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Assessment report for paediatric studies submitted according to Article 46 of the Regulation (EC) No 1901/2006

Zerit

stavudine

Procedure no: EMEA/H/C/000110/P46/063

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5520
Send a question via our website www.ema.europa.eu/contact

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1. Introduction

Stavudine is a nucleoside reverse transcriptase inhibitor (NRTI) used in combination with other antiretroviral (ARV) medications in the treatment of Human Immunodeficiency Virus (HIV) infected adult patients and paediatric patients (over the age of 3 months) only when other antiretrovirals cannot be used.

This document summarizes the results of the final clinical study report for the study entitled, "A cross-sectional study of HIV-negative children, aged 18 to <28 months, born to HIV-1-infected mothers in Europe: A European study sponsored by the Collaborative Committee for Mitochondrial Toxicity in Children (MITOC)"1, and is being submitted in accordance with Article 46 of Regulation (EC) No 1901/2006.

The MITOC was established in 2004, with representatives from each of the marketing authorization holders of NRTIs (Bristol-Myers Squibb [BMS], Gilead Sciences, and GlaxoSmithKline later ViiV Healthcare), following a request from the Committee for Medicinal Products for Human Use to comment on the feasibility of a well-designed cohort study for long-term follow-up of children exposed to NRTIs during pregnancy. The final study protocol was approved by the European Medicines Agency (EMA) on 09 May 2007.

The final clinical study report was submitted by the MITOC to the EMA on 31 May 2015, and is also included in Module 5.3 of this submission.

The objectives of the study:

- To determine the prevalence of neurological clinical symptoms (NCS) of cognitive or motor delay (with or without seizures) in HIV-negative children between the ages of 18 to <28 months born to HIV-1-infected mothers, followed since birth and exposed to antiretroviral therapy (ART), without attribution of cause.
- To categorize these cases of NCS into explained and unexplained NCS and to estimate prevalences accordingly.
- To estimate the proportion of unexplained cases whose symptoms are suggestive (i.e. 'definite', 'probable' or 'possible' by a Data Review Committee (DRC) consensus review) of mitochondrial disorder (or clinically manifested mitochondrial disorder).
- To assess the association between type and duration of ART exposure in utero and/or early neonatal life and unexplained and mitochondrial disorder-related cognitive or motor delay

a The original age of assessment was 18 to 24 months, but the Cohorts requested that children could be assessed up to the last day of 27 months of age. Thus, the range used throughout this report is 18 to <28 month.

A short critical expert overview has also been provided.

2. Scientific discussion

2.1. Information on the development program

Mitochondrial toxicity in children associated with in utero/perinatal exposure to N(t)RTIs was first raised as a specific concern in 1999, following the identification of 8 cases of mitochondrial dysfunction in HIV negative children exposed to zidovudine (ZDV) and other drugs in utero and postnatally, in the French National Epidemiological Network and a clinical study designed to evaluate tolerance to ZDV

and lamivudine (3TC) administered to prevent mother-to-child transmission of HIV-1. Subsequent larger scale screening in the French Perinatal Cohort resulted in an estimated incidence of established mitochondrial dysfunction from birth to 18 months of age of ≥ 0.26% (95% CI: 0.10 to 0.54) among children with perinatal exposure to antiretroviral (ARV) drugs, compared with an incidence of 0.01% for paediatric neuromitochondrial diseases in the general population. A variety of clinical symptoms observed in HIV-1 infected patients receiving long-term N(t)RTI therapy have been attributed to mitochondrial toxicity.

Following a request from the Committee for Medicinal Products for Human Use (CHMP) in April 2001 for further information regarding mitochondrial toxicity in children associated with in utero/postnatal exposure to N(t)RTIs, the individual Marketing Authorisation Holders (MAHs) provided available preclinical and clinical data. The CHMP concluded that the available in vitro studies demonstrated that the individual N(t)RTIs were each capable of causing mitochondrial toxicity to differing extents, and that the available clinical data suggested a causal association between exposure to N(t)RTIs in utero and mitochondrial toxicity in HIV negative children.

A class warning of the risk of mitochondrial dysfunction in children exposed to N(t)RTIs in utero was consequently inserted in Section 4.4 of the Summary of Product Characteristics (SmPC) of each N(t)RTI.

The MAHs were asked to comment on the feasibility of a well-designed cohort study for long term follow-up of children exposed to N(t)RTIs during pregnancy, and the Collaborative Committee for Mitochondrial Toxicity in Children (MITOC) was established in 2004 with representatives from each of the MAHs of N(t)RTIs (Bristol-Myers Squibb, Gilead Sciences, and GlaxoSmithKline – later ViiV Healthcare). The final clinical study report (CSR) (not unanimously approved by the MITOC Scientific Committee) was submitted by prior agreement to the EMA by the MITOC Scientific Committee.

CHMP Comment:

The MITOC study was established following the findings of two studies using data from the French National Epidemiological network and the French National Register (Blanche, 1999, Barret B, 2003). The Barett et al study identified children less than 18 months of age with possible symptoms of mitochondrial dysfunction from their medical records and if mitochondrial dysfunction was a suspected differential diagnosis, specific investigations, adapted on a case -by- case basis to the symptoms were performed, these included haematological markers, lactate concentrations, MRI of the brain, tissue biopsies and enzymological studies of the mitochondrial respiratory chain. The Barett et al study also took into account the patients identified in the earlier study by Blanche et al in their analysis. Overall 12 children were identified as having “established” mitochondrial dysfunction defined in the study as compatible symptoms of significant severity, and a consistent, profound deficit in one or several of the components of the respiratory chain, or characteristic histological findings, all patients had been exposed to zidovudine either pre, post or peripartum. A further 14 children were considered to have “possible” mitochondriopathy (children with unexplained clinical and/or biological symptoms for which a mitochondrial dysfunction could be included in the differential diagnosis). The authors considered these to be an emerging syndrome with three main features – neurological symptoms (stated as mental retardation, seizures and behavioral disturbances), significant abnormalities on cerebral MRI (principally lesions of the white matter and brainstem) and often hyperlactataemia consistent with those described in constitutional mitochondrial diseases with neurological expression. Another study (Tardieu, 2005) found that images observed in children considered to have antiretroviral-induced mitochondrial dysfunction are similar to those observed in congenital mitochondrial diseases. These images were also observed in symptomatic or asymptomatic children without evidence of systemic

mitochondrial dysfunction.

The MITOC study was therefore intended to further investigate mitochondrial toxicity in children associated with in utero/ perinatal exposure to NRTIs.

It is noted that there is discordance between the investigators with regards to the final clinical study report, this is discussed further in Section Report of discordance.

2.2. Clinical aspects

2.2.1. Clinical study

Study Title

A Cross-Sectional Study of HIV Negative Children Aged 18-24 Months Born to HIV-1 Infected Mothers in Europe: A European Study Sponsored by the Collaborative Committee for Mitochondrial Toxicity in Children (MITOC)

Objectives

- To determine the prevalence of neurological clinical symptoms of cognitive or motor delay (with or without seizures) in HIV negative children between the ages of 18 to < 28 months born to HIV-1 infected mothers, followed since birth and exposed to ART, without attribution of cause.
- To categorize these cases of neurological clinical symptoms into explained and unexplained neurological clinical symptoms and to estimate prevalences accordingly.
- To estimate the proportion of unexplained cases whose symptoms are suggestive (ie, "definite", "probable", or "possible" by a DRC consensus review) of mitochondrial disorder (or clinically manifested mitochondrial disorder).
- To assess the association between type and duration of ART exposure in utero and/or early neonatal life and unexplained and mitochondrial disorder-related cognitive or motor delay.

Methods – analysis of data submitted

Study Design

This was a cross-sectional study conducted in HIV-negative children born to HIV-1-infected mothers, exposed to ART in utero and/or neonatally and prospectively followed in established European cohorts since birth.

In order for a medical condition to be confirmed as a primary endpoint, data needed to be collected and reviewed in four stages:

1. Informed consent obtained
2. MITOC screening assessment
3. Neurological evaluation
4. DRC review

In some cohorts, infants were not followed up since birth, and the population consisted of children who were available for analysis at the 18 to <28 months' timepoint.

Participating European Cohorts and Registries were as follows: French Perinatal Cohort Study (ANRS C01 EPF), Spanish Perinatal Cohort Study (NENEXP Project), Swiss Mother and Children HIV Cohort Study (MoCHiV), Italian Register for HIV Infection in Children, German Cohort of Children Born to HIV Positive Mothers (KompNet HIV/AIDS), and Belgian Cohorts (Hôpital St Pierre, and Catholic University of Louvain).

Inclusion Criteria

It was assumed that children enrolled into the MITOC Study would have been exposed to ART. The inclusion criteria for the Study were:

- Born to an HIV-1-infected mother
- Confirmed HIV-negative status (as per cohort/study definition)
- Aged 18 to <28 months at time of initial MITOC assessment
- The mother has signed the informed consent form
- Prospectively followed since birth

Exclusion criteria

- Residence outside of Western Europe (except the French cohort)

CHMP comment:

A limitation of conducting the analysis only during the 18 - 28 month timeframe is that this is a relatively narrow timeframe which may not detect mitochondrial dysfunction symptoms presenting later in life or any potential longer term effects. The cross sectional "snapshot" also limits detection of mitochondrial dysfunction to one point; this would therefore miss presentations occurring after the screening visit, even if they occurred within the 18- 28 month timeframe.

It is acknowledged that it is difficult to identify an optimal age of presentation of mitochondrial disease in this patient population. From the Barret et al study (Barret B, 2003), ages of presentation of the 12 patients with "established" mitochondrial disease ranged from 2 months to 1 year and 6 months. However, in both the Barett and Blanche studies, a question of potential reversibility of any potential effects of ART induced mitochondrial dysfunction once the ART was discontinued was raised. The methodology in the current study may not enable detection of any potential mitochondrial dysfunction symptoms which occurred but resolved before the screening visit.

The rationale for confining the study to Western Europe also potentially limits generalizability, although it is likely that the study was confined to countries where there were existing registers or cohorts to allow pragmatic recruitment. It is noted that divergence from this was allowed within the French cohort.

Variables

Primary endpoint

The primary endpoint is the prevalence of children meeting the case definition of neurological clinical symptoms (NCS) of cognitive or motor delay (with or without seizures) without attribution of cause.

Secondary endpoint

Secondary endpoints were evaluated in the primary population of ART exposed children, and in the secondary populations of all HIV negative children and those with complete data on the duration of ART exposure.

- Prevalence rates after the DRC review

- Suspected and strong evidence for mitochondrial disorders categorized by the DRC as explained mitochondrial disorders

- Neurological abnormalities categorized as unexplained by the DRC, but no evidence of mitochondrial disorder

- Prevalence rates of unexplained NCS after DRC review by type of ART exposure (*in utero*, intrapartum, neonatal), ARV drug class or ARV drug
- Prevalence rates of explainable and unexplained NCS at the MITOC screening Assessment
- Duration of ART exposure by primary endpoint outcome
- Cumulative ART exposure by primary endpoint outcome

Demographics and baseline characteristics

Descriptive summaries of information collected at the cross-sectional screening assessment were tabulated for all enrolled children:

- Age in months at time of screening assessment
- Clinical development since birth (normal/abnormal)
- Previous neurological and non-neurological disorders
- Previous hospitalizations / duration of hospitalizations (by summing up the duration of all hospitalizations documented)
- Physical examination findings (weight, height, head circumference, any abnormalities in physical examination)
- Outcome of the MITOC Assessment

Maternal medical history and characteristics

Information on maternal characteristics (maternal age at delivery, most likely mode of maternal HIV acquisition, injection drug use and drug abuse during pregnancy, alcohol and tobacco use during pregnancy, hepatitis C virus (HCV) and/or hepatitis B virus (HBV) co-infection, classification of HIV disease) were summarized using descriptive statistics for all enrolled children.

Laboratory results (CD4 in number of cells/mm^3, HIV Ribonucleic acid (RNA) viral load in copies/mL) of the last available blood sample collected during pregnancy are presented when available.

ARV drugs

ARV drugs were classified according to drug class as well as by the specific ARV drugs to which the mother/child was exposed. No or unknown ART exposure of the mother or child was documented in the database. ARV drugs given to the mother were classified into the following types of exposure: prepregnancy, *in utero* and intrapartum. ARV drugs given to the child were classified as neonatal. Pre-

pregnancy was defined as having a start date prior to the start of pregnancy. Pre-pregnancy ARV drugs as well as ARV drugs given to the mother after the child's date of birth are not taken into account in any statistical summaries. The duration of *in utero* ART exposure was calculated based on the number of gestational weeks of exposure (using the date of ART initiation and either the date of cessation if drug discontinued during pregnancy or the date of delivery if maternal therapy continued perinatally).

CHMP comments:

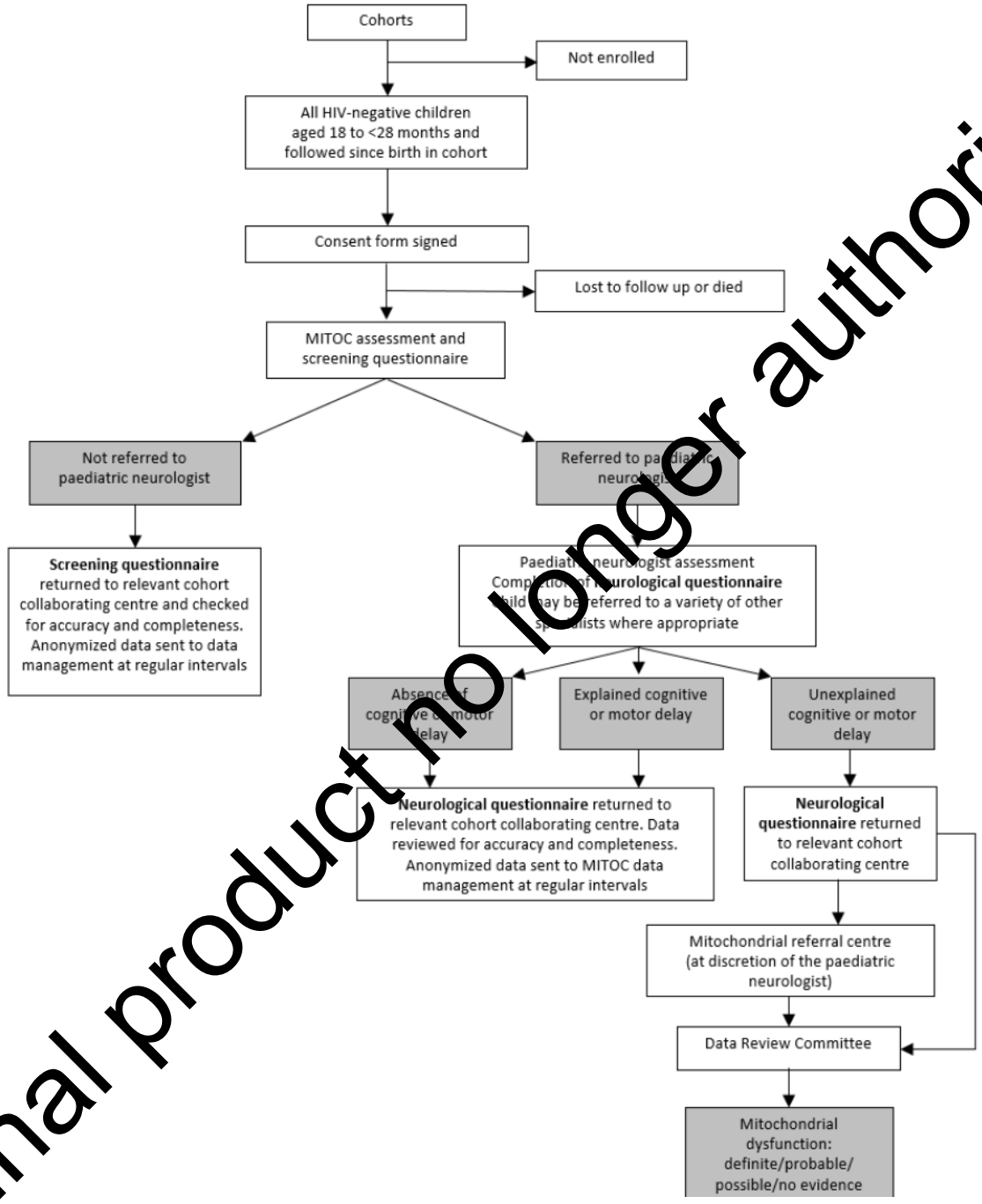
Only neurological manifestations of potential mitochondrial dysfunction were considered to be endpoints. Therefore although the baseline demographic data may have been able to detect any potential non- neurological manifestations, this was not taken into account in the analysis. NRTI associated mitochondrial toxicity is considered to have presentations similar to that of congenital mitochondrial disorders (Brogly SB, 2007) and although the nervous system is the most commonly affected in congenital disease with approximately 45% of children presenting with neurological signs, other signs are considered relatively common in this group such as hepatic, haematological and endocrine (Koenig, 2008) or metabolic signs like hyperlactataemia.

Only the last available laboratory results for maternal HIV disease were taken into account which may not have been reflective of disease control throughout the pregnancy.

No information on family history of mitochondrial disease appears to have been requested as part of the baseline information thereby limiting the ability to distinguish between any potential mitochondrial dysfunction from inherited congenital disease. Information on other risk factors for emergent neurological symptoms in this age group have also not been accounted for such as prematurity and the presence of other congenital/ genetic syndromes. Although congenital syndromes should have been detected as part of the more detailed neurological evaluation, a breakdown for these has not been provided and therefore it is uncertain if these were taken into account. Other drugs/ medicinal products taken in utero have also not been collected as part of maternal history.

For all of the variables (primary and secondary endpoints, demographics and baseline characteristics, maternal medical history and characteristics, ART type of exposure, duration of ART exposure, ARV drug and class), the data should also be stratified by country birth cohort so that differences across cohorts can be better understood.

Study visits



Screening assessment

Children between the ages of 18 to <28 months attended a single screening visit performed by a paediatrician, including a brief clinical assessment and a standard screening questionnaire as part of their standard clinical follow-up, and included a final assessment to identify children with NCS of cognitive or motor delay with or without seizures.

Subjects were categorized as having: (i) absence of neurological clinical symptoms (including cognitive or motor delay); (ii) presence of explainable neurological clinical symptoms (including cognitive or motor delay); or (iii) presence of unexplained neurological clinical symptoms (including cognitive or motor delay).

Children with explainable or unexplained NCS of cognitive or motor delay at the MITOC assessment may have been referred to a paediatric neurologist for additional clinical evaluation according to local clinical practice and/or national recommendations.

Paediatric neurologist assessment

A standard neurological questionnaire was completed, which includes a final assessment from the paediatric neurologist with the classification of the child. At this assessment, children could be categorised as having (i) absence of neurological abnormalities, (ii) Presence of neurological abnormalities due to entities other than mitochondrial disorders, (iii) Suspected mitochondrial disorder (iv) Strong evidence of mitochondria disorder or (v) Unexplained neurological abnormalities but no evidence of mitochondrial disorder.

In the French cohort, the hospital paediatrician following children born to HIV positive mothers may have undertaken the neurological evaluation and recorded these data in the neurological questionnaire without referral to a neurologist.

All subjects with a classification of suspected mitochondrial disorder, strong evidence for mitochondrial disorder or presence of unexplained neurological abnormalities but no evidence of mitochondrial disorder were reviewed by the DRC to confirm the presence of mitochondrial disorder.

Mitochondrial disorder evaluation

Children with suspected or strong evidence for mitochondrial disorder could have been referred by the paediatric neurologist to a specialist in mitochondrial abnormalities for additional evaluations following the neurological assessment visit. The appropriate mitochondrial referral centre was then contacted by the cohort paediatrician and/or the paediatric neurologist pending consent/agreement of the child's parent or main caregiver.

CHMP comments:

The process of evaluation and classification of neurological symptoms in enrolled subjects was potentially very subjective. The screening questionnaire was an open form which largely relied on the judgement of the evaluating paediatrician on whether further neurological examination was needed, this was subject to a large degree of variation depending on local practices (as is highlighted in further detail in the section below).

This screening method would have detected gross neurological abnormalities; however some neurological manifestations can mimic other disorders and may have resulted in non-specific clinical signs (Konig, 2008). These may have been particularly subtle in this age group , for example presenting as poor weight gain, feeding or respiratory difficulties (Chinnery, 2000, (updated 2014)) and may have been difficult to evaluate within a single assessment visit.

There was also not a standard approach following detection of neurological abnormalities at the screening assessment as even subjects with unexplained neurological symptoms may not have been referred on to have further neurological assessment.

There was also variation in the neurological assessment as in some cohorts this may have been performed by the same paediatrician who performed the initial screening assessment, thereby

removing the advantage of a second, independent assessor. This was also subject to variation depending on local practice.

Also, unlike the Blanche or Barett studies (Barret B, 2003 , Blanche, 1999), no other confirmatory investigations such as microscopic examination of biopsies, neurological imaging or enzymatic analyses were necessary to confirm or dismiss a diagnosis of mitochondrial dysfunction. These investigations were left to the discretion of the clinicians and there was no standardisation of the diagnosis of mitochondrial dysfunction within the study. It is acknowledged that diagnosis of mitochondrial dysfunction in children can be challenging and no single investigation can always be applied (Koenig, 2008,Chinnery, 2000 [updated 2014]). Nevertheless variability of local practice make interpretation of findings across cohorts challenging.

Differences between cohorts

The table below highlights some of the differences between the various cohorts, namely relating to enrolment and to patient evaluation:

Belgian cohort (2 sites)	French cohort (25 sites)	German cohort (7 sites)	Italian cohort (18 sites)	Spain, Barcelona (8 sites), Madrid (4 sites)	Swiss cohort (4 sites)
All children were enrolled soon after birth if written consent was obtained	All children born from 01 September 2008 to 30 September 2009 were included in the EPF cohort at birth, except when the mother refused to sign the consent form	Most children were consented after exclusion of HIV-1-infection at the age of 3 -7 months, although some were consented at ≥3 to <28 months before the MITOC screening visit.	Consent was obtained at some sites when HIV vertical infection was excluded. In other sites, consent was obtained at the MITOC screening visit	Barcelona: The consent form was signed after birth or at the MITOC screening visit, depending on the centre Madrid: The consent form was signed when the child was 15-months old	Consent was obtained at the MITOC screening visit
Majority of children were followed up by a single paediatrician All infants with unexplained and explained NCS were referred to the	In most cases, neurological questionnaires were completed by paediatricians Children with seizures, autism and language delay were	All children with unexplained and explained NCS, except those with single febrile convulsions and absence of NCS at the MITOC	All children with unexplained NCS were sent to a neurologist for evaluation Nearly all children with explained NCS	Barcelona: All children with unexplained NCS were referred to a neurologist for evaluation Nearly all children with	All children with unexplained NCS were sent to a neurologist for evaluation Children with

neurologist; most had an MRI. Children with seizures, autism, language delay, etc., were classified as having unexplained NCS	classified as having unexplained NCS	screening visit, were referred to the neurologist for evaluation. This is a different procedure to the other countries.	were sent to a neurologist for evaluation One site referred all HIV-exposed children to a neurologist in the first few months of life	explained NCS were referred to a neurologist for evaluation. Madrid: All children with unexplained and explained NCS were referred to a neurologist for evaluation	explained NCS were not referred to a neurologist Simple febrile seizures were not referred to a neurologist

CHMP comment:

Significant heterogeneity exists in the methodology used between the cohorts. The discrepancy is evident in the time for enrolment. In some cohorts, infants were not followed up from birth, and the population consisted of children who were available for analysis at the 18 to <28 months' timepoint. Differences between the birth cohorts in when the parent consented to be included in the MITOC study could have introduced selection bias into the study particularly in the enrolment of older children where neurological problems may be starting to present.

In addition, there is huge variability between the evaluation of neurological symptoms between cohorts including differences in symptoms/ signs that were considered to be unexplained neurological symptoms, thresholds for conducting further investigations and referring to a paediatric neurologist.

As an example, although febrile seizures are considered to be a symptom of a neurological abnormality according to the screening questionnaire, children with single febrile seizures were not referred for the neurological examination in the German and Swiss cohort. In the French and Belgian cohorts, autism was considered a possible manifestation of mitochondrial dysfunction but not in the other cohorts. In some cohorts, such as the Swiss cohort, no children considered to have explained NCS were referred to a neurologist while in the Spanish, German and Italian cohorts nearly all children were referred to a neurologist regardless of if they had explained or unexplained NCS.

Apart from the differences mentioned in the table above, in the German cohort all *in utero* ARV drugs without a recorded start date were assumed to have been taken since the first day of the pregnancy. i.e. start dates were imputed with the estimated start date of pregnancy for the calculation of the *in utero* duration.

For each of the respective birth cohorts, numbers need to be provided showing the differences in the enrolment process to enable more detailed assessment taking into account these differences.

DRC assessment

The Data Review Committee (DRC) of the Mitochondrial Toxicity in Children and NRTI Exposure during Pregnancy and /or Post-natally Study, (MITOC Study) was formed in 2008 following widespread consultation with experts in the fields of paediatric infectious diseases, paediatric mitochondrial disease and paediatric neuroimaging.

Copies of the individual subject data, including available images (sonography, CT, MRI), for children assessed by their paediatrician and/or neurologist as having 'suspected or strong evidence for mitochondrial disorder' or with 'presence of unexplained neurological abnormalities, but no evidence of mitochondrial disorder', were subject to a DRC review to assess any relationship to mitochondrial disorders.

The DRC operated independently of the individual cohorts and the MITOC Study sponsors. The remit of the DRC was to provide opinion on the quality and accuracy of recorded outcomes from the neurological questionnaires; the DRC was not involved in the concept or design of the MITOC Study or the screening and neurological questionnaires. The initial intention was that the DRC should analyse all the subjects' data, including neuroimaging and neurological questionnaires where outcomes had been recorded as 'suspected' or 'strong evidence for' a mitochondrial disorder, as well as a proportion (10%) of all neuroimaging and neurological questionnaires for the other two groups (absence of neurological abnormalities and neurological abnormalities other than mitochondrial disorders) for data quality assessment purposes. However, as there were so few children who had neurological questionnaires, the data from all these children were reviewed.

The data recorded on each of the anonymised neurological questionnaires was reviewed for accuracy and completeness. The neuroimaging was then reviewed with the assistance of one (or more) of the expert neuroradiologists on the DRC. Based on this review, the DRC reached a consensus decision regarding the relationship to mitochondrial disorder(s). Mitochondrial dysfunction was defined as a compatible clinical syndrome, compatible magnetic resonance imaging findings and/or abnormal biochemistry or muscle biopsy. The DRC assigned the case to one of the following categories (Table 4), which is similar to that used in the Barret *et al.* 2003 report.

Table 4. Classifications available for neurological abnormalities in the DRC review

DRC review consensus classifications
1. Definite mitochondrial dysfunction
2. Probable mitochondrial dysfunction
3. Possible mitochondrial dysfunction
4. No evidence of mitochondrial dysfunction

A DRC data capture form, which included the classifications for the neurological abnormalities (Table 4), was completed and sent to Data Management. A narrative on each case reviewed was also written.

It was not the purpose or responsibility of the DRC to make a diagnosis on the data presented; that was the clinical duty of the paediatric neurologist reviewing the child. To maintain the independence of the DRC, there were no face-to-face meetings with the Cohort representatives. The DRC and MITOC Committee had the same chairman.

If the DRC did not agree with the conclusion of the neurologist, they requested further information from the neurologist to try to understand the difference of opinion.

CHMP Comment:

If the DRC did not agree with the conclusion of the neurologist, they requested further information from the neurologist to try to understand the difference of opinion. The authors should clarify how many discrepancies there were with the conclusion of the neurologist and if there were any classifications made by the neurologist, which were then subsequently changed by the DRC.

The presence of the same chairman for both committees also compromises the independence of each committee.

Medical reviews of the screening questionnaire

Two of the Cohort investigators were appointed as medical reviewers to assess a listing of all unique terms in the following sections of the Screening Questionnaire and flag the neurological conditions which were used in several statistical summaries: hospitalisation, physical examination, Non-neurological disorders, Neurological disorders.

The medical reviewers also reviewed the listings to identify whether there were any children with neurological clinical symptoms that should have undergone a neurological assessment, but were not referred.

CHMP comment:

The authors should provide data on the number of children with neurological symptoms that should have undergone a neurological assessment but were not referred or did not undergo this assessment.

Statistical methods

Analysis of primary endpoint

The percentage of children in the primary population with the primary endpoint was estimated, with associated 95% confidence intervals.

The outcomes of the MITOC and neurological assessment as recorded on the prospective screening questionnaires were compared with each other, to show the consistency of the definitions of neurological clinical symptoms between the two questionnaires. Another comparison was performed on the outcome of the neurological assessment and the DRC consensus, for the same reason.

The prevalence rates of children with the primary endpoint were presented by cohort, type of ART exposure (*in utero*, intrapartum, neonatal), ARV drug class, specific ARV drug and duration of each ARV drug

Univariate and multivariate analyses were performed to identify factors associated with the primary endpoint, for the primary population of ART exposed children (n=2405). These analyses were then repeated for the secondary population of all enrolled HIV negative children (n=2855).

CHMP Comment:

The comparison between the questionnaires to demonstrate the consistency of the definitions of neurological clinical symptoms has not been presented in the study report, this should be provided.

The MAH have not discussed the statistical methods used with respect to the multivariate analyses, the rationale for inclusion of variables in the multivariate analysis, nor the model building approach used; further detail is therefore required.

Selection bias

A selection bias may have been introduced as some eligible children in each participating birth cohort who were potentially eligible for MITOC were not included in the MITOC Study for various reasons: consent was not obtained as the child died or was lost to follow-up before or after consent had been signed. The probability of non-participation in the study was lower when the consent form was signed at the screening assessment than those forms signed at birth.

To assess bias, a sensitivity analysis was performed as a secondary analysis.

CHMP comment:

The authors acknowledge that a selection bias may have been introduced as some eligible children in each participating birth cohort who were potentially eligible for MITOC were not included in the MITOC study for various reasons. The authors also state that the probability of non-participation in the study was lower when the consent form was signed at the screening assessment that those forms signed at birth. The authors should provide data which supports this statement. A discussion should also be provided of the potential impact that the selection bias would have on the results of the study.

Results

All safety analyses in the MITOC Study presented in this submission were performed using the Primary Analysis Population, comprising subjects meeting all criteria for primary analysis (confirmed ART-exposed, HIV negative subjects who were 18 to < 28 months old at the time of the screening assessment).

Participants

Children not enrolled in the MITOC

Each country involved in the MITOC study was asked to provide a cohort of children born to HIV mothers followed prospectively since birth, all potentially eligible for MITOC. Some of these children were not screened at an age eligible for MITOC as they were lost-to-follow-up or died before or after consent proposal, or because of refusal of consent. In France, consent was obtained from all the mothers.

There were 3878 children potentially eligible for the study. Of these, 1013 were not included: 32 children were from Belgium, 5 from France, 548 from Germany, 371 from Italy, 33 from Spain, and 24 from Switzerland. Reasons for not being included in the main study were either absence of informed consent or early death. The details of those children who died are as follows:

- The Belgian cohort had one child who was consented and enrolled, but was not assessed because he died at 6 months of age from a mitochondrial disease, which was confirmed by autopsy and biochemical investigations. The Belgian cohort also had a child who was eligible

for the study, but not enrolled as no consent was received, who had an unexplained sudden death at the age of 2 months.

- The Spanish cohort (Barcelona) had four deaths, three of which were children who were not enrolled in the MITOC study as they were too young to have their consent form signed. The reasons for the deaths were not recorded in three out of the four cases. The child with consent died at 6 weeks of age from diaphragmatic hernia complications and was lost to follow-up.
- The French cohort had five consented children who died: (1) no autopsy and no cause recorded; (2) a premature baby who died at 2 hours of age with no autopsy; (3) a premature infant who died of pulmonary hypoplasia; (4) a premature infant who died of lung infection and pulmonary hypoplasia; (5) an infant who died of cerebral anoxia and multiple organ failure.
- There were no deaths in the Swiss or Italian cohorts.

CHMP Comment:

A sizeable proportion of children that were eligible for enrolment were not included in the main study for a variety of reasons (1013/ 3878 children did not enrol). The MAH should provide a table of the numbers of children who were not consented, were lost-to-follow-up or died stratified by birth cohort.

It is noted there was one death in the Belgian cohort from confirmed mitochondrial disease and several unexplained deaths in the Spanish cohort.

Patient disposition

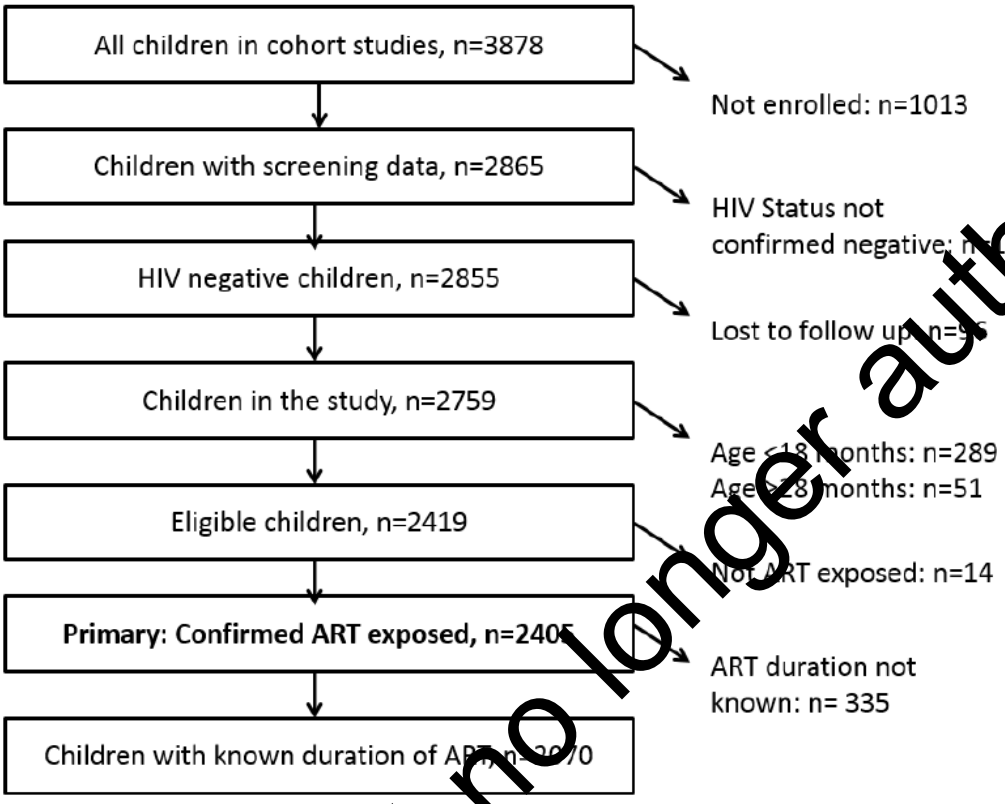
From the 2865 children for whom informed consent was signed and with either retrospective data (medical history) or prospective data (screening assessments) a primary analysis group was defined based on four main criteria:

1. The child was confirmed HIV negative
2. Children needed to have ART data available for analysis
3. Children were between 18 and <28 months of age at the screening assessment
4. ART exposure of the child either during or after gestation was confirmed

The method of enrolment varied between Cohorts and between sites within Cohorts. Consent was obtained either at the time of birth after confirmation of HIV-negative status or at the screening assessment. As a consequence, some children that had been consented at the time of birth could not be included in the Study as they had died or did not turn up for their screening assessment.

Figure 2 shows the stages of enrolment, consent and inclusion of the children in the primary population (2415 children known to have been exposed to ARV drugs).

Figure 2. Definition of the three analysis populations: "All ART exposed" (n=2405, primary), HIV-negative children (n=2855) and "complete ART data" (n=2070)



CHMP comment:

The method of enrolment varied between cohorts and between sites within cohorts. Consent was obtained either at the time of birth, after confirmation of HIV-negative status or at the screening assessment. This raises concerns regarding the potential for selection bias both between cohorts and between sites within cohorts. Children exhibiting neurological symptoms may be more likely to be consented into the study if consent was obtained at the screening assessment that at birth. The MAH should provide a table of data showing the levels of participation stratified by birth cohort, including the number of eligible children, age of child at consent, number of deaths after consent, number of children lost to follow-up after consent, number of children evaluated, number of children with missing HIV status.

Demographic and Baseline Characteristics of Study Population

The Primary Analysis Population contained 2405 subjects (Belgium 289, France 458, Germany 244, Italy 1016, Spain 351, and Switzerland 47).

Baseline characteristics of subjects in the Primary Analysis Population obtained from the screening questionnaire are shown in Table 5. The median age of study subjects was 22.4 months (range: 18.0 to 27.9 months), and the proportions of males and females were approximately equal.

Baseline characteristics of study subjects by cohort are shown in Table 6. Subjects were generally well balanced with regard to age and sex in the different cohorts.

Table 5. MITOC Study: Baseline Characteristics of Study Subjects (Primary Analysis Population)

Baseline characteristic	Number of subjects, n (%)
Age at completion of screening questionnaire, months	
Mean (SD)	22.0 (2.74)
Range	18.0–27.9
Age classification, n (%)	
=18 months	229 (9.4%)
>18 to ≤21 months	799 (33.2%)
>21 to ≤24 months	865 (36.0%)
>24 months	515 (21.4%)
Sex, n (%)	
Male	1222 (50.8%)
Female	1183 (49.2%)
Clinical development of child since birth, n (%)	
Normal	2260 (94.0%)
Abnormal	145 (6.0%)

Table 6. MITOC Study: Baseline Characteristics of Study Subjects by Cohort (Primary Analysis Population)

Baseline characteristic	France n=458	Germany n=244	Italy n=1016	Spain n=351	Switzerland n=47	Belgium n=289
Age at screening, months						
Mean (SD)	23.4 (2.17)	22.0 (2.96)	21.6 (2.80)	21.3 (2.64)	23.9 (1.87)	21.3 (2.5)
Range	18.0–27.9	18.0–27.7	18.0–27.7	18.0–27.5	18.1–26.5	18.0–27.6
Age classification, n (%)						
=18 months	12 (2.6%)	22 (9.0%)	121 (11.9%)	51 (14.5%)	2 (4.3%)	18 (6.2%)
>18 to ≤21 months	66 (14.4%)	96 (39.3%)	364 (35.8%)	129 (36.8%)	[illegible]	141 (48.8%)
>21 to ≤24 months	242 (52.8%)	55 (22.5%)	343 (33.8%)	115 (32.8%)	18 (38.3%)	92 (31.5%)
>24 months	138 (30.1%)	71 (29.1%)	188 (18.5%)	56 (16.0%)	24 (51.1%)	38 (13.1%)
Sex, n (%)						
Male	225 (49.1%)	124 (50.8%)	518 (51.0%)	187 (53.3%)	16 (34.0%)	152 (52.6%)
Female	233 (50.9%)	120 (49.2%)	498 (49.0%)	164 (46.7%)	31 (66.0%)	137 (47.4%)

Descriptive Data for Subjects

Data on birth characteristics of study subjects obtained from the database of each cohort are shown in Table 7. Data were not available for all children in all categories. The median gestational age at birth was 38.0 weeks (range: 22.0 to 43.0 weeks). Most subjects for whom data were available had been delivered by cesarean section (1641 of 2173 subjects, 75.5%).

The median birth weight of study subjects was 2.94 kg (range: 0.6 to 5.0 kg), the median height at birth was 48.0 cm (range: 26.0 to 57.0 cm), and the median head circumference at birth was 33.7 cm (range: 20.0 to 49.0 cm).

Table 7. MITOC Study: Birth Characteristics of Study Subjects (Primary Analysis Population)

Baseline characteristic	Number of subjects, n (%)
Gestational age at birth, weeks	n=2369 (36 not available[a])
Mean (SD)	37.6 (2.07)
Range	26.0–43.0
Type of delivery, n (%)	n=2173 (232 not available[a])
Planned caesarean	1489 (68.5%)
Unplanned caesarean	152 (7.0%)
Vaginal delivery	532 (24.5%)
Weight, kg	n = 2349 (56 not available[a])
Mean (SD)	2.9 (0.5)
Range	0.6–5.0
Height, cm	n = 1221 (1204 not available[a])
Mean (SD)	48.0 (3.16)
Range	26.0–57.0
Head circumference, cm	n = 1374 (1031 not available[a])
Mean (SD)	33.7 (2.10)
Range	20.0 – 49.0

a "Not available" includes data confirmed missing in the cohort databases, and data that were not collected by prior agreement with a cohort.

CHMP comment: In total 6% of children (n=145) were considered to have abnormal clinical development since birth. The mean age at screening was 22 months. The proportions of male and female subjects were approximately equal. Anthropometric data was not available for a substantial number of subjects.

In addition to data presented in this section, the MAHs involved should also provide data on levels of participation stratified by birth cohort, including the number of eligible children, age of child at consent, number of deaths after consent, number of children lost to follow-up after consent, number of children evaluated, number of children with missing HIV status.

The data displayed in table 7 has only been presented for the total study population, but should be stratified by birth cohort in addition.

Although the methodology section states that data on the number of previous hospitalisations and duration were collected, these have not been presented in the table of results. In addition, as mentioned previously, other risk factors for developing neurological symptoms, such as prematurity, complications at birth, neonatal infections, APGAR score do not appear to have been documented. Although a mean gestational age at birth has been provided, insufficient detail has been provided eg: by the WHO definitions of preterm birth to assess the proportion of preterm deliveries.

The data presented in the table have been presented using the mean, standard deviation and range which does not provide sufficient granularity for a full assessment of the characteristics to be made. Therefore, the data should also be presented using standard meaningful categories, particularly for

gestational age at birth, weight, height and head circumference. For example, the WHO categories for birth weight (<1500, 1500-1999, 2000-2499, 2500-3999, 4000kg+), small weight for gestational age, small head circumference for gestational age.

Descriptive Data for Mothers

Maternal history data for the mothers of study subjects were obtained from the database of each cohort (Table 8). Data were not available from all cohorts for all categories. The mean (SD) age at delivery of the mothers for whom data were available was 31.8 (5.77) years (range: 16.0 to 51.0 years). Most mothers for whom data on the likely mode of maternal HIV acquisition were available (1687 of 1853 subjects, 91.0%) had contracted HIV through sexual intercourse. Shared syringes (98 subjects, 5.3%) and blood transfusions (24 subjects, 1.3%) were the other listed causes of infection. Coinfection with hepatitis virus B and/or C was reported for 183 of 1581 mothers (11.6%) for whom data were available. A minority of mothers reported using tobacco, alcohol, injection drugs, or recreational drugs during their pregnancy, although no data were available for these behaviors for the majority of mothers.

Table 8. MITOC Study: Maternal History Characteristics (Primary Analysis Population)

Baseline characteristic	Number of mothers, n (%)
Age of mother at delivery, years	n = 1225 (1180 not available)
Mean (SD)	31.8 (5.77)
Range	(16.0–51.0)
Likely mode of maternal HIV acquisition, n (%)	n = 1853 (552 not available[a])
Sexual intercourse	1687 (91.0%)
Blood transfusion	24 (1.3%)
Shared injection needles/syringes	98 (5.3%)
Other	44 (2.4%)
HBV and/or HCV co-infection status, n (%)	n = 1581 (824 not available[a])
HBV only	41 (2.6%)
HCV only	131 (8.3%)
HBV and HCV	11 (0.7%)
No hepatitis virus infection	1398 (88.4%)
Tobacco use during pregnancy, n (%)	115 / 760 (15.1%) (1645 not available)
Alcohol use during pregnancy, n (%)	24 / 736 (3.3%) (1669 not available)
Injection drug use during pregnancy, n (%)	21 / 894 (2.3%) (1511 not available)
Drug abuse during pregnancy unspecified, n (%)	87/941 (9.2%) (1464 not available)

CHMP Comment:

Mean maternal age has been provided, however the age of the mother at delivery should be displayed using appropriate age categories to allow more granularity, especially as the age range was quite wide (16 – 51 years). It is noted that for a significant proportion of subjects, maternal age at delivery was not available (1180/ 2405).

Where data was available, the majority of maternal HIV infection was acquired via sexual intercourse and the majority were not coinfected with either Hepatitis B or C. Concomitant maternal medication in pregnancy (other than antiretrovirals) was not provided.

Information on substances of abuse and tobacco during pregnancy was not available for a large proportion of subjects (for example, tobacco use unavailable for 68% of mothers, alcohol use unavailable for 69%).

Once again, the data displayed in table 8 should also be stratified by birth cohort to allow comparison across cohorts given the potential for variability.

Although the MAHs/study authors state that data on laboratory results (CD4 counts, HIV RNA viral load) for the mother would also be collected where available, this data has not been presented in the tables of results. This should be presented, although it is noted that only the results from the last available sample in pregnancy were collected, which may not be an accurate reflection of maternal HIV control during gestation.

Exposure

Table 9 summarises ART histories for the primary population of ART-exposed children. Most children were exposed to ART in utero (97%), intrapartum (96%) and during the neonatal period (99.9%).

For children with data available, 99.9% were exposed to at least one NRTI drug. Almost all were exposed to zidovudine (99.8%), at least neonatally, and 55.3% were exposed in utero. Among the 2405 children, 1709 (71.1%) were exposed to at least one PI, and 656 (27.3%) were exposed to at least one NNRTI.

Table 9. Type of ART exposure in the primary population (n=2405)

Type of ART exposure	Number of children, n (%)
In utero	85 unknown / missing 2259 / 2320 (97.4%)
Intrapartum	201 unknown / missing 2107 / 2204 (95.6%)
Neonatal	11 unknown / missing 2392 / 2394 (99.9%)
ARV drug class	
NRTI	2403 / 2405 (99.9%)
NNRTI	656 / 2405 (27.[illegible]%)
Protease inhibitor	1709 / 2405 (71.1%)
Entry inhibitor	[illegible] / 2405 (1.0%)
Integrase inhibitor	[illegible] / 2405 (1.7%)
Unspecified	[illegible] / 2405 (0.7%)

Children may appear in more than one row — Reference Table MITOC 4.3.1

In the primary analysis population, the duration of ART exposure was not available for all children. From the available data, the median duration of *in utero* exposure was 29 weeks (range 0.1-42.4 weeks). Fifty-three percent of mothers already had ART before pregnancy or started ART in the first trimester. (Table 10). The median duration of *in utero* exposure was 169 days for zidovudine (n=1288), 180 days for lamivudine (n=1[illegible]), 250 days for abacavir (n=294) and 197 for tenofovir (n=671).

The median duration of neonatal ART exposure was 6 weeks (range 0.1-16 weeks), with most children (88.8%) being exposed to ART for 4-7 weeks. The median cumulative time that children were exposed to ART was 34 weeks (range 4.0-51.6 weeks). The majority of children were exposed for 28-48 weeks (67%).

Table 10. Duration of ART exposure in children in the primary population (n=2405)

Duration of ART exposure	Number of children, n (%)
In utero ART exposure	
Children with data on duration of *in utero* exposure, n (%)	2139 / 2405 (88.9%)
In utero exposure, gestational weeks	
Mean (SD)	27.6 (10.9)
Range	0.1–42.1
Duration of ART exposure, classification, n (%)	n=2139
≤2 weeks	2 (0.0%)
>2 weeks to ≤12 weeks	220 (10.3%)
>12 to ≤20 weeks	294 (13.7%)
>20 to ≤24 weeks	210 (9.8%)
>24 to ≤28 weeks	267 (12.5%)
>28 to ≤32 weeks	134 (6.3%)
>32 to ≤40 weeks	896 (41.9%)
>40 weeks	96 (4.5%)
Trimester when ART started, n (%)	
First	1126 (52.6%)
Second	771 (36.0%)
Third	242 (11.3%)
Neonatal ART exposure	
Children with data on duration of neonatal ART exposure, n (%)	2221 / 2405 (92.3%)
Neonatal exposure, weeks	
Mean (SD)	5.4 (1.3)
Median (range)	6.0 (0.1–16.3)
Duration of ART exposure, n (%)	n=2221
≤4 weeks	190 (8.6%)
>4 to ≤5 weeks	700 (31.5%)
>5 to ≤6 weeks	361 (16.3%)
>6 to ≤7 weeks	911 (41.0%)
>7 to ≤8 weeks	36 (1.6%)
>8 weeks	23 (1.0%)

Reference Table MITOC 4.3.2

Table 11 shows the results from 2070 children in the primary analysis population who had data available on the duration of *in utero*, intrapartum and neonatal ART exposure. The median cumulative time that children were exposed to ART was 34 weeks (range 4.0-51.6 weeks). The majority of children were exposed for 28-48 weeks (67%).

Table 11. Cumulative ART exposure in the primary population (n=2405)

Duration of ART exposure	Number of children, n (%)
In utero ART exposure	
Children with data on duration of *in utero,* intrapartum and neonatal ART exposure, n (%)	2070 (2405) (86.1%)
Cumulative ART exposure, weeks	
Mean (SD)	32.4 (11.7)
Range	4.0–51.6
Duration of cumulative ART exposure, n (%)	
>2 weeks to ≤12 weeks	153 (7.4%)
>12 to ≤20 weeks	219 (10.6%)
>20 to ≤24 weeks	130 (6.3%)
>24 to ≤28 weeks	168 (8.1%)
>28 to ≤32 weeks	266 (12.9%)
>32 to ≤40 weeks	319 (15.4%)
>40 to ≤48 weeks	808 (39.0%)
>48 to ≤56 weeks	7 (0.3%)

Reference Table MITOC 4.3.3

The majority of children were exposed to at least one NRTI drug, and almost all were exposed to zidovudine (99.8%), at least neonatally. Lamivudine, tenofovir and emtricitabine were also commonly used NRTI drugs. There were 1709 children (71.1%) exposed to at least one PI. The most commonly used PI was KALETRA (lopinavir/ritonavir), to which 1116 of 2405 (46.4%) mothers were exposed.

There were 657 out of 2405 children (27.3%) exposed to at least one NNRTI; the most commonly used NNRTI was nevirapine (23.5%). Other drugs, such as integrase inhibitors (raltegravir) and entry inhibitors (enfuvirtide and maraviroc), were used in a minority of cases. There were 18 children (0.7%) exposed to unspecified ARV drugs.

CHMP comment:

The information in this section should be stratified by birth cohort.

Exposure to ART occurred in almost equally high proportions in utero, intrapartum and during the neonatal period. Table 10 appears to dichotomise data from exposure in utero and data from neonatal exposure. With regards to in utero exposure, 52.6% started ART in the first trimester and the largest proportion of subjects were exposed to ART in utero for ≥32 to ≤40 weeks. For neonatal exposure, the mean exposure was 5.4 weeks with the highest proportions of patients receiving ART for 6- 7 weeks.

Table 11 provides cumulative exposure to ART following exposure from any route (in utero, intrapartum and neonatal). 39% of patients experienced exposure to ARTs from >40 to ≤42 weeks.

As a reminder, there were discrepancies in data collection with regards to exposure, as in the German cohort all *in utero* ARV drugs without a recorded start date were assumed to have been taken since the first day of the pregnancy.

99.9% of exposure occurred to an N(t)RTI, largely to zidovudine (99.8% of the primary population were exposed). 30% and 24.7% of the primary population were exposed to tenofovir and emtricitabine respectively (including all known in utero, intrapartum or neonatal exposure).

Heterogenity in practice/ prescription across the cohorts has not been presented in this section. There was evidence of different practice across the cohorts with regards to prescriptions, for example abacavir use was higher in BE and FR than in the other cohorts.

Screening Assessment

During the MITOC screening assessment, neurological disorders were recorded in predefined categories. These disorders were not primary endpoints at this stage - this was a screening phase to collect all disorders which might be primary endpoints after additional evaluation in the neurological questionnaire. Any relevant additional data from the screening questionnaire, such as neurological conditions in the physical examination, hospitalisation or non-neurological conditions sections, were subsequently classified into the pre-defined categories in the medical review. Results are shown in Table 13.

There were 165/2405 children (6.9%) in the primary population who had at least one neurological disorder reported in the first sections of the screening questionnaire (previous neurological disorders, previous non-neurological disorders, physical examination and hospitalisation); some patients had more than one disorder. Of the pre-defined categories on the previous neurological disorders section of the screening questionnaire, the most common disorders in the primary population were developmental delay (n=64, 2.7%), febrile convulsions (n=35, 1.5%) and motor abnormalities (n=35, 1.5%).

The percentage of children with any neurological disorder recorded ranged from 4.7% (48/1016 children) in the Italian cohort to 15.2% (44/289) in the Belgian cohort.

Table 13. Neurological disorders in the primary population in each cohort recorded in the screening questionnaire[e] (n=2405)

Neurological disorder	France n=458	Germany n=244	Italy n=1016	Spain n=351	Switzerland n=47	Belgium n=289	Total n=2405
Any disorder	25 (5.5%)	12 (4.9%)	48 (4.7%)	33 (9.4%)	3 (6.4%)	44 (15.2%)	165 (6.9%)
Febrile convulsions	8 (1.7%)	2 (0.8%)	14 (1.4%)	5 (1.4%)	1 (2.1%)	5 (1.7%)	35 (1.[illegible]%)
Non-febrile convulsions	1 (0.2%)	1 (0.4%)	3 (0.3%)	3 (0.9%)	0	1 (0.3%)	[illegible] (0.4%)
Developmental delay	9 (2.0%)	5 (2.0%)	19 (1.9%)	11 (3.1%)	1 (2.1%)	[illegible]	64 (2.7%)
Motor abnormalities	6 (1.3%)	4 (1.6%)	9 (0.9%)	6 (1.7%)	0	10 (3.5%)	35 (1.5%)
Behavioural disorders	3 (0.7%)	2 (0.8%)	3 (0.3%)	3 (0.9%)	[illegible]	6 (2.1%)	18 (0.7%)
Other significant cognitive delay or abnormalities	14 (3.1%)	4 (1.6%)	15 (1.5%)	21 (6[illegible]%)	[illegible]	18 (6.2%)	72 (3.0%)

Of the 165 children with at least one neurological disorder at any time reported in the first sections of the screening questionnaire, 43 were classified as having 'presence of explainable NCS' and 39 with 'presence of unexplained NCS' at the MITOC screening assessment at 18 to <28 months of age (table below). There was one additional patient who did not have a neurological disorder recorded, but who was still classified as having an explained NCS in the summary page. This means that the total number of children with an explainable NCS was 44, and the total number of children with any NCS (explainable plus unexplained on the summary page) was 83. There were 82 children who had at least one neurological disorder in the screening questionnaire, but were classified on the summary page as 'absence of neurological conditions' at the time of the screening assessment.

The prevalence of explained plus unexplained neurological clinical symptoms was highest in the Belgian cohort (23/289, 7.96%) and lowest in Italy (14/1016, 1.38%).

The prevalence of explainable NCS was highest in Spain (15/351, 4.27%) and lowest in Italy (8/1016; 0.79%). The prevalence of unexplained NCS was highest in Belgium (15/289, 5.19%) and lowest in Italy (6/1016, 0.59%).

Table 10. MITOC Study: Outcomes from the Screening Questionnaire by Cohort (Primary Analysis Population)

Outcome	Cohort	n / N	Prevalence, %
Presence of any neurological clinical symptoms (explainable plus unexplained on summary page)	France	22 / 458	4.8% (3.0–7.2%)
	Germany	4 / 244	1.6% (0.4–4.1%)
	Italy	14 / 1016	1.4% (0.7–2.3%)
	Spain	18 / 351	5.1% (3.1–8.0%)
	Switzerland	2 / 47	4.3% (0.5–1[illegible]%)
	Belgium	23 / 289	8.0% (5.1–11.[illegible]%)
	Overall	83 / 2405	3.5% (2.8–4.3%)
Presence of explainable neurological clinical symptoms (summary page)	France	10 / 458	2.2% (1.1–4.0%)
	Germany	2 / 244	0.8% (0.1–2.9%)
	Italy	8 / 1016	0.8% (0.3–1.6%)
	Spain	15 / 351	4.3% (2.4–7.0%)
	Switzerland	1 / 47	2.1% (0.1–11.3%)
	Belgium	8 / 289	2.8% (1.2–5.4%)
	Overall	44 / 2405	1.8% (1.3–2.5%)
Presence of unexplained neurological clinical symptoms (summary page)	France	12 / 458	2.6% (1.4–4.5%)
	Germany	2 / 244	0.8% (0.1–2.9%)
	Italy	6 / 1016	0.6% (0.2–1.3%)
	Spain	3 / 351	0.9% (0.2–2.5%)
	Switzerland	1 / 47	2.1% (0.1–11.3%)
	Belgium	15 / 289	5.2% (2.9–8.4%)
	Overall	39 / 2405	1.6% (1.2–2.2%)

CHMP comment:

There was a wide range in the percentage of neurological abnormalities of any kind diagnosed between cohorts (4.7% in the Italian cohort to 15.2% in the Belgian cohort) although it is acknowledged that the number of subjects in each cohort also varied widely.

Although developmental delay was the most commonly cited disorder overall, in each cohort a significant proportion also had unspecified "other significant cognitive delay or abnormalities".

The table above shows that the prevalence of explained and unexplained NCS is quite variable among the various birth cohorts, which suggests there may be some variation of classification of the primary outcome among the birth cohorts. This is not unexpected given the differences in evaluation and actions taken during the screening visits highlighted in Section Study Visits. The authors have discussed classification bias in the discussion on strengths and limitations below.

Although there were 165 children who were initially considered to have some form of neurological abnormality, only 83 children were subsequently classified as having either explained or unexplained neurological symptoms. The MAHs/ authors have not stated why the remaining 82 children with a neurological abnormality were not classified in either of these categories and why these were not considered to require a neurological assessment.

Also, although these 83 children were considered by the authors to have either explained or unexplained neurological symptoms, it appears that these children were initially classified as having an absence of neurological disorders by the assessing clinicians. This discrepancy was presumably identified by the committee appointed medical reviewers who reviewed the assessment questionnaires and it is then unclear if the referral for neurological assessment was then prompted by the committee's medical reviewers.

Nevertheless the findings here indicate uncertainty in the classification of the neurological abnormalities and inconsistencies in evaluation of the subjects at the screening assessment.

Neurological Assessment

Figure 3 shows the number of children with explained or unexplained NCS who were subsequently evaluated by a neurologist or a paediatrician. Neurological questionnaires were completed for 33/44 children with explainable NCS, 37/39 with unexplainable NCS and 4/2322 children who were reported not to have NCS. This resulted in 74 neurological questionnaires being completed.

Any children with suspected mitochondrial disorder, strong evidence of mitochondrial disorder (neurological questionnaire) or unexplained NCS (screening questionnaire) were referred to the DRC to determine whether mitochondrial disorder was present.

Of the 74 children evaluated using the neurological questionnaires, 2 children were classified as having suspected mitochondrial disorder and 25 were classified as having unexplainable neurological abnormalities but no evidence of mitochondrial dysfunction. The clinical data from these 27 children were therefore reviewed by the DRC.

The remaining 47 children had an explainable neurological disorder diagnosed. These children were reviewed by the DRC for quality control purposes only.

Figure 3. Flowchart of the number of children who were evaluated by a paediatric neurologist and the outcome (the reference table for the data is shown in the blue box)

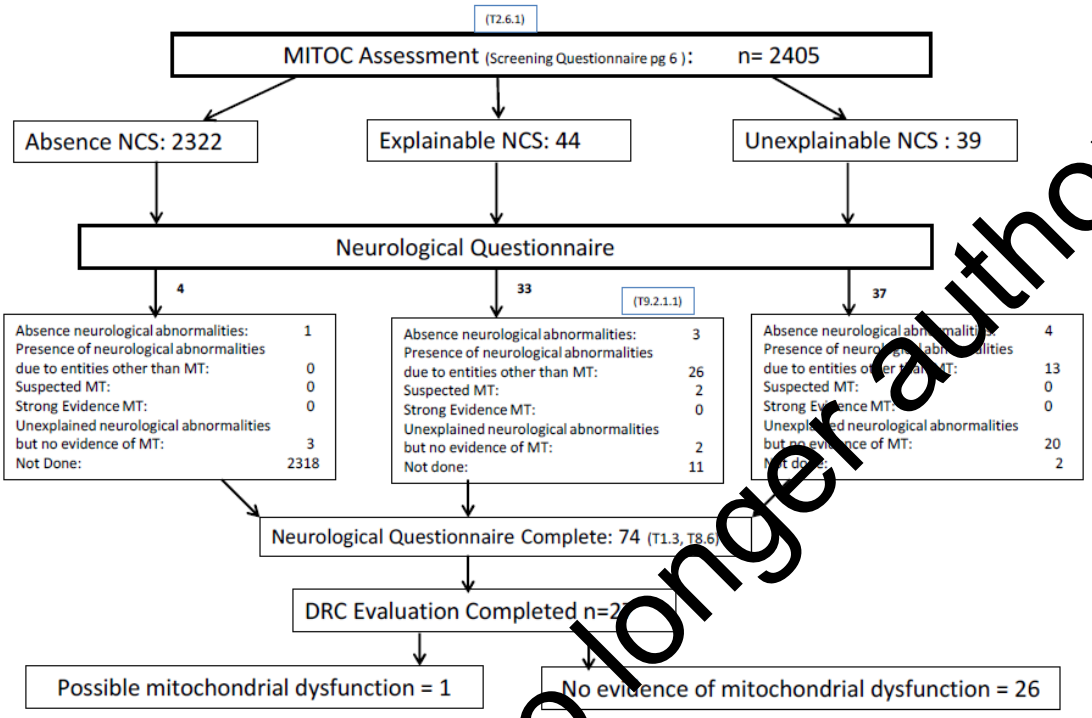


Figure 1.

Table 15 presents the number of screening and neurological questionnaires completed by each cohort in the MITOC study compared with the number of children who had a neurological disorder. Overall, there were 165 children who had at least one neurological disorder recorded in the screening questionnaire. There were 83 children who were referred to a neurologist, and 74 had a completed neurological questionnaire.

Table 15. Number of children in the primary analysis population with screening and neurological questionnaires (n=2405)

Cohort	Number of children with screening questionnaires	Number of children with neurological disorders	Rate of NCS before 18 months	Number of children referred to a neurologist	Number of children with NCS at 18 to <28 months	Number of neurological questionnaires
France	458	25	5.5%	22	25	25
Germany	244	12	4.9%	4	2	2
Italy	1016	48	4.7%	14	11	11
Spain	351	33	9.4%	18	17	17
Switzerland	47	3	6.4%	2	1	1
Belgium	289	44	15.2%	23	18	18
Total	**2405**	**165**	**6.9%**	**83**	**74**	**74**

CHMP comment:

The majority of the 83 subjects that were considered to have either explained or unexplained NCS underwent a neurological assessment (70/83), as well 4 subjects that were not considered to have any symptoms of NCS at all. The reason for these 4 subjects also undergoing neurological assessment is unclear (which is not required according to the protocol).

A number of neurological symptoms were reported as occurring prior to the 18 month point, the highest rate of this occurred in the Belgian cohort.

Neurological questionnaires

Table 16 presents the NCS or abnormalities reported in the 74 children in the primary analysis population with neurological questionnaires. Of these, 60 children (81.9%) had any neurological abnormality at this moment or in the past reported in the neurological questionnaire. The remaining 14 children were reported as no longer having a neurological disorder at the time of the neurological assessment.

The most commonly reported neurological disorders were mental retardation (35.1%) and behavioural disturbances (25.7%). Thirty children (41.7%) had investigations performed for abnormalities.

Table 16. Neurological symptoms or abnormalities reported in all ART-exposed children with neurological questionnaires (n=74)

Neurological symptom / abnormality	Number of children or measure
Any abnormality at this moment or in the past, n (%)	60 / 74 (81.1%)
Any investigations for other abnormalities performed, n (%)	30 / 74 (40.5%)
Mental retardation, n (%)	26 / 74 (35.1%)
Behavioural disturbances, n (%)	19 / 74 (25.7%)
Abnormal stiffness (pyramidal signs), n (%)	6 / 74 (8.1%)
Microcephaly, n (%)	14 / 73 (19.2%)
Microcephaly measure, cm	n=14
Mean (SD)	42.4 (6.36)
Range	28.7–48.5
Abnormal movements, n (%)	6 / 74 (8.1%)
Peripheral neuropathy, n (%)	2 / 74 (2.7%)
Presence of a nystagmus, n (%)	2 / 74 (2.7%)
History of febrile seizures, n (%)	15 / 74 (20.3%)
History of afebrile seizures, n (%)	6 / 74 (8.1%)

Table 17 presents the neurological clinical symptoms or abnormalities reported in the neurological questionnaires of the 27 children with unexplained NCS or suspected mitochondrial dysfunction. All these children were reported to have any neurological abnormality at this moment or in the past reported. The most commonly reported neurological disorders were mental retardation (30%) and behavioural disturbances (22%).

Table 17. Neurological symptoms or abnormalities reported in all ART-exposed children who were reported to have unexplained NCS or suspected mitochondrial dysfunction in their neurological questionnaires (n=27)

Neurological symptom / abnormality	Number of children or measure
Any abnormality at this moment or in the past, n (%)	27 / 27 (100%)
Any investigations for other abnormalities performed, n (%)	10 / 27 (37.0%)
Mental retardation, n (%)	8 / 27 (29.6%)
Behavioural disturbances, n (%)	6 / 27 (22.2%)
Abnormal stiffness (pyramidal signs), n (%)	2 / 27 (7.4%)
Microcephaly, n (%)	1 / 27 (3.7%)
Microcephaly measure, cm Measurement	n=1 45.5cm
Abnormal movements, n (%)	3 / 27 (11.1%)
Peripheral neuropathy, n (%)	1 / 27 (3.7%)
Presence of a nystagmus, n (%)	2 / 27 (7.4%)
History of febrile seizures, n (%)	4 / 27 (14.8%)
History of afebrile seizures, n (%)	2 / 27 (7.4%)

Of the 74 children with neurological questionnaires, there were 35 who underwent magnetic resonance imaging (MRI) procedures as part of their neurological assessment (Table 18). White matter lesions were identified in 8 children and cortical focal lesions in 3. Eight children had a CT scan, of which 6 showed abnormal results. One MRI spectroscopy was performed and showed no lactate peaks.

Few children underwent other procedures as part of the neurological assessment, such as fundoscopy (n=14; 1 abnormal), muscle electrophysiological studies (n=2; both normal), peripheral nerve conduction studies (n=1; normal), and electroretinogram (n=1; normal). Muscle biopsies were indicated for 4 children, but only 2 were performed. The muscle biopsy provided data on histochemistry and/or electronic microscopy, respiratory chain enzymatic studies. Mitochondrial DNA quantification and other mitochondrial specific tests were performed on 1 child. The information available from this skeletal muscle biopsy was conflicting, in that no histological or significant histochemical abnormalities were identified, but a biochemical abnormality of both complexes I and IV of the mitochondrial respiratory chain was identified on spectrophotometry. Moreover, the spectrophotometry and BN-PAGE results are conflicting, the spectrophotometric results are not compatible with the histochemistry, and the BN-PAGE results are at odds with the clinical presentation. The second muscle biopsy showed normal biochemical activity of the mitochondrial respiratory

complexes, but 'swollen' mitochondria were noted on electron microscopy. No mtDNA mutations were identified.

Table 18. All children in the primary analysis population undergoing neurological assessment (n=74) – imaging procedures and muscle biopsies

Outcome	Number of children, n (%)	
Brain MRI	35 / 74 (47.3%)	
	Present	**Absent**
White matter lesions	8	27
Basal ganglia lesions	1	34
Brainstem lesions	2	33
Cortical focal lesions	3	32
Cortical atrophy	2	33
Ventriculomegaly	10	25
MRI spectroscopy	1 / 74 (1.4%)	
	Present	**Absent**
Lactate peak	0	1
Other procedures		
	Normal	**Abnormal**
CT Scan	2	6
Fundoscopy	14	1
Muscle electrophysiological studies	2	0
	Performed	**Not Performed**
Peripheral Nerve Conduction Studies	1 (normal)	48
Electroretinogram	1 (normal)	48

At the end of the neurological questionnaire, the neurologist was asked to assess the likelihood that the child was exhibiting signs of mitochondrial disorder and allocate them to one of five categories (Table 19). There were no cases of 'strong evidence for mitochondrial disorder' and 2/74 (2.7%) children were considered to have 'suspected mitochondrial disorder'.

Table 19. Outcome of neurological assessment for the primary population (n=74)

Primary endpoints shown in bold.

Outcome	Number of children, n (%)
Neurological assessment	74
Suspected mitochondrial disorder	**2 (2.7%)**
Strong evidence for mitochondrial disorder	0
Unexplained neurological abnormalities but no evidence of mitochondrial disorder	**25 (33.8%)**
Absence of neurological abnormalities	8 (10.8%)
Presence of neurological abnormalities due to entities other than mitochondrial disorder	39 (52.7%)

The percentage of children with the primary endpoint was different between cohorts (Table 20). Of the 27 primary endpoints, 22 were diagnosed in either the French or Belgian cohorts.

Table 20. Outcome of neurological assessment for the primary population (n=74) by cohort

	Number of children, n (%)					
Outcome	France	Germany	Italy	Spain	Switzerland	Belgium
Neurological assessment	25 / 458 (5.5%)	2 / 244 (0.8%)	11 / 1016 (1.0%)	17 / 351 (4.8%)	1 / 47 (2.1%)	[illegible]
Suspected mitochondrial disorder	0	0	1	0	0	1
Strong evidence for mitochondrial disorder	0	0	0	0	0	0
Unexplained neurological abnormalities but no evidence of mitochondrial disorder	15	0	3	[illegible]	1	6
Absence of neurological abnormalities	0	0	[illegible]	4	0	3
Presence of neurological abnormalities due to entities other than mitochondrial disorder	10	[illegible]	6	13	0	8
Primary endpoint	**15 / 458 (5.5%)**	**0 / 244 (0.0%)**	**4 / 1016 (0.4%)**	**0 / 351 (0.0%)**	**1 / 47 (2.1%)**	**7 / 289 (2.4%)**

CHMP Comment:

No explanation has been provided to account for the previous neurological symptoms experienced by the14 children that were reported as no longer having a neurological disorder. As mentioned previously, there is a potential for reversibility of possible mitochondrial effects of ART following discontinuation (Blanche, 1999, Barret B, 2003, Morén, 2014).

The explanations for the analyses of the cases referred for neurological assessment and how they were subsequently dismissed as not being related to mitochondrial dysfunction are not clear. For reports of "unexplained NCS" (n=39), summaries of case narratives have been provided in the appendices, some reports have been explained as single occurrences of febrile seizures, others were confounded by prematurity, diagnosed chromosomal abnormalities, hypoxic insults at birth, cerebral palsy, CNS infection, maternal drug abuse during pregnancy. It is worth mentioning that the presence of the alternative explanations do not in themselves exclude the presence of a concurrent mitochondrial dysfunction. However there were also cases within this category where a decision of no evidence of mitochondrial dysfunction had been made without a rationale or alternative explanation for the

symptoms.

26 out of 35 children that underwent a brain MRI had at least one brain abnormality identified, along with 6 out of 8 children that had CT imaging. These abnormalities have not been attributed to mitochondrial dysfunction however no explanations have been provided for them.

25 children were referred to the DRC as they were considered to have unexplained NCS but no evidence of a mitochondrial disorder. The study report does not explicitly explain what evidence for mitochondrial disorders was considered by the clinicians was for these cases or the rationale used to exclude mitochondrial disorders. Only 2 children were considered to have "suspected mitochondrial disorder".

The percentage of children with the primary endpoint was different between the cohorts. Of the 27 primary endpoints, 22 were diagnosed in either the French or Belgian cohorts. This implies there may be classification bias between the cohorts.

Analysis of the Primary Endpoint

Univariate analysis

Univariate analysis of the primary endpoint (the number of subjects in the Primary Analysis Population meeting the case definition of neurological clinical symptoms of cognitive or motor delay, with or without seizures, without attribution of cause), at the neurological assessment showed that unexplained neurological clinical symptoms were significantly associated with the following:

- Sex: boys were 3.4 times more likely to be diagnosed with the primary endpoint than girls.
- Type of N(t)RTI: subjects exposed to abacavir were 2.3 times more likely to be diagnosed with the primary endpoint than subjects not exposed to abacavir.
- Cohort: children in the French plus Belgian cohorts were 9.8 times more likely to be diagnosed with the primary endpoint than subjects in the Italian, German, Spanish, and Swiss cohorts (combined).
- Head Circumference: children with a head circumference less than the median, or with a gestational age less than the median, were less likely to have the primary endpoint.

Univariate analysis demonstrated that there was no difference in relative risk of the primary endpoint by gestational age at birth, body weight at birth, or in utero exposure to ZDV, tenofovir, stavudine or didanosine, and PIs (Table 17).

Overall exposure to abacavir was higher in France and Belgium (125 of 747 subjects, 16.7%) than in Spain, Italy, Switzerland, and Germany combined (169 of 1658 subjects, 10.2%), which may partly explain the univariate correlation between exposure to abacavir and the higher risk of the primary endpoint in the Belgian and French cohorts.

Table 17. MITOC Study: Relative Risk of Neurological Clinical Symptoms of Cognitive or Motor Delay; Univariate Analysis (Primary Analysis Population)

Risk factor	Group 1 n / total	Group 2 n / total	Relative risk (95% CI)
Sex (male vs female)	21 / 1222	6 / 1183	3.39 (1.37–8.37)
Gestational age at birth (<median vs > median)	5 / 955	22 / 1450	0.35 (0.13–0.91)
Head circumference (<median vs > median)	10 / 1614	17 / 791	0.29 (0.13–0.63)
Weight at birth (<median vs > median)	14 / 1226	13 / 1179	1.04 (0.49–2.19)
In utero exposure to zidovudine (yes / no)	14 / 1329	13 / 1076	0.87 (0.41–1.85)
In utero exposure to abacavir (yes / no)	7 / 314	20 / 2091	2.33 (0.99–5.47)
In utero exposure to tenofovir (yes / no)	8 / 72	19 / 1684	0.98 (0.43–2.24)
In utero exposure to protease inhibitors (yes / no)	21 / 1705	6 / 700	1.44 (0.58–3.55)
In utero exposure to stavudine or didanosine (yes / no)	1 / 115	26 / 2290	0.77 (0.10–5.59)
Country of study (France / Belgium vs others)	22 / 747	5 / 1658	9.78 (3.71–25.69)

A sensitivity analysis of the primary endpoint was performed using the secondary analysis population of all potentially eligible subjects for whom informed consent had been obtained, and who were confirmed to be HIV negative, whether or not they had been exposed to ART (the All Enrolled, HIV Negative Subject Analysis Population; N = 2855). Among this secondary population, 35 subjects had the primary endpoint. Risk factors for the primary endpoint in this sensitivity analysis were the same as in the Primary Analysis Population (Table 18).

Table 18. MITOC Study: Relative Risk of Neurological Clinical Symptoms of Cognitive or Motor Delay; Univariate Analysis (All Enrolled, HIV Negative Subject Analysis Population)

Risk factor	Group 1 n / total	Group 2 n / total	Relative risk (95% CI)
Sex (male vs female)	29 / 1467	6 / 1387	4.57 (1.90–10.98)
Gestational age at birth (<median vs > median)	9 / 1074	26 / 1736	0.56 (0.26–1.19)
Head circumference (<median vs > median)	10 / 723	19 / 1003	0.73 (0.34–1.56)
Weight at birth (<median vs > median)	19 / 1388	16 / 1389	1.19 (0.61–2.30)
In utero exposure to zidovudine (yes / no)	17 / 1562	18 / 1293	0.78 (0.40–1.51)
In utero exposure to abacavir (yes / no)	10 / 380	25 / 2475	2.61 (1.26–5.38)
In utero exposure to tenofovir (yes / no)	8 / 830	27 / 2025	0.72 (0.33–1.58)
In utero exposure to protease inhibitors (yes / no)	27 / 2053	8 / 802	1.32 (0.60–2.89)
In utero exposure to stavudine or didanosine (yes / no)	2 / 155	33/2720	1.22 (0.30–5.04)
Country of study (France / Belgium vs others)	30 / 1044	5/1811	10.41 (4.05–26.74)

CHMP comment:

The MAHs/study authors have not explicitly discussed the statistical methods used to conduct the univariate analysis and this should be clarified.

The rationale for aggregating the Belgian and French cohorts and comparing them to all others does not seem to have been pre-specified or adequately justified. Each cohort should be considered individually and compared with a single cohort comparator.

Head circumference and gestational age at birth variables were dichotomised and as indicated previously, these should be categorised so as to provide sufficient granularity to allow for a proper assessment.

Multivariate analysis

The only statistically significant predictors of the primary endpoint by multivariate analysis were the sex and gestational age of subjects, and the cohort in which a subject was enrolled (Table 19). Boys and subjects with a gestational age less than the median were more likely to be diagnosed with the primary endpoint than girls and subjects with a gestational age greater than the median (p = 0.02

and p = 0.05, respectively). Subjects in the French and Belgian cohorts were more likely to be diagnosed with the primary endpoint than subjects in the other cohorts (p = 0.0001). There was no statistically significant independent effect of exposure to any ART drug on the risk of being diagnosed with the primary endpoint.

Table 19. MITOC Study: Relative Risk of Neurological Clinical Symptoms of Cognitive or Motor Delay; Multivariate Analysis (Primary Analysis Population)

Predictive factor	Chi-square	p-value
Cohort (France / Belgium versus other)	15.08	0.0001
Gender (male versus female)	5.09	0.02
Gestational age at birth (<median versus >median)	3.89	0.05

By multivariate analysis of the secondary analysis population (all potentially eligible subjects for whom informed consent had been obtained, and who were confirmed to be HIV negative, whether or not they had been exposed to ART; the All Enrolled, HIV Negative Subject Analysis Population; N = 2855), the only statistically significant predictors of the primary endpoint were the sex of a subject, and the cohort in which a subject was enrolled (Table 20). Boys and subjects in the French and Belgian cohorts were more likely to be diagnosed with the primary endpoint than girls and subjects in the other cohorts (p = 0.003 and p < 0.0001, respectively). There was no statistically significant independent effect of exposure to any ART drug on the risk of being diagnosed with the primary endpoint.

Table 20. MITOC Study: Relative Risk of Neurological Clinical Symptoms of Cognitive or Motor Delay; Multivariate Analysis (All Enrolled, HIV Negative Subject Analysis Population)

Predictive factor	Chi-square	p-value
Cohort (France / Belgium versus other)	15.4	<0.0001
Gender (male versus female)	8.96	0.003

CHMP comment:

Several parameters with known biological associations to the primary endpoint were not included in the multivariate analysis such as prematurity, birth complications, APGAR scores, maternal substance abuse. Markers of maternal viral control, concurrent hepatitis B/C co-infection and concurrent medications which may also influence foetal neurological outcomes (such as antiepileptic medication) also appear not to have been taken into account here.

Of note although data on length of exposure to ART and trimester of exposure was collected, possible associations with these variables and the primary outcome have not been investigated.

As a reminder two of the objectives included a determination of the prevalence of neurological clinical symptoms of cognitive or motor delay without attribution of cause and to assess the association

between type and duration of ART exposure in utero and/or early neonatal life and unexplained and mitochondrial disorder-related cognitive or motor delay. Even though the MAHs/ authors only considered there to be one event of "possible" mitochondrial dysfunction, the prevalence of unexplained neurological disorders should be presented.

In the multivariate analysis no justification has been provided for inclusion of the variables in the model. The multivariate analyses (table 23, 24) do not include adjusted odds ratios which should be presented so as to allow an assessment of the magnitude of risk to be made.

DRC Review

27 subjects were referred to the DRC for review, comprising 2 subjects with suspected mitochondrial disorder and 25 subjects with unexplained neurological clinical symptoms. Of these 27 subjects, 1 subject was identified by the DRC as having possible mitochondrial dysfunction.

Table 21. MITOC Study: Outcome of DRC Review of Subjects Referred Following Neurological Assessment (Primary Analysis Population)

Outcome (neurological questionnaire)	DRC consensus			
	Definite MT dysfunction	Probable MT dysfunction	Possible MT dysfunction	No evidence of MT dysfunction
Suspected MT disorder (n = 2)	0	0	1	1
Unexplained neurological abnormalities but no evidence of MT disorder (n = 25)	0	0	0	25
Total number of children undergoing a DRC review (n=27)	0	0	1	26

The number of subjects meeting the criteria for neurological assessment was markedly less than anticipated. The DRC therefore reviewed all other completed neurological assessment questionnaires (n = 47) and available neuroimaging scans for quality control purposes, to provide a superior data assessment. The DRC was able to request further information from cohorts to aid subject review, which was mostly in relation to the "presence of neurological abnormalities due to entities other than mitochondrial disorder", when little or no information regarding diagnosis of neurological abnormalities had been provided. A sensitivity analysis of the outcome of the DRC review performed on the secondary All Enrolled, HIV Negative Subjects Analysis Population, among whom 35 subjects had the primary endpoint, identified 1 subject with a suspected mitochondrial disorder, as with the Primary Analysis Population

Table 22. **MITOC Study: Outcome of DRC Review of Subjects Referred Following Neurological Assessment (All Enrolled, HIV Negative Subjects Analysis Population)**

Outcome	DRC consensus			
	Definite MT dysfunction	Probable MT dysfunction	Possible MT dysfunction	No evidence of MT dysfunction
Suspected MT disorder (n = 3)	0	0	1	2
Unexplained neurological abnormalities but no evidence of MT disorder (n = 32)	0	0	0	32
Total number of children undergoing a DRC review (n=35)	0	0	1	34

CHMP comment:

At the end of the neurological assessment, 25 subjects were considered to have unexplained neurological symptoms, thereby suggesting that no diagnosis could be made by the clinicians given the available evidence (or lack of it). These subjects were also considered by the DRC to have no evidence of a mitochondrial disorder, however in most cases the evidence used to reach this conclusion was not clear from the case summaries. The documentation of the rationale for excluding mitochondrial disorders is particularly important in the knowledge that mitochondrial dysfunction can present in a wide spectrum of clinical presentations with considerable clinical variability and do not always fit into a particular cluster of clinical features (Chinnery, 2000, (updated 2014), Koenig, 2008, Mattman, 2011) and therefore prone to subjective opinion.

More specific mitochondrial investigations appear to only have been performed in two subjects, and questions have been raised by some of the study investigators on whether the exclusion of mitochondrial dysfunction in the absence of these tests is premature, given that the known clinical spectrum of presentation of mitochondrial disease is wide and indistinct making diagnosis challenging (Rodenburg, 2011) and this continues to change (please refer to Section Report of discordance). The authors themselves acknowledge this in Section Strengths and Limitations.

As an illustration to the assessment of cases from the secondary population (all HIV negative children in the study), the majority of these were cases of cognitive and psychomotor delay or behavioural abnormalities (25/35 identified) sometimes with neuroradiological abnormalities that were not explained. These reports are considered important as the majority of subjects in the original studies by Blanche and Barett reported these symptoms and similar manifestations have also been found with in utero exposure to other medication known to cause mitochondrial dysfunction (Morén, 2014, Lloyd, 2013, Velez-Ruiz, 2015).

It is noted that the DRC disagreed with some of the opinions of the clinicians presented to them. In one of the DRC narratives, a conclusion was that “abnormalities may be due to genetic disease” without any evidence of a clear genetic syndrome or any karyotype testing performed. In addition, of the two subjects that did have muscle biopsies, although abnormalities were detected, these were not

considered significant by the DRC. In some cases the DRC also contested abnormal neuroradiological findings and were in disagreement with the opinion of the clinicians. The committee also dismissed reports of hyperlactataemia on the basis that units were not provided for lactate or concurrent metabolic acidosis was not reported (although the two are not synonymous events). This again highlights the highly subjective nature of the data evaluated and the potential for differences in interpretation. In these situations the validity and reproducibility of the conclusions are brought into question.

For the three cases of suspected mitochondrial dysfunction more detail was provided. In one case, diagnosis was complicated by Hepatitis C infection in the child and a maternal history of epilepsy and drug abuse. A genetic disorder was suspected and on the basis of improving psychomotor delay, lack of parenchymal disease on the MRI and a report by a mitochondrial expert apparently indicating an undisclosed genetic disorder, the case was attributed to "unexplained neurological abnormality." In the remaining two cases, both had mitochondrial abnormalities on biopsy. One was considered "possible mitochondrial dysfunction" as biopsy results were thought by the DRC to be conflicting. In the other the DRC disagreed with the reporting clinician's views on the neuroradiology and excluded mitochondrial disease on the basis mitochondrial respiratory chain analysis was normal.

Strengths and limitations

The strengths of the MITOC Study were that it involved multiple European countries with multiple sites, and had a standard protocol across all the sites. In addition, the Study was a reflection of ART to avoid mother-to-child transmission of HIV-1 in Europe, with most children (99.8%) exposed to zidovudine, 70% exposed to 3TC, 25% to FTC, 20% to tenofovir and 14% to abacavir. It had very low use of ddI/d4T. Seventy percent of children were also exposed to a PI and 27% to an NNRTI.

The MITOC Study was a cross-sectional study that was nested in prospective birth cohorts, and which followed up children that were alive at 18 to <24 months of age. As such, it was not a true prospective study, but rather a 'snap-shot' at a specific time (18 to <28 months old). This means that it is possible that delayed clinical presentation of neurological disorders at 2 to 3 years' old would be missed, especially those that are difficult to ascertain before the age of 3 or 4 years. The pragmatic design of the Study is based on the fact that long-term follow-up of HIV-negative children beyond 2 years' old is rare in most existing European cohorts. Therefore, the Study design is based on the time that mitochondrial dysfunction is most likely to present as unexplained neurological abnormalities. It was assumed that children with these abnormalities would have a more thorough evaluation by a paediatric neurologist.

The primary endpoint was defined according to the neurological questionnaire, which relied upon referral practices in each cohort. The paediatricians, neurologists and DRC were not blinded to the type of ART drugs to which the children and their mothers had been exposed. The study design assumed that unexplained neurological abnormalities would be referred to a local paediatric neurologist, consistent with the local standard of care, and that most of these children would therefore have a more thorough evaluation. Paediatric neurologists were asked to classify cases into absence of neurological abnormalities, presence of neurological abnormalities due to entities other than mitochondrial disorders, suspected mitochondrial disorder, strong evidence for mitochondrial disorder or unexplained neurological abnormalities but no evidence of mitochondrial disorder, and this was the final classification used in the Study. However, the Study cannot exclude the possibility that paediatric neurologists interpreted the Study differently and defined unexplained neurological events differently. In the French cohort, not all patients were referred to the neurology department and the neurological classification was defined by the local paediatrician.

There were significant differences in the prevalence of unexplained NCS between the cohorts. The percentage of children with the primary endpoint differed between countries: 0/244 (0.0%) in Germany, 0/351 (0.0%) in Spain, 4/1016 (0.4%) in Italy, 1/47 (2.1%) in Switzerland, 7/289 (2.4%) in Belgium and 15/458 (5.5%) in France. There was a possibility of classification bias resulting from undiagnosed or unrecorded neurological dysfunction in children misclassified as negative for the primary endpoint, which may underestimate the overall prevalence of neurological dysfunction. Furthermore, medical evaluation that was not thorough enough to determinate the aetiology of the neurological disorder may bias the results. This bias may under or overestimate the prevalence of unexplained NCS and underestimate mitochondrial dysfunction, which is very difficult to confirm without complex investigations, including muscle biopsy. Another cause of bias results from eligible children who were not enrolled into the trial for various reasons, and those children who died before their screening or neurological evaluation could be performed.

The most likely explanation for this range of prevalence of unexplained NCS is the difference of cohort characteristics, such as in consent process, data collection and clinical practices. For example, children with consent forms signed at birth are less likely to attend their screening assessment at 18 to <28 months of age and be enrolled in the MITOC study than those who have consent given at their assessment. The cohort with the highest prevalence (Belgium) was predominantly a single centre, where children were seen prospectively by a single paediatrician, while other cohorts had, at least, several sites. The vast majority of cases (22/27) of unexplained NCS came from two cohorts – France and Belgium.

The DRC was established to review all cases of possible and probable mitochondrial toxicity. As the number of these cases was small, the DRC also examined the available data for all unexplained events to preclude the possibility of misclassification. Although the DRC had access to MR images in the majority of cases, it did not have access to the full neurological evaluation, although further details could be requested. The DRC was not established as a formal endpoint evaluation committee and, per the protocol, unless there was compelling evidence to the contrary, it accepted the classification made by the primary neurologist, which was usually unexplained neurological events with no evidence of mitochondrial dysfunction. In retrospect, a more formal process of full evaluation of these cases might have been wiser; however, this was not chosen as an option give the fact that the Study was designed using existing cohorts and following, as much as possible, standard clinical practice in the individual countries.

CHMP comments:

The authors state that a strength of the MITOC study was that a standard protocol was used across all sites. However, it is the view of the assessor that it is not possible to make such an assertion given the heterogeneity in the consent process across sites, which may have introduced a selection bias, and differences in the clinical diagnosis and assessment of children with possible neurological abnormalities. The authors also highlight that in retrospect, a more formal process of full evaluation of cases might have been wiser and this is supported. The MAHs/study authors should discuss the impact of the potential selection bias.

The pragmatic nature of the study design is noted, however this limits the study evaluation to a relatively narrow timeframe and will have potentially missed events occurring after the screening visit or presenting later in childhood.

The MAHs/study authors explain that the study cannot exclude the possibility that paediatric neurologists interpreted the study differently and defined unexplained neurological events differently. In the French cohort, not all patients were referred to the neurology department and the neurological

classification was defined by the local paediatrician. This therefore does not support the claim that a standard protocol was used across all sites and raises concerns that the prevalence of neurological disorders may have been underestimated in this study.

Given the clear differences in the prevalence of unexplained and explained NCS between cohorts, the MAHs/study authors have acknowledged the possibility of classification bias which may underestimate the overall prevalence of mitochondrial dysfunction. The significant differences between cohorts were not fully taken into account in either the univariate or multivariate analysis.

The MAHs/study authors also mention that medical evaluation was not thorough enough to determine the aetiology of the neurological disorder which may bias the results, as discussed in the section above.

The MAHs/study authors have appropriately recognised that the heterogeneity observed in the prevalence of unexplained NCS is due to differences in cohort characteristics, the consent process, data collection and clinical practices. However, the selection and misclassification biases may have also contributed to this.

The limitations of the DRC in evaluating cases, given the lack of access to the patients themselves and a full neurological investigation and in the absence of some key investigations, is acknowledged.

Generalisability

The MITOC Study was a large study with data on prevalence of unexplained NCS from several European cohorts, who managed patients according to their local processes and standards. Although there was marked heterogeneity in the details of local practice and standards of care across the cohorts, which is a limitation, the fact that there was a broad spectrum of cohorts, which involved patients exposed to the drugs generally and commonly utilised across Europe and other regions for prevention of mother-to-child transmission, means that the findings should be broadly generalizable to the wider population.

Conclusions

In the Primary Analysis Population of 2495 ART-exposed subjects, there were a total of 27 subjects with the primary endpoint, giving a prevalence of 1.04% (95% CI: 0.67 to 1.53). The percentage of subjects with the primary endpoint differed between cohorts: 0 of 244 subjects in Germany; 0 of 351 subjects in Spain; 4 of 1026 subjects (0.4%) in Italy; 1 of 47 subjects (2.1%) in Switzerland; 7 of 289 subjects (2.4%) in Belgium; and 15 of 458 subjects (5.5%) in France.

The classifications of the 27 subjects with the primary endpoint by the cohorts in the neurological questionnaire stated that 25 subjects had unexplained neurological disorders but no evidence of mitochondrial disorder, and 2 subjects had suspected mitochondrial disorders. The review of the 27 subjects with the primary endpoint by the DRC found 1 case of possible mitochondrial toxicity (Subject 60105), who had been exposed to atazanavir, ritonavir-boosted lopinavir, lamivudine, and ZDV – this was one of the 2 subjects with suspected mitochondrial disorder originally classified in the neurological questionnaire). In a sensitivity analysis, consistent results were shown for the secondary analysis population (All Enrolled HIV Negative Subjects), which included all 2855 HIV negative subjects. In this analysis, there were 35 subjects with primary endpoints (1.23%, 95% CI: 0.87% to 1.73%).

Report of discordance

The statements below are lifted from a report of discordance signed by the representatives for the French and Belgian cohorts:

1 – Sources of bias related to the study design

1.1 Selection bias:

a) The enrolment of eligible children into MITOC depends on the consent process, and so the age of the enrolled children differs from one cohort to another: in the Belgian and French cohorts, children were included at birth or soon afterwards, whereas children in the Spanish, German, Swiss and Italian cohorts were included either at birth or at the age of 18 months. Therefore, the number of children eligible but not assessed differs greatly from one cohort to another, and was not clearly presented.

We asked for an additional table presenting the various levels of participation by cohort: Eligible children (birth cohort)/ Number of consents / Number of deaths after consent / Number of children lost-to-follow up after consent / Number of children with missing data on HIV status / Number of children evaluated.

b) The mortality rate: death was reported for eligible children after consent had been obtained but not for deaths that occurred before enrolment. The inter-cohort difference in mortality rate highlights the recruitment bias.

The mortality rate is 4 out of 351 in Spain (1.1%) and 0 out of 1046 in Italy (0%) - a highly significant difference.

CHMP comment:

The assessor agrees that the study may be subject to selection bias due to differences in the consent process between the birth cohorts. The MAHs/ authors should provide the additional tables presenting the various levels of participation by cohort so that the number of children eligible but not assessed stratified by birth cohort is clearly presented.

The difference in mortality rate between the cohorts raises concern, although they are based on relatively small numbers. Further data is required on the characteristics of these children and parents, stratified by birth cohort, as there is likely to be some differences in these characteristics between the countries studied.

1.2 Classification bias:

The primary outcome is based on subjective categorization of NCS symptoms as “explained” or “unexplained” in the screening questionnaire.

The proportion of these “unexplained NCS” (i.e. the primary endpoint) varies markedly from one cohort to another, whereas the prevalence of each reported neurological disorder does not vary so much (see Table 13). In particular, some clinical conditions (such as convulsions) have a similar prevalence in the cohorts but were considered to be “explained” in some cohorts and “unexplained” in others.

These discrepancies lead to classification bias in the estimation of the proportion of “unexplained NCS”. This is not sufficiently emphasized in the “limitations” section of the Discussion.

CHMP comment:

The potential for classification bias is acknowledged given the heterogeneity between the birth cohorts in the prevalence estimates for unexplained and explained NCS. The MAHs/study authors have recognised the prevalence may be an underestimate, but are requested to provide further detailed discussion on this with respect to the individual birth cohorts and the difference in the unexplained and explained NCS results observed in the study.

The assessor is in agreement that the variation in classification is a potential significant limitation of the study.

2 –Results for primary outcome

a) Table 6 presents the baseline characteristics by cohort. We requested a table describing (for each cohort) a number of other key variables of ART exposure (the proportion of infants with *in utero* exposure, and the median duration of exposure to each type of NRTI) and clinical data (birthweight, head circumference, premature delivery, deaths), stating the number of missing data. This can be referred to in the discussion of selection biases (instead of merely hypothesizing about heterogeneity clinical practice).

b) Gestational age, head circumference and weight at birth were categorized as above or below the median, in order to establish whether these variables were associated with the primary endpoint. Given that low birth weight (< 2500g), very low birth weight (<1500gr), and prematurity are classical risk factors for neurological delay, we requested categorization for low birth weight and prematurity. Our request was refused. Moreover "small for gestational age" (which is also associated with poor development) was not analyzed at all.

c) In the multivariable analysis, the rationale for retaining an appropriate, final, multivariate model is not explained, and adjusted odds ratios were not presented.

d) In the analysis, the cohorts with the higher number of primary endpoints (France & Belgium) were pooled, so that these endpoints could be described as risk factors. Given that (i) the number of cases is related to the size of the cohort and (ii) the French cohort is one of the largest, the latter logically contributes a large proportion of the primary endpoints, whereas Belgium had much the same rates as Switzerland and Italy for the primary endpoint**.** This post-hoc categorization was not initially planned and, in our opinion, is unjustified. We asked for the cohort effect to be evaluated in six different categories (with the largest cohort taken as the reference, rather than pooling cohorts with higher outcome rates and comparing them with cohorts with lower outcome rates).

CHMP comment:

a) Failure to stratify results by birth cohort is acknowledged and it is agreed that these should be provided. The MAHs/study authors should submit tables stratified by birth cohort, showing the proportion of infants with in utero exposure, the median and range of duration of exposure to each NRTI, clinical data on the child (birthweight, head circumference, premature delivery, deaths) and number of missing observations.

b) The request for further clinical data on the children is supported and the MAHs/study authors should provide this stratified by birth cohort as requested in the study assessment report. Data on clinical characteristics were dichotomised and presented as above or below the median value. As stated in the study assessment report, the clinical characteristics for gestational age, weight,

height, head circumference should be stratified according to clinically meaningful categories (e.g. (<1500, 1500-1999, 2000-2499, 2500-3999, 4000g+for birthweight), “small for gestational age”, small head circumference for gestational age), so that a more thorough assessment of the children can be made.

c) It is agreed that the rationale for the final multivariate analysis is lacking in detail. The MAHs/study authors have not clearly explained why specific variables were retained in the multivariate model. The authors have only presented the chi squared statistic and not the associated odds ratios, which are required to make an assessment of the magnitude of any risk. The authors should submit tables of results for the multivariate analysis with odds ratios and explain the rationale for the final model.

d) It is agreed that the categorisation of the birth cohorts (where the primary endpoints for France and Belgium were aggregated) has not been adequately justified. The MAHs/study authors should consider the birth cohorts individually and be compared with a single cohort comparator.

3 - *DRC evaluation of mitochondriopathy*

We cannot accept the claim that there is only one “possible case of mitochondriopathy” among the 27 children presenting unexplained neurological problems.

The Evaluation Committee was extremely vigilant in terms of the additional examinations carried out on some children. Indeed the Committee (i) declassified most of the cases of hyperlactataemia, (ii) considered most of the anomalous MRI findings to be artefacts (iii) was highly critical in its interpretation of the two 2 available muscle biopsies (such that only one was considered to be significant).

The great care taken with this analysis was justified, given the known difficulty of diagnosing mitochondriopathies. In contrast, the committee displayed an extraordinarily hasty and superficial attitude in stating that “there is no evidence of mitochondrial dysfunction” for all the other children - without presenting the slightest evidence to back up this conclusion. The case files of these children are very concise, and do not include a serious diagnostic evaluation (of mitochondrial aspects or otherwise). The committee experts are nevertheless, aware that in the absence of *ad hoc* investigations, it is just as difficult to reject the diagnosis as it is to affirm it.

The symptoms associated with mitochondrial dysfunction are not specific, and the list has broadened with time. Recent, well-grounded studies (albeit still subject to debate) have suggested (for example) that some cases of autism have a mitochondrial origin. So, why should this hypothesis be rejected out of hand for children presenting signs resembling autism? Even if one chooses to reject this putative link to autism, the refusal to consider certain observations as “possibly linked to a mitochondrial disease” (in the absence of specific investigations) is questionable.

Below, we give a few examples of cases that could legitimately have been considered as “possible” cases of mitochondriopathy.

- Case 40-10-09, presenting with cognitive delay, muscle weakness and strabismus.

- Case 10-40-29, presenting with cognitive delay and motor impairment.

- Case 10-60-15, presenting with cognitive delay and convulsions.

- Case 10-60-23, presenting with cognitive delay, nystagmus and amblyopia.

At the very least, the committee should have concluded that “no conclusions can be drawn”, which is not at all the same as “no evidence of mitochondrial dysfunction”.

No criteria have ever been validated for the diagnosis of persistent mitochondrial toxicity (and we remain open-minded as to whether or not this phenomenon truly exists). In the absence of better options, the criteria used for constitutional diseases are applied; however, it is possible that this approach needs to be adapted (perhaps even only slightly, and notably with regard to the progressiveness of clinical disease). Thus, it is unsound to immediately rule out a hypothesis of toxic mitochondrial dysfunction in a child with neurological symptoms that improve over time (case 30-90-32).

The gold standard for the diagnosis of constitutional mitochondriopathies remains the enzymatic, histochemical, ultrastructural and molecular analysis of muscle. Normal results for analyses carried out on PBLs and for the routine determination of biochemical markers do not in any way rule out a possible mitochondriopathy. This was clearly demonstrated in the only detailed study to date of children with unexplained neurological problems following exposure to AZT *in utero* (Lancet; 354:2084–89).

Similarly, the absence of mitochondrial DNA depletion some time after drug exposure does not refute a hypothesis of mitochondrial toxicity.

In summary, we think that the only possible conclusions are as follows:

1) One child presented with a “proven” mitochondriopathy.

2) One child presented with a “possible” mitochondriopathy.

3) Several children (at least 5) present clinical signs that are compatible with “possible mitochondriopathy” but have not undergone the rigorous additional investigations that would make it possible to affirm or reject this hypothesis.

4) It is absolutely impossible to draw any firm conclusions on this matter for the other 20 children.

Nevertheless, this major study confirms that some children born to seropositive mothers may present “unexplained neurological symptoms”, which can be severe in some cases.

It would certainly be important to:

1) compare the incidence of these symptoms in the cohort with that in the general population (an extremely difficult task).

2) rigorously explore the various physiopathological hypotheses, with no prior assumptions.

As cohort representatives, we asked to meet the DRC in order to maintain its “independence” but our request was refused. Surprisingly, the independence of the DRC was not challenged, despite the fact that the same person chairs the DRC and the MITOC scientific advisory board. This chairman is neither a paediatrician, a neurologist, nor an epidemiologist.

CHMP comment:

This assessor agrees that it would not have been possible to dismiss cases of mitochondrial dysfunction on the basis of the evaluations performed and the lack of specific key investigations that would normally be employed for diagnosis. There were several cases of unexplained NCS exhibiting symptoms that appear to fit the criteria for potential mitochondrial dysfunction, however lacked sufficient information to either diagnose or exclude it. Also acknowledged is the comment that as there is the potential for symptoms of drug induced mitochondrial dysfunction to be transient/ reversible, the

lack of definitive cases at the time of the one screening visit/ neurological assessment performed cannot conclusively exclude the possibility of events relating to mitochondrial dysfunction occurring prior to the visit. Although information regarding historical events occurring before the screening visit was intended to be gathered, this may not have identified mitochondrial dysfunction as these can present with subtle and non-specific signs that may not have been attributed to mitochondrial dysfunction.

Also of concern are the discrepancies in evaluation between the clinical assessments and investigations and the DRC's opinions. By its own admission, the DRC was not set up to perform the function of a formal endpoint evaluation committee and in many circumstances lacked the information to perform full clinical evaluations, however as per the report of discordance and from some of the case narratives appeared to disagree with some clinicians conclusions and reserved the right to the final decision in the case evaluations. As mentioned previously, this highlights the highly subjective nature of the data evaluated and the potential for differences in interpretation. In these situations the validity and reproducibility of the conclusions are brought into question.

Finally, it is acknowledged that the presence of the same chairman presiding over both DRC and MITOC scientific committees diminishes the independence of either committee.

4 – Discussion

The discussion suggests that the observed heterogeneity may result from inter-cohort differences in clinical practice. However, no data on putative differences in clinical practice are presented.

In view of our comments on the study limitations and the inability to draw any definite conclusions, we requested the inclusion of the following text in the Conclusion section:

"In view of the inter-cohort heterogeneity in terms of participation, data collection and neurological evaluation, and to the limited age period for this pragmatic screening programme, the study data are not able to provide an accurate estimate of the prevalence of unexplained neurological dysfunction in uninfected children born to HIV-infected mothers.

Moreover, given the absence of comprehensive clinical, biochemical and imaging assessments for most of the children reviewed by the DRC, it is not possible to conclude that none of these unexplained events were not due to some form of mitochondrial damage. Hence, it is not possible to draw firm conclusions with regard to the two main questions addressed by the present study (i.e. the incidence of unexplained neurological symptoms and the incidence of mitochondriopathy)."

CHMP comment:

It is agreed that the study has numerous limitations and this throws into question the study's ability to provide an accurate estimate of the prevalence of unexplained neurological dysfunction in uninfected children born to HIV-infected mothers and this has been highlighted in the assessment report.

The MAHs/study authors are requested to provide an interpretation of the prevalence estimated by this study given the limitations and biases highlighted.

2.2.2. Discussion on clinical aspects

Overall there are concerns with the validity of the study conclusions due to several limitations of the study as raised also by representatives of two cohort representatives (FR and BE).

The main issues are summarised as follows:

Methodology

- The relatively narrow “snapshot” timeframe presented by this study would not have enabled detection of symptoms which occurred but resolved before the 18 month timeframe/ screening visit. Potential reversibility of mitochondrial dysfunction was raised in the original Blanche/ Barett studies and in other studies evaluating drug induced mitochondrial effects. It would also not have detected longer term effects occurring after 28 months/ the screening visit.
- Significant heterogeneity exists in the methodology used between the cohorts, particularly in the time of enrolment and evaluation of neurological abnormalities during the screening visit and neurological assessment. This may have introduced selection and classification biases.
- Unlike the Barett et al study, no confirmatory or specific mitochondrial investigations were necessary to confirm or dismiss a diagnosis of mitochondrial dysfunction. These investigations were left to the discretion of the clinicians and there was no standardisation of the diagnosis of mitochondrial dysfunction within the study.

Results

- A sizeable proportion of children that were eligible for enrolment were not included in the main study for a variety of reasons (consent not obtained, lost to follow up, died).
- The degree of heterogeneity of results between cohorts was not made clear, despite the fact that results indicate that significant heterogeneity exists. This was seen the potential differences in baseline characteristics, evident differences in prescribing practice between cohorts, the variable prevalence of explained and unexplained NCS between cohorts.
- The rationale for the final multivariate analysis is lacking in detail. The MAHs/study authors have not clearly explained why specific variables were retained in the multivariate model. The categorisation of the birth cohorts (where the primary endpoints for France and Belgium were aggregated) has also not been adequately justified.
- The rationale for dismissing reports as not having mitochondrial dysfunction at any stage of the evaluation process was not made clear for the majority of cases. In the vast majority of cases more specific investigations for mitochondrial dysfunction were not available.
- There were discrepancies in evaluation between the clinical assessments and investigations and the DRC's opinions. This highlights the subjective nature of the data evaluated and the potential for differences in interpretation. There was a lack of independence between the DRC and MHAC committees as they both had the same chairman.

Therefore, as mentioned in the report of discordance, at present it is not possible to draw firm conclusions with regard to the two main questions addressed by the present study (i.e. the incidence of unexplained neurological symptoms and the incidence of mitochondrial disorders).

3. Overall conclusion and recommendation

There are concerns with the validity of the study conclusions due to several limitations of the study as raised also by representatives of two study cohort representatives (FR and BE) in the report of discordance. Therefore, at present it is not possible to draw firm conclusions with regard to the two main questions addressed by the present study (i.e. the incidence of unexplained neurological symptoms and the incidence of mitochondrial disorders).

☒ Not fulfilled:

Based on the data submitted, the MAH should provide additional clarifications as part of this procedure. (see section "Additional clarification requested")

4. Additional clarification requested

Based on the data submitted, the MAH should address the following questions as part of this procedure:

1. For all of the variables (primary and secondary endpoints, demographics and baseline characteristics, maternal medical history and characteristics, ART, type of exposure, duration of ART exposure, ARV drug and class), the data should also be stratified by country birth cohort so that differences across cohorts can be better understood.
2. With regards to the differences in the enrolment process for each of the respective birth cohorts, numbers of subjects affected by these differences in each cohort should be provided to enable more detailed assessment taking into account those differences.
3. Clarification should be provided on the number of discrepancies there were between the conclusions of the DRC and clinicians and which of those subsequently resulted in a change of classification by the DRC.
4. Data should be provided on the number of children identified by the medical reviewers with neurological symptoms that should have undergone a neurological assessment but were not referred or did not undergo this assessment.
5. The comparison between the conclusions of the screening visit questionnaires with the neurological assessment questionnaires to demonstrate the consistency of the definitions of neurological clinical symptoms has not been presented in the study report, this should be provided.
6. Tables of results for the multivariate analysis with odds ratios and the rationale for the final model should be presented. The categorisation of the birth cohorts (where the primary endpoints for France and Belgium were aggregated) should be justified. The birth cohorts should be considered individually and be compared with a single cohort comparator.
7. With regards to selection bias, the study report states that the probability of non-participation in the study was lower when the consent form was signed at the screening assessment that those forms signed at birth, data should be provided in support of this statement. A discussion should be provided on the potential impact that any selection bias would have on the results of the study.
8. To further establish the differences in reasons for potential subjects not enrolled in the study by cohort, and missing information, a table of data showing the levels of participation stratified by birth cohort, including the number of eligible children who were not consented, age of child at

consent, number of deaths after consent, number of children lost to follow-up after consent, number of children evaluated, number of children with missing HIV status.

9. Tables of descriptive data eg: tables 7 -12 in the study report should be presented according to birth cohort. The numbers of subjects with missing observations in these tables should also be presented.

10. Data in table 7 should also be presented using standard meaningful categories, particularly for gestational age at birth, weight, height and head circumference. For example, the WHO categories for birth weight (<1500, 1500-1999, 2000-2499, 2500-3999, 4000kg+), small weight for gestational age, small head circumference for gestational age.

11. Data on maternal laboratory results (CD4 counts, HIV RNA viral load) should also be presented in the tables of results.

12. Although there were 165 children who were initially considered to have some form of neurological abnormality, only 83 children were subsequently classified as having either explained or unexplained neurological symptoms. The MAHs/authors have not stated why the remaining 82 children were not classified in either of these categories and why these subjects were not considered to require a neurological assessment.

13. In the univariate analysis the rationale for aggregating the Belgian and French cohorts and comparing them to all others does not seem to have been pre-specified or adequately justified. Each cohort should be considered individually and compared with a single cohort comparator.

14. The MAHs/authors should provide a discussion on the points raised in the report of discordance, with respect to the conclusions that the study data is not able to provide an accurate estimate of the prevalence of unexplained neurological dysfunction in uninfected children born to HIV-infected mothers, and that it is not possible to draw firm conclusions with regard to the incidence of unexplained neurological symptoms and the incidence of mitochondrial disorders.

5. Assessment of the responses to the request for supplementary information

1. For all of the variables (primary and secondary endpoints, demographics and baseline characteristics, maternal medical history and characteristics, ART type of exposure, duration of ART exposure, ARV drug and class), the data should also be stratified by country birth cohort so that differences across cohorts can be better understood.

Summary of MAH's Response (for brevity only a selection of the data highlighting differences between the cohorts provided is shown here):

We have stratified the main tables from the Study Report by cohort (tables provided in Annex I of the AR).

Baseline characteristics

The Swiss cohort had the largest difference in gender distribution with 34% male and 66% female study participants. Abnormal clinical development since birth ranged from 1.6% of children assessed in the German cohort to 9.0% in the Belgian cohort.

Birth characteristics

Planned caesarean delivery ranged from 93.7% in Italy to 0.0% in France. The highest levels of unplanned caesarean and of vaginal delivery were observed in the French cohort, 32.8% and 67.2% respectively. The lowest level of vaginal delivery was observed in the Italian cohort, at 0.5%. Mean birth weight of children was 2.9 kg, ranging from 0.6 kg in Belgium to 5.0 kg, also in Belgium. Mean height was 48 cm, ranging from 26.0 cm in France to 57.0 cm in both Germany and Spain. Mean head circumference was 34 cm, ranging from 20.0 cm in Germany to 49.0 cm, also in Germany.

Descriptive data

The maternal age at delivery was only available for 1225/ 2405 mothers. Of the 1180 records not available for mother's age, 917 (78%) of these unavailable records were from Italy.

Of the mothers with results of tests for HBV/HCV coinfection available (n=2069), 9% were co-infected with hepatitis virus B or C. HCV was particularly prevalent in the Spanish cohort (22%) compared to all other cohorts (all under 6%). 95% of the Italian cohort were clear of HBV or HCV coinfections.

A minority of mothers (amongst those for whom the information was available) reported using tobacco (115/760, 15.1%), alcohol (24/736, 3.3%), injection drugs (21/894, 2.3%) or recreational drugs (87/854, 10.2%) during pregnancy. However, the majority of mothers had no data available in these categories (1464/2405, 61%). Of the available data, Spanish mothers showed the highest rates of tobacco use (38% of the Spanish cohort), injection drug use (11.8%) and drug abuse (15.2%) during pregnancy.

ART histories

Intrapartum exposure was particularly low in Switzerland (19.1%) and also low in Germany (42.6%), compared to the other country cohorts (all others above 93%). In utero exposure was also lowest in Germany (86.5%), compared to the other country cohorts (all others above 90%).

For children with data available (n=2403), 99.9% were exposed to at least one NRTI drug. In all countries except Spain this was 100% of the country cohort. Among the 2405 children, 1709 (71.1%) were exposed to at least one PI (ranging from 55.3% in Germany, up to 81.7% in Belgium and 88.6% in France). 656 (27.3%) were exposed to at least one NNRTI (ranging from 14.5% in Belgium to 39.0% in Spain).

The majority of children were exposed to at least one NRTI drug (99.9%), and almost all were exposed to zidovudine (99.8%), at least neonatally (100% of all exposed children in France, Germany, Spain, Switzerland and Belgium, 1 exposed child in Italy was not exposed to zidovudine). Lamivudine (71.2%), tenofovir (30.0%) and emtricitabine (24.7%) were also commonly used NRTI drugs. In Germany, lamivudine, tenofovir and emtricitabine were used at similar rates (5.3%, 4.8% and 4.2%), in comparison to the other countries that favoured lamivudine more strongly over tenofovir and emtricitabine.

Neurological disorders

There were 165/2405 children (6.9%) in the primary population who had at least one neurological disorder reported in the first sections of the screening questionnaire (previous neurological disorders, previous non-neurological disorders, physical examination and hospitalisation); some patients had more than one disorder. 26.7% of these children were from the Belgian cohort (44/289, 15.2% of the cohort). 48 and 25 of these children were from the Italian and French cohorts (respectively), yet these represented a smaller proportion of their cohorts (4.7% and 5.5% respectively). The percentage of

children with any neurological disorder recorded ranged from 4.7% (48/1016 children) in the Italian cohort to 15.2% (44/289) in the Belgian cohort.

Around 6% of both the Spanish and Belgian cohorts (6.0% and 6.2% respectively) showed other significant cognitive delay or abnormalities, not categorised by the study.

The prevalence of explainable NCS was highest in Spain (15/351, 4.3%) and lowest in Italy (8/1016;0.8%). The prevalence of unexplained NCS was highest in Belgium (15/289, 5.2%) and lowest in Italy (6/1016, 0.6%).

The prevalence of explained plus unexplained NCS was highest in the Belgian cohort (23/289, 8.0%) and lowest in Italy (14/1016, 1.4%).

Belgium showed the highest rate of NCS before 18 months, at 15.2%. The next highest rate was Spain at 9.4%, whilst the lowest rate was observed in Italy at 4.7%. The number of children with NCS at 18 to 28 months was lower than the number of children referred to a neurologist in all countries, except for France (25 children with NCS, 22 referred).

CHMP comment:

From the data presented there are evident differences between the study cohorts. The Swiss cohort had a very small number of subjects compared to the others (n=47) and therefore it is not known if any findings from this cohort were due to chance. There was a significant range in subjects reported as having abnormal clinical development since birth (from 1.6% in the German cohort to 9.0% in the Belgian cohort), however as the background incidence of neonatal/childhood neurological abnormalities is unknown in these countries, the reason for these differences is unknown. There was a large amount of missing data for example on birth characteristics (particularly anthropometric data), maternal substance abuse and data on cumulative ART exposure in particular from Belgium. As noted previously, the proportion of neurological abnormalities detected at the screening questionnaire was higher in Belgium, however it is noted that this group also had higher rates of abnormal clinical development since birth. From the descriptive data presented it is not possible to infer the reasons for some of the apparent differences between the groups, such as the different rates of planned caesarean section and in utero administration of ARTs which may be due to local variations in clinical practice or to different disease demographics amongst the patients in the cohorts. The impact of these differences have also not been analysed in the study.

This point is considered resolved.

2. With regards to the differences in the enrolment process for each of the respective birth cohorts, numbers of subjects affected by these differences in each cohort should be provided to enable more detailed assessment taking into account these differences.

MAH's response:

There were 3878 children in the Cohorts, but of these, 1013 were not enrolled (32 from Belgium, 5 from France, 548 from Germany, 371 from Italy, 33 from Spain and 24 from Switzerland). The MITOC Protocol states that the screening assessment is performed once informed consent has been obtained. Data on children who were not consented are therefore not available. Furthermore, the reason that consent was not given was not available for all Cohorts. The French Cohort had to re-consent their patients, so every child in their Cohort was also in the MITOC Study, except those that were not

eligible. Nevertheless, although we do not have detailed information on those children not enrolled, the number of children with primary endpoints was consistently low across the Cohorts, regardless of the method of enrolment.

For those children with consent, 10 had an unknown HIV status and 96 were lost to follow-up from the cohort. There were 289 consented children aged <18 months at the screening visit and 51 aged over 28- months old at the screening visit. These children were not eligible for the MITOC Study as they were not 18 to <28 months old at the time of the screening assessment and were therefore excluded. Fourteen children were not exposed to any ARV drug at any stage. Using data available, the reasons that children were lost to follow-up have been summarised in Table 18.

Table 18. Summary of reasons for lost to follow-up by cohort using data available

Reason	France	Germany	Italy	Spain	Switzerland	Belgium
Pulmonary hypoplasia	2					
Preterm birth	1					
Lost at birth	4					
Lost to follow-up	12					
Sudden infant death syndrome	1					
Brain anoxia + multi-organ failure	1					
Patient did not attend visit		25	20	9		12
Unknown		2				
Too old at screening visit		1				
HIV positive			1			
Died				1	1	
Withdrawn by parent				1		

CHMP comment:

The MAH has clarified that data on children who were not consented was not collected and is therefore not available, data presented in this section were instead of subjects who were lost to follow up during the course of the study. As no further data can be provided on this, **this point is considered resolved.**

3. Clarification should be provided on the number of discrepancies there were between the conclusions of the DRC and clinicians and which of those subsequently resulted in a change of classification by the DRC.

MAH's response

There were 27 children referred to the DRC for review. The results from the neurological questionnaire showed two children with suspected mitochondrial disorder (subject numbers 601057 and 309032) and 25 with unexplained abnormalities (a total of 36% of children with completed neurological assessments). The assessments from the neurological questionnaire and DRC review are shown in Table 19, below. The DRC could request additional data from investigators in order to make their classifications.

Of the 25 children who had unexplained abnormalities but no evidence of mitochondrial dysfunction according to the neurological questionnaire, all 25 were classified as having no evidence of

mitochondrial dysfunction by DRC consensus. Thus, there was 100% agreement between the two review processes for these 25 children.

Of the 2 children who had suspected mitochondrial disorders by the neurological questionnaire, the DRC classification was one case of possible mitochondrial disorder (subject number 601057) and one case with no evidence of mitochondrial dysfunction. Therefore, there was one apparent discrepancy between the classification from the neurological questionnaire and the DRC consensus. It should be noted that, although this case was initially classified by the Italian cohort as suspected mitochondrial disorder, subsequent clinical review at a national referral centre for mitochondrial disease also concluded that there was no evidence of mitochondrial dysfunction.

The number of cases meeting the criteria for a neurological assessment was markedly less than anticipated.

The DRC therefore reviewed all other completed neurological questionnaires (n=47) and available neuroimaging for quality control purposes, thereby providing an additional level of review. This review found no additional cases of mitochondrial dysfunction. A sensitivity analysis on the outcome of the DRC consensus was performed on the secondary population of all 2855 HIV-negative children in the study. There were a total of 35 children reviewed in this larger population, with an additional 8 children compared with the primary analysis. Raw data for this analysis was not given by country cohort.

Very similar levels of consensus were seen when comparing the outcomes of the neurological questionnaire and the DRC review for the secondary population of all HIV-negative children:

Table 20. Sensitivity analysis – outcome of DRC consensus in the secondary population of all HIV-negative children (n=2855)

Outcome (neurological questionnaire)	Definite MT dysfunction	Probable MT dysfunction	Possible MT dysfunction	No evidence of MT dysfunction
Suspected MT disorder (n = 3)	0	0	1	2
Unexplained neurological abnormalities but no evidence of MT disorder (n = 32)	0	0	0	32
Total number of children undergoing a DRC review (n=35)	0	0	1	34

CHMP comment:

The MAH has confirmed that there was only one case in which the DRC disagreed with the views of the clinicians and that case was subsequently reviewed at a national referral centre for mitochondrial disease which concluded that there was no evidence of mitochondrial dysfunction. There was general consensus between the outcomes of the neurological questionnaires reviewed and the DRC conclusions.

This point is considered resolved.

4. Data should be provided on the number of children identified by the medical reviewers with neurological symptoms that should have undergone a neurological assessment but were not referred or did not undergo this assessment.

MAH's response:

The MITOC Protocol states that:

'All examinations including imaging procedures and laboratory tests are at the discretion of the paediatric neurologist and will not be required specifically for the MITOC assessment.'

The investigator's decision to refer a child with neurological symptoms to a paediatric neurologist was thus based on their routine clinical practice and was not a requirement of MITOC. This means that not all children with neurological symptoms had a neurological assessment. The French Cohort did not follow the MITOC Protocol as they did not refer children to a paediatric neurologist, but instead, the children were evaluated by a hospital paediatrician who completed the neurological questionnaire.

Table 21 shows the number of ART-exposed children who had a conclusion of absence of neurological clinical symptoms on their MITOC screening questionnaire, although a neurological symptom was identified by the medical reviewers in the following sections of the MITOC screening questionnaire – hospitalization, previous neurological disorders, physical examination and non-neurological disorders. The symptoms included language delay, delay in mobility, non-febrile convulsions, febrile convulsions and trisomy 21.

Table 21. The number of ART-exposed children in each cohort that were identified as having neurological symptoms in the hospitalization, previous neurological disorders, physical examination and non-neurological disorders sections of the MITOC screening questionnaire, but with a conclusion of absence of neurological symptoms (each child is only listed in one section)

Cohort	Previous hospitalizations	Previous neurological disorders	Physical examination at 18 to <28 months old	Previous non-neurological disorders
France		2	1	
Germany	1	6		3
Italy	12 (6 also had a neurological disorder)	19	1	1
Spain	7 (2 also had a neurological disorder)	8	2	3 (1 also had a disorder found at the physical examination)
Switzerland		1		
Belgium	3	5	14	
Total	**24**	**41**	**18**	**7**

CHMP comment:

The study report states that "*medical reviewers also reviewed the listings to identify whether there were any children with neurological clinical symptoms that should have undergone a neurological assessment, but were not referred*."

As a reminder, given the open nature of the protocol, the referral for a neurological assessment was left to the discretion of the physician at the screening assessment. The MAH have provided detail on subjects identified by the MITOC medical reviewers who had neurological symptoms identified in the screening questionnaire from episodes prior to the date of the screening assessment, but who had been given a conclusion of "absence of neurological symptoms", it is inferred that this is the closest approximation of the subjects who would have been eligible for a neurological assessment but did not receive one, totalling 90 children.

This point is considered resolved.

5. The comparison between the conclusions of the screening visit questionnaires with the neurological assessment questionnaires to demonstrate the consistency of the definitions of neurological clinical symptoms has not been presented in the study report, this should be provided.

MAH's Response:

During the MITOC screening assessment, neurological disorders were recorded in pre-defined categories.

Any relevant additional data from the screening questionnaire, such as neurological conditions in the physical examination, hospitalisation or non-neurological conditions sections, were subsequently classified into the pre-defined categories in the medical review.

There were 165/2405 children in the primary population (6.9%) who had at least one neurological disorder reported in the first sections of the screening questionnaire (previous neurological disorders, previous non-neurological disorders, physical examination and hospitalisation). Some patients had more than one disorder. Among the secondary population of 2855 HIV negative children there were 199 (7.0%) with at least one neurological disorder.

In the primary population, of the pre-defined categories on the previous neurological disorders section of the screening questionnaire, the most common disorders were developmental delay (n=50, 2.1%), febrile convulsions (n=35, 1.5%) and motor abnormalities (n=33, 1.4%). The percentage of children with any neurological disorder ranged from 3.3% (8/244 children) in the German cohort to 9.7% (28/289) in the Belgian cohort.

In the Protocol for the MITOC study, which was agreed with the EMEA on 9 May 2007, the investigators had to decide how to classify each child according to the neurological conditions observed: either absence of neurological conditions, presence of an explainable condition and presence of an unexplained neurological condition. Table 22 shows this information by cohort. The percentage of children with neurological disorders in the screening questionnaire ranges from 4.7% in Italy to 15.2% in Belgium. The rate of referral to a neurologist differed between countries. For example in Italy, 14/48 children with a neurological disorder were referred to a neurologist, versus 23/44 in Belgium.

Table 22. Number of children in the primary analysis population with screening and neurological questionnaires (n=2405)

Cohort	Number of children with screening questionnaires	Number of children with neurological disorders in the screening questionnaire	Rate of NCS before 18 months	Number of children referred to a neurologist
France	458	25	5.50%	22
Germany	244	12	4.90%	4
Italy	1016	48	4.70%	14
Spain	351	33	9.40%	18
Switzerland	47	3	6.40%	2
Belgium	289	44	15.2%	23
Total	2405	165	6.90%	83

CHMP comment:

The response presented has not fully addressed the question. The original aim of this question was to obtain the results of the analysis performed by the study authors assessing the consistency of the outcomes between the screening and the neurological questionnaires as stated in Section 10.9.2 of the report body : "*The outcomes of the MITOC and neurological assessment as recorded on the prospective screening questionnaires were compared with each other, to show the consistency of the definitions of neurological clinical symptoms between the two questionnaires*." However, no data has been provided in this response to demonstrate auditable consistency between symptoms identified in the screening questionnaire, whether these were picked up in the subsequent neurological assessment and the reasoning ascribed to their presence or absence. The data provided does confirm that a much higher rate of NCS was reported in the Belgian cohort as occurring before the 18 month screening assessment. It is likely that no additional data is available on this question is available. Given that the lack of information on this issue is unlikely to be available and will not change the general assessment of the study at this point, no further information on this will be requested.

This point is considered resolved.

6. Tables of results for the multivariate analysis with odds ratios and the rationale for the final model should be presented. The categorisation of the birth cohorts (where the primary endpoints for France and Belgium were aggregated) should be justified. The birth cohorts should be considered individually and be compared with a single cohort comparator.

Summary of MAH's response (for brevity only a selection of the data is provided here):

The responses to questions 6 and 13 have been combined.

Of the 27 primary endpoints, 22 (81%) were diagnosed in either the French or Belgian cohorts. The results from the French and Belgian cohorts were combined in the multivariate analyse because of the high number and percentage of children diagnosed with these medical conditions in these cohorts. This

was not a pre-planned analysis, but was designed because of the differences in rates of diagnosis between the country cohorts.

There are too few endpoints in the individual country cohorts to conduct analyses of each one in isolation. The prevalence of the endpoint is too variable between the cohorts to look at a single cohort comparator – it is not clear what this would be, given the differences between the cohorts. The same analyses were performed for the secondary population of all HIV-negative children. Very similar results were seen in these analyses.

In the multivariate analysis of the primary population of all ART-exposed children, the only statistically significant predictors of the primary endpoint were the gender of the child and the cohort in which the child was enrolled (Table 26). Boys were significantly more likely to be diagnosed with the primary endpoint than girls (p=0.02. Children in the French and Belgian cohorts were significantly more likely to be diagnosed with the primary endpoint (p=0.0001). Children with a lower gestational age were more likely to be diagnosed with the primary endpoint (p=0.05), but this was at borderline significance. In the multivariate analysis, there was no independent effect of any ARV drugs on the risk of being diagnosed with the primary endpoint (the p-value was not significant (ns) for each comparison).

Table 26. Multivariate analysis – factors associated with the primary endpoint in the primary ART exposed population

Predictive factor	Chi-square	p-value	MV Odds Ratio (95% C.I.)
Cohort (France / Belgium versus other)	15.08	0.0001	10.42 (2.42 – 43.47)
Gender (male versus female)	5.09	0.02	2.82 (1.10 – 7.24)

In the multivariate analysis of the secondary population of all HIV-negative children, the only statistically significant predictors of the primary endpoint were the gender of the child and the cohort in which the child was enrolled. Boys were more likely to be diagnosed with the primary endpoint than girls (p=0.003), and children in the French and Belgian cohorts were significantly more likely to be diagnosed with the primary endpoint (p<0.0001). In this multivariate analysis, there was no independent effect of any ART drug received on the risk of being diagnosed with the primary endpoint (p= ns for each comparison).

Table 27. Multivariate analysis – factors associated with the primary endpoint in the HIV negative population

Predictive factor	Chi-square	p-value	MV Odds Ratio (95% C.I.)
Cohort (France / Belgium versus other)	15.4	<0.0001	10.31 (2.44–43.47)
Gender (male versus female)	8.96	0.003	3.66 (1.48–9.09)

CHMP comment:

The difficulty in obtaining a suitable cohort for comparison at this stage is acknowledged. Existing European cohort collaborations/ databases for HIV tend to collect data from HIV positive individuals, therefore it may have been difficult to identify a readily available or suitable comparator group. In addition, neurological symptoms are multifactorial, therefore simply comparing the prevalence of

neurological symptoms to the general population of children of a similar age will not be clinically meaningful.

The odds ratios and confidence intervals for the multivariate analyses have been presented here as requested. It is noted that for both the analyses in the primary and secondary populations, the CIs are wide for both predictive factors, particularly for the French/Belgian combined cohort factor.

The combination of the French and Belgian cohorts was due to the differences in diagnostic rates in these cohorts as stated by the MAH.

This point is considered resolved.

7. With regards to selection bias, the study report states that the probability of non-participation in the study was lower when the consent form was signed at the screening assessment that those forms signed at birth, data should be provided in support of this statement. A discussion should be provided on the potential impact that any selection bias would have on the results of the study.

MAH's response:

The statement in the study report should have stated that there was a lower probability of non-participation when the consent form was signed at birth compared with consent being given at the screening assessment.

Data on children who were not consented are not available, and therefore, the selection bias could not be determined. Please refer to the response to question 2 for the information available on children lost to follow-up.

CHMP comment:

The MAH have clarified the statement in the study report. As discussed in Question 2, further information on children who were not consented is not available.

This point is considered resolved.

8. To further establish the differences in reasons for potential subjects not enrolled in the study by cohort and missing information, a table of data showing the levels of participation stratified by birth cohort, including the number of eligible children who were not consented, age of child at consent, number of deaths after consent, number of children lost to follow-up after consent, number of children evaluated, number of children with missing HIV status.

MAH's response:

There are no data available for those patients who did not have a signed consent form. Please refer to the response to question 2 for the information available on children lost to follow-up.

CHMP comment:

As with the response to the previous question, no information was available on children not consented.

This point is considered resolved.

9. Tables of descriptive data eg: tables 7 -12 in the study report should be presented according to birth cohort. The numbers of subjects with missing observations in these tables should also be presented.

MAH's response:

Please refer to the response to question 1.

CHMP comment:

Please refer to the assessment of question 1.

This point is considered resolved.

10. Data in table 7 should also be presented using standard meaningful categories, particularly for gestational age at birth, weight, height and head circumference. For example, the WHO categories for birth weight (<1500, 1500-1999, 2000-2499, 2500-3999, 4000kg+),small weight for gestational age, small head circumference for gestational age.

MAH's response:

The results from the multivariate analyses of the primary endpoint did not show an association with birth weight, height or head circumference. In the multivariate analysis of the primary endpoint, there was a borderline significant association between the primary endpoint and lower gestational age at birth (below the median). However this association was not seen in the analysis of the secondary population of all HIV negative children. The primary aim of the MITOC study was to investigate associations between neurological abnormalities and antiretroviral treatment. Therefore we consider that it is out of scope for this analysis to further investigate predictive factors for the primary endpoint, using alternative categorisations, when these factors have not shown significant associations in the current analysis, and are unrelated to the primary objectives of the MITOC study.

CHMP comment:

The use of non-standardised categories here is suboptimal as only the mean values had been presented and most probably used in the multivariate analyses. Although this was not the primary aim of the study, use of standard categories in the multivariate analysis affords a better understanding of concurrent risk factors and potential confounding variables of the primary endpoint of NCS without attribution of cause, especially since there were at least 27 children with unexplained neurological symptoms reviewed by the DRC. However, in light of other methodological limitations which preclude conclusions regarding the association between the more commonly used antiretrovirals and the primary endpoint, the lack of categories here is unlikely to influence the outcome of this assessment. No further data is therefore requested.

This point is considered resolved.

11. Data on maternal laboratory results (CD4 counts, HIV RNA viral load) should also be presented in the tables of results.

MAH's response:

These data were not collected as part of the MITOC study, and so cannot be reported.

CHMP comment:

Additional data was requested but is not available. **This point is considered resolved.**

12. Although there were 165 children who were initially considered to have some form of neurological abnormality, only 83 children were subsequently classified as having either explained or unexplained neurological symptoms. The MAH / authors have not stated why the remaining 82 children were not classified in either of these categories and why these subjects were not considered to require a neurological assessment.

MAH's Response:

According to the MITOC Protocol, it was the investigator's decision to refer a child with neurological symptoms to a paediatric neurologist and was not a requirement of MITOC. The only cohort that did not follow the Protocol regards referral was the French Cohort as their children were evaluated by a hospital paediatrician rather than a paediatric neurologist. Thus, not all the 165 children who were initially considered to have some form of neurological abnormality went on to have a neurological assessment.

There were 165/2405 children in the primary population (6.9%) who had at least one neurological disorder reported in the first sections of the screening questionnaire (previous neurological disorders, previous non neurological disorders, physical examination and hospitalisation). Some patients had more than one disorder.

Please refer to the responses to questions 4 and 5 for further information.

CHMP comment:

The response provided to this question does not address the reason for 82 out of 165 children who were initially considered to have a form of neurological abnormality not being classified as having either explained or unexplained neurological symptoms. The outcome for these children is therefore unclear. It is inferred from this that the information is not available, as this information was also not provided in the responses to questions 4 and 5, no further data is therefore requested.

This point is considered resolved.

13. In the univariate analysis the rationale for aggregating the Belgian and French cohorts and comparing them to all others does not seem to have been pre-specified or adequately justified. Each cohort should be considered individually and compared with a single cohort comparator.

MAH's Response:

Please refer to the response to question 6 as the responses to questions 6 and 13 have been combined.

CHMP comment:

As stated in the response to question 6, results from the French and Belgian cohorts were combined in the multivariate analysis because of the high number and percentage of children diagnosed with these medical conditions and was not pre-planned.

This point is considered resolved.

14. The MAH/authors should provide a discussion on the points raised in this report of discordance, with respect to the conclusions that the study data is not able to provide an accurate estimate of the prevalence of unexplained neurological dysfunction in uninfected children born to HIV-infected mothers, and that it is not possible to draw firm conclusions with regard to the incidence of unexplained neurological symptoms and the incidence of mitochondrial disorders.

MAH's response:

The MITOC study does, in fact, provide an estimate of unexplained neurological events in HIV-negative infants born to HIV-infected mothers who were assessed for neurological dysfunction between 18 and 27 months of life. There are a total of 27 unexplained neurological events in this study giving a prevalence of 1.04 % (95% CI 0.67-1.53).

It is important to understand the nature of this study. Children were initially assessed by general paediatricians who determined the presence or absence of neurologic symptoms at that single assessment time point. *This was NOT the study endpoint.* Children were then supposed to be referred to a paediatric neurologist who would more carefully assess the neurological symptom for cause and would classify the child. The assessment of the paediatric neurologist was the final assessment. A child classified by the paediatric neurologist as having 'unexplained neurological events' met the study endpoint.

The reason for some discordance among the investigators reflects the significant differences between sites in events. At the assessment by the general paediatrician, the rate of any neurologic symptoms varied from 1.4% to 8.0% by cohort, and the rate of any unexplained neurologic symptom varied from 0.6% to 5.2%.

The most likely explanation for this difference is site characteristics. The vast majority of cases of unexplained neurologic syndrome came from two cohorts – France and Belgium. The cohort with the highest prevalence (Belgium) was predominantly a single centre, where children were seen prospectively by a single paediatrician.

As noted, the study design assumed that unexplained neurologic abnormalities would be referred to local paediatric neurologists. We cannot preclude the possibility that paediatric neurologists interpreted the study differently and defined unexplained neurologic events differently. More importantly, in the French cohort, not all patients were referred to neurology and the neurological classification was defined by the local paediatrician – thus, the additional verification by a specialist in paediatric neurology was not provided in these cases.

The fact that two cohorts (France and Belgium) contributed 22/27 events (82%) does confound the results and suggests significant ascertainment bias. Whether this reflects under-diagnosis in four countries or overdiagnosis in two is impossible to determine, but there is no evidence that the cohorts in any country did not adhere to the study as designed. The only exception to this is the French Cohort in which children were evaluated neurologically by a paediatrician, rather than a paediatric neurologist.

It is not possible from a study such as this to definitively conclude that none of these unexplained events were not due to some form of mitochondrial damage; however, none were identified as possible mitochondrial toxicity by experienced paediatric neurologists, who were specifically asked to determine if mitochondrial toxicity might be present. Thus, we feel it is reasonable to assume that mitochondrial toxicity is an unlikely cause for these unexplained events.

CHMP comment:

As discussed in the CHMP comments to the previous questions, the prevalence of neurological clinical symptoms, and by extension mitochondrial toxicity in this study is uncertain due to methodological limitations discussed above and in the main assessment report circulated previously, eg. 82/165 children with neurological symptoms were unaccounted for, a lack of consistency to diagnostic criteria. As discussed by the MAH, the study results are confounded by the large contribution of events from the Belgian/ French cohorts combined. Although the MAH concludes that it can be assumed that mitochondrial toxicity is unlikely, this is mainly an assumption, as the strength of the evidence presented in this study casts significant doubts on the conclusions made by the study. The discussion provided here does not alter the overall assessment of the study.

This point is considered resolved.

6. Updated discussion

Following the assessment of the responses to the request for supplementary information, there are no further outstanding points. Data requested could not be provided on several points and overall the responses provided do not greatly alter the findings of the previous assessment, which is that no firm conclusions can be made with regard to the main questions addressed by the MITOC study (i.e. the prevalence of unexplained neurological symptoms and the association between NRTI exposure in utero or the post natal period and mitochondrial disorders).

It is acknowledged that the diagnosis of mitochondrial dysfunction can be subjective as reflected in the variability in diagnostic rates across cohorts, making detection or exclusion of these events very difficult, especially in the absence of any clear diagnostic criteria. This, in addition to the short time frame, snapshot view of the data and potential for reversibility of mitochondrial toxicity related neurological symptoms preclude any confident assertions from the study findings. It is acknowledged that diagnosis of neurological related mitochondrial dysfunction in children much more challenging compared to previously reviewed topics related to mitochondrial dysfunction ie: lipodystrophy and lactic acidosis.

The findings of the previous EMA reviews in general found some NRTIs eg: tenofovir disoproxil fumarate and emtricitabine to have a lower propensity for mitochondrial toxicity, however, it should be noted that these were performed mainly using study data in adults. In addition to stavudine and didanosine, zidovudine was one of the substances considered in the previous reviews for lipodystrophy/ lactic acidosis to be at a higher risk for mitochondrial dysfunction, and all cases referred for neurological assessment had been exposed to zidovudine. In cases reported with NRTIs perceived to be less toxic to mitochondria, eg: tenofovir, all had also been administered with zidovudine. It is

acknowledged that mitochondrial dysfunction in children was originally detected with use of zidovudine and that this warning was subsequently applied on to other NRTIs as a potential class effect, however it is impossible to distinguish the effects of individual components of ART that were administered together from this study.

The number of children exposed to stavudine and didanosine is far lower than that of other NRTIs. The numbers exposed were: stavudine 36 subjects - 1.5%, didanosine 79 subjects - 3.3% vs zidovudine 2399 subjects – 99.8%. Therefore the sample size involving these substances may not have been large enough to detect neurological abnormalities and the lack of reported cases does not necessarily provide re-assurance that there is no causal association.

It is likely that no matter how well designed the study, owing to the subjective nature of the diagnosis, the absence or presence of mitochondrial toxicity induced neurological symptoms is unlikely to be conclusively proven. Without agreeing a predefined criteria for diagnosis of mitochondrial dysfunction, which often includes subjecting children to potentially invasive procedures, it may be challenging to conduct a study which will provide sufficient meaningful evidence on the association between NRTI exposure and mitochondrial dysfunction in children. It is acknowledged that there is no "gold standard" for diagnosis of mitochondrial dysfunction which will also make consensus on study criteria difficult. In order to attempt to determine an association, any such study design should also incorporate methodology to allow this, such as a comparator cohort. It may, however, be challenging to identify sufficient numbers for comparators as zidovudine appears to be the predominant substance used in these circumstances, and this will also affect the potential for distinguishing between the effects of the individual NRTIs. Therefore, despite the lack of confirmatory evidence from the MITOC study, it is acknowledged that the realistic possibility of conducting a further study which will gather sufficient meaningful information to guide further regulatory action is small.

Sources other than the MITOC study (eg: reports from safety databases, the published literature and the findings from the previous reviews on lipodystrophy and lactic acidosis as other manifestations of mitochondrial dysfunction) should be used in considering whether the proposed amendments to warnings in the product information are justified, as the findings from the MITOC study are unlikely to be robust enough on their own to support any changes. In addition, as the study focused on neurological manifestations of mitochondrial dysfunction, the data from this study cannot provide comment on the non-neurological manifestations such as haematological abnormalities.

7. Overall conclusion and impact on the benefit/risk balance

The SmPCs for all Nucleotides/Nucleotides reverse transcriptase inhibitor (Ns/tRTI) currently include a class labelling on mitochondrial dysfunction in section 4.4 (Special warnings and precautions for use) and in section 4.6 (Pregnancy and Lactation).

It is expected that developing embryo/foetus would have particular vulnerability to mitochondrial dysfunction even if the duration of exposure is limited, given the periods of maturation and growth of all organs. Moreover, neurological manifestations of mitochondrial dysfunction could have deleterious consequences for the growing child.

As regards the clinical findings of relevance as part of the "Cross-sectional study of HIV-negative children, aged 18 to <28 months, born to HIV-1-infected mothers in Europe: a European study sponsored by the Collaborative Committee for Mitochondrial dysfunction in Children (MITOC)" were particularly expected. The objectives of this study were to determine the prevalence of neurological disorders (clinical symptoms, cognitive or motor delay) HIV-negative children aged 18 to <28 months born to HIV-1 infected mothers and enrolled in one of the 6 European participating cohorts (Belgian,

French, German, Italian, Spanish and from Switzerland) and to estimate the incidence of unexplained neurological disorders suggestive of mitochondrial dysfunction. This study was considered as the cornerstone to determine the incidence of long-term neurological disorders in this population.

Major issues have been raised regarding the validity of the study conclusions by representatives of 2 participating cohorts (French and Belgian investigators) and a list of questions has been raised in consistency by the PRAC on the MITOC study results.

While clarifications on the MITOC study results were provided during the review process, data requested could not be provided on several points and overall the PRAC stated that no firm conclusions can be made with regard to the main questions addressed by the MITOC study (i.e. the prevalence of unexplained neurological symptoms and the association between NRTI exposure in utero or the post natal period and mitochondrial disorders).

However, the situation cannot be regarded as strictly superimposable.

Indeed, the large clinical experience accumulated in HIV infected patients (vast majority of adults) under combination antiretroviral therapies has enabled to substantiate the clinical impact of mitochondrial dysfunction in terms of risk of lipoatrophy and lactic acidosis and thus further substantiating the ranking of NRTIs on mitochondrial dysfunction originally based on in vitro and in vivo data, with ZDV, d4T and ddI being regarded as particularly inducers of mitochondrial dysfunction. However, the relevance of such a clinical experience is more limited when now considering the potential impact on mitochondria of in utero Ns/tRTI exposure. It cannot be ruled out that even limited impact on mitochondria with other Ns/tRTIs might have clinical translation in neonates. Developing embryo/foetus could indeed have particular vulnerability to mitochondrial dysfunction given the in process maturation and growth of organs, with particular concern on CNS.

Finally, as a critical issue, while the recent MITOC study was expected to be the main body of evidence to substantiate the clinical relevance of in utero Ns/tRTI exposure, significant limitations preclude any firm conclusion to be drawn.

Therefore, the removal of the class labelling for the risk of mitochondrial dysfunction with Ns/tRTI in utero exposure cannot be foreseen. It is important to underline that this class labelling already encompasses the notion of a variable degree of mitochondrial damage. PRAC on March 2016 recommended the maintenance of the class labelling with adjustment in order to better reflect the current knowledge highlighting that zidovudine, stavudine and didanosine are the most inducers of mitochondrial dysfunction:

"*Section 4.4 for all Ns/tRTI*

Mitochondrial dysfunction following exposure in utero

Nucleos(t)ide analogues may impact mitochondrial function to a variable degree, which is most pronounced with stavudine, didanosine and zidovudine. There have been reports of mitochondrial dysfunction in HIV negative infants exposed in utero and/or postnatally to nucleoside analogues; these have predominantly concerned treatment with regimens containing zidovudine. The main adverse reactions reported are haematological disorders (anaemia, neutropenia) and metabolic disorders (hyperlactatemia, hyperlipasemia). These events have often been transitory. Late onset neurological

disorders have been reported rarely (hypertonia, convulsion, abnormal behaviour). Whether such neurological disorders are transient or permanent is currently unknown. These findings should be considered for any child exposed in utero to nucleos(t)ide analogues, that present with severe clinical findings of unknown etiology, particularly neurologic findings. These findings do not affect current national recommendations to use antiretroviral therapy in pregnant women to prevent vertical transmission of HIV.

The following harmonized statement has to be considered in all PILs of Ns/tRTI (except those of medicinal products for pediatric use only):

" If you have taken XXX during your pregnancy, your doctor may request regular blood tests and other diagnostic tests to monitor the development of your child. In children whose mothers took NRTIs during pregnancy, the benefit from the protection against HIV outweighed the risk of side effects."

The benefit-risk balance of Zerit, remains positive.

Scientific Summary for the EPAR

Nucleos(t)ide analogues may impact mitochondrial function to a variable degree, which is most pronounced with stavudine, didanosine and zidovudine. There have been reports of mitochondrial dysfunction in HIV negative infants exposed in utero and/or postnatally to nucleoside analogues; these have predominantly concerned treatment with regimens containing zidovudine. The main adverse reactions reported are haematological disorders (anaemia, neutropenia) and metabolic disorders (hyperlactatemia, hyperlipasemia). These events have often been transitory. Late onset neurological disorders have been reported rarely (hypertonia, convulsion, abnormal behaviour). Whether such neurological disorders are transient or permanent is currently unknown. These findings should be considered for any child exposed in utero to nucleos(t)ide analogues, that present with severe clinical findings of unknown etiology, particularly neurologic findings. These findings do not affect current national recommendations to use antiretroviral therapy in pregnant women to prevent vertical transmission of HIV.

8. Final overall conclusion and recommendation

Based on the final study results of the HIV study 'A cross-sectional study of HIV-negative children, aged 18 to <28months, born to HIV-1 infected mothers in Europe: A European study sponsored by the Collaborative Committee for Mitochondrial Toxicity in Children (MITOC)'. The MAH is requested to update the SmPC as described in section 8. These revisions are in line with the ones concluded for other nucleoside/nucleotide reverse transcriptase analogues.

☒ Not fulfilled.

The MAH is requested to submit a variation application to implement the requested SmPC changes to conclude the revision of the class labelling related to mitochondrial dysfunction.

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