This supplement contains the following items:

1. Original protocol, final protocol, summary of changes.
2. Original statistical analysis plan, final statistical analysis plan, summary of changes.
Amendments from the initial version (v1) of the protocol to the final version (v7) provided were as follows:

- **Amendment #1** (v2) approved by CPP ("comité de protection des personnes") 30/06/2008 and AFSSAPS (French drug agency) 28/07/2008:
  - Changes in blood sampling for ancillary studies.
  - Change of the principal investigators in 3 recruiting centers.
  - Change in wording of the consent form.
  - No change in statistical analysis plan.

- **Amendment #2** (v3) approved by CPP, 17/03/2009 and AFSSAPS, 16/04/2009:
  - Change in wording of the consent form.
  - Changes in data recorded (vital signs, concomitant treatments and adverse events).
  - Change in severe adverse events list.
  - Addition of a novel recruiting center.
  - No change in statistical analysis plan.

- **Amendment #3** (v4) approved by CPP, 05/10/2009 and AFSSAPS, 16/09/2009:
  - Change in severe adverse events list.
  - Modifications of the rules associated with SAE notification.
  - No change in statistical analysis plan.

- **Amendment #4** (v5) approved by CPP, 04/10/2010 and AFSSAPS, 15/10/2010:
  - Decision by the steering committee of scale to be used in the 2-year follow-up: Revised Brunet Lézine scale (global score and subscores) at 18-24 months of corrected age.
  - Decision from the DSMB regarding ACTH test (48h following the end of the treatment): stop.
  - Change of the principal investigator in one recruiting center.
  - No change in statistical analysis plan.

- **Amendment #5** (v6) approved by CPP, 03/08/2011:
  - Change of the principal investigator in two recruiting centers.
  - Change in wording of the consent form (blood sampling).
  - Extension of the recruitment period.
  - No change in statistical analysis plan.

- **Amendment #6** (v7) approved by CPP, 08/07/2013:
  - Addition of a novel recruiting center.
  - Extension of the recruitment period.
  - No change in statistical analysis plan.
Early Low-Dose Hydrocortisone to Improve Survival without Bronchopulmonary Dysplasia in Extremely Preterm Infants: a Randomised, Double-Blind, Placebo-controlled, Multicenter Trial

P060250

Sponsor
Assistance Publique- Hôpitaux de Paris

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Signature page of a biomedical research protocol

Code of biomedical research: P 060250

**Title:** « Early Low-Dose Hydrocortisone to Improve Survival without Bronchopulmonary Dysplasia in Extremely Preterm Infants ». PREMILOC trial

Version 7.0 of 03/06/2013
Research will be conducted in accordance with the protocol and laws and regulations.

Principal investigator & coordinator:
Pr Olivier BAUD
Date: ................../........./.......... Signature :

Service de Néonatologie
Hôpital Robert Debré
Address 48 Bd Sériurier
75935 PARIS cedex 19

Executive DRCD:
Christophe MISSE
Date: ................../........./.......... Signature :

Assistance publique – hôpitaux de Paris
Délégation Interrégionale à la Recherche Clinique
Hôpital Saint Louis
1 Av. Claude Vellefaux
75010 PARIS

This version has received a favourable opinion of the CPP Ile de France II dated 08/07/2013
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1. **ABSTRACT**

**Titre:** Early Low-Dose Hydrocortisone to Improve Survival without Bronchopulmonary Dysplasia in Extremely Preterm Infants: PREMILOC trial

**Code project:** P060250

**PI & Coordinator:** Pr Olivier BAUD, Service de Néonatologie, Hôpital Robert Debré

**Sponsor:** AP-HP, D.R.C.D.

**Hypothesis:**
The administration of low doses of hydrocortisone hemisuccinate (HSHC) for 10 days may induce a pulmonary anti-inflammatory effect in very premature neonates born in the context of perinatal inflammation, a risk factor for neonatal mortality and respiratory and neurological morbidities. This anti-inflammatory effect during the first postnatal days could lead to reduced incidences of neonatal mortality and bronchopulmonary dysplasia.

**Primary objective:**
To test the ability of early administration of HSHC to reduce neonatal mortality and the occurrence of bronchopulmonary dysplasia, defined as the need for any ventilatory support with positive pressure and/or administration of oxygen, at 36 weeks of postmenstrual age, in very premature babies born in a context of perinatal infection.

**Molecule tested:**
Hydrocortisone hemisuccinate, initiated as soon as possible before the 24th hour of life, at a dose of 0.5 mg / kg / 12h IV for 7 days, and then 0.5 mg / kg / 24h for 3 days, versus placebo.

**Inclusion criteria:**
- Initial medical examination
- Any neonate with a gestational age between 24+0 and 27+6 weeks of gestation born in a context other than those specified in the exclusion criteria.
- Newborns whose parental authority holders signed a consent form
- Newborns whose parental authority holders are covered by the social security system or ("couverture médicale universelle" CMU).

**Exclusion criteria:**
- Preterm infants with a gestational age ≥ 28 weeks of gestation
- Congenital malformation and/or heart diseases other than patent ductus arteriosus or foramen ovale
- Rupture of membranes before 22 + 0 weeks of gestation
- Newborns from a pregnancy with > 3 fetuses
- Newborns whose birth weight is < 500g
- IUGR <3rd percentile according to customised French curves (AUDIPOG)
- "Outborn" neonates
- Newborns whose parental authority holders are minor
- Newborns who will not be able to receive the full treatment (chromosomal abnormalities, severe birth asphyxia)
- Newborns whose parental authority holders are not beneficiaries of social security coverage.

**Maximum number of subjects required:** 786.

Expected number of participating centres: 24 (France)

**Search time and duration of participation for each patient**
Duration of participation per patient: 2 years.
Expected duration of the entire study: 6 years, including 4 years of inclusion.

**Methodology:**
Multicenter, randomised, placebo-controlled, double-blind trial with a sequential analytical design.

**Primary endpoint:**
All premature babies surviving without bronchopulmonary dysplasia at 36 weeks of gestation will be considered "success."
All premature babies that died or that are surviving with an FiO₂ > 21% or dependent on any positive-pressure ventilation (whatever the FiO₂) at 36 weeks of gestation will be considered "failure".

**Tests required specifically for research and centralised at the Robert Debré Hospital Paris**

- **Collection of cord blood**
  Blotter Guthrie type for subsequent assay of interleukins (IL1, 6, 8, 9) and TNFα,
  Serum bank for subsequent hormonal assays,
  DNA banking

- **Venous blood samples**

- **Sample obtained during the first 24 hours of life of the newborn:**
  Total cortisol assay

  Sample obtained 48 hours after the end of treatment (day 12)
  T4 and TSH assays
INTRODUCTION OF LITERATURE DATA AND RATIONALE FOR RESEARCH

1.1. **Evolution of the clinical presentation of bronchopulmonary dysplasia**

Despite improved perinatal management of preterm labour and the reduction of neonatal mortality in the most immature children, neurological and respiratory morbidity related to prematurity remains high. The presentation of bronchopulmonary dysplasia (BPD) has largely changed over the last 20 years (Bancalari et al., 2003). Before the era of surfactant therapy, pulmonary inflammation, fibrosis induced by oxygen toxicity and barotrauma induced by mechanical ventilation were the main features of BPD (O’Brodovich and Mellins, 1985). More recently, lung maturation enhanced by antenatal glucocorticoid administration, administration of exogenous surfactant, and the widespread use of non-invasive ventilatory support have been accompanied by a marked decrease in the impact of the severity of the initial respiratory disease and have profoundly changed the presentation of the chronic lung disease, leading to a “new form” of BPD. The lungs of premature infants with BPD are characterised by a simplification of the alveolar architecture and a reduction in microvasculature. According to Jobe and Ikegami, the new form of BPD consists of a combination of inflammation related to the circumstances of birth, and a disorder of angiogenesis resulting in severe insult to the developing lung (Jobe and Ikegami 1998). However, recent changes in the perinatal management of preterm labour do not seem to attenuate the move towards this new form of BPD. Neither antenatal steroids nor surfactant administration is associated with a significant reduction in the incidence of DPB at 36 weeks of postmenstrual age (PMA) (Crowley, 2000; Bancalari et al., 2003; Stevens et al., 2004). For example, in two studies conducted in North America in the 90’s, 23-26% of children with birth weight < 1500 g developed BPD (Le et al., 2000; Lemons et al., 1996). More recently, Bancalari et al. report an incidence of BPD between 50% and 10% in infants with a birth weight between 500g and 1250g (Bancalari et al., 2003).

The incidence of BPD at 36 weeks of PMA in Europe is summarised in Table 1:

**Table 1:** Incidence of BPD at 36 weeks of PMA in Europe

<table>
<thead>
<tr>
<th>Population-based study / country</th>
<th>BPD at 36 weeks of PMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPILAGE 1997 (Egreteau et al., 2001)</td>
<td>39% (&lt;28 wks)</td>
</tr>
<tr>
<td>EPIBEL 2000 (Dr Vanhaesebrouck)</td>
<td>45% (&lt;26 wks)</td>
</tr>
<tr>
<td>Germany 2002 (Dr Hummler)</td>
<td>17-27% (24 - 27 wks)</td>
</tr>
<tr>
<td>England 2001-2002 (Dr Field)</td>
<td>23-59% (25 - 28 wks)</td>
</tr>
<tr>
<td>Spain 2002 (Dr Sanchez-Luna)</td>
<td>8-49% (500 - 1250g)</td>
</tr>
<tr>
<td>Finland (Dr Hallman)</td>
<td>10-47% (500 - 1500g)</td>
</tr>
<tr>
<td>MOSAIC Ile-de-France (2003)</td>
<td>15% (22 - 31 wks)</td>
</tr>
</tbody>
</table>

Table 2 depicts eligible patients, neonatal mortality rate and BPD incidence in 2003-2005 in the main recruiting centres.

<table>
<thead>
<tr>
<th>Center</th>
<th>Expected eligible patients / year</th>
<th>Neonatal mortality</th>
<th>BPD at 36 wks</th>
<th>Survival without BPD at 36 wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>37</td>
<td>38%</td>
<td>29%</td>
<td>44%</td>
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<td>B</td>
<td>37</td>
<td>22%</td>
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<td>C</td>
<td>32</td>
<td>28%</td>
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<td>O</td>
<td>22</td>
<td>nc</td>
<td>40%</td>
<td>nc</td>
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Pathophysiological concepts of bronchopulmonary dysplasia

The new form of BPD is linked to preterm delivery and its association with perinatal inflammation, disrupting alveolarisation and angiogenesis and leading to the arrest of lung development (Jobe and Ikegami, 1998). Antenatal inflammation initiates the subsequent development of BPD lesions, as evidenced by extensive epidemiological, biological and experimental data. Not only is chorioamnionitis a more common circumstance of birth at lower gestational ages, it is also a major risk factor for BPD (Watterberg et al., 1996; Baud et al., 2000; L Foix l’Hélias et al., 2005, Viscardi et al., 2004). On the other hand, neonates born to mothers who have given birth prematurely have elevated inflammation markers in the serum and lung (Watterberg et al., 1996). An increase in macrophage infiltration and increased expression of adhesion factors and IL8 in the bronchoalveolar epithelium is observed following intrauterine infection (Speer, 2004). In cases of inflammation / antenatal infection, several experimental findings support increased pulmonary inflammation and vascular remodelling characteristic of BPD (Kallapur et al., 2004). These histological findings are associated with a decrease in the expression of endothelial NO synthase (eNOS) and VEGF, both molecules having a critical role in angiogenesis, and therefore in lung alveolarisation. Thus, perinatal inflammation may disrupt lung development by disrupting angiogenesis and alveolar septal formation, characteristic of the new form of BPD, according to Jobe et al. This antenatal inflammatory disease is extended by postnatal inflammatory insults in the form of by mechanical ventilation, oxygen and infections, other risk factors for BPD.

In parallel to the concept of perinatal inflammation involved in the development of BPD, the association between chronic lung disease and the adrenocortical axis (or hypothalamic-pituitary-adrenal axis, HPA) has been largely developed by Watterberg et al. (Watterberg et al., 1995, 1996, 1999, 2004). Adrenal insufficiency is a well-known response to a situation of acute stress. Patients in shock have normal basal adrenal function but are unable to adequately respond to severe stress, leading to high mortality and cardiovascular instability (Lamberts et al., 1997; Cooper and Stewart, 2003; Joosten et al. 2000). Very preterm infants are likely to be exposed to this phenomenon due to the immaturity of their HPA axis. According to Mesiano and Jaffe, there is a deficit of 3β-hydroxysteroid dehydrogenase (3βHSD), an enzyme catalysing the conversion of cholesterol to cortisol, in fetuses during pregnancy (Mesiano and Jaffe, 1997). Antepartum, the production of cortisol by the fetus and is almost null before 23 weeks of gestation and becomes significant from 30 weeks of pregnancy. Cortisol necessary for the survival of the fetus is produced in the placenta from progesterone. After a premature delivery before 30 weeks of gestation, the newborn is thus deprived of placental glucocorticoid synthesis and the low activity of 3βHSD in the neonate does not allow it to adapt normally to the intense stress associated with very preterm birth. Rates of low cortisol and an inadequate adrenal response after stimulation have been reported in premature infants with a high index of morbidity including the requirement for significant ventilatory support and / or hemodynamic failure (Huysman et al., 2000; Scott and Watterberg, 1995; Ng et al., 2004). In addition, there is an association between low cortisol levels and a high incidence of mortality in this population of very preterm infants (Scott and Cimino, 2004). Premature babies who will develop BPD have lower cortisol levels during the first week of life than control populations free of any respiratory disease (Watterberg et al., 1999). These children have a poor response to ACTH stimulation, showing the inadequate reactivity of their HPA axis. Adrenal insufficiency has also been reported to be associated with an exaggerated inflammatory response (Chrousos, 1995; Goujon et al., 1996). Finally, low levels of cortisol are associated with the presence of inflammatory markers in the lungs and a higher incidence of patent ductus arteriosus, another risk factor for BPD (Watterberg et al., 1995). Thus, there is an association between the presence of a deficit in the

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Table 2: Recruitment of neonates born between 24 and 27 + 6 weeks of gestation, mortality rate and BPD incidence at 36 weeks of PMA in 2003-2005 in the main recruiting centres.
endogenous production of glucocorticoids by adrenocortical insufficiency during the early neonatal period and the subsequent occurrence of BPD and/or its risk factors.

1.2. Postnatal corticosteroid therapy and incidence of BPD

The first study analysing the impact of postnatal glucocorticoid therapy in premature infants was published in 1956 and evaluated the effect of this treatment on the incidence of respiratory distress syndrome (RDS) in newborns of diabetic mothers (Haddad et al., 1956). The first controlled study was reported in 1972 by Baden (Baden et al., 1972), on hydrocortisone compared to placebo administered within 12 hours of life to premature infants with RDS. This protocol showed no respiratory benefit at the time. In the 1980s and 1990s, many controlled studies showed that the postnatal administration of dexamethasone was associated with a decrease in the duration of supplemental O₂ and mechanical ventilatory support, thus promoting the widespread use of this treatment in neonatal intensive care units. The pathophysiological basis for these results comes from the observation of inadequate synthesis of glucocorticoids in response to postnatal stress in very premature infants (Watterberg et al., 2001). Halliday et al. (2003a, b, c) conducted a meta-analysis of all randomised trials testing postnatal dexamethasone administered to preterm infants at risk for BPD. In its most recent version, 37 studies were split into 3 categories according to the schedule of the regimen (early, moderately early, and delayed). Whatever the type of treatment used, postnatal dexamethasone reduced the need for mechanical ventilation at 28 days of age and at 36 weeks of PMA. A significant reduction in the use of supplemental steroids was also observed in the 3 protocols. However, early or moderately early regimens were not associated with a reduction in supplemental O₂ at term-equivalent of age and none of them were associated with a reduction in neonatal mortality (Halliday et al., 2003).

The respiratory benefits documented in these studies must be weighed against the side effects observed during postnatal treatment with dexamethasone. Hyperglycemia and hypertension are relatively mild and treatable complications. More severe are intestinal complications including haematemesis and gastrointestinal perforation (Stark et al., 2001). But the most serious side effect is the increase in the incidence of white matter damage and/or neurological impairment (Yeh et al., 1998 and 2004. Shinwell et al., 2000; O’Shea et al., 1999; Baud, 2001; Barrington, 2001; Halliday, 2002; Spinillo et al., 2004). This effect on brain growth and injury is more common when gestational age at birth is lower and when glucocorticoid treatment is administered early. A follow up of a cohort of children treated early for 4 weeks with dexamethasone showed a reduction in head circumference, motor performance and developmental performance at school age (Yeh et al., 2004). A study focused on behaviour and spontaneous mobility showed significant changes in the amplitude and speed of movement in treated children, disturbances correlated with the severity of brain injury and the subsequent occurrence of cerebral palsy (Bos et al., 1998). Considering the brain anatomy of the children treated, Murphy et al. used volumetric MRI performed at term equivalent of age to quantify the influence of the postnatal administration of dexamethasone on brain growth and cortical development in children having no white matter lesions or intraventricular haemorrhage (Murphy et al., 2001). He showed that dexamethasone induces a decrease in brain growth affecting both the cortex and basal ganglia. Similarly, repeated courses of prenatal glucocorticoids have been shown to decrease brain surface and the gyration index of the neonatal brain in antenatally exposed infants (Modi et al., 2001).

These recent data showing an obvious deleterious effect of dexamethasone on neurological development have pushed down the use of glucocorticoids in preterm infants. However, the prevention of BPD remains an unsolved challenge. Indeed, while dexamethasone has undeniable short-term benefits at the respiratory level, it is not possible to use because of its deleterious effects on the developing brain during the period when it would be most effective (the 1st week of life) and in the most immature infants.

1.3. Is there an alternative to dexamethasone in the prevention of BPD?

The therapeutic impasse observed with dexamethasone seems specific to this molecule, and its carrier and/or genomic and non-genomic targets could account for its toxicity (Baud et al., 2000, 2001, 2005). Indeed, all the side effects of perinatal steroids reported in the literature have mainly been observed with the parenteral administration of dexamethasone. Watterberg et al. looked for an alternative to this molecule, using a physiological glucocorticoid 30-fold less potent than dexamethasone: hydrocortisone hemisuccinate (HSHC). This molecule was initially tested as a “replacement” molecule in the prevention of BPD in very premature infants with adrenal insufficiency, characteristic of this population.

A pilot study demonstrated a significant protective effect of HSHC administration during the first 15 days of life (Watterberg et al., 1999). This was followed on a larger scale by the multicenter, randomised "PROPHET" trial ("prophylaxis of early adrenal insufficiency to prevent bronchopulmonary dysplasia")
In this trial, preterm neonates with a birth weight of 500-999 g, aged between 12 and 48 h and requiring mechanical ventilation were randomised to a "replacement" dose of hydrocortisone (0.5 mg / kg / 12 h for 12 days, followed by 0.25 mg / kg / 12 h for 3 days) vs. placebo. The primary endpoint was supplemental oxygen at 36 weeks of PMA. The trial was stopped prematurely after 360 infants were included due to a significant increase in gastrointestinal perforation in the treated group (9.4% vs. 2.2%, p <0.01). Survival without BPD (35.2% vs. 33.7%) and mortality rate (16.7% vs. 18.3%) were both identical between the HSHC and placebo groups. Nevertheless, HSHC was associated with a significant decrease in the incidence of BPD in children born with histological chorioamnionitis (n = 149; 38.4% vs. 23.7%, p <0.005). In the same sub-population, neonatal mortality was significantly reduced by HSHC (10% vs. 18%). However, the data from these post hoc analyses must be taken with caution. The significant increase in the incidence of gastrointestinal perforations early was explained by the combination of hydrocortisone with indomethacin given prophylactically before 24 hours of life. This was a highly questionable decision (Roberts, 2004). Indeed, the benefit / risk balance does not seem so unfavourable if one considers that that only 21/360 perforations were observed, and led to only 3 deaths, without any change in overall mortality between the HSHC and placebo groups (15% vs. 16% respectively). The increased risk of gastrointestinal perforation is known to be linked to the use of glucocorticoids in preterm infants and assessed by the Cochrane Database at 3% (Halliday et al., 2003). This risk is increased by the concomitant administration of a nonsteroidal anti-inflammatory drug (NSAID), itself a risk factor for gastrointestinal perforation and widely used in the treatment of patent ductus arteriosus, a risk factor of BPD (Fujii et al., 2002). This effect is even clearer when the NSAID treatment is administered early. In the PROPHET trial, most premature infants received prophylactic indomethacin within 24 hours of life. It is highly likely that the administration of an NSAID to patients in this trial even before HSHC administration is responsible for an increase in the incidence of gastrointestinal perforation. Data showing that there is no increased incidence of gastrointestinal perforations in other situations (indomethacin alone or HSHC only) reinforce this hypothesis.

The long-term outcome of children treated with HSHC is not yet known. However, some preliminary data suggest that HSHC is better tolerated than dexamethasone by the developing brain. Indeed, it has been reported that postnatal treatment with HSHC is associated neither with reduced head circumference at the age of theoretical term (Watterberg et al., 2004) nor a reduction in brain volume on MRI as previously described with the administration of dexamethasone (Lodygensky et al., 2005).

Results from the "PROPHET" trial suggest that HSHC could be of benefit in the prevention of BPD in newborns from pregnancies complicated by histological chorioamnionitis. However, this finding requires confirmation through a new randomised trial designed to answer the following specific question: "Can HSHC increase BPD-free survival in extremely preterm infants?" The current trial would establish the validity of a treatment based on a robust pathophysiological background, with an overall improvement in survival and morbidity in very preterm infants. This trial is also essential to limit off-label use of glucocorticoids during the neonatal period in very premature infants.

1.4. Expected benefits and foreseeable risks to the patient

Expected benefits:
- Hemodynamic stabilisation,
  - Attenuation of the course of early respiratory disease,
  - Decrease in the incidence of patent ductus arteriosus,
  - Reduction in the duration of ventilation,
  - Reduction of supplemental oxygen at term,
  - Improvement of neurocognitive performance at 2 years of age

Foreseeable risks:
- Hyperglycemia,
- Hypertension,
- Gastrointestinal perforation (the PREMILOC trial has been designed to reduce this risk by 75-90% risk compared to previous published studies on hydrocortisone use).

The gastrointestinal toxicity of HSHC was highlighted by the PROPHET trial (Watterberg et al., 2004). This toxicity was found to be due to the combination of indomethacin and HSHC. Indeed, in this study, the administration of HSHC or indomethacin alone was not associated with an increased incidence of gastrointestinal perforations compared to the untreated population. In addition, 2/3 of premature infants enrolled in the trial received indomethacin before 24 postnatal hours. More than 50% of them received indomethacin as a prophylactic treatment for patent ductus arteriosus. The prophylactic use of NSAIDs within 24 hours of life is not a common practice in participating centres in France and will be not
permitted for the overall duration of the PREMILOC trial. In addition, according to unpublished data, it no similar increase of the incidence of gastrointestinal perforations has been noted in a cohort of children treated with HSHC according to the same protocol than that used by Watterberg et al. (Drs Véronique Zupan-Simunek and Laurence Caymaex, CHU Antoine Béclère). Recommendations for the treatment of patent ductus arteriosus in patients enrolled in the PREMILOC trial are based on curative regimen of patent ductus arteriosus with hemodynamic consequences, beyond 24 hours of life. Introducing minimal enteral feeding with breast milk in very small amounts (<15ml / kg), as soon as possible after birth is recommended to reduce intestinal vulnerability. Finally, knowing that premature infants enrolled in the PROPHET trial who experienced gastrointestinal perforation had very elevated serum cortisol levels at D0, we propose to measure serum cortisol concentrations in the 100 first inclusions to look for a potential correlation between serious adverse events (including gastrointestinal perforation) and abnormally high serum levels (> +2 SD) of total cortisol. If such a correlation is observed, the values would be taken as a reference to not include subsequent patients with such high cortisol levels. In addition, the exclusion of neonates born of pregnancies characterised by IUGR aims to minimise the risk of serious adverse gastrointestinal events while targeting the population that could potentially benefit from HSHC administration, i.e. premature rupture of membranes, chorioamnionitis or spontaneous preterm labour. A sequential analysis will be conducted to identify any additional risk related to the treatment and stop the trial if necessary, according to the recommendations of the Data Safety Monitoring Board (DSMB).

1.5. Feasibility and expected results

The proposed clinical trial involves the participation of 24 level III perinatal centres in France. A preliminary demographic study estimates that 842 premature infants <28 weeks of gestation are admitted to these perinatal centres per year (Table 2). Among them, 80 to 85% of births occur in a context of perinatal inflammation (chorioamnionitis and/or prolonged membrane rupture and/or preterm labour), which allows us to estimate the annual number of eligible patients at 680. Consistently, it has been reported that 50-65% of preterm deliveries before 28 weeks of gestation have an infectious/inflammatory origin (Lahra and Jeffery, 2004) and premature rupture of amniotic membranes has been reported to be complicated by an intrauterine infection in 33% of cases (Gonçalves et al., 2002).

The expected outcomes of this clinical trial in a population of extremely preterm infants are:

- Reduction of the incidence of neonatal mortality,
- Reduction in the incidence of BPD defined as ventilatory dependence and / or persistent supplemental oxygen at 36 weeks of PMA,
- Reduction of patent ductus arteriosus occurrence,
- Reduction of the duration of primary and secondary hospitalisation,
- Reduction of costs associated with the management of specifically evaluated preterm infants (Pr Isabelle Durand Zaleski, Public Health, Henri Mondor Hospital).

The objectives of this trial are to test HSHC as a new treatment in the routine care of extremely preterm infants born in the context of perinatal infection or inflammation. A reduction of inflammation could lead to a reduction in neonatal mortality, the incidence of BPD at 36 weeks of PMA and functional sequelae associated with brain lesions in a context of chorioamnionitis and neonatal inflammation (Wu and Colford, 2000). Several subsequent long-term studies will be conducted on the cohort included in this clinical trial:

- Impact of HSHC on lung function assessed at 7 years of age,
- Impact of HSHC on the incidence of neuropsychological disorders at 18-24 months of corrected age and 7 years of chronological age,
- Ancillary studies detailed in the secondary objectives.

This research will be conducted in accordance with the present protocol, good clinical practices and the applicable laws and regulations in force.

2. RESEARCH PLAN: HYPOTHESIS AND OBJECTIVES

Low doses of hydrocortisone hemisuccinate (HSHC) for a limited period of time (10 days) may induce a pulmonary anti-inflammatory effect in extremely premature babies born in the context of perinatal infection or inflammation. Children born in the context of placental vascular pathology (IUGR <
3rd percentile) are excluded from this study to select for a very high risk of preterm histological chorioamnionitis births (encompassing the diagnosis of clinical chorioamnionitis, rupture of membranes, and preterm unexpected labour). Among these inflammatory criteria, the diagnosis of chorioamnionitis will subsequently be confirmed by standardised histological analysis of the placenta. The anti-inflammatory effect of HSHC administered shortly after birth (within 24h of life) may induce:
A decrease in neonatal mortality,
A decrease in inflammatory lung disease related to very premature birth and / or ventilatory support during the first days of life, and promote lung growth. Indeed, a growth arrest and alveolar septal defects characterise new forms of DBP (Bancalari et al., 2003).

2.1. Main objective
The main objective of this study was to test the utility of early administration of low dose HSHC in extremely premature babies born in the context of perinatal infection on reducing neonatal mortality and the occurrence of BPD, defined as the need for ventilatory support and / or administration of supplemental oxygen, at 36 weeks of PMA, as physiologically defined by Walsh et al.

2.2. Secondary objectives
Secondary objectives are:

• Evaluate the impact of HSHC on neonatal mortality and respiratory morbidities:
  o According to the gestational age at birth (2 strata: 24-25 weeks and 26-27 weeks)
  o Neonatal mortality at 36 weeks of PMA and before discharge,
  o Severity of BPD at 36 weeks of PMA,
  o Early respiratory complications (air leaks, pulmonary haemorrhage, persistent pulmonary hypertension),
  o The duration of ventilatory support, supplemental oxygen and final date for weaning,
  o The use of systemic and inhaled postnatal corticosteroid treatment beyond the treatment period with HSHC,

• Evaluate the impact of HSHC on extra-respiratory morbidities:
  o Patent ductus arteriosus and its treatments ¹,
  o Necrotising enterocolitis of grades > IIA according to Bell's classification (Bell et al., 1978),
  o gastrointestinal perforation,
  o cerebral white matter lesions and intraventricular haemorrhage,
  o late-onset sepsis,
  o insulin requirement during the first two weeks of life,

• The number and duration of readmissions beyond 36 weeks of PMA, determined at 1 and 2 years of chronological age (in days, all causes and respiratory causes)

• The safety of early administration of HSHC will be assessed on:
  o The incidence of gastrointestinal perforation,
  o The incidence of systemic Candida infections,
  o The incidence of severe brain lesions (including high-grade intraventricular haemorrhage and cystic periventricular leukomalacia),
  o The function of the HPA axis after treatment,
  o The incidence of severe retinopathy of prematurity,

Other ancillary studies:
  o Prospective analysis of the value of cord blood CRP, interleukins and procalcitonin concentrations as diagnosis markers of chorioamnionitis,
  o Thyroid function following the period of HSHC (or placebo) treatment

¹ Definition of patent ductus arteriosus: ductus arteriosus observed in echocardiography and at least two of the following criteria: a right atrium / aorta ratio > 0.48, null or retrograde diastolic flow in the anterior cerebral artery, pulsatile flow in the ductus arteriosus, diastolic flow in the pulmonary artery, velocity > 20 cm/s (Gournay et al., 2004)
3. RESEARCH DESIGN

3.1. Methodology

3.1.1. Type of study
The proposed study is a multicenter, randomised, double-blind, placebo controlled clinical trial. It tests the effect of parenteral administration of HSHC in extremely premature babies born in the context of inflammation / perinatal infection. This trial will benefit from a sequential analysis design justified in the statistical methods.

3.1.2. Justification of the study design
A controlled trial will attempt to demonstrate if a large premature population can safely benefit from early parenteral administration of HSHC. A multicenter study is needed to recruit an adequate number of patients and to take into account differences in management of preterm infants between level III perinatal centres. The treatment given is hidden in order to minimise the risk of bias in the administration of treatment and data collection. A placebo control group was chosen as the most appropriate way to objectively judge the effect of HSHC over time and between different participating centres.

3.1.3. Selection of the study population
All babies inborn in participating centres will be evaluated in terms of eligibility to be randomised. Their clinical characteristics will be compared to inclusion and non-inclusion criteria before inclusion in the study.

3.1.4. Number of patients expected
Given an incidence of 25% of overall neonatal mortality in the target population and an incidence of BPD in surviving infants at 36 weeks of PMA of 25%, the predetermined number of patients to be included to increase the proportion of BPD-free survival from 56% to 66% is 786. The rationale for the number of subjects to be included is detailed in the statistical methods.

3.1.5. Diagram of the trial

Step 1: Inclusion visit (as soon as possible and in all cases before the first 24 hours of life)

- Verification of inclusion criteria,
- Collection of informed signed consent,
- Collection of demographic and perinatal data including antenatal antibiotics, glucocorticoids, circumstances of birth, ethnicity, gestational age, sex, number of fetuses,
- Collection of the date and time of birth,
- Collection of postnatal time of randomisation and injection treatments,
- Concomitant treatments,
- Clinical examination (gestational age, ethnicity, weight, height and head circumference at birth, APGAR score at 1 and 5 min)
- Vital signs\(^2\), ventilation parameters\(^3\), including oxygen saturation, FiO\(_2\), ventilatory support, mean arterial pressure, blood glucose,

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\(^2\) CRIB score, mean arterial pressure (MAP), maximum glucose capillaire before inclusion, intravenous fluids or vasopressors, administration of exogenous surfactant

\(^3\) FiO\(_2\), inspiratory and expiratory pressure, mean airway pressure
• Cranial ultrasound (if possible before the initiation of treatment, and in any case within the first 72 hours of life),
• Sampling of blood before initiation of the treatment for determination of total serum cortisol levels.

Cortisol measurements will be done centrally at the Robert Debré hospital (Department of Biochemistry and Hormonology).
A correlation between serious adverse events and serum cortisol concentrations will be analysed for the first 100 patients. This analysis will indicate a threshold below which the risk of gastrointestinal perforation or other adverse effects associated with potentially excessive glucocorticoid exposure will be very low (adding, if necessary, a criterion for non-inclusion).
This decision will be based on the recommendations of the DSMB.

• **Collection of cord blood:**
  o Guthrie blood sample for subsequent assay of interleukins (IL1α, 6, 8, 9) and TNFα,
  o serum bank for subsequent hormone assays,
  o DNA library,
  o C-reactive protein, procalcitonin (assays performed locally at Robert Debré hospital).

• Reference chest X-ray before treatment,
• Histological analysis of the placenta.

**Step 2: Administration of treatment at a dose of 0.5 mg / kg / 12h starting before the 24th hour of life for 7 days and then 0.5 mg / kg / 24h for an additional 3 days.**

H12, H24 after the start of treatment: measurement of vital signs and ventilation, concomitant treatments and side effects.

**Step 3: Visits during and after the treatment period**

*During the treatment period (from D2 to D10):*
  o Daily vital signs, ventilation parameters
  o Echocardiography prior to D10

*End of the treatment (D12 or 48 hours after the end of treatment):*
  o Clinical examination
  o Vital signs, ventilation parameters
  o Measurement of thyroid function (T4 and TSH)

*At D21:*
  o vital signs, ventilation parameters
  o Cranial ultrasound,

*At D28:*
  o Clinical examination
  o Vital signs, ventilation parameters

*Visit at 36 weeks of PMA:*
  o Clinical examination
  o Vital signs, ventilation parameters, vital status
  o Cranial ultrasound
  o Oxygen reduction test according to Walsh et al. and BPD status according to physiological definition (cf. annexe 1)

*Patients transferred to another hospital before 36 weeks of PMA*
If patients are to be transferred to other hospitals before 36 weeks of PMA (other than recruiting centres), data related to this visit will be recovered in the following manner:
The data collection form for the visit at 36 weeks of PMA from the eCRF and Walsh O₂ reduction test are printed and sent together with the patient to the receiving centre.

- When the patient is transferred, these documents are attached to the medical record.
- The centre that receives the patient must complete, date, sign and return these documents to the centre where the patient was initially recruited.
- These documents will then be stored in the patient record.
- Meanwhile, reports of hospitalisation will be recovered.

End of hospitalisation date and status of patient information in the report forms

At 40 +/- 1 weeks of PMA: brain MRI with dedicated sequences.

After hospital discharge:
Parents will be contacted by phone every 4 months for news of their children, until the planned visit at 2 years.

Visit at chronological age of 1 year:
- Clinical examination,
- Vital parameters, vital status.

Visit at chronological age of 2 years:
- Clinical examination,
- Vital parameters, vital status,
- Standardized neurological examination and neurocognitive evaluation using revised Brunet Lézine scale.

Tests required specifically for research and centralised at the Robert Debré Hospital Paris

- Collection of cord blood:
  - Guthrie blood sample for subsequent assay of interleukins (IL1, 6, 8, 9) and TNF-α,
  - Serum bank for subsequent hormone assays,
  - DNA library.

- Other venous blood samples:
  - Total cortisol within the first 24 hours of life and before initiation of the treatment,
  - Dosage of T4 and TSH 48 hours after the end of treatment.

3.2. Overview of visits
<table>
<thead>
<tr>
<th>Examinations / Date</th>
<th>T0</th>
<th>H12 after initiation of treatment</th>
<th>H24 after initiation of treatment</th>
<th>D2 - D10</th>
<th>D21</th>
<th>D28</th>
<th>36 wks PMA</th>
<th>40PMA</th>
<th>Visit 1 yr</th>
<th>Visit 2 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acceptable time</td>
<td>+/- 1 h</td>
<td>+/- 8h</td>
<td>+/- 1 day</td>
<td>+/- 3 days</td>
<td>+/- 1 week</td>
<td>+/- 3 months</td>
<td>+/- 3 months</td>
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<td>Clinical examination</td>
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<tr>
<td>Ventilation parameters (FiO₂, O₂)</td>
<td>During the study period, at baseline and every 24 hours until the end of treatment</td>
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<td>Echocardiography (between D2 and D10)</td>
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<td>Cranial ultrasound</td>
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<tr>
<td>Sampling for determination of total cortisol BEFORE THE 1st INJECTION</td>
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<tr>
<td>Determination of T4 and TSH</td>
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<td>Creative assay protein, procalcitonin, interleukins</td>
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<td>Blood bank, DNA library</td>
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<td>Concomitant treatments</td>
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<td>Adverse events and severe adverse events</td>
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</tbody>
</table>
| Neurocognitive evaluation using the Revised Brunet Lazine scale | X                       | X                           | X                   | X             | X               | X               | X               | X*
3.3. **Blood samples and banking**

**Samples before the 24th hour of life (before the initiation of treatment)**

Venous blood
- Measurement of total serum cortisol concentrations: 1 ml in sterile dry tube.

Cord blood
- serum banking: 1 ml in sterile dry tube
- DNA banking: 5 ml in EDTA tube
- CRP and Procalcitonin: 1 ml in sterile dry tube
- Guthrie blotting type: 1 ml in heparinised tube

The histological analysis of the placenta will be performed by the fetopathology department of each centre with a standardised report.

D12 samples of venous blood
- Measurement of thyroid hormone (T4 and TSH) serum concentrations: 1 ml in sterile dry tube.

**Shipping of the samples**

- Measurements of CRP and procalcitonin will be performed by the biochemistry laboratory of each recruiting centre.

- The Guthrie blotting type will be addressed in a dedicated envelope provided for this purpose to the Laboratory of Biochemistry and Hormonology, Robert Debré Hospital.

- The other samples will be frozen at -20 °C and stored at each site. Removal will take place every three months at the request of the URC Paris-Nord for centralised analysis at the Robert Debré hospital.
  
  • The DNA banking will be performed at the Laboratory of Biochemistry and Genetics (Dr Suzanne KUZBARI, Tel.: 01 40 03 40 67)
  • The other assays will be carried out at the Laboratory of Biochemistry and Hormonology (Dr Jean-François BENOIST, Tel.: 01 40 03 40 42)

3.4. **Expected duration of the study**

The expected duration of the study is 6 years, including 4 years of inclusion. The duration of participation per patient is 2 years.

The patient cannot participate in another biomedical research project before 40 weeks of PMA.

3.5. **Randomisation**

Randomisation will be stratified by gestational age. Two lists will be established: for the first stratum, 24+0 to 25+6 weeks of gestation and the second stratum, 26+0 to 27+6 weeks of gestation.

Randomisation lists will be produced by the Clinical Epidemiology Unit of the Robert Debré hospital in four original copies signed and dated
  
  • 1 copy for the sponsor DRCD, APHP
  • 1 copy for the Biostatistician
  • 1 copy for Fernand Widal hospital
  • 1 copy for the AGEPS

A comprehensive document describing the randomisation procedure will be kept confidential and filed at the Centre and the DRCD.
**Procedure of randomisation and masking**

After verification of inclusion and non-inclusion criteria and obtaining consent, the newborn will be electronically randomised by computer. It will then be assigned a randomisation number (Randomisation No. = Treatment No. = No. of patient).

The number assigned to each patient randomisation is the first treatment number available at the centre for each stratum. For each list, a streamlined inventory management of medications will be implemented while maintaining a balance of randomisation centre.

Given the multicenter trial design, randomisation will be done via a dedicated website (Cleanweb®). At randomisation, an email will be sent to the Clinical Epidemiology Unit of the Robert Debré hospital in order to keep pace with the inclusions. The inclusion form will always include the initials of the newborn, date and time of birth, date of inclusion, the randomisation stratum and the identification of the physician who included the patient.

**Blinding lifting**

Before unblinding, the investigator will contact the study coordinator. In case of an absolute necessity to unblind, a procedure has been established for this protocol and the Fernand Widal anti-poison centre Paris or doctor can be reached by phone 24h/24h (01 40 05 48 48). These details will be specified on the patient card for participation in a clinical trial, which will be provided to parents upon their child's inclusion in the study. The date, time and reason for unblinding will be specified.

**3.6. Stopping rules**

The test may be stopped for the following reasons:

- Significant excess of gastrointestinal perforations in the treatment arm compared to the placebo arm and risk-benefit ratio of the test considered unfavourable by the independent monitoring committee (DSMB),

- Inhibition of adrenocortical axis imposing the pursuit an alternative treatment beyond the period of the treatment protocol in > 10% of children included in the first interim analysis,

- Detection of cerebral white matter lesions at 40 weeks of gestation whose incidence is twice that expected in this population at interim analyses (10 to 15%),

- Significant excess of intraventricular haemorrhage (IVH) of grade 3 and 4 or any other serious adverse events in the treatment arm compared to the placebo arm and risk-benefit ratio of the test considered unfavourable by the DSMB.

The trial safety will be evaluated by the DSMB at each interim analysis or when additional analyses are requested by the sponsor, the steering committee or by the Supervisory Committee.

To respond to ethical concerns about the study in a population at risk, and potential adverse effects of immediate HSHC, it was decided to analyse the data sequentially using a triangular plan. This will provide stopping rules (if the rate of success in the HSHC arm is at least 66% at any given moment, the conclusion of the trial will be obtained while reducing the number of patients by about 1/3 compared to the predetermined fixed number (approximately 520 children effective)).

**3.7. Procedure for premature discontinuation of the treatment**

- Severe digestive disorders: severe enteropathy, hematemesis, rectal bleeding, intestinal perforation or radiological pneumatisis before the end of treatment (see side effects of treatment). In all cases, the children will be followed until the end of the protocol.

- When treatment with HSHC outside the trial field seems necessary or essential to the investigator, he can stop the study treatment and continue to collect medical data on the child.
• A similar procedure will be followed in treatment by NSAIDs is deemed necessary before 24 hours of life in the event of persistent ductus arteriosus or in case of acute related morbidity.

3.8. **Patients leaving study**

• Withdrawal of consent

**Modality to replace these patients:** Patients leaving the study will not be replaced. A number assigned to a patient during processing, even in unused, cannot be reassigned.

Regarding patients whose parents have withdrawn their consent, they will be followed in the usual way according to the practice of each centre.

4. **SELECTION AND EXCLUSION CRITERIA TO RECRUIT PATIENTS**

4.1. **Inclusion criteria**

• Initial medical examination,
• Every newborn with a gestational age between 24+0 and 27+6 weeks of gestation born in a context other than those specified in the exclusion criteria,
• Neonates whose parental authority holders have signed consent forms,
• Neonates whose parental authority holders are covered by the social security system or CMU*

4.2. **Exclusion criteria**

• Preterm infants with a gestational age $\geq$ 28 weeks of gestation
• Congenital malformation and/or heart diseases other than patent ductus arteriosus or foramen ovale
• Rupture of membranes before 22+0 weeks of gestation
• Newborns from a pregnancy with > 3 fetuses
• Newborns whose birth weight is $<$ 500g
• IUGR <3rd percentile according to customised French curves (AUDIPOG)
• “Outborn” neonates
• Newborns whose parental authority holders are minor
• Newborns who will not be able to receive the full treatment (chromosomal abnormalities, severe birth asphyxia)
• Newborns whose parental authority holders are not beneficiaries of social security coverage.

**Justification of inclusion criteria:**
In the randomised trial "PROPHET" of Watterberg et al. (Watterberg et al., 2004), administration of HSHC was associated with a significant reduction in neonatal mortality and the incidence of BPD at 36 weeks of PMA in premature infants whose placentae were characterised by chorioamnionitic lesions. In the same study and in personal communications by the same author, 81% (29/36) of mothers with clinical chorioamnionitis had histological chorioamnionitis. In contrast, among 139 placentas with histological chorioamnionitis lesions, only 29 showed clinical signs of chorioamnionitis. Thus, limiting the inclusion criteria to only clinical chorioamnionitis may pass over true histological chorioamnionitis. Furthermore, according to Watterberg et al., 85% of ruptures of membranes $> 24h$ were associated with histological chorioamnionitis. Conversely, 75% of pregnancies with histological chorioamnionitis were not associated with a rupture of membranes $> 2h$. Thus, the presence of one or more criteria such as "clinical chorioamnionitis", "prolonged rupture of membrane" and "spontaneous preterm labour" are intended to detect the majority of histological chorioamnionitis.
5. TREATMENT GIVEN

5.1. Description of treatments

5.1.1. Studied treatment (HSHC)
Lyophilisate for injection containing 100 mg of hydrocortisone sodium succinate (DCI) / 2 ml (corresponding to HYDROCORTISONE UPJOHN® 100 mg injection) in a format compatible with a double-blind trial.

5.1.2. Placebo
Vials will be identical to those containing HSHC in accordance with Good Manufacturing Practice for Medicinal Products for Clinical Trials appearance. Lyophilisate and formula will be guaranteed for safety (similar qualitative and quantitative formula as active ingredients of the studied drug with the exception of the absence of hydrocortisone sodium succinate, replaced by a small amount of non-pyrogenic mannitol).

5.1.3. Dosage and administration
Treatments will be administered by slow intravenous infusion of an equivalent volume of HSHC or placebo. Hydrocortisone hemisuccinate dosage will be of 0.5 mg / kg / 12hours for 7 days, then 0.5 mg / kg / 24hours for 3 days (slow injection after dilution in a final concentration of 1 mg/ml).

Treatment should be initiated as soon as possible before the 24th hour of life.

5.2. Pharmaceutical circuit

5.2.1. Presentation of treatment boxes
Each box, pre-numbered according to the randomisation list for each of two groups of gestational age, and sealed contains:
- 20 vials of lyophilised HSHC 100 mg (corresponding to the commercial product HYDROCORTISONE Upjohn 100 mg for injection) or 20 vials of lyophilised PLACEBO of identical appearance according to Good Manufacturing Practices guidelines.
- 20 ampoules of 2 ml of water for injection.

The vials are for single use, with 3 extra bottles per box. The diluted solutions are used extemporaneously, residue to be destroyed.
Records of Use / continuation sheets administrations will be provided with the patient box.
Pursuant to the requirements of Good Manufacturing Practices dated 26 May 2006, a patient card will be given to parents.

5.2.2. Supply centres
An initial stock will be sent to each centre by AGEPS at the request of the URC.
Pharmacies within the centres will distribute boxes to the care service, allowing rapid implementation of treatment.

5.2.3. Replenishment centres
Replenishments of centres will be requested of the AGEPS by the URC according to the rhythm of inclusions at each centre.

5.2.4. Method of accounting for treatment units
No opened vials or empty vials will be kept empty for accounting but will be immediately destroyed at the care facility according to the usual procedures.
Empty boxes or boxes containing unused patient vials will be returned to the internal-use pharmacy at each participating centre and will be retained until the passage of the clinical research assistant
The CRA will conduct an accounting of treatment units when monitoring recruiting centres. After accounting, boxes will be returned to the AGEPS for destruction following completion of the final test report.

5.3. Permitted and prohibited drug treatments during the protocol

Prohibited drugs:

- Any steroid administered parenterally during the treatment period
- NSAIDs (ibuprofen or indomethacin) during the first 24 hours of life

Other treatments are permitted depending on the practices of each centre.

If hypotension occurs, the use of dopamine or volume expansion is allowed. However, if HSHC treatment appears necessary or essential to the investigator, he can stop the study treatment and continue to collect medical data concerning the child. The same is true if treatment by NSAIDs is deemed necessary before 24h of life in case of significant patent ductus arteriosus.

6. ÉVALUATION CRITERIA

6.1. Primary endpoint

All premature babies surviving without BPD at 36 weeks of PMA will be a considered as "success." All premature babies who die or survive with supplemental O₂ or dependent on any invasive or noninvasive positive-pressure ventilation, whatever the FiO₂, up to 36 weeks of PMA will be considered "failure".

A combined criterion was chosen because it allows an increase in the statistical power of the trial and thus the possibility of obtaining positive findings from a smaller number of patients than if only one criterion is chosen (Freemantle et al., 2003). Each of the criteria in the combined criterion will be assessed as a secondary endpoint as recommended by Freemantle et al.

Definition of BPD at 36 weeks of PMA

The diagnosis of BPD will be determined at 36 weeks of PMA +/- 3 days based on the following criteria:

- Any child under ventilatory support, such as mechanical ventilation or noninvasive positive-pressure ventilation,
- Any child under spontaneous ventilation with an FiO₂ > 0.30 (see Table 1 in Appendix 1),
- Any child under spontaneous ventilation with an FiO₂ between 0.22 and 0.30 (see Table 1 in Appendix 1) and requiring oxygen as evidenced by the oxygen reduction test of Walsh et al. (see Appendix 1).

6.2. Secondary endpoints

The impact of HSHC on the following predetermined secondary end-points will be assessed:

- BPD at 28 days of life and 36 weeks of PMA,
- Neonatal mortality at 36 weeks of PMA and before discharge,
- Early respiratory complications (air leaks, pulmonary haemorrhage, persistent pulmonary hypertension),
- The duration of ventilation, supplemental oxygen and date at final weaning before discharge,
- The use of systemic or inhaled postnatal corticosteroids (HSCH or other steroids) beyond the treatment period,
- Impact of HSHC according to the detection/presence of chorioamnionitis
- Extra-respiratory morbidities assessed as secondary outcomes:
  - Patent ductus arteriosus and its treatments,
  - Necrotising enterocolitis of grades > IIA according to Bell’s classification (Bell et al., 1978),
• gastrointestinal perforation,
• cerebral white matter lesions and intraventricular haemorrhage,
• secondary sepsis,
• insulin requirement during the first two weeks of life,
• The number and duration of re-hospitalisations beyond 36 weeks of PMA, determined at 1 and 2 years of chronological age (in days, all causes and respiratory causes),

The safety of early administration of HSHC will be assessed on the basis of:
• The incidence of gastrointestinal perforation,
• The incidence of systemic Candidiasis or other late-onset sepsis,
• The incidence of severe retinopathy of prematurity,
• Thyroid function after the treatment period,
• Brain MRI at term-equivalent age (40 +/-1 weeks of PMA),
• Neurocognitive functions at 18-24 months of corrected age using the revised Brunet Lézine scale, and standardized neurological examination.
• Estimates for the total hospital costs.

7. SAFETY ASSESSMENT

7.1. Definition of parameters for assessing safety

• Adverse event
Any harmful event occurring in a person who is enrolled in biomedical research whether or not this event is related to the research or product being researched.

• Adverse event of an investigational drug
Any noxious and unintended responses to an investigational drug at whatever dose.

• Serious Adverse Event (SAE)
Any event or adverse reaction which results in death, endangers the life of the person enrolled in the trial, requires hospitalisation or prolongation of existing hospitalisation, causes persistent or significant disability, or results in an anomaly or a birth defect, and in the case of a drug, regardless of the dose administered.

• Suspected Unexpected Serious Adverse Reaction (SUSAR)
Any adverse reaction, the nature, severity or outcome of which is not consistent with the information contained in the summary of product characteristics when the drug is authorised, and in the investigator's brochure when not authorised.

• Developments
Any new security data that could lead to a reassessment of the benefits and risks of research or an experimental drug, or may be sufficient to consider changes in the administration of the investigational drug in the conduct of research.

7.2. Specific research committees

Steering Committee
It will consist of the physicians initiating the project, the biostatistician in charge of the project, the representatives of the developer and the URC appointed for this research.

It will define the general organisation and conduct of the research and coordinate information. It initially determines the methodology, monitors the progress of the research, in particular with regard to tolerance and adverse events.

Independent Oversight Committee
It has an advisory function when the sponsor appeals to it regarding medical issues such as tolerance and adverse events. It consists of people outside the research and recruiting centres who are necessarily specialists in the field, a pharmacologist and a methodologist/biostatistician.
It will meet at the request of the sponsor or the coordinating investigator. At its first meeting, the Committee will establish its rules of procedure and decision.

In particular, in the PREMILOC trial, the committee will meet and analyse the correlation between serious and abnormally high levels of cortisol in the first 100 patients. This analysis will be used for subsequent inclusions to set the threshold below which the risk of gastrointestinal perforation or other adverse effects related to glucocorticoid overdose should be the lowest. This decision will be implemented as recommended by the committee.

The sponsor will inform the Committee in real time of any SAEs that could lead to the premature termination of the trial, whether or not these SAE could be considered to be related to the experimental or drug treatment arms (arms A or B).

The sponsor shall transmit to the Committee every three months a summary table listing the SAEs that have occurred since the beginning of the trial by treatment arm.

The Committee will have the opportunity to ask the sponsor for the unblinding of one or several enrolled patients.

7.3. Procedures for the recording and reporting of adverse events

• Non-serious adverse events:
  All non-serious adverse events observed up to the 36 weeks of PMA visit must be entered in the case report form in the section provided for this purpose.

  A single event should be reported per item. The event may be a symptom, a diagnosis or supplementary examination results considered to be significant. All clinical and paraclinical evidence required to best describe the event must be reported.

• Serious adverse events (SAE):
  
  Investigators should immediately notify the AP-HP sponsor of SAEs as defined above. Complete investigator SAE forms (from the observation research notebook) and send them to DRCD fax 01 44 84 17 99 within 48 hours (if possible after an immediate phone call to 01 44 84 17 23 in the event of death or life-threatening complications).

  SAEs will have to be reported to the sponsor for up to 36 weeks of PMA, except death or any SAEs potentially related to experimental drug, which must be notified up to 2 years of age.

  For each SAE, the investigator shall issue an opinion as to the cause of the event with regard to the experimental drug or other possible treatments.

  Obtaining information on the description and evaluation of an adverse event may not be possible in the time allotted for the initial declaration.

  Also, any clinical findings and diagnostic examinations and / or laboratory results, or any other information allowing an adequate analysis of cause must be reported:

  On the initial SAE declaration if it is readily available.
  Or later and as soon as possible by faxing a new completed SAE declaration (specifying that it is a follow up of an SAE previously reported, with the tracking number).

  All statements made by the investigators must identify each research participant using the unique code number, which is the same as the inclusion number assigned to each individual.

  If reporting the death of a participating subject, the investigator shall provide the sponsor with all additional information requested (report of hospitalisation, autopsy results ...)

  Any event that occurs in clinical or experimental research or new data in the literature must be communicated to the sponsor.

  Reporting of SAEs to health authorities
This will be carried out by the “Division of Pharmacovigilance DRCD” after assessing the severity of the causal link between the SAE and the experimental drug or any other treatments as well as the unexpected side effects of these treatments.

All suspected unexpected serious adverse reactions (SUSARs) will be reported by the sponsor to the competent authorities within the statutory time.

Any safety data or any developments that may significantly alter the evaluation of the benefits and risks of an investigational drug, or research, or that could lead to the consideration of changes regarding the administration of medication or driving research will be provided by the sponsor to the competent authorities, the “Comité de Protection des Personnes” and investigators of the research. For example:

a) Any clinically significant increase in the frequency of expected SAEs;
b) SUSARs occurring among participants who completed the trial and communicated by the investigator to the sponsor, as well as any follow-up reports;
c) Any developments related to the procedure of the clinical trial or drug development, where the new development is likely to affect the safety of patients. Examples:
   • A serious adverse event possibly related to investigations and diagnostic procedures of the trial and that could change the course of this trial,
   • A significant risk to the patients enrolled in the trial such as a lack of efficacy of the drug used in the treatment,
   • Significant safety results from a pre-clinical study recently completed (such as a carcinogenicity study),
   • Early termination or a temporary interruption for safety reasons of a trial carried out with the same drug in another country,
   • A SUSAR to a non-experimental drug needed to carry out the trial (e.g. “challenge agents’ rescue therapy)
d) the recommendations of the independent monitoring committee, when relevant to the safety of patients,
e) Any SUSAR transmitted to the sponsor by another sponsor of another clinical trial conducted on the same drug in another country.

7.4. **Terms and duration of follow up after the occurrence of adverse events**

Any patient with an adverse event should be followed until resolution or stabilisation thereof.

- If the event does not matter, changes will be noted on the page of the report form in the space provided for this purpose.
- If the event is serious, follow-up of the SAE will be sent to the DRCD.

8. **DATA MANAGEMENT AND STATISTICS**

8.1. **Sample size calculation**

The primary outcome is survival without BPD at 36 weeks of PMA. The calculation of the sample size (number of subjects required) is based on the combined incidences of BPD and death at 36 weeks +/- 3 days PMA. The percentage of expected deaths in the studied population is 25% and the occurrence of BPD 25% in surviving children at 36 weeks +/- 3 days PMA. Overall, the percentage of expected events (death or BPD) is 44% (among 100 newborns, 25 will die and 19/75 of survivors will develop BPD = 44/100 events). It is assumed that the HSHC will decrease neonatal mortality from 25% to 20% and reduce the incidence of BDP from 25% to 18% of survivors at 36 weeks +/- 3 days PMA (leading to a total of 34/100 events).

In total, a decrease of the proportion of total events from 44% to 34% is expected, corresponding to a success rate of 56% in the placebo arm and 66% in the treatment arm. By setting the alpha risk at 5%, and the beta risk at 20% in bilateral formulation, and balancing randomisation (1/1), 393 subjects should be included per group to show an absolute difference of 10% between the success rate in the HSHC group (66% success) compared to the placebo group (56% success).
To address the ethical concerns of studying a high-risk population (extremely preterm neonates), potential immediate SAEs of HSHC, and the anticipated length of time of inclusion (at least 2 years), the number of premature infants planned for a fixed analysis (n = 786) and the endpoint measured at a fixed date (36 weeks of PMA, meaning no later than 12 weeks after inclusion), we decided to adopt a sequential data analysis method using a triangular plan as described below. This will provide stopping rules. If the success rate in the HSHC arm is at least 66% at any interim point, the conclusion of the trial will be obtained with a reduction in the number of patients by about 1/3 compared to the original fixed number (i.e. approximately 520 children).

8.2. Statistical stopping rules

Sequential plan

In the PREMILOC randomised placebo-controlled trial, analysis will be done after every 100 patients reach the primary outcome on the basis of intention to treat, using a stratified triangular test (Whitehead and Stratton, 1983). This triangular test design tests sequentially if the success rate (π) with placebo treatment is 56% (p₀, H₀ null hypothesis) or different from 66% in absolute value (pₐ, alternative hypothesis) where p₀ and pₐ are two constants of interest in the context of the disease and the treatments studied. Stopping rules will be constructed from the triangular sequential plan.

The first step is to determine the alpha and beta risks, p₀, pₐ and the number of interim analyses. Conventionally, the alpha and beta risk are determined to be 5% and 20%. P₀ is the success rate below which the study treatment is considered "ineffective" and does not warrant further study; it is set to 56%. Pₐ is the success rate in the HSHC arm that is considered to be beneficial; it is set at 66%. Interim analyses are planned for every 100 enrolled children and evaluated consecutively.

Based on these assumptions (α = 5%, β = 20%, interim analyses for every 100 included children, the probability of success of 56% in the placebo arm and 66% in the HSHC arm) the boundaries of the triangular are the following:

• The upper boundary, rejection of H₀: Z = 10.1 + 0.15V
• The lower boundary, non-rejection of H₀: Z = -10.1 + 0.45V

V is a schematic measure of the number of included patients; Z is the difference between the observed and expected success rates under H₀. The coordinates of the intersection of the two lines (apex of the triangle) are obtained by solving the two previous equations. In this case Zₐpex = 20.2 and Vₐpex = 68,1
According to this design, the number of patients needed to meet the assumptions and calculated for a fixed analysis will be reached at the 8th interim analysis. This experimental design should help to stop the trial earlier either because of a favourable effect of the new treatment (if the sample path of the score statistic has crossed the upper boundary of the test continuation region), or because of the lack of effect of the new treatment being tested (if the sample path of the score statistic has crossed the lower boundary of the test continuation region) and would help to reduce the number of patients needed on average by $1/3$, that is, it would be possible to stop after the inclusion of 520 children and the 5th or 6th interim analysis. In case of an unfavourable situation (error on the initial assumptions) to exit the triangle for the same reasons as mentioned above, the number of inclusions will be greater ($n = 822$) than that calculated for a fixed analysis at the end of the trial ($n = 786$).

### Statistical analysis

Statistical analysis will be conducted under the supervision of Pr. Corinne Alberti, Clinical Epidemiology Unit, Hôpital Robert Debré.

### Descriptive analysis

Qualitative variables are described as numbers and percentages and quantitative variables as means (standard deviation) or medians (first and third quartiles) according to the distribution. Comparisons will use parametric or non-parametric tests depending on the nature of the variables. The tests will be bilateral with a significance level of 5%. SAS v9.3 software will be used.

### Primary analysis plan

#### Sequential analyses

A statistical analysis is performed each time a group of 100 consecutive premature infants is evaluated. It is performed on the primary endpoint. It consists of determining the total number of successes observed since the beginning of the trial in both arms and estimating the values of $V$ and $Z$. The point defined by the coordinates $(V, Z)$ is shown in the triangular area. The trial is pursued as long as the sample path consisting of the points defined by the coordinates $(V, Z)$ remains inside the continuation region. At each interim analysis, the data base is frozen. If the upper boundary of the triangle is reached, the trial is stopped because the HSHC treatment is beneficial. If the lower boundary of the triangle is reached, the trial is stopped because the HSHC treatment is harmful or
because the treatment has no effect and there is no significant difference between the 2 arms. At the end of each inconclusive interim analysis, the only result given will be the non-crossing of the boundaries.

At each planned interim analysis, the rate of adverse effects corresponding to the stopping rules outlined above will be estimated.

Terminal analysis

The final statistical analysis will be conducted according to the principle of intention to treat (i.e. analysis of all patients included in the study regardless of the treatment received) after termination of inclusions (see section on stopping rules). It will be performed on all neonates enrolled in the trial, including those who did not participate in the triangular test during an interim analysis. The difference between the success rates observed in both arms along with its 95% confidence interval will be estimated. The comparison of success rates between the 2 arms will be performed using the stratified triangular test. We will estimate the NNT (number needed to treat: i.e. the number of premature babies treated with HSHC to observe one beneficial effect of this treatment) and its 95% confidence interval. An adjustment by logistic model will be performed to take into account potential confounding factors.

Secondary pre-defined analyses

At the termination of the trial, the following pre-defined secondary analyses will be conducted:
- Correlation between cortisol levels and serious adverse effects in the 100 first inclusions,
- Comparison between the two treatment arms:
  - mortality rate at 36 weeks of PMA and before discharge,
  - HSHC effect according to the stratum of gestational age (24-25 weeks vs. 26-27 weeks)
  - the rate of BPD among children surviving at 36 weeks of PMA,
  - the final weaning date from ventilatory support and supplemental oxygen,
  - effect of HSHC on the subpopulation exposed to chorioamnionitis,
  - extra-respiratory morbidity including patent ductus arteriosus and its treatments, necrotising enterocolitis, gastrointestinal perforation, severe brain damage,
  - detection of cerebral white matter lesions on brain MRI at 40 +/- 1 weeks of PMA,
  - number and duration of readmissions beyond 36 weeks of gestation, determined at 1 and 2 years of chronological age (in days, all causes and respiratory causes),
  - tolerance of early administration of HSHC assessed by the incidence of bowel perforations and the function of the HPA axis after treatment,
  - the incidence of severe forms of retinopathy of prematurity (grade > III),
  - neurocognitive development at 18-24 months of corrected age using a revised Brunet-Lézine scale and a standardized neurological examination.

8-3 Ancillary studies also involved in the PREMILOC trial

- Evaluation of the diagnostic value of CRP and procalcitonin in biological detection of chorioamnionitis. The presence of chorioamnionitis will be confirmed by a pathological examination. The diagnostic value of CRP and procalcitonin will be reviewed by the ROC curves, sensitivity, specificity and likelihood ratios.
- Study costs (Pr Isabelle Durand-Zaleski, Public Health, Henri Mondor Hospital)

The costs will be estimated from the perspective of the hospital for a period of 2 years. For the initial admission, the costs will be estimated from the length of hospitalisation and cost accounting figures and unit consumption data collected in the case report form. The valuation will be made from the purchase price and unit cost of returns to the AP-HP. Hospital costs will be estimated from the costs of GHS (“groupe homogène de séjour”, Homogeneous Group Stay) and GHM (“groupe homogène de malade”, Homogeneous Group III). Non-hospital costs will not be considered.
- Study the impact of treatment with cerebral HSHC evaluated by MRI at 40 +/- 1 weeks of PMA, centrally reviewed by 2 radiologists blind to the treatment and primary outcome.

Method for dealing with missing and unused data

Missing data will not be replaced. If the primary endpoint is involved in these missing data, it will be encoded as a “failure” to preserve the intent-to-treat analysis.
9. **RIGHT OF ACCESS TO DOCUMENTS AND DATA SOURCE**

People with direct access in accordance with laws and regulations, including articles and L.1121-3 R.5121-13 code of public health (e.g., investigators, those responsible for quality control, monitors, clinical research assistants, auditors and all those called on to collaborate in testing) take all necessary precautions to ensure the confidentiality of information relating to experimental drugs, tests, people who are suitable and particularly with regard to their identity and the results obtained. The data collected by these people during quality checks and audits are then anonymised.

10. **CONTROL AND QUALITY ASSURANCE**

The research will be framed according to the standard operating procedures of the promoter. The progress of research in the study centres and support issues will be reported in accordance with the Declaration of Helsinki and Good Practices guidelines.

10.1. **Monitoring procedures**

CRAs, as representatives of the sponsor, will conduct visits at recruiting centres at a rate that matches the level of risk D (highest risk level) attributed to the research with the pattern of monitoring patients in the protocol and the inclusion rates at the various centres.
- Initial visit at the opening of a centre: Before inclusion, for implementation of the protocol and getting to know the various parties in biomedical research.
- On subsequent visits, case report forms will be reviewed to monitor the progress of research by CRA. The principal investigator of each centre as well as other investigators who enrol patients in the trial agree to receive specific training and meet the CRA at regular intervals.

During these site visits and in accordance with Good Clinical Practice, the following items will be reviewed:
- Compliance with the protocol and procedures defined for research,
- Verification of informed consent of patients
- Review of sources and comparison with instructions regarding data accuracy, missing data, data consistency according to the rules based on DRCD procedures.
- Closing visit: recovery of consent forms, pharmacy records, biomedical research documents, archiving.

10.2. **Transcription of data in the electronic case report form (e-CRF)**

The research data will be collected and monitored using the Workbook Observations Electronics CleanWEB, under the Public Contract concluded between the AP-HP and TELEMEDICINE TECHNOLOGIES SA, published on 17/11/2003 and referenced under the No. 033845. These data will be hosted at a centralised Department of Operational Services (DSO) of the AP-HP, 67 boulevard Bessières server, 75017 PARIS.

Data analysis will be performed at Robert Debré UEC under the responsibility of Pr. Corinne Alberti. SAS software will be used.

A first version of the e-CRF can be downloaded online and tested after acceptance of the Promotion of Research agreement by the DRCD, the release of funds and placement of the order with the TELEMEDICINE company. After the agreement of the Coordinator and Project Manager as to the final version of the e-CRF, the DRCD Coordinator gives permission to start research (standard letter Inv. 14), and the e-CRF is produced.

All information required by the protocol must be provided in the e-CRF and the investigator must explain all missing information.

Both clinical and paraclinical data will be transferred to the e-CRF in real-time.

The anonymity of the subjects will be protected with a coded number and the initials of the person in charge of retrieving documents necessary for research, and the deletion by appropriate means of personal data on copies of source documents, for the documentation of the research.

Computerised data on file will be declared to the CNIL using the appropriate procedure.
11. LEGAL AND ETHICAL CONSIDERATIONS

The sponsor is defined by the law 2004-806 of 9 August 2004. In this research, AP-HP is the sponsor and the Department of Clinical Research and Development (DRCD) ensures regulatory tasks.

11.1. Request authorisation from the AFSSAPS (Agence Française de Sécurité Sanitaire des Produits de Santé)

Before starting the research, AP-HP as a sponsor must submit a permit application to the competent authority, the AFSSAPS. The competent authority, as defined in Article L.1123-12, pronounces as to the safety of people who are suitable for biomedical research, especially considering the safety and quality of the products used during the research under, where appropriate, the references in force, the conditions of use and the safety of persons in respect of acts performed and methods used as well as arrangements for tracking people.

11.2. Request for an opinion to the Committee of People Protection (“Comité de Protection des Personnes”, CPP)

In accordance with Article L.1123-6 of the Code of Public Health, the research protocol will be submitted by the sponsor to the Committee of People Protection (CPP) Ile de France II. The competent authority is notified of opinion of the committee by the sponsor before the research can start.

11.3. Changes

The DRCD must be informed of any proposed changes to the protocol by the coordinating investigator.

The changes must be described as significant or non-significant.

A significant change is defined as a change that could modify the guarantees given to subjects participating in Biomedical Research (modification of an inclusion criterion, extension of the period of inclusion, participation of new recruiting centres, ...).

After the beginning of the research, any substantial change proposed by the sponsor must obtain a favourable opinion from the Committee and approval of the competent authority prior to its implementation. In this case, if necessary, the committee ensures that a new consent of research is collected from participants.

Furthermore, any extension of the research (substantial modification of the regimen or populations included, or extension of treatment and therapeutic procedures not originally planned in the protocol) should be considered as a new research protocol.

Any substantial change necessitates a reapplication for authorisation by the AFSSAPS and / or a new review by the CPP.

11.4. CNIL declaration

The law requires that a statement regarding the computerised filing of personal data collected for the research be submitted before the beginning of the research.

Specific methods for the processing of personal data associated with biomedical research are defined by the law 2004-806 of 9 August 2004 within the scope of Articles L.1121-1 and following the Code of Public Health established by the CNIL in January 2006.

This methodology allows a simplified declaration procedure when the nature of the data collected in research is consistent with a list provided by the CNIL in its reference document.

When the research protocol is within the scope of this simplified procedure CNIL, the DRCD, as sponsor, asks the project manager to assume the responsibility for respecting this methodology MR06001.

The collection of data "ethnicity" is justified by significant differences already reported in the literature or expected and related to BPD, treatment response and prognosis of the population studied.
11.5. **Information of the patient and consent**

Written consent must be obtained from the holders of parental authority before inclusion of patients.

When the father is absent:

- Written consent is systematically obtained from the mother **BEFORE** the inclusion of the neonate(s) and treatment administration.
- Oral consent should be obtained by telephone contact with the father, after explanation of the protocol.
- The written consent of the father will be collected as soon as possible.
- The procedure for obtaining consent will be described in the source file of the child, where the date and time of collection of oral consent will be specified.

11.6. **Final research report**

The final research report will be written in collaboration with the coordinator and biostatistician for this research. This report will be submitted to each of the investigators for review. Once a consensus has been obtained, the final version must be endorsed by the signature of each of the investigators and sent to the sponsor as soon as possible after the effective end of the research. A report must be submitted to the competent authority as well as the CPP within one year after the end of the research, meaning the last follow-up visit of the last enrolled patient. This period is fixed at 90 days in case of early termination of the trial.

12. **DATA PROCESSING AND STORAGE OF DOCUMENTS AND DATA RELATED TO THE RESEARCH**

Documents related to any biomedical research must be stored by all parties for a period of 15 years after the end of the study.

This includes indexed archiving:

- Successive versions of the protocol (identified by the version number and version date)
- By the coordinating investigator: certificates of authorisation from the competent authority of the CPP for research concerned
- Correspondence with the sponsor
- Signed consents of persons undergoing research under seal (in the case of minors signed by the holders of parental authority) with a list or register of inclusion.
- The printout of the completed and validated clinical observation of each subject included (automatically dated), signed by the Principal Investigator or investigators involved in the research
- The audit trail
- Manual Data Handling, document in which the e-CRF is described accurately
- All specific research annexes
- The final research report from the statistical analysis and research quality control (transmitted in duplicate to the sponsor). During the closing visit, the CRA will take with him/her an external CD burner drive to record on CD-ROM the following documents:
  - Patient CRFs in pdf format, with fax randomisation generated by CleanWEB
  - Emails relating to research
  - The audit trail and electronic requests for correction.

This CD-ROM will be filed at the Investigator's center, along with the other documents.

The database used for statistical analysis will also be stored by the principal methodologist.

13. **INSURANCE AND SCIENTIFIC COMMITMENT**

13.1. **Insurance**

The Assistance Publique - Hôpitaux de Paris is the sponsor of this research. In accordance with the Law on biomedical research, it has taken out insurance with the company GERLING KONZERN for the duration of the research, ensuring its own liability as well as that of any parties (doctors or staff involved in the implementation of the research) (Act No. 2004-806, Art L.1121-10 CSP).

The Assistance Publique - Hôpitaux de Paris reserves the right to interrupt the research at any time for medical or administrative reasons; in this case, a notification will be provided to the investigator.
13.2. **Scientific commitment**

Each investigator will undertake to meet the requirements of the law and to conduct research according to Good Clinical Practices, and in accordance with the terms of the Helsinki declaration. To do this, a copy of the scientific commitment (document DRCD document template) dated and signed by the principal investigator of each recruiting centre will be given to the representative of the sponsor.

14. **RULES FOR ADVERTISING**

The Assistance Publique - Hôpitaux de Paris owns the data and no use or transmission to a third party may be made without prior consent.

The first signatories of publications will be those individuals who actually took part in the preparation and conduct of the protocol as well as in the writing up of results. The Assistance Publique - Hôpitaux de Paris should be mentioned as the sponsor of the biomedical research, and financial support if applicable. The terms "Assistance Publique - Hôpitaux de Paris" must appear in the authors' affiliations.

15. **RÉFÉRENCES**

- Fujii AM, Brown E, Mirochnick M, O'Brien S, Kaufman G. Neonatal necrotizing enterocolitis with intestinal perforation in extremely premature infants receiving early...


• Stevens TP, Blennow M, Stoll RF. Early surfactant administration with brief ventilation vs selective surfactant and continued mechanical ventilation for preterm infants with or at risk for respiratory distress syndrome. Cochrane Database Syst Rev. 2004;(3):CD003063.
16. ANNEXES

ANNEXE 1: Reduction Oxygen Test (Walsh et al., 2004)
ANNEXE 2: Guidelines for management of very preterm infants
ANNEXE 3: MAP Patient
ANNEXE 4: SAE grid for BioMedical Research
ANNEXE 5: Declaration of a serious adverse event (SAE)
ANNEXE 6: Investigators’ coordinates
ANNEXE 7: Classification of intraventricular haemorrhage according to Papile et al.
ANNEXE 8: Bell Classification (Bell et al., 1978)

The children will be positioned on their backs with a pulse oxymeter sensor (ideally Nelcor N-200) correctly attached to the end of a member, and administered their usual O₂ concentration 30 minutes after a meal.

The test consists of four phases: basal state, reduction phase in air, observation phase, then back to the usual concentration of oxygen.

**Basal state:** heart rate, respiratory rate, oxygen saturation, and frequency of apnoea (defined as the cessation of breathing for 20 seconds) and bradycardia (defined as heart rate <80 / min for ≥ 10 seconds) will be monitored continuously and recorded by direct observation every 60 seconds for 15 minutes. Any artefact linked to movement (defined as a visible movement of the child coupled with the loss of the plethysmographic signal displayed on the monitor) will be indicated and the corresponding oxygen saturation value will not be taken into account. If basal SpO₂ > 90% in FiO₂ ≤30%, the child can be subjected to Oxygen Reduction Test.

**Oxygen reduction phase:**
- Children under a hood: FiO₂ reduction in 2% increments every 5 minutes up to 21%, under continuous monitoring of clinical signs and SpO₂.
- Children receiving oxygen by nasal cannula:
  1. If the FiO₂ is controlled by an Air-Oxygen blender: FiO₂ reduction in 2% increments every 5 minutes, while nasal flow is maintained. Once the child is in air, the flow will be reduced by 0.5L / min increments every 5 minutes from 2 to 1 L/min, then 0.1 L/min from 0.9 to 0.1 L/min. The nasal cannula will be removed from the nostrils of the child, but left attached to the face so as not to disturb the child by the occurrence of discomfort.
  2. If the FiO₂ is adjusted only by pure oxygen flow (FiO₂ 100%) through the nasal cannula (see table below), the O₂ flow is progressively reduced by steps of 0.5 L/min every 5 minutes from 2 to 1 L/min, then 0.1 L/min from 0.9 to 0.1 L/min. The nasal cannula will be removed from the nostrils of the child, but left attached to the face so as not to disturb the child by the occurrence of discomfort.

**Observation phase/conclusions of the test:**
1. If the child tolerates breathing in air for a period of 30 minutes = No BPD.
2. If the child desaturates to < 90% in air for 5 consecutive minutes or < 80% for 15 seconds during this period of 30 minutes = BPD.

**Back to basic oxygen:**
At the end of the oxygen reduction test, the child may be given oxygen again.
If a single failure criterion is met at any time, oxygen therapy should be re-established immediately.

Determination of FiO₂ actually applied (%) when 100% O₂ is administered through a nasal cannula at the flow rates indicated (in L/min):
Table 1: Correction factor depending on flow rate and weight

<table>
<thead>
<tr>
<th>Flow O₂ (L/min)</th>
<th>Weight (kg)</th>
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<th>2</th>
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Table 2: Effective FiO₂ according to the correction factor and the concentration of oxygen

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<th>Concentration (%)</th>
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16.2. **ANNEXE 2 : Suggested guidelines for the management of extremely preterm neonates**

Preliminary Note: The following data are only recommendations and not rules for the management of extremely preterm neonates.

**Respiratory care**

**Alarms:**
- SpO₂: between 85% and 92% from D0
- TcpCO₂ > 40 and <60 mmHg

**Exogenous surfactant:**
Prophylactic systematically proposed in the study population.

**Ventilation parameters:**
- **CPAP:**
  - Expiratory pressure: 5-6 cm H₂O
  - FiO₂ ≤ 40%
- **Mechanical ventilation (CMV):**
  - Frequency between 30 and 60 / min
  - Expiratory pressure between 3 and 5 cm H₂O
  - Max Inspiratory Pressure <20 cm H₂O
  - Inspiratory time <0.35 sec
  - Flow between 8 and 12 L / min.
- **High frequency ventilation:**
  - Peak to peak amplitude or to obtain efficient chest vibration
  - Mean pressure corresponding to the average pressure in CMV + 2 cm H₂O then in increments of 1 cm H₂O until satisfactory lung recruitment.

**Extubation criteria**
According to investigator's practices in each recruiting centre:
- FiO₂ <30%
- And an Inspiratory pressure <15 cm H₂O,
- And a frequency <30 / min,
- And a PaCO₂ > 40 and <60 mmHg,
- In the absence of hemodynamic disorders and severe neurological damage (grades III-IV IVH, cystic PVL).

**Criteria for reintubation**
- Recurrent apnea (> 12 episodes / 24h) with bradycardia <80 / min
- Or PaCO₂ > 70 mmHg and pH <7.20

**Systemic and inhaled corticosteroid use**
- Any child requiring the administration (not recommended) of another corticosteroid (oral, parenteral or inhaled) during the duration of the HSHC administration will be removed from the study.
- Any supplemental steroid use after the study period is not recommended but left to the clinical judgment of the participating teams.

**Haemodynamic care**

**Management of patent ductus arteriosus**
- No prophylactic treatment using NSAIDs before 24 hours of life. Treatment of patent ductus arteriosus according to the practices of each centre from 24-48h using ibuprofen (10 mg / kg for 1 day and 5 mg / kg 2 days).
- Surgical ligation if failure of medical closure.

**Support for initial hemodynamic disorders**
- Volume expansion using saline (10 ml / kg) if mean arterial pressure less than appropriate for gestational age in weeks during the first week of life.
• Possible use of dopamine according to the practices of each centre.

**Enteral feeding protocol**
• Initial minimal enteral feeding with breast milk in gastric bolus of 1 ml per 3h from 24 hours of life according to the practices of each centre.
• No concomitant increase if ibuprofen is used.
• Daily increase of 15 ml / kg / day maximum.
Please keep this card with you at all times

Name:........................................... First name: .....................................
My child is participating in the following biomedical research: PREMILOC, promoted by the AP-HP

My child has received the following treatment for 10 days: placebo or hydrocortisone hemisuccinate
Dosage: 0.5 mg / kg / 12 hours for 7 days and 0.5 mg / kg / 24 hours for 3 days (Start date of treatment: ....../....../........)

Under patient number N°: ....................

My child is being followed by Dr .................................................................
At the Hospital ............................................................................................
Tel. : ............................................

In case of need for emergency unblinding, contact:
Pr Olivier Baud at 01 40 03 31 30
Or Fernand Widal (if unblinding) at 01.40.05.48.48
Trial for the prevention of bronchopulmonary dysplasia by early postnatal hydrocortisone in extremely premature infants = PREMILOC, P060250

DO NOT NOTIFY THE SPONSOR BY FAX (Do not fill the SAE reporting form) but see Adverse Events CRF pages

The investigator must IMMEDIATELY NOTIFY the sponsor (Send the SAE reporting form by fax to 01 44 84 17 99) and refer to Adverse Events CRF pages

<table>
<thead>
<tr>
<th>Other events:</th>
<th>Unexpected Serious Adverse Effects</th>
<th>Expected Events and Serious Adverse Effects</th>
<th>Unexpected Serious Adverse Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Known to be linked: to experimental drug or research procedures.</td>
<td>- Severe Hyperglycemia</td>
<td>This column will fill up as notifications by investigators.</td>
</tr>
<tr>
<td></td>
<td>Pulmonary haemorrhage WITHOUT significant change in ventilation</td>
<td>- Air leaks</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pulmonary hypertension untreated (by inhaled nitric oxide)</td>
<td>- Pulmonary haemorrhage leading to increased ventilation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sepsis not requiring ventilatory treatment and / or hemodynamic</td>
<td>- Hypertension treated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patent ductus arteriosus treated medically</td>
<td>- Persistent pulmonary hypertension treated with inhaled nitric oxide</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ROP grades 1-2</td>
<td>- Hypotension treated (expansion volume of&gt; 20 ml / kg or vasoactive drugs &gt; 24h)</td>
<td></td>
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<tr>
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<td>Inguinal hernia (operated or not)</td>
<td>- Late onset sepsis requiring ventilatory treatment and / or hemodynamic</td>
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<tr>
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<td>Intraventricular haemorrhage grades 1 and 2</td>
<td>- Intestinal Perforation</td>
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<td>Hypertension untreated</td>
<td>- Necrotising enterocolitis</td>
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<td></td>
<td>- Intraventricular haemorrhage grades 3 and 4</td>
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<td>- Hydrocephalus</td>
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<td>- Periventricular Leukomalacia</td>
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<tr>
<td></td>
<td></td>
<td>- ROP grades 3-4</td>
<td></td>
</tr>
</tbody>
</table>

SAE will have to be reported to the developer for up to 36 weeks of gestation except death and any potentially related to SAE experimental drug that will notify up to 2 years of the child.

Name and signature of the coordinating investigator: Pr. BAUD

Name and signature of the head of the CRU: Pr. ALBERTI

Name and signature of Project Leader: Dr. OUSLIMANI

Name and signature of the pharmacovigilance official: Dr. BROCVIELLE

Name and signature of the medical coordinator: Dr. CHASSANY

Grille PV, version 4.0 du 04/08/2010
### ANNEXE 5 : List of Investigators

<table>
<thead>
<tr>
<th>PRINCIPAL INVESTIGATOR</th>
<th>SERVICE</th>
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<tbody>
<tr>
<td>Pr. Olivier BAUD</td>
<td>Néonatologie</td>
<td>HOPITAL ROBERT DEBRE, APHP, PARIS</td>
</tr>
<tr>
<td>Pr. Alexandre LAPILOSS</td>
<td>Médecine Néonatale</td>
<td>HOPITAL SAINT VINCENT DE PAUL, APHP, PARIS</td>
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<tr>
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</tr>
<tr>
<td>Dr. Véronique ZUPAN</td>
<td>Réanimation Néonatale</td>
<td>HOPITAL ANTOINE BECLERE, APHP, CLAMART</td>
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<tr>
<td>Dr. Stéphane LE BOUEDEC</td>
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<td>CHU D’ANGERS</td>
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<td>CHU BESANCON</td>
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16.5. **ANNEXE 6 : Classification Papile**

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<tr>
<th>GRADE</th>
<th>sonographic appearance</th>
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<tr>
<td>I</td>
<td>Subependymal hemorrhage</td>
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<tr>
<td>II</td>
<td>Intraventricular hemorrhage without ventricular dilatation</td>
</tr>
<tr>
<td>III</td>
<td>Intraventricular hemorrhage with ventricular dilatation</td>
</tr>
<tr>
<td>IV</td>
<td>Intraventricular hemorrhage associated with hemorrhagic lesion in the adjacent parenchyma</td>
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</table>

16.6. **ANNEXE 7 : Classification Bell**

<table>
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<tr>
<th>Stage</th>
<th>General signs</th>
<th>Intestinal signs</th>
<th>Radiological signs</th>
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<tr>
<td>NECOTISING ENTEROCOLITIS DOUBTFUL</td>
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<tr>
<td>IA suspected</td>
<td>apathy or agitation, apnea-bradycardia, thermal dysregulation, ± CRP</td>
<td>Small gastric residues, vomiting, painless abdominal distension ± stool changes, no macroscopic blood</td>
<td>Normal appearance or dilated bowel loops, moderate ileus (fixed handle)</td>
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<tr>
<td>IB probable</td>
<td>Same as above</td>
<td>Same as above + red blood in stools</td>
<td>Same as above</td>
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<tr>
<td>ECUN proven</td>
<td>II low to medium severity</td>
<td>Same as above ± metabolic acidosis ± thrombocytopenia</td>
<td>Same as above + silent bloat ± green waste ± defense ± plastron FID ± inflammatory wall</td>
</tr>
<tr>
<td>III serious</td>
<td>Same as above ± Hypotension, oliguria, electrolyte disturbances, ± mixed acidosis ± neutropenia ± CIVD ± SDRA</td>
<td>Same as above + large bloating, painful contracture</td>
<td>Same as above ± pneumoperitoneum large cavity or localised</td>
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