STUDY TITLE: Low and Intermediate Risk Neuroblastoma European Study
EudraCT NUMBER: 2010-021396-81
PROTOCOL NUMBER: LINES

SPONSOR NAME AND ADDRESS: Instituto de Investigación Sanitaria La Fe "Fundación para la Investigación del Hospital Universitario y Politécnico de La Fe de la Comunidad Valenciana"
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TRIAL STATUS: ended

Purpose: *This document presents the final clinical-trial results for European Low and Intermediate Risk Neuroblastoma (LINES) trial. Results are submitted by the sponsor for posting in EudraCT and must be read together with the authorised protocol and protocol amendments.*

Approved by the sponsor:

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1. Clinical trial information

1.1 Clinical trial identification

Title of trial: Low and Intermediate Risk Neuroblastoma European Study
Protocol number: LINES
EudraCT Nº: 2010-021396-81
ClinicalTrials.gov Identifier: NCT01728155

1.2 Sponsor details

Sponsor of the international clinical trial: LINES

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1.3 LINES trial committee members

The LINES study was designed and the protocol developed by an expert committee comprising members of SIOPEN (the International Society of Paediatric Oncology Europe Neuroblastoma Group), bringing together extensive clinical and scientific expertise in the management of neuroblastoma. Individual committee members are not identified in this document.

Trial governance: The sponsor maintained scientific, statistical, biological, pathological and radiotherapy oversight through the LINES trial committees and SIOPEN reference structures

1.4 Lay summary of the LINES trial

The LINES study is a large European trial designed to improve the treatment and survival of children with low and/or intermediate-risk neuroblastoma, a type of childhood cancer. These children are considered "non–high-risk," meaning their disease usually has a good chance of being cured. The goal of LINES is to make treatment safer, less intensive when possible, and more effective when needed.

Why this study is important

Children with neuroblastoma do not all need the same treatment. Some can recover with very little therapy, while others need from more treatment burden. LINES brings together children from many European countries into a single protocol so doctors can:

- Reduce unnecessary treatments for children who are likely to recover with minimal therapy
- Increase treatment burden to children with features that make the disease more likely to relapse

- Collect biological samples to better understand the disease and improve future treatments
- Understand how often different types of neuroblastoma appear across Europe

How the study works

Children are placed into different groups based on several factors, including:

- Age
- Stage of the disease (how far the cancer has spread)
- Whether certain genes in the tumour are changed
- Whether the tumour can be removed safely with surgery
- Whether the child has symptoms that threaten their health

Based on these factors, patients are assigned to Low Risk or Intermediate Risk groups.

Low Risk Study

This group includes infants and young children who have neuroblastoma without *MYCN* gene amplification (a change that makes tumours more aggressive). It includes:

- Children 18 months or younger with a tumour that cannot be safely removed at diagnosis.
- Children 12 months or younger with disease that has spread, but *not* to the bones, lungs/pleura and/or central nervous system.

Main goals of the Low-Risk Study

- Give as little treatment as possible while keeping excellent survival rates
- Give chemotherapy early to children whose tumours show specific genetic changes linked to a higher chance of the cancer coming back
- Collect and analyze tumour samples and follow patient outcomes to learn more about this group

Intermediate Risk Study

This group includes:

- Children over 18 months with localised tumours that cannot be safely removed
- Children 12 months or younger with disease that has spread to bones, lungs/pleura, and/or central nervous system.
- Children with a localised tumour that was fully removed but has *MYCN* amplification

Main goals of the Intermediate Risk Study

- Reduce chemotherapy for children whose tumours show signs of becoming more mature, since they often do very well.
- Increase treatment (such as radiotherapy or retinoic acid) for tumours that look more aggressive by pathologic features in tumor biopsy.

- Improve outcomes for children with *MYCN*-amplified tumours that were completely removed.
- Maintain the excellent survival seen in previous European studies.
- Collect and analyze tumour samples and follow patient outcomes to learn more about this group.
- Understand how frequent intermediate-risk cases are across Europe.

Special group: Newborns with adrenal masses

A third part of the study includes newborn babies (under 90 days old) who have adrenal masses. These newborns and infants are registered and carefully observed without surgery at first, since many of these masses disappear on their own.

1.5 General data

LINES is an academic clinical trial designed by a multidisciplinary group of experts who are members of SIOPEN (the International Society of Paediatric Oncology Europe Neuroblastoma Group). LINES trial was initiated at the sponsor's site in June 2011. In summary, the trial was opened in Australia (AU), Austria (AT), Belgium (BE), Denmark (DK), France (FR), Greece (GR), Ireland (IE), Israel (IL), Italy (IT), Lithuania (LT), Norway (NO), Portugal (PT), Spain (ES), Sweden (SE), and Switzerland (CH).

The study was conducted according to the following timeline:

First ethics committee approval	06-06-2011
First visit first patient (FVFP)	13-01-2012
End of recruitment	31-12-2022
End of study	15-11-2024

Medical condition: Low and Intermediate risk Neuroblastoma

Population age; infants, toddlers, adolescents under 18 years

Trial protocol: ended

1.6 Global substantial modifications

Detailed changes are provided in the first section of each protocol version. A summary of the main changes submitted with each amendment is provided below:

Amendment 1: protocol version 4.0 (5/01/2012)

- To improve the clarity of the protocol, multiple typographical corrections were made throughout the document.
- Because of the need to clarify treatment initiation for low-risk patients with LTS and L2 patients ≥ 18 months without *MYCN* amplification, they may receive the first two cycles of VP/carbo while waiting for reviews.

Amendment 2: protocol version 5.0 (1/10/2015)

- To improve the clarity of the protocol, multiple typographical corrections were made throughout the document.
- For clarity, there is an option to include patients who need to start chemotherapy as soon as possible.

Amendment 3: protocol version 6.0 (31/01/2018)

- To improve the clarity of the protocol and the informed consent documents, multiple typographical corrections were made throughout the text, and the approved changes were incorporated.
- Enrolment was extended for further five years (2021).
- Study Group 1 closed *Stage L2, ≤ 18 months, MYCN non-amplified with a NCA genomic profile, with no life-threatening symptoms)*: Randomization between up front chemotherapy and observation (with chemotherapy at progression if needed) has been closed due to the very low randomization rate.
- Due to very low accrual, trial group 9 (*INSS stage 1 neuroblastoma, MYCN amplified, any age)* was closed.
- NAM study was closed because target accrual was reached.
- Sections were changed to reflect the potential for allergies (peanut or soya) to the excipients contained in the 13-cis retinoic acid preparation.
- Clarification has been added to ensure that male and female patients of reproductive potential are instructed about methods of birth control.

Amendment 4: protocol version 7.0 (5-07-2019)

- For all groups that have reached accrual or are modified at any point, a registry group to follow the incoming patients is needed. When a LR and IR group is closed, patients can still be enrolled in LINES trial into the corresponding Registry Group with parents'/patient's consent and the appropriate information submitted via SIOPEN-R-NET. The full data set will be collected on them but they will be analyzed separately in the trial. All the registered patients will benefit from full Biology studies. Our goal is to improve the overall knowledge about neuroblastoma and to improve treatment and outcomes for all children with neuroblastoma and estimate the incidence of NB groups across Europe and participating countries.
- Appendix 7: Biology review section has been updated to reflect the most current analysis techniques and to improve clarity about sample types. A sentence highlighting the importance of mandatory biomaterial collection for all patients, specially for patients with NB Stage L2 > 18 months has been added.
- Prospective radiotherapy review: new procedures according to QUARTET.
- Changes have been made to the Parent and Patient Information Form Templates

Amendment 5: protocol version 8.0 (8-06-2021)

- To improve the clarity of the protocol, multiple typographical corrections were made throughout the text.
- Target accrual for Group 8 was increased to 85 patients.
- The final date for inclusion of all patients into this study was extended to 31-12-2022.
- Changes have been made to the Parent and Patient Information Form Template

Nº Amendment	**Summary of changes**
1	New amended protocol version v4.0 5-01-2012
2	New amended protocol version v5.0 04-09-2015
3	New amended protocol version v6.0 12-02-2018
4	New amended protocol version v7.0 5-07-2019
5	New amended protocol version v8.0 30-07-2021

2. Paediatric regulatory details

This trial does not involve the introduction of new drugs for the treatment of neuroblastoma. It relies on medications that are already well-established and commonly used for this condition. Consequently, this clinical trial is not currently part of a Paediatric Investigation Plan (PIP).

3. General information about the clinical trial

3.1 Trial design

The European LINES study (*Low and Intermediate Risk Neuroblastoma European Study*) brings together, in a single protocol, the treatment of patients with non-high-risk neuroblastoma (NB), stratified into two substudy groups: low risk (LR) and intermediate risk (IR). Patients were stratified according to INRG disease stage (L1, L2, M, or MS), age (using cut-offs of 12 and 18 months), tumour genomic profile (numerical chromosome alterations [NCA] or segmental chromosome alterations [SCA]), resectability, the absence or presence of life-threatening symptoms (LTS), *MYCN* non-amplified tumour status, and histology, resulting in 10 risk groups. Central biology and pathology reviews were established. Enrolment was to be completed within 6 weeks of diagnosis. The third substudy concerned neonatal adrenal masses (NAM). Infants younger than 90 days were registered and observed without initial surgery. A brief description of the trial design is provided below (Figure 1).

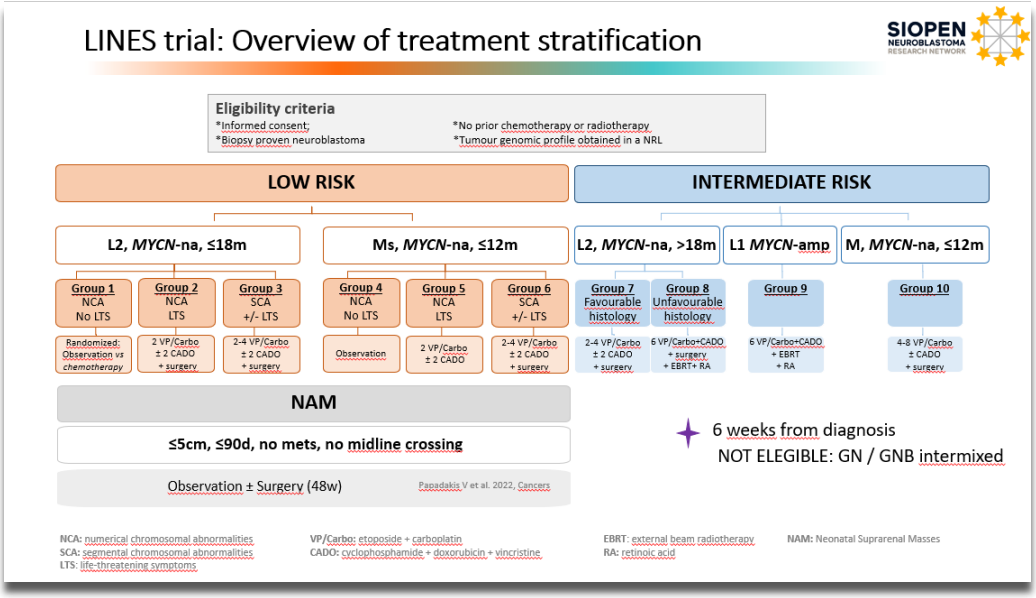


Figure 1: Description of the LINES TRIAL. LINES (NCT01728155) includes ten separate therapeutic groups, one of them randomised, who will be stratified according to stage, age, resectability using a structured algorithm. Risk-adapted therapies are tailored according tumour genotyping and Life-Threatening Symptoms (LTS) for Low Risk (L2 stage below 18 month; Metastatic Special (MS) below 12month) and histology for Intermediate risk (L2 stage older than 18 months; Metastatic below 12 months). Genomic profile was studied in a SIOPEN reference laboratory. *NCA : numerical chromosome alterations; SCA : segmental chromosome alterations; LTS: life threatening symptoms. GN; ganglioneuroma; GNB; ganglioneuroblastoma.*

3.2 Patient cohorts

A brief description of the population being studied according to eligibility criteria is below (Table 1).

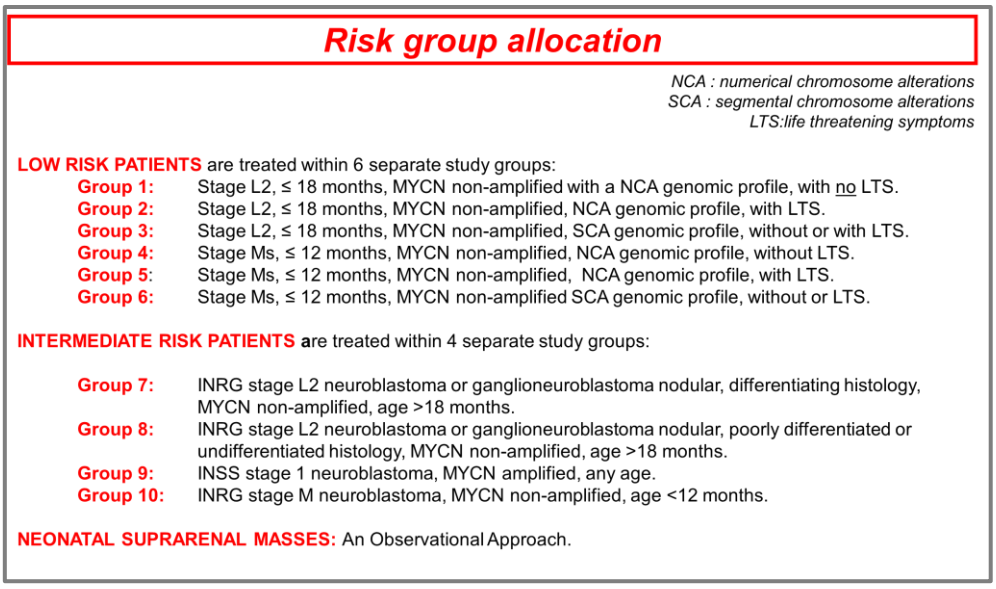


Table 1: Description of the LINES population
NCA : numerical chromosome alterations; SCA: segmental chromosome alterations; LTS:life threatening symptoms

3.3 Description of study treatment.

Depending on risk-group assignment (Table 1), treatment ranged from observation alone to several cycles of chemotherapy, or to the addition of radiotherapy and 13-cis-retinoic acid to the chemotherapy backbone. Chemotherapy was also adapted according to response and included two to four courses of carboplatin/etoposide (VP/Carbo), followed, in the event of an inadequate response, by up to two to four courses of cyclophosphamide/doxorubicin/vincristine (CADO). Group 1 patients were randomised to observation or chemotherapy. The primary tumour was to be resected after chemotherapy when feasible.

For **Low and Intermediate risk substudy**, treatment is described below: If chemotherapy is assigned to be given to eligible patients, three different regimens might be applicable: CO, VP/Carbo, or CADO. The chemotherapy regimens applicable in this protocol are described as follows:

Group 1: Randomisation between observation and 2-6 courses of chemotherapy (CO x 2-4 ± VP/Carbo x 2) ± surgery.
Group 2: Two-four courses of chemotherapy (VP/Carbo x 2 ± CADO x 2) ± surgery.
Group 3: Four courses of chemotherapy (VP/Carbo x 2-4 ± CADO x 2) ± surgery.
Group 4: Observation only.
Group 5: Two-four courses of chemotherapy (VP/Carbo x 2 ± CADO x 2).
Group 6: Four courses of chemotherapy (VP/Carbo x 2-4 ± CADO x 2) ± surgery.
Group 7: Four courses of chemotherapy (VP/Carbo x 2 + CADO x 2 or VP/Carbo x 2) ± surgery.
Group 8: Six courses of chemotherapy (VP/Carbo x 2, CADO x 2, VP/Carbo x 1 + CADO x 1 or CADO x 2), ± surgery, radiotherapy, and 6 courses of 13-cis-RA.
Group 9: Six courses of chemotherapy (VP/Carbo x 2, CADO x 2, VP/Carbo x 1, CADO x1) followed by radiotherapy and 6 courses of 13-cis-RA.
Group 10: Four-eight courses of chemotherapy (VP/Carbo x 2-4 ± CADO x 2-4), ± surgery.

It is very important to note that according to patient weight, the final drug dose might be calculated based on:

- Body surface area (BSA) for patient weighing>10 kg or
- Weight for patients weighing ≤ 10 kg
- For infants weighing below 5 kg, chemotherapy drug doses should be reduced by a further 33%.

CO (Cyclophosphamide and vincristine)

Courses of CO are given at 14 day intervals

DAY	1	2	3	4	5
Cyclophosphamide	•	•	•	•	•
Vincristine	•				

DRUG	Dose (mg/kg)	(mg/m^2)	Administration
Cyclophosphamide PO)	5.0 mg/kg	150 mg/m^2	Slow bolus injection dailyx5 (or
Vincristine	0.05 mg/kg	1.5 mg/m^2	Slow bolus injection day 1 only

VP/Carbo (Etoposide {VP16} and carboplatin)

Courses of VP/Carbo are given at 21 day intervals

DAY	1	2	3
Carboplatin	•	•	•
Etoposide	•	•	•

DRUG	Dose (mg/kg)	Dose (mg/m^2)	Administration
Carboplatin hr daily x 3	6.6 mg/kg	200 mg/m^2	5% dextrose (5 ml/kg) over 1
Etoposide (VP16) 2 hrs daily x 3	5.0 mg/kg	150 mg/m^2	0.9% saline (12.5 ml/kg) over

CADO (Cyclophosphamide, doxorubicin and vincristine)

Courses of CADO are given at 21 day intervals

DAY	1	2	3	4	5
Cyclophosphamide	•	•	•	•	•
Doxorubicin				•	•
Vincristine	•				•

DRUG	Dose (mg/kg)	Dose (mg/m^2)	Administration
Cyclophosphamide x 5 days	10 mg/kg	300 mg/m^2	5% dextrose (5 ml/kg) over 1 hr, daily
Doxorubicin 5	1 mg/kg	30 mg/m^2	0.9% saline over 6 hours on days 4 and
Vincristine 5	0.05 mg/kg	1.5 mg/m^2 (max 2 mg)	Bolus injection on days 1 and

Neonatal adrenal masses (NAM): Following diagnosis, close monitoring was requested, without further therapeutic intervention. Two conditions interrupted the observation strategy and prompted surgical complete excision; 1. ≥40% increase of the original tumor volume, with or without an increase in urine catecholamines and 2. Mass persistence following 48-week observation period. Isolated catecholamines increase, without increase in mass size did not prompt surgery.

4. Protocol summary.

The European study, LINES, groups together in a single protocol the treatment of all patients with "non high risk" neuroblastoma (NB), with stratification into two groups: low risk and intermediate risk. These two separate cohorts are included in one single protocol to enable patient data from these two groups to be entered into a common database, as the current prognostic classifications determining treatment may evolve further with subsequent more detailed molecular analysis of the tumours.

Low Risk Study

The low risk group of patients includes NB patients without *MYCN* amplification with or without life threatening symptoms in the following clinical situations:

- Children aged ≤ 18 months with localised neuroblastoma associated with image defined risk factors precluding upfront surgery (stage INRG L2).
- Children aged ≤ 12 months with disseminated neuroblastoma without bone, pleura, lung or CNS disease (stage INRG Ms)

The Low Risk Study is proposed in order to:

- minimise the amount of treatment (chemotherapy and surgery) for all appropriate low risk patients, who in previous studies have been shown to have an excellent long-term outcome (as in the SIOPEN 99.1-2 infant neuroblastoma studies where the overall survival was greater than 97%).
- improve the EFS and maintain the OS in L2 and Ms patients with a SCA genomic profile tumour (presence of any segmental chromosomal change (SCA)) by electively treating these patients with chemotherapy despite the absence of symptoms.
- to collect tissue/biologic information and follow outcome in Low-risk patients.
- to estimate the incidence of NB risk groups across Europe and participating countries.

Intermediate Risk Study

The intermediate risk group of patients includes NB patients in the following clinical situations:

- Children aged >18 months with localised neuroblastoma without *MYCN* amplification, associated with image defined risk factors precluding upfront surgery (stage INRG L2).
- Children aged ≤12 months with disseminated neuroblastoma involving bone, pleura, lung and/or CNS (stage INRG M), without *MYCN* amplification.
- Children with localised resected NB (stage INSS 1) with *MYCN* amplification.

The Intermediate Risk Study is proposed in order to:

- reduce the amount of chemotherapy for differentiating histology INRG stage L2 NB and ganglioneuroblastoma nodular patients who in previous SIOPEN study have been shown to have an excellent long-term outcome;
- increase the amount of treatment (radiotherapy and 13-cis-RA) for poorly differentiated or undifferentiated histology INRG stage L2 NB or ganglioneuroblastoma nodular patients in order to improve the EFS registered in the previous SIOPEN study;
- improve the EFS of *MYCN* amplified INSS stage 1 NB patients with the introduction of adjuvant treatment.
- maintain the very good results obtained in previous SIOPEN study for INRG stage M infants with a moderate treatment.
- to collect tissue/biologic information and follow outcome in Intermediate-risk patients.
- to estimate the incidence of NB risk groups across Europe and participating countries.

5. Aims of the trial

5.1 Low risk study

Primary aim
To demonstrate through a randomisation between observation and chemotherapy that you can safely reduce treatment in a subgroup of L2 low risk patients (those without life threatening symptoms (LTS) and without any segmental chromosomal changes (SCA) by giving less treatment than has been given historically while maintaining an excellent OS of 100%.

Secondary aims
- To maintain a 2 year EFS of at least 90% and an OS of at least 95% in L2 patients with LTS without SCA (Study Group 2).
- To maintain the 2 year EFS of 85% and an OS of at least 98% in Ms patients without SCA (Study Groups 4 and 5).
- To improve the 2 year EFS to at least 90% and maintain the OS of close to 100% in L2 patients with SCA (Study Group 3) and improve the 2 year EFS to over 70% in Ms patients with SCA (study group 6).
- To evaluate adherence to the protocol recommendations regarding LTS.
- To reduce surgical morbidity by promoting strict adherence to IDRFs to determine surgical resectability.
- To define the long term follow-up and natural history of the Stage L2 non-resected masses that have remained IDRF positive at the end of treatment (study groups 1-3).
- To collect tissue/biologic information and follow outcome in Low-risk patients.
- To estimate the incidence of NB risk groups across Europe and participating countries.

5.2 Intermediate risk study

Primary aim
To improve the EFS to 70% with an OS of 90% of INRG stage L2 patients over the age of 18 months, with poorly differentiated or undifferentiated tumour histology (INPC criteria), by the

addition of radiotherapy and 13-cis-RAcompared to historical conventional treatment) (Study group 8).

Secondary aims

- To confirm in a larger patient cohort the excellent OS of 95% in stage M NB without MYCN amplification, less than 12 months of age, when treated with moderate therapy (study group 10) (as achieved in INES 99.3)
- To maintain the excellent results of 3 year EFS of 90% and 3 year OS of 100% in stage L2 patients over the age of 18 months, with differentiating neuroblastoma or differentiating ganglioneuroblastoma nodular, despite a treatment reduction (study group 7).
- To improve 3 year EFS to at least 50% and the 3 year OS to 80% in INSS stage I patients with MYCN amplified NB by the addition of adjuvant treatment (study group 9).
- To evaluate the impact of the tumour genomic profile on patient outcome, in order to consider its role in the treatment stratification of these intermediate risk patients (all groups).
- To collect tissue/biologic information and follow outcome in Intermediate-risk patients.
- To estimate the incidence of NB risk groups across Europe and participating countries.

5.3 Neonatal suprarenal masses

Primary aim

To maintain a 3-year event free survival over 80% with a non-operative therapeutic approach (serial monitoring, surgery if warranted) in infants with a localised suprarenal mass discovered ante or neonatally.

Secondary aims

- To manage infants with suprarenal masses discovered ante or neonatally with a uniform approach in Europe in a multicentre setting.
- To maintain an excellent overall survival with a non-operative therapeutic approach (serial monitoring, surgery if warranted) in infants with a localised suprarenal mass discovered ante or neonatally.
- To determine the 3-year surgery-free survival in infants with suprarenal masses discovered ante or neonatally and managed conservatively (non-initial surgery).
- To find out the natural history of perinatal suprarenal masses, according to the definitions set up for the study.
- To study the kinetics of regression in those suspected suprarenal neuroblastomas in infants with suprarenal masses discovered ante or neonatally and managed conservatively (non-initial surgery).
- To collect tissue from those suprarenal masses excised in order to perform standard and investigational pathological and biological studies (INPC, *MYCN*, 1p, 11). To collect frozen plasma from all patients included in the study in order to perform research.

6. Patient cohorts description

From the recruitment of the first patient in January 2012 through 31 December 2022, 1,028 patients were registered, although not all completed enrolment. A total of 327 patients did not successfully complete the enrolment process and were recorded under the categories -1/NA. Total accrual by country is shown in Table 2.

	AT	AU	BE	CH	DK	ES	FR	GR	IE	IL	IT	LT	NO	PT	SE	TOTAL
TOTAL SCREENED	47	11	44	36	12	137	317	9	5	46	336	0	13	3	12	1028
LR SUBSTUDY	15	7	12	21	0	55	130	6	4	13	125	0	9	0	7	404
IR SUBSTUDY	8	2	9	8	0	22	57	1	0	4	55	0	1	0	0	167
NAM SUBSTUDY	1	0	1	0	0	18	45	0	0	3	62	0	0	0	0	130
GROUP NA/-1	23	2	22	7	12	42	85	2	1	26	94	0	3	3	5	327

Table 2. The number of patients assigned to each substudy per country: Austria (AT), Australia (AU), Belgium (BE), Switzerland (CH), Denmark (DK), Spain (ES), France (FR), Greece (GR), Ireland (IE), Israel (IL), Italy (IT), Lithuania (LT), Norway (NO), Portugal (PT) and Sweden (SE).

The study population was approximately balanced by sex. Across the low- and intermediate-risk substudies, 49% of participants were female and 51% were male. Neuroblastoma disease is most commonly diagnosed between 1 and 2 years of age. Based on INRG NB staging, NB Low risk groups are all aged below 18 months for localized disease and below 12 months for metastatic disease and NB Intermediate risk groups are older than 18 months. For neonatal adrenal masses, 130 children (60.9% males /39.1 % females) with median age at diagnosis of 3 days (range, 0-87) were enrolled. Patient characteristics are described in detail below:

Common Inclusion criteria for all the studies:

- Informed consent and follow-up warranted
- Group assignment completed within 6 weeks from diagnosis
- No prior chemotherapy* or radiotherapy

Common exclusion criteria for all the studies:

- Diagnosis of ganglioneuroma or ganglioneuroblastoma intermixed

The specific eligibility criteria for low risk Neuroblastoma patients are outlined below.

LOW RISK STUDY	**ELEGIBILITY**	
	INCLUSION CRITERIA	**EXCLUSION CRITERIA**
STAGE		
L2 *Locoregional tumour with presence of one or more IDRFs*	• Biopsy proven neuroblastoma • Tumour genomic profile obtained in a NRL according to guidelines • *MYCN* non-amplified • ≤ 18 months	• Any metastatic site • *MYCN* amplification • >18 months
Ms *Metastatic disease confined to skin and/or liver and/or bone marrow (or even other sites such as lymph nodes and/or testes), but NOT bone, lung, pleura or CNS, in infants ≤12 months. MIBG or technetium scintigraphy uptake to the skeleton can occur but without bone lesions documented by X-ray and/or CT scan*	• Biopsy proven neuroblastoma • Tumour genomic profile obtained in a NRL according to guidelines • *MYCN* non-amplified • ≤12 months	• Bone, Pleura/lung, and/or CNS metastasis • *MYCN* amplification • >12 months

The specific eligibility criteria for intermediate Neuroblastoma patients are outlined below.

INTERMEDIATE RISK STUDY	ELEGIBILITY	
	INCLUSION CRITERIA	**EXCLUSION CRITERIA**
STAGE		
(L1) INSS1 *Localised tumours with complete gross excision, with or without microscopic residual disease; representative ipsilateral lymph nodes negative for tumour microscopically (nodes attached to and removed with the primary tumour may be positive)*	• Biopsy proven neuroblastoma • Tumour available for genomic profiling according to guidelines • *MYCN* amplified	• *MYCN* non-amplified • INSS stages 2, 3, 4, 4s
L2 *Locoregional tumour with presence of one or more IDRFs*	• Biopsy proven neuroblastoma and confirmed in a NRL: differentiating, poorly differentiated, undifferentiated NBor ganglioneuroblastoma nodular • Tumour available for genomic profiling according to guidelines • *MYCN* non-amplified • >18 months	• Neuroblastoma NOS • *MYCN* amplification • ≤ 18 months
M *Distant metastatic disease with positive MIBG (except Stage Ms). In infants ≤12 months, MIBG or technetium scintigraphy uptake to the skeleton to be confirmed with a bone abnormality visible on X-ray and/or CT scan.*	• Biopsy proven neuroblastoma • Tumour available for genomic profiling according to guidelines) • *MYCN*non-amplified • ≤ 12 months	• *MYCN* amplification • > 12 months

Inclusion criteria for each cohort:

- **Group 1:**

Eligible patients: Stage L2, ≤ 18 months, MYCN non-amplified with a NCA genomic profile, with no life-threatening symptoms

- **Group 2:**

Eligible patients: Stage L2, ≤ 18 months, MYCN non-amplified, NCA genomic profile, with life threatening symptoms

- **Group 3:**

Eligible patients: Stage L2, ≤ 18 months, MYCN non-amplified, SCA genomic profile, without or with life threatening symptoms.

- **Group 4:**

Eligible patients: Stage Ms, ≤ 12 months, MYCN non-amplified, NCA genomic profile, without life threatening symptoms

- **Group 5:**

Eligible patients: Stage Ms, ≤ 12 months, MYCN non-amplified, NCA genomic profile, with life threatening symptoms

- **Group 6:**

Eligible patients: Stage Ms, ≤ 12 months, MYCN non-amplified SCA genomic profile, without or with life threatening symptoms.

- **Group 7:**

L2, Age >18 months, differentiating neuroblastoma or differentiating ganglioneuroblastoma nodular

- **Group 8:**

L2, Age >18 months, undifferentiated and poorly differentiated neuroblastoma or undifferentiated and poorly differentiated ganglioneuroblastoma nodular

- **Group 9:**

INSS stage 1, MYCN amplified neuroblastoma

- **Group 10:**

Eligible patients: Infants (≤12 months) with stage M neuroblastoma, without MYCN amplification

Regarding the Neonatal adrenal masses substudy:

Inclusion criteria

- Age less than or equal to 90 days when the suprarenal mass is discovered.
- Suprarenal mass detected by ultrasound and/or MRI. The suprarenal mass may be cystic and/or solid, but it cannot reach the midline and should measure ≤ 5 cm at the largest diameter.
- No regional involvement: MRI scan does not show evidence of positive ipsi/contralateral lymph nodes or other spread outside the suprarenal gland.
- No metastatic involvement.
- Frozen plasma available.
- Informed consent.
- Availability to do the adequate follow-up

Exclusion criteria

- Age older than 90 days.
- Suprarenal mass bigger than 5 cm.
- Regional involvement.
- Metastatic involvement.
- Inability to undertake mandatory diagnostic studies (biological markers, US, MRI, MIBG).
- Follow-up not guaranteed by parents/guardians.

7. Statistics

Group 1 is a Phase III randomized, prospective, multi-centre clinical trial comparing chemotherapy versus observation with chemotherapy initiated if progression occurs. 150

patients were to be included to demonstrate, with 85% power, a 25% reduction in the 1-year initiation of VP/Carbo in the observation arm, the rate being approximately 55% in the chemotherapy arm, with a two-sided test of 5% (Freedman method). Following an independent Data Monitoring Committee (IDMC) recommendation, the trial was closed due to the slow accrual rate. Primary analysis was then modified and focused on estimation of event free survival (EFS) and overall survival (OS) as for the two other trials, as no comparison was statistically reliable.

Groups 2 to group 10 are multi-centre, non-blinded, single-arm prospective arms. When designing the protocol, it was recognized that, even with wide multinational collaborations, the number of patients within the various subgroups would not be sufficient to base sample size calculations on conventional methods of test size and power. Hence the general objective was to obtain as many patients as possible within 5 years, and thereby to estimate the endpoint statistics (2 year-EFS) for LR substudy and (3 year-EFS) for IR substudy, with a two-sided 95% confidence interval. Due to the slow accrual rate, the enrolment was extended for further five years.
The date of diagnosis is defined as being the date of diagnostic biopsy/surgery. Event free survival was defined as the time from diagnosis to the first event (relapse, progression or death) or the date of last contact. Overall survival was defined as the time from diagnosis to death or the date of last contact. OS and EFS were analysed using the Kaplan-Meier method and survival estimates were reported at 2 years for low-risk and 3 years for intermediate risk. The analysis of OS or EFS were performed as intention to treat. The survival curves were compared using the log-rank test. Results with a p-value < 0.05 were considered statistically significant. Language R (v4.5.2) was used for all statistical analyses.

Due to the expected low incidence of NAM patients, a single arm design was chosen, aiming for descriptive statistics in a patient cohort recruited over five years, and estimate target accrual of 100 patients. Overall survival (OS) and Event Free Survival (EFS) at 3 years were estimated using the Kaplan-Meier method. Event was defined a >40% increase in mass size (volume) and/or progression to higher neuroblastoma stage (L2, M or MS). The time to event was defined as the time from the date of diagnosis to the first occurrence of an event, or until the time of last contact, if no event occurred. Language R (v4.5.2) was used for all statistical analyses.

8.Results

8.1 Low and Intermediate risk substudy groups

- **Group 1:** L2**,** NCA genomic profile, No LTS

Randomisation will be stratified according to country.
Objectives: To maintain an OS of 100% with less treatment than has been given historically. The treatment burden will be less in some patients who will only be given chemotherapy if progression occurs and chemotherapy may be avoided completely in others. The primary endpoint is the time of initiation of VP/Carbo administration during the first year following diagnosis
Outcome:
Randomization between up front chemotherapy and observation (with chemotherapy at progression if needed) for group 1 was closed in 2017 due to the very low randomization rate (n=33). The primary endpoint is the time of initiation of VP/Carbo administration during the first year following diagnosis and could not be assessed due to sample size.

Chemotherapy: 2-year EFS (100%); 2-year OS (100%)

Observation: 2-year EFS: 65% (95% CI, 0.4-0.9); 2-year OS: 100%

- **Group 2: L2, NCA, with LTS**

Single arm:
Objective: To maintain a 2-year EFS of at least 90% and an OS of at least 95%
Outcome: 2-year EFS: 83% (95% CI, 0.7-0.9); 2-year OS: 100%

- **Group 3: SCA genomic profile, irrespective of LTS**

Single arm:
Objective: To improve the 2-year EFS to at least 90% and maintain the OS of close to 100%
Outcome: 2-year EFS: 87% (95% CI, 76-99); 2-year OS: 97% (95% CI, 91-100)

- **Group 4: Ms, NCA genomic profile, No LTS**

Single arm:
Objectives: Maintain 2-year EFS over 85 % and OS of at least 98%.
Outcome: 2-year EFS: 75% (95% CI, 0.6-0.8); 2-year OS: 100%

- **Group 5: Ms, NCA genomic profile, with LTS**

Single arm:
Objective: Maintain a 2-year EFS over 85% and OS of at least 98%
Outcome: 2-year EFS: 88% (95% CI,0.8-0.9); 2-year OS: 98% (95% CI, 0.9 -1.0)

- **Group 6: Ms, SCA genomic profile, irrespective of LTS**

Single arm:
Objectives: Aim to improve 2-year EFS to over 70%. Retrospective data show EFS of 60% in this group. It would be clinically worthwhile to improve this 2 year EFS to 70% with the addition of upfront VP/Carbo chemotherapy.
Outcome: 2-year EFS: 80% (95% CI, 0.7-0.9); 2-year OS: 100%

- **Group 7: L2, Age >18 months, differentiating neuroblastoma or differentiating ganglioneuroblastoma nodular**

Single arm:
Objectives: Maintain 3-year EFS of 90% with 3-year OS at 100%
Outcome: 3-year EFS 69% (95% CI, 0.49-0.96); 3-year OS 94% (95% CI, 0.83-1.00).

- **Group 8: L2, Age >18 months, undifferentiated and poorly differentiated neuroblastoma or undifferentiated and poorly differentiated ganglioneuroblastoma nodular**

Single arm:
Objectives: To improve 3-year EFS to 70% with 3-year OS at 90%
Outcome: 3-year EFS 68% (95% CI, 0.58-0.78); 3-year OS 90% (95% CI, 0.84-0.97).

- **Group 9: INSS stage 1, MYCN amplified neuroblastoma**

Single arm:
Objectives: To improve 3-year EFS at least to 50% with 3-year OS at 80%.
Due to very low accrual, trial group 9 was closed in 2017 with 2 patients enrolled.

- **Group 10: M, Age < 12 months**

Single arm:
Objectives: Maintain 3-year EFS of 85% with 3-year OS at 95%
Outcome: 3-year EFS: 84% (95% CI, 0.7-0.9); 3-year OS: 100%

8.2 Neonatal adrenal masses substudy

- **Neonatal adrenal masses (NAM)**

Single arm: Interventional. Serial monitoring based on imaging and laboratory studies in order to avoid initial surgery in small suprarenal masses.
Objectives: Maintain 3-year EFS of 80% with 3-year OS over 95%.
Outcome: 3-year EFS: 87% (95% CI, 81-93); 3-year OS: 100%
No infant had detectable MYCN amplification in the peripheral blood.

The expected accrual was achieved on January 2016. Results from the neonatal adrenal masses substudy have already been shared with the scientific community and were published in 2022. The abstract and a summary of the results from the manuscript are provided below:

"Suprarenal Masses in Very Young Infants: Is It Safe to Watch and Wait? Report of a SIOPEN Observational Study Results. Papadakis V, Segura V, Conte M, Plantaz D, Di Cataldo A, Schleiermacher G, Wheeler K, Bermúdez JD, Ash S, Brichard B, Ladenstein R, Combaret V, Sarnacki S, Fagnani AM, Granata C, Cañete A. Cancers (Basel). 2022 Aug 19;14(16):4007. doi: 10.3390/cancers14164007."

8. Adverse events information

This trial does not intend to use new drugs in neuroblastoma treatment. This is based on previously and commonly used drugs for this disease. All drugs (Vincristine, doxorubicin, carboplatin, cyclophosphamide, etoposide and 13 cis-retinoic acid) involved in the trial are commercially available marketed drugs. The treatment given within this trial has well-known side effects. Therefore, for the purposes of this trial only serious adverse events (SAE) will be reported.

8.1 Evaluation of risks

All investigational drugs being used in this clinical trial have been granted a marketing approval and no significant post-marketing data and/or any new safety concerns have arisen during the trial. Risks remain the same as described per SmPC for all drugs being used in this study. At present, the risk of use and dosage of all study drugs is well-known as per routine practice.

8.2 Summary of Adverse event

During the trial period no newly identified safety concerns have arisen different than the well-known toxicity profiles of all drugs used in this clinical trial. During the reporting period, 120 Serious Adverse Events or Serious Adverse Reactions occurred. During this reporting period, none dropouts related to adverse events have been reported. No case of medication error has been notified to the Sponsor as a SAE. Overall, 25 deaths have occurred 5 in the Low-risk substudy and 20 in the intermediate-risk substudy. With the exception of two deaths unrelated to neuroblastoma disease progression, all events were attributed to disease progression.