- Clinical follow-up and pharmacovigilance monitoring (new events and review those ongoing)

11.5 Day 5

- Azithromycin 10mg/kg or placebo, once daily intravenously
- ETA for *Ureaplasma* spp. colonisation status, if intubated; NPA sample, if not intubated. Day 4-6 is acceptable.
- Stool sample (one sample opportunistically). Day 4-6 is acceptable.
- Clinical follow-up and pharmacovigilance monitoring (new events and review those ongoing)

11.6 Days 6-9

- Azithromycin 10mg/kg or placebo, once daily intravenously
- Clinical follow-up and pharmacovigilance monitoring (new events and review those ongoing)

11.7 Day 10

- Azithromycin 10mg/kg or placebo, once daily intravenously
- ETA for *Ureaplasma* spp. colonisation status, if intubated; NPA sample, if not intubated. Day 8-12 is acceptable.
- Clinical follow-up and pharmacovigilance monitoring (new events and review those ongoing)

11.8 Days 11-13

- Pharmacovigilance monitoring (new events and review those ongoing)

11.9 Day 14 (up to day 21)

- ETA for *Ureaplasma* spp. colonisation status, if intubated; NPA, if not intubated (one sample opportunistically). Between day 14 and day 21 is acceptable.
- Stool sample (one sample opportunistically between days 14-21)
- Pharmacovigilance monitoring (new events and review those ongoing)

11.10 36 weeks post-menstrual age (or discharge home or death, whichever is soonest)

- Clinical follow-up and pharmacovigilance monitoring (new events and review those ongoing)
- Assessment of CLD
- Final review of pharmacovigilance events
- Number of days of endotracheal intubation
- Number of days of non-invasive positive pressure ventilation (CPAP, High flow nasal oxygen)
- Number of days on oxygen
- Length of stay: number of days receiving level I, II, or III care
- Requirement for home oxygen (on discharge home)
- Development of complications of prematurity: Nosocomial infection (Microbiologically confirmed or treated with antibiotics for 5 days or more, not including trial treatment) severe intraventricular haemorrhage (grade III/IV); necrotising enterocolitis (Bell stage II and above);

diagnosis and treatment for retinopathy of prematurity (including stage); diagnosis and treatment for patent ductus arteriosus; liver and renal function

- Contact details for later follow up (mother, father, alternative contact)- independent sub-study (see section 16)

In event of death:

- Likely cause of death (discharge summary/death certificate post-mortem report)

11.11 On discharge home, or death (post 36 weeks post menstrual age)

- Final review of pharmacovigilance events
- Number of days of endotracheal intubation
- Number of days of non-invasive positive pressure ventilation (CPAP, High flow nasal oxygen)
- Number of days on oxygen
- Length of stay: number of days receiving level I, II, or III care
- Requirement for home oxygen (on discharge home)
- Development of complications of prematurity: severe intraventricular haemorrhage (grade III/IV); diagnosis and treatment for retinopathy of prematurity (including stage); diagnosis and treatment for patent ductus arteriosus

In event of death:

- Likely cause of death (discharge summary/post-mortem report)

11.12 Schedule of assessments

PROCEDURES	Screening	Baseline	Day 1 of treatment	Day 2 of treatment	Day 3 of treatment	Day 4 of treatment	Day 5 of treatment	Day 6 of treatment	Day 7 of treatment	Day 8 of treatment	Day 9 of treatment	Day 10 of treatment	Day 14 of treatment	Day 21 of treatment	36 weeks PMA (or discharge if sooner)
Assessment of Eligibility Criteria	X														
Signed Informed Consent	X														
Review of Medical History	X														
Review of Concomitant Medications	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination	X														
Eligibility confirmation		X													
Stool sample		X**					X†						X‡		
ET/NP aspirate		X					X§					X§	X*		
Randomisation		X													
Administration of trial medication			X#	X	X	X	X	X	X	X	X	X			
Assessment of CLD															X
Cardiorespiratory monitoring	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Pharmacovigilance reporting		X	X	X	X	X	X	X	X	X	X	X	X	X	X
ECG			XΔ												

**First sample opportunistically, as available

First dose within 72 hours of birth at the latest

†Approximately day 5 (±1 day), opportunistically as sample available

‡Between days 14and 21 of treatment , opportunistically as sample available

§ Day 5 of treatment: opportunistically when suction required, ±1 day. Day 10 of treatment: opportunistically when suction required, ±2 days. If not intubated, nasopharyngeal sample will be collected

* Between days 14 and 21 days of life. Opportunistically when suction required. If not intubated, nasopharyngeal sample will be taken

Δ Formal ECG to be conducted only if alterations in cardiac rhythm are noted, with a follow-up ECG to be conducted 10 days following cessation of treatment

11.13 Assessment of primary outcome

The primary outcome is defined as a composite outcome of death by 36 weeks of post-menstrual age, or CLD (moderate/severe) at 36 weeks postmenstrual age.

The assessment will be severity-based, with a consideration of oxygen dependency. Since the requirement for supplementary oxygen is subjective, this will be confirmed using an oxygen reduction test (Quine 2006). This will be documented at 36 weeks postmenstrual age, or at discharge home if sooner.

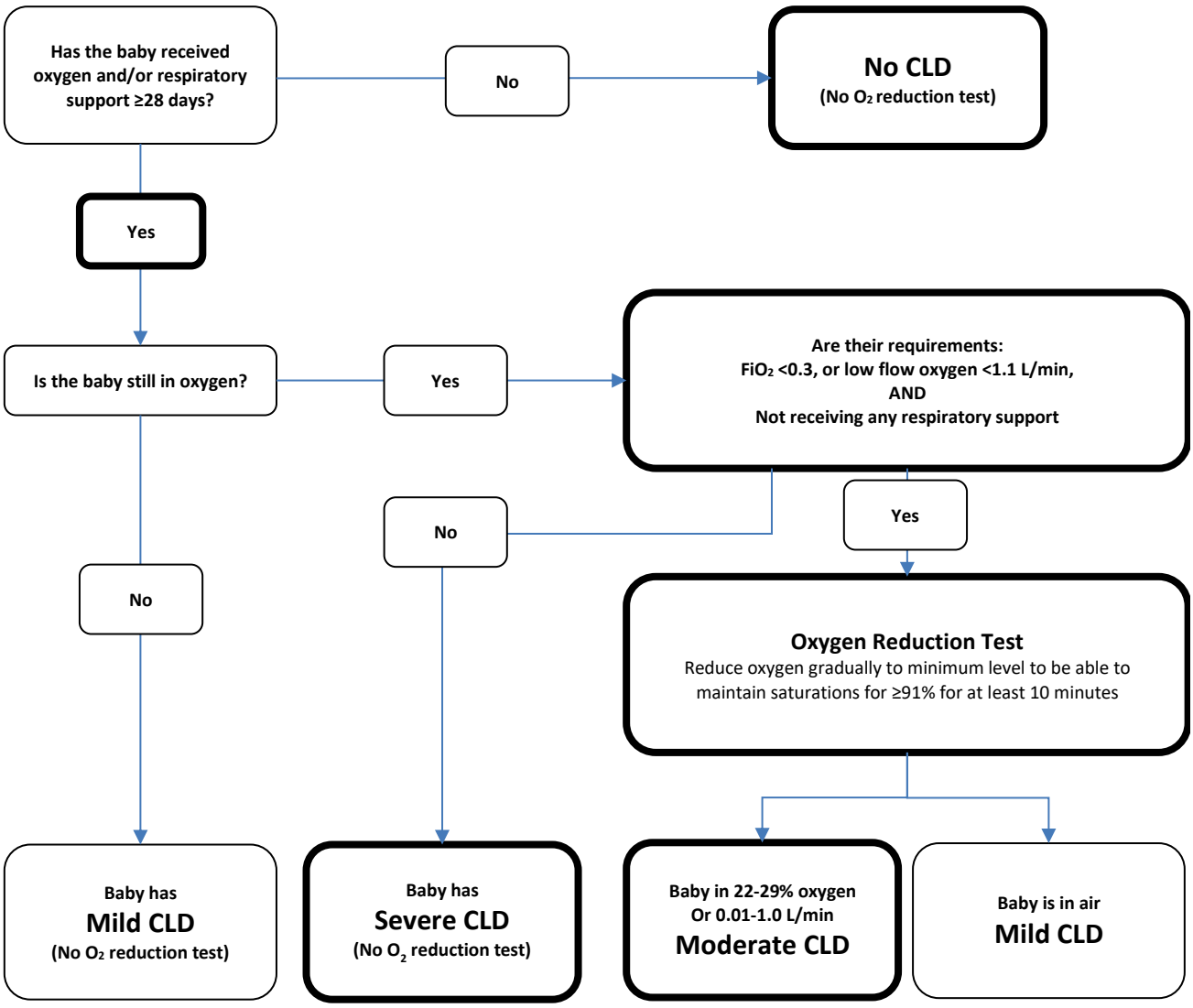


Figure 8: Schematic of CLD assessment based on Quine 2006

12 Pharmacovigilance

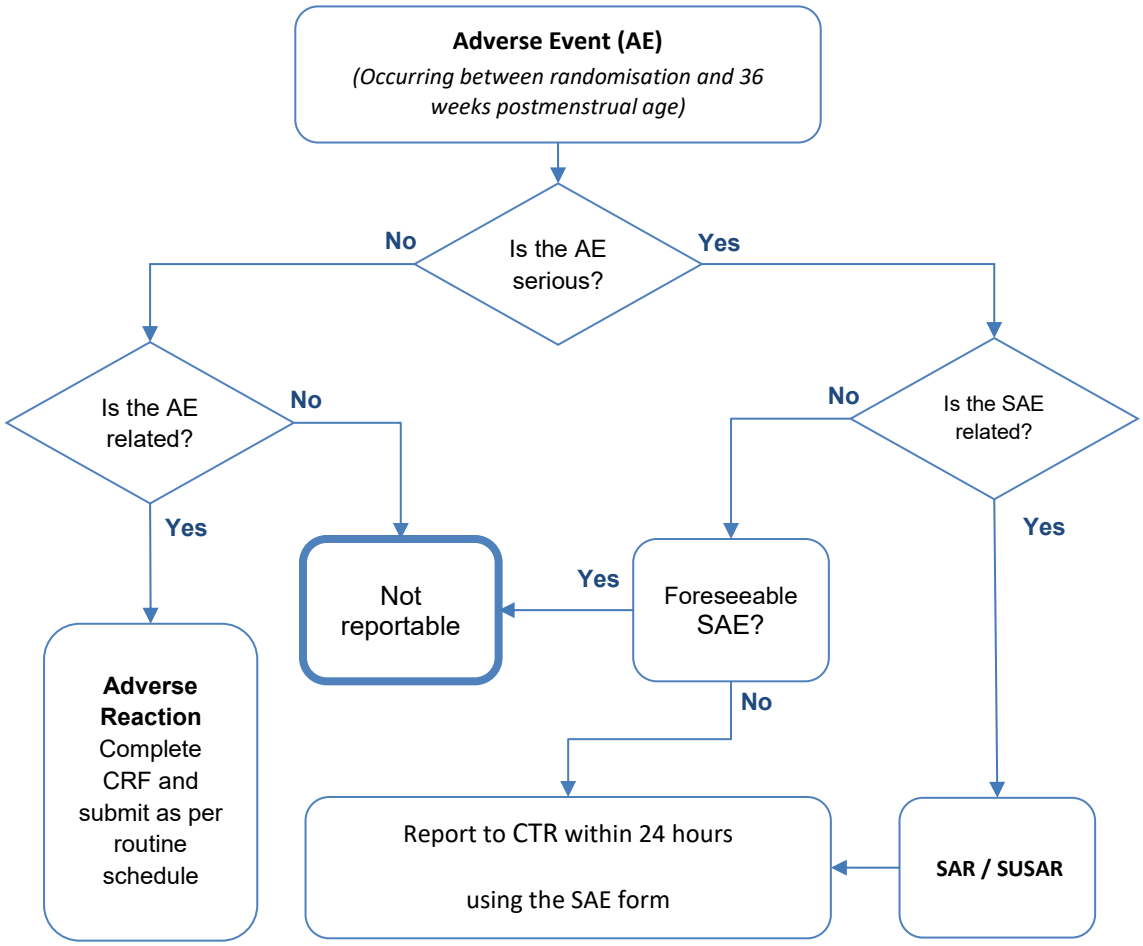


Figure 9: Pharmacovigilance reporting procedure

The above figure demonstrates the pharmacovigilance reporting process for the AZTEC trial. The PI is responsible for ensuring that all site staff involved in this trial are familiar with the content of this section.

All SAEs must be reported immediately (and within 24 hours of knowledge of the event) by the PI at the participating site to the CTR PV and Safety Specialist unless the SAE is specified as not requiring immediate reporting (see section 12.2.1).

12.1 Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant or clinical trial participant administered a medicinal product and which are not necessarily caused by or related to that product
Adverse Reaction (AR)	Any untoward and unintended response in a clinical trial participant to an investigational medicinal product which is related to any dose administered to that participant
Serious Adverse Event (SAE)	Any adverse event that - • Results in death • Is life-threatening* • Required hospitalisation or prolongation of existing hospitalisation** • Results in persistent or significant disability or incapacity • Consists of a congenital anomaly or birth defect • Other medically important condition***
Serious Adverse Reactions (SARs)	Any SAE occurring in a clinical trial participant for which there is a reasonable possibility that it is **related** to the IMP at any dose administered.
Suspected Unexpected Serious Adverse Reactions (SUSARs)	A SAR, the nature and severity of which is not consistent with the Reference Safety Information (RSI) for the IMP.

***Note:** The term 'life-threatening' in the definition of serious refers to an event in which the trial participant was at risk of death at the time of the event or it is suspected that used or continued used of the product would result in the subjects death; it does not refer to an event which hypothetically might have caused death if it were more severe.

**** Note:** Hospitalisation is defined as an inpatient admission, regardless of the length of stay, even if the hospitalisation is a precautionary measure for continued observation. Pre-planned hospitalisation e.g. for pre-existing conditions which have not worsened, or elective procedures, does not constitute an SAE.

***** Note:** other events that may not result in death, are not life-threatening, or do not require hospitalisation, may be considered as an SAE when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

12.2 Trial specific SAE reporting requirements

12.2.1 Foreseeable Serious Adverse Events

The following are serious adverse events that could be reasonably anticipated to occur in this population of infants during the course of routine care and treatment and as such do not require immediate reporting to the CTR if the investigator considers that there is no causal relationship between study medication and the event:

- Anaemia requiring blood transfusion[+]

- Intracranial abnormality (haemorrhage or focal white matter damage) on cranial ultrasound scan or other imaging
- Chronic lung disease of prematurity (or bronchopulmonary dysplasia)
- Coagulopathy requiring treatment[+]
- Culture-proven infection or sepsis
- Death (unless unforeseeable in this population)
- Fluid retention
- Fractures
- Gastrointestinal bleeding
- Haematuria
- Hydrocephalus
- Hyperbilirubinemia necessitating phototherapy and/or exchange transfusion[+]
- Hypercalcemia
- Hypocalcaemia
- Hyperglycaemia
- Hypoglycaemia
- Hypertension
- Hypotension treated with inotropes[+]
- Impaired renal function (urine output <0.5 ml/kg/hour and/or serum creatinine defined as >100 µmol/L)
- Left ventricular hypertrophy on echocardiography
- Low serum sodium level/hyponatremia (defined as sodium <130 mmol/L)
- Liver failure, clinically significant
- Necrotising enterocolitis or gastrointestinal perforation
- Neutropenia (defined as $<1x10^9$/L)
- Patent ductus arteriosus (PDA)
- Pneumothorax requiring treatment
- Pulmonary haemorrhage, significant
- Pulmonary hypertension requiring treatment with pulmonary vasodilator[+]
- Respiratory failure
- Retinopathy of prematurity
- Seizures requiring treatment[+]
- Thrombocytopenia

Events marked with [+] that do not require treatment will not be deemed serious

Any events occurring ***not* included** on the above list are considered to be ***unforeseeable*** and if they meet the criteria to be defined as an SAE should be immediately reported as outlined in section 12.3.

12.2.2 **Notes on reporting of deaths**

The AZTEC trial population is extremely preterm neonates. There is a continuous and high risk of SAEs, including death, independent of the study procedures and treatment. Death (mortality) forms part of the efficacy end point of the trial and is also foreseeable (approximately 20%) in relation to the disease under study (extremely preterm birth).

Thus, death will not be routinely be reported though the SAE reporting channel. Rather, data regarding death as an outcome (including likely cause) shall be recorded on the eCRF.

For avoidance of doubt, any serious adverse event which results in death and which is *unforeseeable* (not listed in 12.2.1) **must be immediately notified to the CTR PV & Safety Specialist as an unforeseeable SAE, as described in 12.3, regardless of whether causality with the IMP is suspected.**

If a death is suspected to have any causal relationship to the study medication or procedures it immediately must be immediately notified to the CTR PV & Safety Specialist as a SAR as described in 12.3.

12.2.3 Cardiac monitoring

Review of the available safety information did not raise any risks requiring specific assessment of safety. However, due to the reported risk of increased cardiac-related mortality being associated with azithromycin administration in high risk populations (adults with cardiovascular disorders), any noted alterations in cardiac rhythm shall be investigated with a formal ECG including assessment of QTc interval, with repeat ECG 10 days later. Reporting of forthcoming SAEs will be as per the pharmacovigilance procedure described in section 12.

12.2.4 Causality

Causal relationship will be assessed for the IMP:

IMPs: Azithromycin (Zedbac™)
nIMPs: None
Procedures: None

The PI (or another delegated medically qualified doctor from the trial team) and CI (or another medically qualified doctor from the Trial Management Group who has signed the delegation log for this activity) will assess each reported SAE to determine the causal relationship:

Relationship	Description	Reasonable possibility that the SAE may have been caused by the IMP?
Unrelated	There is no evidence of any causal relationship with the trial/intervention	No
Unlikely	There is little evidence to suggest there is a causal relationship with the trial/intervention (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment).	No
Possible	There is some evidence to suggest a causal relationship with the trial/intervention (e.g. because the event occurs within a reasonable time after administration of	Yes

	the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments).	
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.	Yes
Definite	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.	Yes

The causality assessment given by the PI (or delegate) cannot be downgraded by the CI (or delegate), and in the case of disagreement both opinions will be provided.

12.2.5 **Expectedness**

The CI (or another delegated appropriately qualified individual) will assess each SAR to perform the assessment of expectedness.

The expectedness assessment should be made with reference to the current Reference Safety Information (RSI) for each IMP. Expectedness decisions must be based purely on the content of the RSI; other factors such as the participant population and participant history should not be taken into account. Expectedness is not related to what is an anticipated event within a particular disease.

SARs which add significant information on specificity or severity of a known, already documented adverse event constitute unexpected events. For example, an event more specific or more severe than that described in the RSI is considered unexpected.

The table below lists the RSIs that should be referenced:

IMP	RSI to be used for expectedness assessment	Relevant section to be used for expectedness assessment
Azithromycin	SPC: Zedbac^{TM} 500mg	Section 4.8

Reference Safety Information (RSI) on any CTR trial will be reviewed regularly according to CTR procedures.

12.2.6 **Severity grading**

The assignment of the severity grading for each reportable SAE should be made by the investigator responsible for the care of the participant using the definitions below.

Mild: does not interfere with routine activities

Moderate: interferes with routine activities

Severe: impossible to perform routine activities

A distinction is drawn between serious and severe AEs. Severity is a measure of intensity (see above) whereas seriousness is defined using the criteria in section 10.1, hence, a severe AE need not necessarily be a Serious Adverse Event.

12.3 SAE Reporting procedures

12.3.1 Participating Site Responsibilities

The PI (or delegated medically qualified doctor from the trial team) should sign and date the SAE CRF to acknowledge that he/she has performed the seriousness and causality assessments. Investigators should also report SAEs to their own health boards or trust in accordance with local practice.

A completed SAE form for all events requiring immediate reporting should be submitted via email to CTR within 24 hours of knowledge of the event. A separate form must be used to report each event, irrespective of whether or not the events had the same date of onset.

A separate form must be used to report each event, irrespective of whether or not the events had the same date of onset.

The participant will be identified only by Study ID, date of birth and initials. The participant's name should not be used on any correspondence.

It is also required that sites respond to and clarify any queries raised on any reported SAEs and report any additional information as and when it becomes available through to the resolution of the event. Additionally, CTR/pharmaceutical companies may request additional information relating to any SAEs/SARs and the site should provide as much information as is available to them in order to resolve these queries.

Serious Adverse Event (SAE) email address:

CTR-Safety@Cardiff.ac.uk

SAE Fax number:

0203 0432 376

Serious adverse events should be reported from time of signature of informed consent, throughout the treatment period up to until 36 weeks' postmenstrual age, or discharge home from hospital (whichever is soonest).

An SAE form is not considered as complete unless the following details are provided:

- Full participant trial number
- An Adverse Event / Adverse Reaction
- A completed assessment of the seriousness, and causality as performed by the PI (or another appropriately medically qualified doctor registered on the delegation log).

If any of these details are missing, the site will be contacted and the information must be provided by the site to the CTR within 24 hours.

All other reportable events (see flowchart in 14.2) should be reported on the CRF following the CRF procedure described in Section 15.3.

12.3.2 The CTR responsibilities

Following the initial report, all SAEs should be followed up to resolution wherever possible, and further information may be requested by the CTR. Follow up information must be provided on a new SAE form.

The CTR should continue reporting SAEs until 36 weeks' postmenstrual age, or discharge home from hospital (whichever is soonest).

Once an SAE is received at the CTR, it will be evaluated by staff at the CTR and sent to the CI (or their delegate) for an assessment of expectedness and causality.

Investigator reports of suspected SARs will be reviewed immediately and those that are identified as SUSARs are reported to the MHRA, REC and Aspire.

12.4 SUSAR reporting

Cardiff University is undertaking the duties of trial Sponsor and has delegated to the CTR the responsibility for reporting SUSARs and other SARs to the regulatory authorities (MHRA and REC) and to Aspire as follows:

SUSARs which are fatal or life-threatening must be reported to the MHRA and REC within 7 calendar days of receipt at the CTR. If report is incomplete, then additional follow-up information should be reported within a further 8 calendar days of submitting the initial report.

SUSARs that are not fatal or life-threatening must be reported to the MHRA and REC within 15 days of receipt at the CTR. Any additional, relevant information must be reported within a further 15 days.

12.5 Unblinding for the purposes of SUSAR reporting

To enable processing of a SAR in this blinded trial, expectedness should be assessed initially using the assumption that the IMP has been given to the trial participant.

If it is assessed as unexpected as per the RSI of the IMP, the SUSAR will be unblinded by the CTR safety group prior to reporting to the MHRA and REC. The blind will only be broken centrally for suspected SUSARs once assessed by the clinical reviewer.

If after unblinding it is evident that the trial participant received the placebo, this event will not require expedited reporting to the MHRA and REC.

12.6 Safety Reports

A list of all SARs (expected and unexpected) will be reported annually to the MHRA, REC, trial sponsor and Aspire Ltd. in the form of a Development Safety Update Report (DSUR). This report must be submitted within 60 days of the anniversary of the MHRA CTA approval date.

The CTR will report a list of all SARs (expected and unexpected) and any other safety recommendations to all PIs annually throughout the course of the trial. This frequency may be reviewed and amended as necessary. This reporting will be done via the Investigator safety report (ISR).

12.7 Urgent Safety Measures (USMs)

An urgent safety measure is an action that the Sponsor, CI or PI may carry out in order to protect the subjects of a trial against any immediate hazard to their health or safety. Any urgent safety measure relating to this trial must be notified to the MHRA and Research Ethics Committee immediately by telephone, and in any event within 3 days in writing, that such a measure has been taken. USMs reported to the CTR will be handled according to CTR processes.

13 Statistical considerations

13.1 Randomisation

Babies will be remotely randomised using an online computerised randomisation system called Sortition created by the University of Oxford Primary Care Health Sciences Primary Care Clinical Trials Unit (PCCTU). The system will be operational 24 hours a day.

Randomisation will be performed by delegated members of the local trial team only after the participant has signed the Consent Form and completed the baseline assessments.

The delegated individuals will be provided with an individual login ID to the online system. Participants will be randomised to either azithromycin or placebo. Babies from multiple births will be randomised individually.

As AZTEC is a double-blind trial, the infant's family, clinicians, nurses and trial team (including the data manager and statistician) will be unaware of the arm to which the participant has been allocated for the duration of the trial.

Each treatment pack will be labelled with a unique identification number (Pack ID). Sortition will allocate a Pack ID for each participant. The treatment pack will contain 12 days' supply of treatment.

The participant Study IDs and Pack IDs will be linked in the randomisation file, which will only be accessible by a statistician who is independent of the trial.

13.2 Blinding

Treatment allocation of azithromycin or placebo will be blinded such that the allocation will not be known by clinicians, the baby's family or the trial outcome assessors.

13.3 Unblinding of individual participants during the trial

Unnecessary or unintended decoding of the blinding should not occur during the trial. Unblinding is only performed in emergency situations where knowledge of the identity of the trial drug is

considered absolutely necessary for the clinical management of the participant, or if there is another compelling medical or safety reason to do so.

The Site PI is responsible for requesting emergency unblinding. Where authority for requesting unblinding is delegated to another member of the research team this should be clearly identified in the site delegation. Where possible, permission to unblind an individual case should be requested via the TM, who will then seek the agreement of the CI (or delegate). If the treating doctor/ healthcare professional is not the PI, the PI must be informed of the unblinding as soon as possible and the reasons for the actions taken, however, the allocation does not need to be revealed to the PI, unless required for the participant's medical treatment.
If unblinding is deemed necessary by authorised members of the local investigational team, the following procedure should be followed.

If unblinding is deemed necessary by authorised members of the local investigational team, the following procedure should be followed:

1. A web-based unblinding system will be made available to the local investigational team. Access will be controlled using a unique username and password.
2. Where possible (during office hours), consent for individual unblinding should be made via the TM at the CTR who will seek the opinion of the CI
3. An authorised member of the investigational team will log on to the unblinding system. On entering the required information, including the Pack ID of the infant, the treatment allocation will be revealed. The allocation will be transmitted to the person primarily responsible for their care.
4. A delegated member of the research team will complete the AZTEC Unblinding CRF, to be returned to the CTR within 24 hours of the event.
5. The trial treatment allocation should not be included on the CRF and the allocation should not routinely be revealed to CTR staff.

In the event the randomisation system is not available, during office hours the site may contact the CTR safety team, who will arrange for unblinding to be performed by a member of staff who is independent from the project team.

13.4 Sample size

Relevant interventional studies investigating CLD as an outcome (including studies using macrolides) in preterm infants are shown in Table 3. The Ballard data (Ballard 2011) showed a survival rate without CLD of 50%, including death rate of 20% and 30% rate for development of CLD. Similarly, Table 3 shows that, in general, national and international studies consistently show rates of survival without CLD of 50 – 60% (those with lower rates are due to highly selected groups of sicker participants). Thus, adopting a conservative approach using 50% survival without developing CLD is reasonable. Similarly, the absolute differences in effect size ranges from 10% to 20% in most studies. A range of sample size calculations in which the size of effect varies from 10% to 15% have been considered in Table 4. Powering to detect a 15% improvement would miss smaller but still clinically important differences. However, a smaller effect size of 10% would substantially increase recruitment time and costs of delivering the trial with this size of effect being unlikely to be accepted universally in the clinical arena. Thus, an effect size of 12% is a reasonable minimal, clinically worthwhile difference that would be convincing in the clinical arena and impact routine use. Furthermore, an improvement of 12% (50% to

62%) in survival without CLD with a power of 0.90 and significance level of 5% would require 796 subjects (including a dropout rate of 10%) recruitment of which is highly feasible as shown by several similar UK studies in this population. Since the trial will involve formal assessment with an oxygen challenge test in both tertiary units and in step-down units, a dropout rate of 10% has been estimated.

Study	Status	Treatment	Gestation included	Baseline % of Survival without CLD	Expected effect of treatment	sig. level	Power	sample size per group
Waterberg	Ongoing	Hydrocortisone	<30	25-35%	?	0.05	0.80	400
Collins	Published	Docosahexaenoic acid	<29	51.4%	10% absolute, 19% relative	0.05	0.90	622
Bassler (NEUROSIS)	Published	Budesonide	≤27	50%	20%	0.05	0.80	404
Mercier (EUNO)	Published	Nitric Oxide	≤28	64%	20%	0.05	0.80	400
OSCAR	Ongoing	Indomethacin	≤28	60%	12% absolute, 20% relative	0.05	0.90	365
TINN2	Incomplete	Azithromycin	≤28	50%	10%	0.05	0.90	385
AZTEC	**Proposed**	**Azithromycin**	**<30**	**50%**	**12%**	**0.05**	**0.90**	**398**

Table 3 Summary of recent relevant interventional studies of preterm infants

P1	P2	Effect size	Power	Sig. level	Sample Size	+10% dropout
0.60	0.50	0.10	0.90	0.05	1038	1153
0.62	**0.50**	**0.12**	**0.90**	**0.05**	**716**	**796**
0.65	0.50	0.15	0.90	0.05	454	504

Table 4 sample size calculation using two group chi-square test for different relevant effect size

13.5 Feasibility (attaining recruitment targets)

An internal pilot will be conducted to assess feasibility in 5 tertiary neonatal units. The primary outcome will not be available for up to 13 weeks after recruitment and this along with the predicted site initiation and recruitment rates have determined the duration of the pilot to be 12 months. By month 9 of recruitment, we expect 37 randomisations on which the primary outcome should be observed by month 12 of recruitment.

Willingness to participate:

We anticipate that consent will be obtained from around 50% of parent(s)/legal guardian(s) of eligible infants:

- If consent rate is greater than or equal to 50%, then proceed to main trial.
- If consent rate is 30-49%, then the reasons why parent(s)/legal guardian(s) do not want their infant to participate will be investigated to identify any aspects amenable to change. Reasons for decline will be collected from parent(s)/legal guardian(s) willing to provide a reason. We shall liaise with our Patient & Public Involvement (PPI) group to explore methods to increase recruitment rates.

- If consent is less than 30% and no clear solution can be determined, then we would stop the trial without proceeding to the full study.

Willingness of clinician to recruit:

- When sites agree to participate, they will be asked to submit monthly logs documenting the number of eligible babies, number approached and those randomised:
- If screening logs identify that greater than or equal to 80% of parent(s)/legal guardian(s) of eligible infants are approached for consent, then proceed with the full study.
- If screening logs identify that 50-79% of parent(s)/legal guardian(s) of eligible infants are approached for consent, then we shall investigate any aspects of screening process that are amenable to change.
- If screening logs identify that <50% of parent(s)/legal guardian(s) of eligible infants are approached for consent and no obvious solution can be identified, then we would stop the trial without preceding to the main study.

Primary outcome completeness:

Since many babies are transferred to step down units after initial ITU/HDU management, we shall document any issues with completion of data in both the tertiary and in the step-down units:

- If primary outcome completeness is greater than or equal to 90% then proceed to main trial
- If primary outcome completeness is between 70-89% then identify and introduce any remedial factors. Contact fortnightly to reassess.
- If primary outcome completeness is less than 70%, and no obvious solutions exist then stop the trial without proceeding to the main study.

Recruitment:

- If based on the recruitment achieved in the internal pilot the predicted recruitment period is 30 months or less, proceed to main trial
- If based on the recruitment achieved in the internal pilot the predicted recruitment period is 31-36 months then look to identify ways to improve recruitment (consider replacing underperforming sites, additional sites, training needs, review of eligibility criteria, consent rates)
- If based on the recruitment achieved in the internal pilot the predicted recruitment period is greater than 36 months and no obvious solution exists, then do not proceed to the full trial.
- We shall also closely scrutinise IMP distribution, storage, delivery, adherence to treatment protocol (specifically age at commencing 10 days i.v. treatment), and sample collection. Any plans to revise the trial will be discussed with the IDSMC and TSC before being submitted to the funder.

13.6 Missing, unused & spurious data

Detail provided in the Statistical Analysis Plan (SAP).

13.7 Procedures for reporting deviation(s) from the original SAP

These will be submitted as substantial amendments where applicable and recorded in subsequent versions of the protocol and SAP.

13.8 Termination of the trial

Progression criteria for the internal pilot phase is described in section 13.5. There is potential for the study to terminate early if our funder assesses the trial as not being feasible following an assessment of progress against our targets at the end of the internal pilot with input from our TSC and IDMC.

13.9 Inclusion in analysis

All randomised participants will be included in the intention to treat analysis as primary analysis. The ineligible/unevaluable participants need to be excluded in secondary analyses (per protocol, CACE etc.).

14 Analysis

14.1 Main analysis

The trial will be analysed and reported using the 'Consolidated Standard of Reporting Trials' (CONSORT) and the ICH E9 guidelines. A separate and full statistical analysis plan (Gamble 2017) will be developed prior to database lock. The SAP will be agreed by the TSC and IDSMC. The main features of these planned statistical analyses are included here.

The principle of intention-to-treat will be applied as far as practically possible including all participants in the primary analysis in the group to which they were randomly allocated. All analyses will use a 5% level of statistical significance and 95% confidence intervals will be used throughout. The primary outcome will be analysed using logistic regression fitting treatment group, gestational age at birth, and any randomisation stratification variables. Dichotomous secondary outcomes will be analysed using the same approach. Number of days of respiratory support will be analysed as a time to event outcome allowing for competing risk of death. All models will account for any clustering effects of multiple births within the same mother. Statistical tests will not be used on safety and tolerability outcomes. These outcomes will use descriptive statistics only.

14.1.1 Sub-group & interim analysis

To explore the extent to which there may be a differential treatment effect by presence of *Ureaplasma spp.*, the model fitted for the primary analysis will be extended by including a main and treatment group interaction term for *Ureaplasma spp.* colonisation at baseline. Estimates from the statistical models (main effects and interaction terms) will be presented alongside 95% confidence intervals and p-values.

No formal interim analysis will be conducted.

15 Data Management

The source data for AZTEC trial will be from a variety of sources. Data will be collected using an electronic system with paper CRF back up. There will also be data collected from participant's medical

notes. Source data from the Badgernet database and laboratory data will also be utilised. Derived data from these sources will be entered into the trial database.
Training for completion of study eCRFs will be provided to the appropriate trial staff prior to trial commencement at site initiation.

Source Data is defined as *"All information in original records and certified copies of original records of clinical findings, observations or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents."* There is only one set of source data at any time for any data element, as defined in the source data verification form.

15.1 Data collection

15.2 Completion of CRFs

All assessments and data collection will be completed using web-based eCRFs. All data will be stored in accordance with Cardiff University and CTR policies and procedures and in line with Good Clinical Practise (GCP). Identifiable data will be encrypted and stored separately from non-identifiable data in a SQL Server database. All data will be stored on secure servers in Cardiff University and backed up daily. Access to the database and the server is restricted to named & appropriately trained personnel only.

If the web-based system is not accessible, paper CRFs will be used to record data. The data will then be inputted into the web-based system once it is accessible.

In accordance with the principles of GCP, the PI is responsible for ensuring accuracy, completeness, legibility and timeliness of the data reported to the CTR in the CRFs.

CRF pages and data received by the CTR from participating trial sites will be checked for missing, illegible or unusual values (range checks) and consistency over time.

If missing or questionable data are identified, a data query will be raised on a data clarification form. The data clarification form will be sent to the relevant participating site. The site shall be requested to respond to the data query on the data clarification form. The case report form pages should not be altered.

All answered data queries and corrections should be signed off and dated by a delegated member of staff at the relevant participating site. The completed data clarification form should be returned to the CTR and a copy retained at the site along with the participants' CRFs.

The CTR will send reminders for any overdue data. It is the site's responsibility to submit complete and accurate data in timely manner.

A full Data Management Plan (DMP) will accompany this protocol and will be stored in the TMF.

16 Follow-up study

Subject to funding, an independent sub-study will be performed (with separate REC and relevant approvals) to determine if azithromycin alters neurodevelopmental and respiratory outcomes in infancy at 1 and 2 years of age. Parents will be asked to report respiratory symptoms by validated respiratory and neurodevelopmental questionnaires via post. Routine data will be utilised which will

be collected from the recruiting centres and national databases (National Neonatal Research Databse, NHS Digital or equivalent in the devolved nations). Prior permission from the database custodians will be obtained before data linkage to AZTEC data is performed.

17 Protocol/GCP non-compliance

The PI should report any non-compliance to the trial protocol or the conditions and principles of Good Clinical Practice to the CTR in writing as soon as they become aware of it. The CTR will assess the nature and severity of any issues of non-compliance in accordance with their SOPs.

18 End of Trial definition

The end of the trial is defined to be the date on which data for all participants is frozen and data entry privileges are withdrawn from the trial database

Sponsor must notify the MHRA and main REC of the end of a clinical trial within 90 days of its completion or within 15 days if the trial is terminated early.

19 Archiving

The TMF and TSF containing essential documents will be archived at an approved external storage facility for 25 years. The CTR will archive the TMF and TSFs on behalf of the Sponsor. The PI is responsible for archival of the ISF at site on approval from Sponsor. Essential documents pertaining to the trial shall not be destroyed without permission from the Sponsor.

20 Regulatory Considerations

20.1 Clinical Trials Authorisation

This trial has Clinical Trials Authorisation (CTA) from the UK Competent Authority: MHRA.

The outcome of the study (e.g. completed) will be reported to the MHRA within 90 calendar days of study closure. In the event of the study being prematurely terminated a report will be submitted to the MHRA within 15 calendar days.

A summary of the results will be submitted to the MHRA within one year of completion of study closure

20.2 Ethical and governance approval

This protocol has approval from a Research Ethics Committee (REC) that is legally “recognised” by the United Kingdom Ethics Committee Authority for review and approval.

This trial protocol will be submitted through the relevant permission system for global governance review (Health and Care Research Wales Permissions Coordinating Unit).

Approval will be obtained from the host care organisation who will consider local governance requirements and site feasibility. The Research Governance approval of the host care organisation must be obtained before recruitment of participants within that host care organisation.

The Sponsor will provide an opinion on whether an amendment meets criteria to be considered substantial. All substantial amendments must be approved by the REC responsible for the study, in addition to approval by NHS Research and Development (R&D). Non-substantial amendments will not require prior approval by the REC.
If the study is stopped due to adverse events or an urgent safety measure it will not be recommenced without reference to the REC responsible for the study.

The outcome of the study (e.g. completed) will be reported to the REC responsible for the study within 90 calendar days of study closure. In the event of the study being prematurely terminated a report will be submitted to the REC responsible for the study within 15 calendar days.

A summary of the results will be submitted to the REC responsible for the study within one year of completion of study closure.

20.3 Data Protection

The CTR will act to preserve participant confidentiality and will not disclose or reproduce any information by which participants could be identified, except where specific consent is obtained. Data will be stored in a secure manner and will be registered in accordance with the General Data Protection Regulation 2016. The data custodian and sample custodian for this trial is the CI.

Participants will always be identified using their unique study identification number and any additional identifiers. This includes collection of NHS number (or equivalent – e.g. CHI number in Scotland), name and postcode to register and trace participants with NHS digital (or equivalent e.g. NHS Wales Informatics Service). Collection of NHS number or equivalent is also required to utilise NHS data for future research through the National Neonatal Research Database.

20.4 Indemnity

- Non-negligent harm: This trial is an academic, investigator-led and designed trial, coordinated by the CTR. The PI, local Investigators and coordinating centre do not hold insurance against claims for compensation for injury caused by participation in a clinical trial and they cannot offer any indemnity. The Association of the British Pharmaceutical Industry (ABPI) guidelines will not apply.
- Negligent harm: Where studies are carried out in a hospital, the hospital continues to have a duty of care to a participant being treated within the hospital, whether or not the participant is participating in this trial. Cardiff University does not accept liability for any breach in the other hospital's duty of care, or any negligence on the part of employees of hospitals. This applies whether the hospital is an NHS Trust or not. The Sponsor shall indemnify the site against claims arising from the negligent acts and/or omissions of the Sponsor or its employees in connection with the Clinical Trial (including the design of the Protocol to the extent that the Protocol was designed solely by the Sponsor and the Site has adhered to the approved version of the Protocol) save to the extent that any such claim is the result of negligence on the part of the Site or its employees.

All participants will be recruited at NHS sites and therefore the NHS indemnity scheme/NHS professional indemnity will apply with respect to claims arising from harm to participants at site management organisations.

20.5 Trial sponsorship

Cardiff University will act as Sponsor for trial. Delegated responsibilities will be assigned to the sites taking part in this trial. A memorandum of understanding will be in place between the Sponsor's office and the CTR, outlining the responsibilities which have been delegated.

20.6 Funding

This trial is funded by the NIHR Health Technology Assessment Programme through a competitive grant award (16/111/106). It has been adopted to the NIHR portfolio (39385).

21 Trial Management

21.1 Project team (PT)

The Project Team (PT) will meet fortnightly and will include the CI, TM, Data Manager, Statistician, Administrator and other research staff directly employed to the trial. The project team will discuss all day-to-day management issues and will refer any key management decisions to the Trial Management Group (TMG).

21.2 TMG (Trial Management Group)

The TMG will consist of the CIs, Co-Applicants, Collaborators, TM, DM, TS and TA. The role of the TMG will be to help set up the trial by providing specialist advice, input to and comment on trial procedures and documents (information sheets, Protocol, etc.). They will also advise on the promotion and running of the trial and deal with any issues that arise. The group will normally meet monthly throughout the course of the study. TMG members will be required to sign up to the remit and conditions as set out in the TMG Charter.

21.3 TSC (Trial Steering Committee)

A Trial Steering Committee (TSC), consisting of an independent chair, and three other independent members including a parent representative, will meet at least annually. The first meeting will be before the trial commences to review the Protocol and arrange the timelines for the subsequent meetings. If necessary, additional/more frequent meetings may occur. The TM and TS will attend as observers. The TSC will provide overall supervision for the study and provide advice through its independent chair. The ultimate decision for the continuation of the study lies with the TSC. TSC members will be required to sign up to the remit and conditions as set out in the TSC Charter.

21.4 IDMC (Independent Data Monitoring Committee)

In order to monitor accumulating data on safety and any trial intervention benefit, an IDMC will be established. The Committee will consist of an independent chair and two/three other independent members. The first meeting will take place before the trial commences in order to review the

Protocol and agree on timelines for interim analyses to take place. The main role of the IDMC is to review the data periodically and makes recommendations to the TSC.
IDMC members will be required to sign up to the remit and conditions as set out in the IDMC Charter which will be filed in the TMF.

22 Quality Control and Assurance

22.1 Risk assessment

A Trial Risk Assessment has been completed to identify the potential hazards associated with the trial and to assess the likelihood of those hazards occurring and resulting in harm. This risk assessment has been completed in accordance with the MRC/DH/MHRA Joint project guidance document 'Risk-adapted approaches to the management of Clinical Trials of Investigational Medicinal Products' and includes:

- The known and potential risks and benefits to human subjects
- How high the risk is compared to normal standard practice
- How the risk will be minimised/managed

This trial has been categorised as a TYPE B where the level of risk is somewhat higher than the risk of standard medical care. A copy of the trial risk assessment may be requested from the TM. The trial risk assessment is used to determine the intensity and focus of monitoring activity (see section 22.2).

22.2 Monitoring

The clinical trial risk assessment has been used to determine the intensity and focus of central and on-site monitoring activity in the AZTEC trial. Moderate levels will be employed and are fully documented in the trial monitoring plan. Investigators should agree to allow trial related monitoring, including audits and regulatory inspections, by providing direct access to source data/documents as required. Participant consent for this will be obtained.

Findings generated from on-site and central monitoring will be shared with the Sponsor, CI, PI & local R&D.

22.3 Audits & inspections

The trial is participant to inspection by the MHRA as the regulatory body. The site must inform the CTR of any MHRA inspections.

The trial may also be participant to inspection and audit by Cardiff University under their remit as Sponsor.

The CI or PI organisations/institution(s) will permit trial-related monitoring, audits, REC/ IRB review, and regulatory inspection(s), providing direct access to source data / documents. The study may be audited by NHS Digital Audit Team.

23 Publication policy

Standard principles in accordance with the policy of the International Committee of Medical Journal Editors will be followed. The CI co-ordinate dissemination and writing of data from the main study. Authorship at the head of the primary results paper will take the form "[author1], [author2] and [author3] on behalf of the 'The AZTEC Collaborative Group'", (latter if acceptable for journal policy). All other contributors will be listed at the end of the main paper, with their contribution identified. All publications and presentations relating to the trial will be authorised by the Trial Management Group in accordance with the trial publication policy. It will be ensured the relevant information centres are acknowledged (e.g. NHS digital) as per the data sharing agreement for any applicable publication.

The results of the trial will be disseminated regardless of the magnitude or direction of effect, via a full report to the funder and in peer-reviewed journal publications. Parent(s)/ legal guardian(s) who have provided consent on behalf of infants will be able to view a summary of trial publications if they wish, which will contain full references on the trial website.

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24 Appendices

None