CLINICAL STUDY REPORT

Version 1

APRIL 21ST 2021

PHASE II FEASIBILITY STUDY USING CH14.18/CHO ANTIBODY AND SUBCUTANEOUS INTERLEUKIN 2 AFTER HAPLOIDENTICAL STEM CELL TRANSPLANTATION IN CHILDREN WITH RELAPSED NEUROBLASTOMA

Sponsor Study Number: ch14.18-IL2 1021
EudraCT Number: 2009-015936-14
Date of Protocol, version 1.2, 24 April 2012

CLINICAL STUDY DATES

First patient enrolled: 09-NOV-2010
Last patient completed: 21-OCT-2018
Last patient visit included in analyses: 27-MARCH-2019

Coordinating Investigator: Peter Lang, MD, PhD

This study was performed in full compliance with applicable Good Clinical Practices (GCP) and regulations, including the archiving of essential documents

2. SYNOPSIS

Title of Study:

Phase II feasibility study using ch14.18/CHO antibody and subcutaneous interleukin 2 after haploidentical stem cell transplantation in children with relapsed neuroblastoma

Study Number: ch14.18-IL2 1021

Investigators:

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Study Centers:

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Publication (reference): n.n

Study Period (Years): First Patient Enrolled to Last Patient Completed 09-Nov-2010 - to 21-Oct-2018 Last patient follow-up: 27-March-2019	**Clinical Phase:** II

Objectives:

Primary:

Evaluation of safety and feasibility of the chimeric 14.18 anti-GD2 monoclonal antibody (ch14.18/CHO) in combination with subcutaneous aldesleukin (IL-2, interleukin-2)

Secondary:

Evaluation of the anti-tumor responses resulting from this immunotherapy regimen through clinical assessments (radiographic and clinical measurements, including bone marrow immunohistochemistry for those research participants with marrow involvement).

Evaluation of the pharmacokinetics of the ch14.18/CHO.

Evaluate the changes in natural killer (NK) cell activation and proliferation (immunological monitoring).

Methodology:

The six-cycle regimen consisted of an 8-hour infusion (ch14.18/CHO 20 mg/m²/day) for five consecutive days administered every 4 weeks. If there was evidence of response after 6 cycles, patients could receive another 3 cycles.

Interleukin-2 was added to cycles 4-9 at days 6, 8, 10 (1 x 10^6 IU/m²/day subcutaneously).

Participants were pre-medicated with an intravenous antihistamine and ranitidine within approximately 30 minutes prior and during the infusion of the study agent.

Pain as an anticipated side effect of ch14.18/CHO was managed by a standard pain prophylaxis with morphine hydrochloride.

Disease status was evaluated by computed tomography/magnetic resonance imaging (CT/MRI) or metaiodobenzylguanidine (mIBG) as well as catecholamine excretion in urine and serum neuron-specific enolase (NSE) levels after 3 and 6 cycles and after 1 year.

In addition, patients who received 9 cycles of treatment had disease status evaluated after their 9th cycle.

Number of Patients (planned and analyzed): 68 patients planned for this report, 68 patients analyzed

Diagnosis and Main Criteria for Inclusion:

Diagnosis of relapsed neuroblastoma.

Inclusion Criteria:

1. Less than or equal to 21 years of age.
2. Histologically confirmed neuroblastoma.
3. Refractory to standard treatment (i.e. refractory disease) or relapse after previous autologous or allogeneic stem cell transplantation.
4. Patient had undergone haploidentical stem cell transplantation prior to antibody infusion according to appendix IV [of the study protocol] at least 60 days prior to starting immunotherapy.
5. Serum glutamate pyruvate transaminase (SGPT) less than 2.5 times the upper limit of normal for age and total bilirubin less than 2 times the upper limit of normal for age. D-Dimers less than 2 times the upper limit of normal.
6. Creatinine clearance or radioisotope glomerular filtration rate (GFR) greater than or equal to 40 ml/min/1.73 m^2.
7. Cardiac shortening fraction greater than or equal to 20% by echocardiogram.
8. Karnofsky/Lansky performance score (age appropriate) of greater than or equal to 50.
9. Females of childbearing potential must have had a negative pregnancy test. Patients of childbearing potential must have agreed to use an effective birth control method. Female patients who were lactating must have agreed to stop breast-feeding.
10. Written informed consent was obtained, and for minors a written agreement by parents or legal guardian.
11. All institutional and national requirements for human studies were met.

Exclusion Criteria:

1. Marked baseline prolongation of QT/QT_c interval (e.g. demonstration of a QT_c interval >450 milliseconds).
2. Patients with symptoms of congestive heart failure or uncontrolled cardiac rhythm disturbance.
3. Patients with significant psychiatric disabilities or uncontrolled seizure disorders.
4. Patients with active infections or active peptic ulcer, unless these conditions were corrected or controlled.
5. Patients with acute Graft-versus-host disease (GvHD) Grade 3 or 4 or extensive chronic GvHD.
6. Patients with clinically significant, symptomatic, pleural effusions.
7. Patients who have had major surgery, (i.e. laparotomy or thoracotomy) within two weeks of screening.
8. Patients who were more than 12 months post haploidentical stem cell transplantation at the time of starting the first cycle of immunotherapy.
9. Prior administration of ch14.18 antibody after allogeneic stem cell transplantation (SCT)
10. Human immunodeficiency virus (HIV) or hepatitis B surface antigen (HB_S Ag) positive. The presence of either may have influenced the ability of the immune system to be stimulated by this treatment.

Test Products, Doses and Mode of Administration, Batch Number:

Ch14.18/CHO : 4.5 ± 0.25 mg/mL; intravenous infusion, Lot T651204-A, T900310-A, T1101012-A

Interleukin-2 (IL-2): $1 \bullet 10^6$ IU/m^2; subcutaneous injection (commercially available IL-2 was used)

Duration of Treatment and Study Participation:

The duration of treatment per individual patient was up to 36 weeks

The duration of study participation per individual patient was 1 year

Criteria for Evaluation:

Primary Safety Endpoint:

Primary endpoint was "success of treatment" defined as a patient receiving the full protocol treatment, still alive 180 days after treatment without progression and without unacceptable toxicity and acute GvHD ≥ Grade 3 or extensive chronic GvHD.

Primary Safety Outcome Measure:

Rate and type of dose limiting toxicity

Pharmacokinetics

Serum samples were obtained prior to initiating treatment at the “on study” time, which was within 10 days of starting treatment, and on days 1, 5, and 10 of cycles 1, 3, 4, 6 just prior to the ch14.18/CHO infusion. These samples will be assessed by enzyme-linked immunosorbent assay (ELISA) for ch14.18/CHO.

Statistical Methods:

All patients that were enrolled in the study were included in the analysis following considerations equivalent to the intention-to-treat principle in randomized trials.

Exact 95% confidence intervals for the rate of patients with treatment success were determined. Time to events data were analyzed using the Kaplan-Meier method.

Patients were allowed to receive three more treatment cycles with ch14.18/CHO and IL-2 for their own potential benefit. These patients were further evaluated for the secondary objectives (pharmacokinetics and immune response).

SUMMARY OF RESULTS

Disposition and Baseline/Demographic Characteristics:

Pediatric or adult patients (N=68) with histologically confirmed neuroblastoma refractory to standard treatment or relapse after previous autologous or allogeneic SCT were enrolled into this study.

Patients had a median age of 7,5 years, and included more males than females (67,6% vs. 31%). Most patients (94,1%, i.e. distant relapse, distant and local relapse, refractory metastatic disease) had evidence of distant relapse, particularly metastases to the skeleton and bone marrow. Most patients (67,6%) were negative for N-myc proto-oncogene. Nearly all (98,5%) had chemotherapy, more than half of the patients (57,4%) had surgery, 38,2% of the patients had radiation therapy and approximately 60% of the patients (63,2%) had ^{131}I-radiolables mIBG therapy for treatment of recurrent neuroblastoma.

Most patients (>80%) were concomitantly treated with analgesic drugs and antihistamines. Ongoing previous supportive medications taken most frequently were antibiotic drugs, antiviral drugs, antifungal medication, and vitamins.

Efficacy Results:

Primary Efficacy Outcome:

Primary efficacy endpoint of the study was "Success of treatment" defined as a patient receiving the full protocol treatment, still alive 180 days after treatment without progression and without unacceptable

toxicity and acute GvHD ≥Grade 3 or extensive chronic GvHD. Fourty of the 68 patients (58.8%) were considered to have reached the primary efficacy endpoint

Safety Results:

Primary Safety Outcome:

Overall, combination immunotherapy after haploidentical SCT was manageable in most patients. Sixteen out of 68 patients (23,5%) experienced life-threatening, disabeling or fatal adverse events (see table severe adverse events). The most frequent undesirable effects of ch14.18/CHO treatment combined with aldesleukin were pain, capillary leak syndrome, hypotension, and hypersensitivity reactions. Undesirable effects on red and white blood cells as well as changes in transaminases were common laboratory adverse events.

Secondary Safety Outcomes

Overall secondary safety outcomes were as follows:

- one fatal possibly immunotherapy related severe adverse event was reported during this study;
- fluid retention and infections were the most frequent non-serious TEAEs;
- one patient (0.3%) experienced severe GI-GvHD (grade III) during the antibody treatment.

CONCLUSIONS:

* Combination treatment of ch14.18/CHO with IL-2 initiated not earlier than 60 days after haploidentical SCT was shown to be feasible. Despite the occurrence of considerable but expected toxicity (hypersensitivity reactions, gastrointestinal disease, neurotoxicity, infections, fever, hypotension, neutropenia, anemia, increased transaminases) treatment was manageable. Treatment-related pain was common and required concurrent opioid administration.
* Immunotherapy does not seem to enhance the risk for GvHD after haploidentical SCT over a treatment period of nine cycles of ch14.18/CHO and six cycles of combination therapy with IL-2.
* In conclusion, ch14.18/CHO mAb showed an overall favorable human toxicity profile at the selected dose-level in pediatric patients with relapsed neuroblastoma after haploidentical

Date of the Report: 21-April-2021, Version 1: 21-April-21

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4. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADCC	Antibody dependent cellular cytotoxicity
AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransaminase
BP	Blood pressure
BSA	Body surface area
CBC	Complete blood cell count
CDC	Complement dependent cytotoxicity
CHO	Chinese hamster ovary
CI	Confidence interval (Exact 95% confidence interval)
CNS	Central nervous system
CPCS	Center for Pediatric Clinical Studies
CR	Complete response
CRF	Case report form
CRP	C-reactive protein
CT	Computed tomography
CTC	Common Toxicity Criteria (National Cancer Institute, United States)
DLT	Dose-limiting toxicity
ECG	Electrocardiogram
EFS	Event-free survival
FAS	Full analysis set
G-CSF	Granulocyte-colony stimulating factor
GFR	Glomerular filtration rate
GvHD	Graft-versus-host disease
HB_s Ag	Hepatitis B surface antigen
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HVA	Homovanillic acid
IEC	Independent Ethics Committee
IgG	Immunoglobulin G
IL-2	Interleukin-2
IMP	Investigational medicinal product
i.v.	Intravenous
LDH	Lactate dehydrogenase
mAb	monoclonal antibody
MedDRA	Medical Dictionary for Regulatory Activities
MHC	Major histocompatibility complex
MRI	Magnetic resonance imaging
mIBG	Metaiodobenzylguanidine (iobenguane)
Na	Sodium
NCI	National Cancer Institute (United States)
NK	Natural killer (cell(s))
NSE	Neuron-specific enolase
OS	Overall survival
PD	Progressive disease
PR	Partial response
PT	Prothrombin Time

QT/QT_c	Interval from start of Q wave to end of T wave/ and corrected for heart rate
rIL-2	Recombinant interleukin-2
SAE	Serious adverse event
SAP	Statistical analysis plan
s.c.	Subcutaneous
SCT	Stem cell transplantation
SD	Standard deviation
SGPT	Serum glutamate pyruvate transaminase
sIL2r	Soluble IL-2 receptor
SIOPEN	[Société Internationale d'Oncologie Pédiatrique European Neuroblastoma]; Society of Pediatric Oncology European Neuroblastoma Network)
SOC	System Organ Class
T	Temperature
TEAE	Treatment-emergent adverse event
VMA	Vanillylmandelic acid
VOD	Veno-occlusive disease

5. ETHICAL AND REGULATORY REQUIREMENTS

5.1 INDEPENDENT ETHICS COMMITTEE

The regulatory permission to perform the study was obtained in accordance with applicable regulatory requirements. The Independent Ethics Committee (IEC) approved the protocol, the amendments, the patient information sheets, the informed consent form (ICF), and their updates. Regulatory and ethical approvals were obtained before patients were exposed to any study-related procedure, including screening tests to determine eligibility.

This interim report has been written based on version 1.2 of the study protocol.

The sponsor was responsible for informing the IEC and the health authority of any serious adverse event (SAE) and/or major amendments to the protocol as per local requirements. All correspondence with the committee was filed by the investigator.

5.2 ETHICAL CONDUCT OF THE STUDY

The study was conducted in accordance with the Declaration of Helsinki, 2008 and standard research practice at the Institutions, Good Clinical Practice (GCP) and applicable local regulatory requirements.

5.3 PATIENT INFORMATION AND CONSENT

It was the responsibility of the Principal Investigator at each site to obtain written informed consent for each patient either from the patient or the patient's parents/legal guardian before performing any treatment related procedures.

The study patients and their parents/legal guardian/s were given full and adequate verbal, as well as written, information regarding the objectives and procedures of the study, as well as possible risks involved in study participation. For children this information was provided in a style and language suitable for the age of the patient. The patient information and informed consent document was written in a non-technical and easily understandable manner. These documents were supplied to potential patients prior to inclusion into the study. Duplicates of the written information, as well as the signed consent form, were given to the patients and filed in their medical record.

Patients were informed that participation in the study was voluntary, that they had the right to withdraw at any point and that withdrawal from the study would not affect subsequent medical treatment or their relationship with the treating investigator. Throughout the trial the patient and parent or legal guardian had the opportunity to ask questions about the trial and any new information that may have been relevant to the patient's continued participation was shared with them in a timely manner.

The investigator ascertained that no patient was subjected to any study-related procedures before the informed consent was signed and dated.

Each patient authorized in writing that his/her source records and source data related to the study may be reviewed by a monitor, an auditor or a regulatory inspector, in accordance with applicable regulatory requirements.

Data protection was handled in accordance with the national laws of the countries in which the study took place.

Appendix 16.1.3 contains a copy of a sample informed consent form.

6. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Four sites are participating in this study. The coordinating Principal Investigator is Prof. Peter Lang, MD, PhD, University Children's Hospital Tuebingen, Germany. The study coordinator is Tim Flaadt, MD, University Children's Hospital, Tuebingen.

Initial monitoring and data management was performed by the Center for Pediatric Clinical Studies (CPCS), University Children's Hospital Tuebingen, Germany.

The consulting Biometrician is Ulrike Pötschger, Children's Cancer Research Institute, St. Anna Children's Hospital, Vienna, Austria.

The Laboratory used for FACS (fluorescence activated cell sorting) and cytokines was located in the University Children's Hospital Tuebingen, Germany.

Bone marrow samples were analyzed at Labdia Labordiagnostik GmbH (Vienna, Austria) and the hematologic-oncologic reference laboratory of the German Neuroblastoma Study Group (University Children's Hospital Cologne, Cologne, Germany.

The safety board consists of Rupert Handgretinger, MD, PhD, University Children's Hospital Tuebingen, Germany; Ruth Ladenstein, MD, PhD, MBA and Ulrike Pötschger (for statistical issues) at St. Anna Children's Hospital, Vienna, Austria; Isaac Yaniv, MD, Schneider Children's Medical Center of Israel; Cathérine Paillard, MD, Centre Hospitalier Universitaire, France; Jacek Toporski, MD, PhD, Lund University Hospital Sweden; and Holger Lode, MD, University Hospital Greifswald, Germany. The purpose of this safety board is to protect patients' safety. The safety board is informed immediately about all serious adverse effects, life threatening events or fatalities of a patient.

Appendix 16.1.4 contains a list of investigators and their affiliations as well as other important staff who materially affect the conduct of the study. The curricula vitae of the principal investigators are attached.

7. INTRODUCTION

7.1 BACKGROUND

In childhood, the most common GD2-ganglioside expressing tumor is neuroblastoma. This disease remains one of the major challenges in pediatric oncology. Most patients with neuroblastoma are young (median age at diagnosis of 18 months) and commonly present with metastatic disease. Approximately 60% of patients have high-risk tumors that are likely to be incurable. Standard treatment uses multi-modal therapeutic approaches comprising chemotherapy, surgical de-bulking or excision of the primary tumor, radiotherapy, differentiating agents such as 13-cis-retinoic acid and autologous bone marrow transplantation [1]. Such therapy drastically reduces the tumor load during induction and consolidation, and can result in apparent complete remission of disease. However, after a variable period of remission, most patients with high-risk tumors progress from this status of minimal residual disease to relapse with metastatic foci resistant to multiple drugs. To date, no curative option exists for patients with relapsed neuroblastoma after previous autologous stem cell transplantation. Further chemotherapy or second autologous transplantation results in a median survival of about 3 years.

In the development of novel immune therapies for high-risk cancers including neuroblastoma, one goal is to find tumor targets that are not widely shared by normal cells. One such target is the surface protein GD2. Several very high-risk tumors express the GD2 protein, making it an attractive target for relatively tumor-specific therapies such as antibody therapy. Therapies using various anti-GD2-antibodies have been assessed in Phase I, Phase II and Phase III trials, and their safety profile has been established.

The first version of monoclonal antibody 14.18 directed against disialoganglioside GD2 of murine immunoglobulin G (IgG) 3 isotype was developed in the 1980s [15]. As murine IgG3 isotypes are difficult to handle, a murine IgG2a class switch variant of 14.18, called 14.G2a, was prepared.

Murine antibody 14.G2a was tested in Phase I clinical trials and showed anti-tumor responses. Because anti-tumor activity was demonstrated, a human/murine chimeric monoclonal antibody (mAb) ch14.18 [16] was generated using the murine variable genes of 14.18 and the human constant IgG1 and κ genes, known to effectively mediate antibody dependent cellular cytotoxicity (ADCC) and to maintain complement dependent cytotoxicity (CDC). The antibody was produced in SP2/0 non-secreting murine hybridoma cells (ch14.18/SP2/0) and subjected to preclinical evaluation. It has been shown that ch14.18/SP2/0 antibody induces killing of neuroectodermal tumor cells in vitro mediated by ADCC and CDC.

The antibody producing cell lines originally used to generate recombinant ch14.18 antibodies were SP2/0 and NS0 cell lines, which are both non-secreting murine myeloma cells commonly used for antibody production. However, as these murine cell lines carry murine xenotropic retrovirus in contrast to cells of hamster origin [2], the production cell line was switched to Chinese hamster ovary (CHO) cells. Identical binding of ch14.18/CHO to the nominal antigen disialoganglioside GD2 in vitro compared to ch14.18/SP2/0 and

ch14.18/NS0 has been demonstrated. The functional properties of ch14.18/CHO were determined in CDC and ADCC reactions against GD2 positive neuroectodermal tumor cell lines in vitro. There was no difference in CDC mediated specific tumor cell lysis among the three different ch14.18 antibody preparations. The efficacy of ch14.18/CHO was evaluated in the NXS2 neuroblastoma model in vivo. Importantly, the ch14.18/CHO preparation was effective in suppression of experimental liver metastasis in this model. In vivo depletion of natural killer (NK) cells completely abrogated this effect, suggesting that the mechanism involved in the ch14.18/CHO induced anti-neuroblastoma effect is mediated by NK-dependent ADCC. Safety and tolerability of ch14.18/CHO was determined in a bridging study in patients with recurrent neuroblastoma. No Common Toxicity Criteria (CTC) grade 4 toxicity was observed, grade 3 CTC observed only for fever in two instances and once for nausea. PK data revealed no significant difference between ch14.18 produced in SP2/0 or CHO cells. Thus, it was concluded the use of this antibody is safe and well tolerated and its toxicity profile similar to previous antibody preparations.

The application of interleukin-2 (IL-2) by subcutaneous (s.c.) injection was determined in a multi-center, observer-blind, randomized phase II parallel study comparing three doses of s.c. recombinant IL-2 (rIL-2) in stage 4 neuroblastoma. In this study, a safe dose of s.c. rIL-2 was established that induced a sustained increase of NK cells in an outpatient setting for stage 4 neuroblastoma patients. Dose levels of 3, 6 and $9x10^6$ IU rIL-2/m² were given s.c. in six 5-day cycles every 2 weeks. Repeated increase of NK cells was achieved in 93% of cycles with >100% over baseline and/or >1G/L in 58%. At dose level 6 $x10^6$ IU rIL-2/m², the median increase of absolute NK cells was 1.38 G/l in 93% of all 87 cycles, corresponding to a median relative NK cell increase over baseline of 721%. Fever was frequent, but controllable with adequate supportive care. Hospitalization was as low as 6.5% for all patients. Local pain was moderate and acceptable.

Furthermore, IL-2 has been already used in pediatric patients after allogeneic stem cell transplantation (SCT) with doses between 6x and 24x 10^6 IU/m²/d [3, 4].

In a recent study, IL-2 was administered s.c. at a lower dose of $1x10^6$ IU/m²/d for 14 days after both HLA matched and HLA mismatched (haploidentical) family donors without major side effects. Observed side reactions were: fever, local inflammation, rash and transient limited chronic Graft-versus-Host Disease (GvHD) [5].

7.2 STUDY RATIONALE

Rationale for studying the ch14.18 mAb after haploidentical stem cell transplantation

Haplo-identical SCT is now a well-established method in patients with leukemia and several non-malignant diseases that lack an identical donor [6, 7]. Alloreactive effects have been in particular observed in adult patients with myeloid leukemia [8], which improve the event free survival. Furthermore, in pediatric lymphatic leukemia, alloreactive effects could be also demonstrated in vitro.

An investigator initiated study with haploidentical SCT with CD3/CD19 depleted grafts in children with solid tumors was evaluated in the University Children's Hospital Tuebingen. Those stem cell grafts included high numbers of donor derived NK cells, which are able to kill several tumors in a non-major histocompatibility complex (MHC) restricted manner. Anti-tumor activity exerted by donor derived NK cells was demonstrated in these patients post-transplant. Furthermore, a clear increase of the cytotoxic activity was observed by using appropriate antibodies (ADCC) against antigens expressed on the target cells. This was true for chimeric anti CD19 mAb and B-lineage acute lymphoblastic leukemia blasts [9] and also for chimeric or humanized ch14.18 mAbs and neuroblastoma cell lines [10].

Thus, the donor derived immune system post-transplant may be a basis for further immunotherapeutic approaches. The use of ADCC in particular has been shown to exert anti-tumor effects after haploidentical transplantation in vitro and this study evaluated the feasibility of antibody application also *in vivo*.

8. STUDY OBJECTIVES

8.1 PRIMARY OBJECTIVES

The primary objective of this study was to determine the safety and feasibility of the chimeric 14.18 anti-GD2 monoclonal antibody produced in CHO cells (ch14.18/CHO) in combination with subcutaneous aldesleukin (IL-2) after haploidentical stem cell transplantation in pediatric patients with relapsed neuroblastoma.

8.2 SECONDARY OBJECTIVES

The secondary objectives of this study were to:

- Evaluate the anti-tumor responses that resulted from this immunotherapy regimen through clinical assessments (radiographic and clinical measurements, including bone marrow immunohistochemistry for those research participants with marrow involvement)
- Evaluate pharmacokinetics of the ch14.18/CHO
- Evaluate changes in NK cell activation and proliferation (immunological monitoring)

9. METHODS AND INVESTIGATIONAL PLAN

9.1 OVERALL STUDY DESIGN AND PLAN

This ongoing open label, phase II study was designed to evaluate the safety, clinical toxicity, antitumor activity and *in vivo* immunological effects of ch14.18/CHO in combination with s.c. IL-2 after haploidentical stem cell transplantation in patients with relapsed neuroblastoma

This study comprised 2 parts. In the first part up to 9 patients were to be enrolled and, if an acceptable safety profile was shown in these patients, a second, confirmatory part, including another 26 patients, would be added. The results showed an acceptable safety profile in the first 9 recruited patients; therefore, the additional 26 patients were enrolled. This confirmatory part is also being extended with another 25 patients, as described in Section 9.8. In this interim analysis the first 35 patients enrolled in the study were evaluated. These initial 35 patients have completed the study treatment period, 11 patients died, but the follow-up of the other 24 patients is still ongoing.

The treatment period consisted of up to 6 consecutive cycles of 4 weeks each. During each cycle an 8-hour infusion of 20 mg/m^2 ch14.18/CHO was administered on 5 consecutive days (Days 1 to 5).

Interleukin-2 was added to Cycles 4-6 on Days 6, 8 and 10 (1 x 10^6 IU/m^2/d s.c.).

Following the initial cycle, patients were not eligible for subsequent cycles if toxicities had not recovered to <Grade 2 within 2 weeks or to <Grade 1 after 4 weeks and/or if progression occurred after the 3rd cycle.

Patients with stable disease and no requirement for treatment of disease related symptoms or with regression after the 3rd cycle were eligible to receive the 4th, 5th and 6th cycle of therapy.

Patients with stabilization or regression of disease after the 6th cycle of treatment were eligible to receive 3 additional cycles of treatment, with similar treatment regimen as for Cycles 4-6.

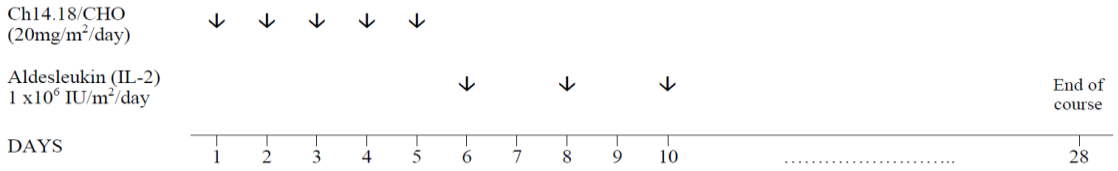
The overall study design is presented in Table 9-1 and Table 9-2

Table 9-1: Study Design, Cycles 1-3[a]

Infusion day	Participant study activity
	
Day 1	ch14.18/CHO infusion 1 of 5 (20 mg/m²) over 8 hours
Day 2	ch14.18/CHO infusion 2 of 5 (20 mg/m²) over 8 hours
Day 3	ch14.18/CHO infusion 3 of 5 (20 mg/m²) over 8 hours
Day 4	ch14.18/CHO infusion 4 of 5 (20 mg/m²) over 8 hours
Day 5	ch14.18/CHO infusion 5 of 5 (20 mg/m²) over 8 hours

[a] Administration of all 5 doses of the study agent is referred to as a cycle.

Table 9-2 Study Design, Cycles 4-6[a]

Infusion day	Participant study activity
	
Day 1	ch14.18/CHO infusion 1 of 5 (20 mg/m²) over 8 hours
Day 2	ch14.18/CHO infusion 2 of 5 (20 mg/m²) over 8 hours
Day 3	ch14.18/CHO infusion 3 of 5 (20 mg/m²) over 8 hours
Day 4	ch14.18/CHO infusion 4 of 5 (20 mg/m²) over 8 hours
Day 5	ch14.18/CHO infusion 5 of 5 (20 mg/m²) over 8 hours
Days 6, 8, 10	Subcutaneous injections of recombinant human IL-2 (1 x 10^6 IU /m² /d s.c.)

[a] Administration of all 5 doses of the study agent is referred to as a cycle.

Further information on the design of the study can be found in the clinical study protocol and its amendments. These documents are presented in Appendix 16.1.1, and a sample case report form (CRF) is provided in Appendix 16.1.2.

9.2 DISCUSSION OF STUDY DESIGN, INCLUDING THE CHOICE OF CONTROL GROUPS

This was a Phase II, open-label, uncontrolled, multi-center safety and exploratory efficacy study of ch14.18/CHO mAb plus IL-2 given in a single dose group not earlier than 60 days and maximally 1 year after haploidentical SCT to relapsed neuroblastoma patients Due to the exploratory nature of this trial, no active control treatment was administered in parallel to a comparator group.

Two-part design

The 2-part design of the study was used to ensure an acceptable safety profile, to be shown in the first 9 patients, before the confirmatory cohort was enrolled. The confirmatory cohort was performed to provide further evidence for the feasibility of the approach. This interim report includes the results for the first 35 patients. Please note that Version 1.3 of the protocol will allow a further 25 patients to be treated.

Number of cycles

Patients were to receive 3 cycles of treatment before being assessed for tumor response. If eligible, patients would then receive up to 3 further cycles of treatment. If there was evidence of response after 6 cycles, patients could receive another 3 cycles for their own benefit (these cycles were only evaluated for secondary objectives).

Rationale for administration schedule

A schedule of ch14.18/CHO administration was chosen that is similar to other studies using this antibody. The German Neuroblastoma Phase III Trials NB90 and NB97 included 334 evaluable patients, of whom 166 received a ch14.18 mAb. The mAb was administered over a period of one year at a schedule of 20 mg/m²/day for 5 days every 2 months (6 cycles total). The results of these studies showed that the overall survival was superior in the ch14.18 group, as compared to groups receiving maintenance chemotherapy or no further therapy. Based on this study, it was decided to administer ch14.18/CHO as a 6-cycle regimen consisting of an 8-hour infusion for five consecutive days every 4 weeks.

Ch14.8/CHO-mediated tumor cell destruction relies on ADCC to kill tumor cells and strong effector functions are required. Preclinical data indicated that ADCC can be more effective when appropriate effector cells are activated by cytokines. IL-2 is thus used to prime NK cells before administration of ch14.8/CHO to augment effector cell function and improve the overall antibody therapy efficacy. Similar treatment regimens have been proved beneficial in other clinical trials.

In the present study IL-2 was added as of Cycles 4 to 6 to minimize the risk of GvHD. For the same reason IL2 was given in lower doses than in other Société Internationale d'Oncologie Pédiatrique European Neuroblastoma (SIOPEN) studies.

Graft-versus-Host Disease

A potential risk of allogeneic SCT is GvHD. Immunotherapy could promote the induction of GvHD by increasing pro-inflammatory cytokines. To address this potential risk, patients with acute GvHD Grade 3 or 4 or extensive chronic GvHD were not eligible to enter the study. In addition, the occurrence of acute GvHD (≥ grade 3), or extensive chronic GvHD during antibody infusion, was defined within the protocol (Section 9.3.3.1, Appendix 16.1.1) as "unacceptable toxicity" which constituted a formal "stopping rule".

Patients were therefore carefully monitored for any occurrence of GvHD throughout the study (refer also to Section 9.5.1.2).

9.3 SELECTION OF STUDY POPULATION

The study population for this study included male and female patients aged less than or equal to 21 years, with relapsed neuroblastoma, who had previously received an allogeneic haploidentical SCT.

9.3.1 INCLUSION CRITERIA

Patients had to meet the following criteria to be included in the study:

Inclusion Criteria:

- Less than or equal to 21 years of age.
- Histologically confirmed neuroblastoma.
- Refractory to standard treatment (i.e. refractory disease) or relapse after previous autologous or allogeneic stem cell transplantation (SCT).
- Haplo-identical SCT prior to antibody infusion according to appendix IV of the study protocol (Appendix 16.1.1) at least 60 days prior to starting immunotherapy.
- Serum glutamate pyruvate transaminase (SGPT) less than 2.5 times the upper limit of normal for age and total bilirubin less than 2 times the upper limit of normal for age. D-dimers less than 2 times the upper limit of normal.
- Creatinine clearance or radioisotope glomerular filtration rate (GFR) greater than or equal to 40 mL/min/1.73 m^2.
- Cardiac shortening fraction greater than or equal to 20% by echocardiogram.
- Karnofsky/Lansky performance score (age appropriate) of greater than or equal to 50.
- Females of childbearing potential must have had a negative pregnancy test. Patients of childbearing potential must have agreed to use an effective birth control method. Female patients who were lactating must have agreed to stop breast-feeding.
- Written informed consent was obtained, and for minors a written agreement by parents or legal guardian.
- All institutional and national requirements for human studies were met.

9.3.2 EXCLUSION CRITERIA

Patients who had any of the following criteria were to be excluded from the study:

- Marked baseline prolongation of QT/QTc interval (e.g. demonstration of a QTc interval >450 milliseconds).
- Symptoms of congestive heart failure or uncontrolled cardiac rhythm disturbance.
- Significant psychiatric disabilities or uncontrolled seizure disorders.
- Active infections or active peptic ulcer, unless these conditions were corrected or controlled.

- Acute GvHD Grade 3 or 4 or extensive chronic GvHD.
- Clinically significant, symptomatic, pleural effusions.
- Major surgery, (i.e. laparotomy or thoracotomy) within two weeks of screening.
- More than 12 months post haploidentical SCT at the time of starting the first cycle of immunotherapy.
- Prior administration of ch14.18 antibody after allogeneic SCT
- Human immunodeficiency virus (HIV) or hepatitis B surface (HB_S) Ag positivity. The presence of either may influence the ability of the immune system to be stimulated by this treatment.

9.3.3 REMOVAL OF PATIENTS FROM THERAPY OR ASSESSMENT

9.3.3.1 Criteria for discontinuation

Patients who had progression of disease after the third cycle did not receive subsequent cycles of therapy.

Patients with unacceptable toxicity despite dose reduction were taken off protocol therapy.

Definitions of unacceptable toxicity and stopping rules were as follows:

Unacceptable toxicities were life threatening adverse reactions defined as Grade 3 or 4 toxicity using the National Cancer Institute (NCI) CTC version 4.0 which were in causal relationship with the study treatment.

There was the possibility that infusion of the ch14.18/CHO mAb could induce GvHD by increasing inflammatory cytokines. Occurrence of acute GvHD (≥grade 3) or extensive

chronic GvHD during antibody infusion was therefore also defined as unacceptable toxicity.

The following exceptions were not considered unacceptable toxicity. These exceptions are based on the known, transient, reversible, non-dose limiting toxicities of ch14.18/SP2/0:

a) Grade 3 nausea and vomiting;

b) Grade 3 fever;

c) Grade 3 skin toxicity that improved with treatment, e.g. intravenous (i.v.) diphenhydramine (Benadryl), within 24 hours.

d) Grade 3 electrolytes, especially hyponatremia ≤124 mEq/L in the absence of central nervous system (CNS) symptoms and sequelae which improved with treatment within 24 hours.

e) Grade 3 hypotension and hypertension;

f) Grade 3 hepatic toxicity which was present for <3 days or returned to Grade 1 or less prior to the time for next ch14.18/CHO dose;

g) Grade 3 neurotoxicity, i.e. interference with function plus objective weakness, if transient and reversed within three days of stopping ch14.18/CHO. Subjective findings, e.g. tingling, hot or cold hands, etc., were expected and were not unacceptable toxicity.

h) Grade 4 hematologic toxicity which improved to at least Grade 2 or baseline pre-therapy values within one week following ch14.18/CHO treatment;

i) Grade 3 and 4 allergic reactions, i.e. anaphylaxis, readily controlled with supportive measures;

j) Grade 3 performance (Lansky/Karnofsky Score 30 - <50%, see Appendix I of the protocol [Appendix 16.1.1]); or

k) Grade 3 capillary leak syndrome that did not persist for more than one week

l) All grade 1 and 2 toxicities (incorrectly stated as grade 1 and 3 in the study protocol)

Any patient who demonstrated unacceptable toxicity had treatment with ch14.18/CHO stopped. If toxicity resolved, treatment could be resumed for that patient at 50% ch14.18/CHO dose (see criteria in Section 9.5.1.2). If toxicity did not resolve, the patient was taken off study. If GvHD ≥grade 1 occurred, Interleukin 2 administration was stopped and ch14.18/CHO was administered as a single drug. If GvHD ≥grade 3 occurred,

ch14.18/CHO infusion was also stopped. If all toxicities recovered to ≤grade 1 within 4 weeks (including GvHD), IL-2 and ch14.18/CHO could be given in the next cycle.

Patients with inter-current illness that, in the judgment of the investigator, affected assessments of clinical status to a significant degree, or required initiation of treatment with excluded medications or therapies, were removed from protocol therapy.

Patients who went off protocol therapy were followed until they met the criteria for off study.

Off Study Criteria

1. Death
2. Lost to follow-up
3. Entry onto another therapeutic study.

The Sponsor had the right to terminate this study, either at an individual site, or in its entirety, at any time for the following reasons:

1. If unexpected life threatening (grade 4) side effects or severe acute GvHD (Grade 3-4) occurred in ≥5 out of 9 patients, despite dose reductions.
2. Lack of efficacy

9.3.3.2 Voluntary discontinuation by a patient

Patients who requested withdrawal were removed from the study.

9.3.3.3 Replacement

Discontinued patients were not replaced.

9.4 STUDY TREATMENT

9.4.1 TREATMENTS ADMINISTERED

The study medication ch14.18/CHO was administered as an 8 hour i.v. infusion from Day 1 to Day 5 of each 4 week cycle. The ch14.18/CHO was given on an inpatient basis so that recipients could be carefully monitored for any adverse reactions. Research participants were pre-medicated within approximately 30 minutes prior to and during the infusion of the study medication with an i.v. antihistamine (diphenhydramine) and with morphine sulfate.

Recombinant human Interleukin-2 (IL-2; aldesleukin) was given by s.c. injection on Days 6, 8 and 10, starting from Cycle 4 and extending to Cycle 9.

9.4.2 IDENTITY OF INVESTIGATIONAL MEDICINAL PRODUCTS

9.4.2.1 Packaging, labelling and storage

Ch14.18/CHO and IL-2 are considered investigational medicinal products.

ch14.18/CHO

Product name:	Mouse-human chimeric monoclonal anti-GD2 IgG1 antibody (ch14.18/CHO)
Dosage form:	Concentrate of solution for infusion (reconstituted in 0.9% sodium chloride solution, 1% human albumin)
Route of Administration:	intravenous infusion
Total daily dose:	20 mg/m² (total amount per cycle: 100 mg/m²)
Frequency:	8-hour i.v.. infusion, at a dose of 20 mg/m²/day over 5 days, every 4 weeks over 5 cycles
Active ingredient:	ch14.18/CHO
Inactive ingredient:	10 mM sodium phosphate, 150 mM sodium chloride, 20 mM histidine, 5% sucrose, 0.01% Tween 20, water for injection
Batch no.:	Lot T651204-A, T900310-A, T1101012-A
Packaging:	Single-use glass vials closed by a rubber stopper
Strength:	4.5 ± 0.25 mg/mL
Content:	4.5 ± 0.25 mL

For more details of dose preparation and specification please refer to Section 10.1.4 of the protocol (Appendix 16.1.1).

Ch14.18/CHO is produced and released according to current European GMP regulations. Ch14.18/CHO was stored at 2-8°C protected from direct sunlight.

Aldesleukin (IL-2)

Product name:	Aldesleukin (IL-2)
Dosage form:	White-to-off white lyophilized cake to be reconstituted with sterile water
Route of Administration:	Subcutaneous injection
Total daily dose:	$1x10^6$ IU/m^2
Frequency:	Once daily in Cycles 4-9 at Days 6, 8, 10
Active ingredient:	Interleukin-2
Inactive ingredient:	Mannitol, sodium dodecyl sulfate, sodium phosphates

Packaging:	Supplied in 5 mL rubber stoppered single-use clear glass vials containing 22 x 10^6 IU IL-2
Batch no.:	Commercially available IL-2 is used
Storage:	Refrigerated at 2-8°C

9.4.3 METHOD OF ASSIGNING PATIENTS TO TREATMENT GROUPS

This was an open-label study with only one treatment arm.

9.4.4 SELECTION OF DOSES USED IN THE STUDY

The dose of ch14.18/CHO used in this study was 20 mg/m²/day, and the dose of IL-2 was 1 x 10^6 IU/m²/day.

Dose reduction to 50% could be used when necessary due to unacceptable toxicity in a previous treatment cycle. This reduced dosage was then used in any further treatment cycles. (For further information on dose reductions in managing toxicity please see Section 9.5.1.2 of this report.)

Rationale for dose selection

The primary concern in this study was patient safety. The ch14.18 antibody has been used at doses as high as 50 mg/m^2/day. However, in this study a dose of 20 mg/m^2/day was used. This dose is less than 1/2 of the maximum tolerated dose (MTD) of any clinical trial with this investigational medicinal product (IMP) and was also already used as a standard dose in a SIOPEN phase I study with ch14.18/CHO.

The rationale for the dose of IL-2 used in the study was based on a recent study that administered IL-2 s.c. at a dose of 1x10^6 IU/m²/d for 14 days after both HLA matched and HLA mismatched (haploidentical) family donors without major side effects [5].

9.4.5 SELECTION AND TIMING OF DOSE FOR EACH PATIENT

Infusion of ch14.18/CHO started no earlier than 60 days after haploidentical SCT. The treatment was started within the first 6 months but not later than 1 year post-transplant.

Patients were admitted to the hospital for the antibody infusion. Ch14.18/CHO was given as an 8 hour i.v. infusion on Days 1, 2, 3, 4 and 5. Each treatment cycle lasted 5 days and was administered every 4 weeks for up to 6 treatment cycles.

Interleukin 2 was added to Cycles 4-6 on Days 6, 8 and 10 (1 x 10^6 IU/m²/day, s.c.)

Patients were able to receive another 3 cycles of ch14.18/CHO, if there was evidence of response after 6 cycles. Patients were eligible to receive additional IL-2, if no severe acute GvHD (Grade 3-4) or extensive chronic GvHD was present.

9.4.6 BLINDING

Not applicable, this was an open label study.

9.4.7 PRIOR AND CONCOMITANT MEDICATION

Concomitant Medication

Management of symptoms due to the antibody infusion:

Pruritus or urticaria were treated with diphenhydramine or dimetindene as clinically indicated.

Anaphylactic precautions were undertaken. Dexamethasone and epinephrine were available, along with equipment for assisted ventilation. A free-flowing intravenous line was established at all times.

Participants were pre-medicated with an intravenous antihistamine (diphen-hydramine 1 mg/kg i.v. or dimetindene 0.1mg/kg i.v.) and ranitidine (1 mg /kg i.v.) within approximately 30 minutes prior and during the infusion of the study agent (3 doses/day and 2 doses/day, respectively).

Pain as an anticipated side effect

This was managed by a standard pain prophylaxis as follows:

Morphine hydrochloride

0.1 – 0.5 mg/kg bolus (just prior to the start of infusion of the antibody)

0.5-1.0 mg/kg/day continuous infusion.

Dosing could be adapted for complete analgesic activity. Previous experience has revealed that the morphine hydrochloride dose to achieve complete analgesia was lower when started on the second day of each cycle. The morphine dose could be halved during the night. After the fifth antibody infusion, morphine was tapered within 24 hours (halving the dose every 6 hours).

If pain could not be managed, pain therapy was escalated with:

- Metamizol 10 mg/kg (3 doses/day), up to 15 mg/dose.
- Paracetamol 10-15 mg/kg in addition, when necessary.

Hypotension

Hypotension treatment guidelines (Grade 3 or 4 not responsive to fluid challenge) included the following as clinically indicated: stopping the infusion, supporting blood pressure with intravenous fluids (and vasoactive drugs pressors/inotropes if necessary).

Prohibited medications

Immunoglobulins for substitution were not permitted to be used directly associated to antibody cycles since ADCC may be blocked.

9.4.8 TREATMENT COMPLIANCE

Treatment compliance measurements were not applicable; all treatments were given in a hospital setting.

9.5 STUDY MEASUREMENTS AND VARIABLES

9.5.1 EFFICACY AND SAFETY MEASUREMENTS ASSESSED AND FLOW-CHART

Table 9-3 below details the schedule of treatment administration and clinical assessments performed for each patient:

Table 9-3 Plan of Investigational Events: Clinical Assessment for Each Treatment Cycle

Activity	Pre-Therapy / Screening	Days 1-10									
		1	2	3	4	5	6	7	8	9	10
Informed consent	X										
Complete History	X										
Physical Exam	X	X	X	X	X	X	X				X
Body Weight	X	X	X	X	X	X	X				X
Vital signs (T, BP, HR, RR) [1]	X	X	X	X	X	X	X				
Level of pain (per appropriate pain scale)[1]		X	X	X	X	X					
Performance Status	X	X	X	X	X	X	X				X
Ch14.18/CHO 8h i.v. application (Cycles 1 to 6)		X	X	X	X	X					
rhIL-2 application s.c. (1×10^6 IU / m^2)- 2 hours after ch14.18/CHO infusion stop (ONLY cycles 4 to 6)							X		X		X
CBC, Differential	X	X	X	X	X	X	X				X
Blood Chemistry[2]	X	X	X	X	X	X	X				X
PT/PTT	X	X	X	X	X	X	X				X
Urinalysis	X										
Tumor measurement (disease evaluation)[3]	X										
HIV Test/HB_sAg	X										
Bone Marrow Aspirate[4]	X										

ALT = alanine aminotransferase, AST = aspartate aminotransaminase, BP = blood pressure, CBC = complete blood cell count, Cl = chloride, CO_2 = carbon dioxide, CT = computed tomography, HB_sAg = hepatitis B surface antigen, HIV = human immunodeficiency virus (1, 2), HR = heart rate, K = potassium, mIBG = metaiodobenzylguanidine, MRI = magnetic resonance imaging, Na = sodium, PT = prothrombin time, PTT = activated partial thromboplastin time, RR = respiratory rate, T = temperature.

1 Every 4 hours on Day 1, then every 8 hours on Days 2-5

2 Chemical survey to include: Na, K, Cl, CO_2, Urea, Creatinine, Bilirubin, ALT, AST, Albumin

3 Patients disease status was evaluated following the 3rd, 6th and, if applicable, the 9th cycle by mIBG, MRI/CT and tumour markers

4 Bone Marrow Aspirates are required after 3rd, 6th and, if applicable, the 9th cycle for patients with known prior marrow involvement

For screening, all scans including ultrasounds, CT scans, MRIs and X-rays were to be performed within two weeks prior to enrolment. CBC with platelets, human anti-chimeric antibodies and chemistries were to be done ≤2 weeks before study registration and meet eligibility criteria.

9.5.1.1 Efficacy Variables

9.5.1.1.1 Tumor measurement (disease evaluation)

Tumor response was evaluated after the 3rd and 6th courses, after the 9th course if applicable, and after one year. Evaluations included mIBG scan and MRI/CT scan as well as catecholamine excretion in urine and serum neuron-specific enolase (NSE) levels.

Definitions used for Assessment of Disease Status:

Measurable Disease

Bi-dimensionally measurable lesions with clearly defined margins by: 1) plain X-ray, with at least one diameter 0.5 cm or greater (bone lesions not included); or 2) CT, MRI or other imaging scan, with both diameters greater than the distance between cuts of the imaging study.

Objective Status

This was recorded at each evaluation. If an organ had too many measurable lesions at each evaluation, three were chosen to be followed before the patient entered the study. The remaining measurable lesions in that organ were considered evaluable for the purpose of objective status determination. Unless progression was observed, objective status could only be determined when all measurable and evaluable sites and lesions were assessed.

Non-Measurable Disease Evaluated for Response Assessment

Uni-dimensionally measurable lesions, masses with margins not clearly defined, lesions with both diameters less than 0.5 cm, lesions on scan with either diameter smaller than the distance between cuts, palpable lesions with either diameter less than 2 cm, bone disease.

Complete Response (CR)

Complete disappearance of all measurable and evaluable disease. No new lesions. No disease-related symptoms. No evidence of evaluable disease, including normalization of markers and other abnormal lab values. All measurable, evaluable, and non-evaluable lesions and sites were assessed using the same technique as baseline.

Partial Response (PR)

Applied only to patients with at least one measurable lesion. Greater than or equal to 50% decrease under baseline in the sum of products of perpendicular diameters of all measurable lesions. No progression of evaluable disease. No new lesions. All measurable and evaluable lesions and sites were assessed using the same techniques as baseline.

Stable/No Response

Did not qualify for CR, PR, or progression. All measurable and evaluable sites were assessed using the same techniques as baseline.

Progressive Disease (PD)

Defined as at least one of the following - Twenty-five percent (25%) increase or an increase of 10 cm^2, whichever is smaller, in the sum of the products of the perpendicular diameters of all measurable lesions over smallest sum observed (over baseline if no decrease) using the same techniques as baseline; clear worsening of any evaluable disease; reappearance of any lesion that had disappeared; appearance of any new lesion/site; failure to return for evaluation due to death; or deteriorating condition unless clearly unrelated to this cancer. For “scan-only” bone disease, increased uptake did not constitute clear worsening. Worsening of existing non evaluable disease did not constitute progression.

Exception: Lesions that appeared to increase in size due to presence of necrotic tissue were not considered to have progressed.

Unknown

Progression had not been documented and one or more measurable or evaluable sites had not been assessed.

Non evaluable disease did not affect objective status except in determination of CR (all disease must be absent - a patient who otherwise had a CR, but who had non-evaluable disease present or not assessed, was classified as having a PR) and in determination of progressive disease (if new sites of non-evaluable disease develop). Patients with only non-evaluable disease could not be assessed for response.

Best Response

Note that the "Best Response" analysis has not been performed as described in the protocol for this interim analysis. Instead the best response observed at any time point for each individual patient. It was considered as the patient’s best overall response on this study. To evaluate response, the following methods were used: mIBG scintigraphy, bone marrow, Ultrasound, CT/MRT.

9.5.1.2 Safety Variables

9.5.1.2.1 Adverse Events

Patients were monitored closely for adverse events (AE) from the time of informed consent signing to 30 days after the last administration of the study medication. Grading of toxicities on this study was performed using the NCI common toxicity criteria version 4.0.

Only suspected unexpected serious adverse reactions (SUSAR) required expedited reporting (defined as within 24 hours of knowledge of the event). Expected SAEs, i.e. those consistent with the profile of expected toxicities in this trial (see below), unless those were, in the opinion of the local investigator, unexpectedly severe, did not need expedited reporting.

Expected SAEs arising from study medication treatment were:

a) Grade 3 nausea and vomiting;

b) Grade 3 fever;

c) Grade 3 skin toxicity that improved with treatment, e.g. i.v. Benadryl, within 24 hours.

d) Grade 3 electrolytes, especially hyponatraemia ≤124 mEq/L in the absence of CNS symptoms and sequelae which improved with treatment within 24 hours.

e) Grade 3 hypotension and hypertension;

f) Grade 3 hepatic toxicity which was present for <3 days or returned to Grade 1 or less prior to the time for next ch14.18/CHO dose;

g) Grade 3 neurotoxicity, i.e. interference with function plus objective weakness, was not considered a dose-limiting toxicity (DLT) if transient and reversing within three days of stopping ch14.18/CHO. Subjective findings, e.g. tingling, hot or cold hands, etc., are expected and will not be DLT.

h) Grade 4 hematologic toxicity which improves to at least Grade 2 or baseline pre-therapy values within one week following ch14.18/CHO treatment;

i) Grade 3 and 4 allergic reactions, i.e. anaphylaxis, readily controlled with supportive measures;

j) Grade 3 performance (Lansky Score 30 - <50%, see Section 16 Appendix 1 of the Study Protocol [Appendix 16.1.1]; or

k) Grade 3 capillary leak syndrome that does not persist for more than one week

The following events were also exempted from expedited reporting: relapse, signs and symptoms of disease progression, death from disease progression and secondary malignancy.

All AEs that were exempted from expedited reporting were still reported in the appropriate toxicity forms in the CRF.

The local investigator was required to report all SAEs requiring expedited reporting within 24 hours of knowledge of the event. The sponsor was responsible for reporting the relevant SAEs to the regulatory authorities and IECs and Reports were sent to Peter Lang, MD, PhD at University Children´s Hospital Tuebingen, Germany.

The following data should have been reported:

- Institution Patient identification number
- Presenting diagnosis
- Laboratory data supporting event
- Total dose(s) of suspected drug
- Time to event. Type and grade of adverse event

- Methods used to recognize and characterize the effect

9.5.1.2.2 Grading of Toxicities and Dose Modification

Grading of toxicities on this study was performed according to NCI common toxicity criteria version 4.0.

Toxicities that were monitored closely included the constitutional symptoms associated with IL-2 treatment and the neuropathic symptoms occasionally associated with ch14.18/CHO treatment.

Moreover, occurrence of GvHD and hematological toxicity (neutropenia, anemia, thrombocytopenia) were also closely monitored.

Constitutional symptoms, hypotension, fevers

If Grade 4 constitutional symptoms, hypotension, or fevers occurred while the patient was receiving ch14.18/CHO and resolved to ≤1 Grade above baseline, administration of ch14.18/CHO could be resumed at 50% of the dose for all subsequent courses.

ch14.18 associated Neuropathy

Occasional reports of peripheral neuropathy and hyponatraemia have been reported with the ch14.18 mAb. These were closely monitored and patients who experienced any objective prolonged weakness attributable to ch14.18/CHO administration or patients with either symptomatic hyponatraemia, persistent (>48 hrs) sodium less than 125 nmol/L, or severe hyponatraemia without symptoms, sodium less than 120 nmol/L, were taken off protocol therapy and received no further ch14.18/CHO, and the sponsor was notified by phone.

9.5.1.2.3 Management of Toxicity

Hematologic Toxicity

All patients were transfused as needed to maintain an adequate hemoglobin level and platelet count. If the patient experienced neutropenia (<500 neutrophils /µl) while receiving the ch14.18/CHO mAb and IL-2, treatment would not be interrupted. Use of granulocyte-colony stimulating factor (G-CSF) was recommended to treat neutropenia. However, if the neutropenia had not resolved by the start of the next course of treatment, then the next course was delayed for up to two weeks until the neutropenia resolved and the dose of ch14.18/CHO mAb for future courses was reduced to 50%.

If the patient experienced thrombocytopenia (<20 000/µl), IL-2 treatment was interrupted until platelet substitution was not necessary any more.

Hepatic Toxicity, including Veno-occlusive Disease (VOD)

Alkaline phosphatase: No dose interruption or dose modifications were to be made for elevated alkaline phosphatase, as this alone is not a good indicator of hepatic toxicity and occurs commonly. All other hepatic toxicities, except bilirubin, were to be handled as indicated below.

Bilirubin: If bilirubin increased to >3 times normal, the ch14.18/CHO infusion was delayed until the bilirubin returned to normal. Then the ch14.18/CHO infusion was re-started at 50 % dose reduction.

In case of bilirubin elevations, gain of weight and severe thrombocytopenia, a VOD was to be excluded by ultrasound. Treatment was stopped if this diagnosis was confirmed. Patients with severe VOD were to be taken off protocol therapy.

Cardiac Toxicity

Any evidence of cardiac abnormalities required an immediate electrocardiogram (ECG) evaluation. Evidence of ischemia required immediate discontinuation of therapy. Patients with evidence of asymptomatic atrial irregularities, related to an elevated temperature, but no evidence of ischemia or clinically significant hypotension, were monitored but continued therapy. Patients experiencing Grade 3 or worse cardiac toxicity were to be taken off protocol therapy.

Complications of fluid overload may have been seen. Patients with clinical problems related to fluid overload would be treated with furosemide provided they had <40 mm Hg decrease in systolic blood pressure from the baseline systolic blood pressure.

Treatment should be stopped for a sustained decrease in blood pressure below 80 mm Hg systolic that is at least 20 mm Hg decreased from baseline systolic blood pressure and has not been restored with brief fluid or albumin challenge, i.e. 20 cc/kg of Normal Saline. Intravenous vasopressor was used when clinically indicated. Treatment was restarted at 50% of the dose of the ch14.18/CHO that caused the toxicity, after the blood pressure returned to 80 mm Hg systolic.

Dyspnea

Patients who experienced dyspnea and whose oxygen saturation was less than 90% may have received brief oxygen supplementation. If the oxygen saturation did not then improve to over 90% off of oxygen supplementation, treatment would have been discontinued and restarted at 50% dose reduction.

Neurotoxicity

Patients who experienced Grade 3 or worse neurotoxicity, except confusion would have been taken off protocol therapy.

Performance Status

Treatment would have been stopped for a performance status of 20. If performance status improved to ≥ 40, ch14.18/CHO treatment could be restarted at 50% dose reduction.

Temperature Elevations

Ch14.18/CHO and IL-2 treatment would be delayed for persistent temperature elevations (six hours) of 40°C or greater not responding to antipyretics. Temperature elevations >38°C

would be treated with metamizole. No dose modifications would be made for temperature elevations (unless the temperature persisted >40°C).

Confusion

Confusion which was clearly not temperature related or related to supportive care medicines, benadryl, morphine, etc., would have resulted in the interruption of treatment. Confusion related to temperature elevations would be managed by aggressive use of antipyretics and cooling blankets. Persistent confusion (>6 hours) of any cause required the discontinuation of therapy, with subsequent re-initiation of treatment at 50% dose reduction if reversal of this toxicity occurred prior to the next scheduled course.

Pain

Patients experiencing pain due to the ch14.18/CHO despite pain prophylaxis would be treated with additional morphine or similar analgesics, as needed, and have their pain graded according to the common toxicity criteria (CTC).

Nephrotoxicity

Adequate renal function was an eligibility requirement. If renal function worsened, but did not reach Grade 3, other nephrotoxic drugs, such as indomethacin, were to be avoided.

Coagulation disorders and endothelial damage

Treatment was stopped if subsequent increase of D-Dimers occurred together with elevation of lactate dehydrogenase (LDH). This might indicate endothelial damage and can result in VOD or Morbus Moscowitz. Treatment was continued with 50% dose reduction, if all signs of endothelial damage were resolved. Patients with two events would be taken off protocol therapy.

GvHD

If GvHD ≥ grade 1 occurred, Interleukin 2 administration was stopped and ch14.18/CHO was administered as a single drug. If GvHD ≥ grade 3 occurs, ch14.18/CHO infusion was also stopped.

If all toxicities recovered to ≤ Grade 1 within 4 weeks (including GvHD), IL-2 and ch14.18/CHO could be given in the next course.

9.5.2 PRIMARY ENDPOINT

The primary endpoint was "success of treatment", defined as a patient receiving the full protocol treatment, still alive 180 days after treatment, without disease progression, without unacceptable toxicity and without acute GvHD ≥ Grade 3 or extensive chronic GvHD.

9.5.3 DRUG CONCENTRATION MEASUREMENTS

To determine pharmacokinetics, serum samples (3 mL of blood) were collected but not analyzed yet.

9.5.4 PHARMACODYNAMIC MEASUREMENTS

Blood samples were analyzed for NK cell activation and cytokine levels (including soluble IL-2 receptor, IL-6 and TNF-alpha). These analyses are reported in a separate pharmacodynamics report (Appendix 16.1.13).

9.6 DATA QUALITY ASSURANCE

Participating sites were regularly monitored by staff from Center for Pediatric Clinical Studies (CPCS), Tuebingen, Germany. Audits were performed regularly.

Initial data management was performed by CPCS.

Radiological central reviews were performed.

9.7 STATISTICAL METHODS PLANNED IN THE PROTOCOL AND DETERMINATION OF SAMPLE SIZE

9.7.1 STATISTICAL AND ANALYTICAL PLANS

A full description of the statistical analyses that were performed for this Interim Report together with the planned tables and figures is given in the Statistical Analysis Plan (SAP), dated 12 March 2015, which was developed and filed with the Sponsor. Any deviation(s) from the final SAP are described and justified in this clinical study report. The SAP is attached in Appendix 16.1.9 of this report.

The Full Analysis Set (FAS) consisted of all patients for whom at least one treatment cycle was documented in the data. The Safety Analysis Set (SAF) was identical to the FAS.

All analyses were performed using SAS®. Data is presented in individual listings and summarized – if appropriate over time - according to data type:

- continuous data by mean, standard deviation, minimum, first quartile (Q1), median, third quartile (Q3), maximum.
- qualitative (nominal) data by absolute and/or relative frequencies.

Baseline was defined as the date of first haplo-transplantation.

Missing data was not replaced, and data from all centers will be analyzed together.

Baseline patient characteristics include a summary of the following:

- Patient demographics
- Baseline disease characteristics
- Prior therapies
- Concomitant medications

Other patient characteristics were summarized as deemed appropriate. A description of all donor related data was only provided in a listing.

Treatment duration was described by the number of initiated cycles, the number of cycles with full antibody treatment and the number of completed cycles. A course was considered as completed if the full dose of antibodies was administered and if, in courses 4 to 9, 3 doses of IL-2 were given. For the combined variable "Success of treatment", full protocol treatment is defined as 85 % of 600 mg/m² (= 510 mg/m²) or greater of ch14.18/CHO treatment in cycles 1 to 6. Exposure to study drugs will also be described by cumulative doses overall (for ch14.18/CHO in mg and in mg/m²) and by cycle as well as by the number of days study drugs were given.

9.7.1.1 Efficacy

The analysis of all efficacy variables was based on the FAS population. Tabular listings of efficacy outcome including response and disease status were provided.

The primary endpoint "**Success of treatment**" was defined as a patient receiving the full protocol treatment, still alive 180 days after treatment without progression and without unacceptable toxicity and acute GvHD (≥Grade 3) or extensive chronic GvHD. Thus, a composite variable was used as the primary endpoint. Treatment was defined as successful if a patient did not experience:

1. Unacceptable toxicities
2. Acute GvHD ≥ Grade 3 or extensive chronic GvHD
3. Other toxicities that did not recover to ≤Grade 1 within 4 weeks or
4. Progressive disease after 6 cycles or
5. Death within treatment after SCT
6. Withdrawal due to other reasons.

For the evaluation of treatment success, disease progression at the end of the 6th cycle was judged by the investigator at the end of the study. In two cases (01-12 and 01-17) patients were judged to have a treatment success although PD was noted at the end of the 6th cycle. Immunotherapy was continued for further 3 cycles (cycles 7 to 9) despite PD and tumor assessments showed a CR in both patients at the end of the 9th cycle. Exact 95% confidence intervals for the rate of patients with treatment success were given.

Overall response: Tabular listings over time of overall response were provided for all patients and in addition for the subgroups of patients with evidence of disease before haplo-transplantation and for patients with evidence of disease after haplo-transplantation. The analysis includes the summary for the last cycle of each patient and the analysis of best response.

Survival analysis: In order to measure the fraction of subjects living for a certain amount of time after treatment, survival analysis using Kaplan-Meier methods was used. Analysis was done for overall survival (OS) and event-free survival (EFS). EFS was calculated as number of days from starting the antibody therapy until relapse or disease progression was observed. The date of progression/relapse was documented for the annual follow-up visits. If progression/relapse was already observed during the evaluations at the end of the treatment cycles, the date of last antibody administration was considered as date of event

in these cases. For the disease assessments at the end of treatment cycles no exact evaluations dates were available. Death was considered an event for EFS, too. In both cases, start of the observation period was the date of first antibody intake. In cases of no events, data was censored with the last available date from study drug application or dates of annual follow-up visits.

9.7.1.2 Safety

Safety assessments were performed for the FAS.

Adverse events were assessed for Treatment Emergent Adverse Events (TEAE), i.e. AEs which occurred after the first dose of study medication within 30 days after the last study drug application or - if pre-existent - deteriorated after the first dose of study medication. AEs were coded using Medical Dictionary for Regulatory Activities (MedDRA).

The incidence and type of **TEAE**s (SAEs, related AEs, etc.) was summarized overall and by preferred term and system organ class (SOC). Separate analyses were performed for all TEAEs, for TEAEs possibly related to study drug, serious TEAEs and for serious TEAEs possibly related to study drug. AEs possibly related to study drug included AEs with relationship to study drug coded as possible, probable, definite or with missing relationship. They were analyzed globally (all cycles) as well as by time interval, where cycles 1 to 3 and cycles 4 to 9 were considered separately to distinguish between ch14.18/CHO alone and ch14.18/CHO in the combination with IL-2 (only given in cycles 4 to 9). Incidences and number of events were given overall and by maximal severity (CTC grading). Listings of adverse events (probable, possible, definite or unknown study drug related serious TEAE, TEAEs leading to discontinuation, TEAEs leading to death and non-treatment emergent AEs) and listings of deaths are provided.

Toxicities were summarized by CTC-grade within each treatment cycles and overall with maximal grading.

in this patient population, unless clinical considerations suggested otherwise.

9.8 CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

9.8.1 CHANGES TO THE CONDUCT OF THE STUDY

The following amendments were made to the approved protocol:

Amended study protocol version 1.2, dated 24 April 2012, changed the inclusion criterion “prior administration of ch14.18 antibody” to “prior administration of ch14.18 antibody after allogeneic stem cell transplantation”.

Amended study protocol version 1.3, dated 30 November 2013, has been approved in the meanwhile, permitting inclusion of further 25 patients and allowing to extend the infusion

time to 24 hours in patients whose ch14.18/CHO dose was decreased to 50% after experiencing unacceptable toxicities.

10. STUDY PATIENTS

10.1 DISPOSITION OF PATIENTS

Four European centers enrolled 68 patients between 2010 and 2017 (date of HSCT). Participating hospitals were the Children's University Hospitals Tuebingen, Greifswald, (Germany), Graz and Vienna (Austria). Most patients (n= 52; 76,5 %) received the antibody treatment and the stem cell transplantation at the center in Tuebingen.

25 deaths were recorded the throughout the study start, relapse was a major cause of death (n=21, 84,0%).

Of the 68 patients, five patients died, resulting in a cumulative incidence of non-relapse mortality of 7,4%. Three patients died while receiving immunotherapy: Two patients because of infections, one patient with HHV-6 infection with encephalitis and pneumonitis 134 days after HSCT and one fatal bacterial infection (E.coli) 463 days after HSCT. One patient died three weeks after the second antibody cycle (189 days after HSCT) with signs of encephalitis and/or PRES. One patient died of an acute lymphoblastic leukemia 648 days after HSCT / 230 days after the end of the antibody treatment, one patient 656 days after HSCT because of a E. coli sepsis.

10.2 DEMOGRAPHIC DATA AND OTHER BASELINE CHARACTERISTICS

10.2.1 DEMOGRAPHY AND BASELINE CHARACTERISTICS

A summary of the demographic characteristics for study patients is given in Table 10-1 below.

68 patients with 1st to 5th relapse were eligible for the study, two patients were excluded (one pre-screening failure, one patient with progressive disease after HSCT). The median age at study entry was 6,5 years (range 3-20) with a predominance of male participants (n=46; 67,6%). The median time from initial diagnosis to haploidentical stem cell transplantation was 33 months (range 6–171 months)

10.2.2 MEDICAL HISTORY AND PREVIOUS TREATMENT FOR ILLNESS

All patients suffered from a stage IV relapse of a histologically confirmed neuroblastoma, most patients had also metastatic disease at initial diagnosis (94,1%)

Most patients enrolled into this study had distant relapse (n=41; 60.3%) and about 30 percent of the patients (n=20; 29.4%) had a combination of distant and local relapse. In most cases the distant relapses were metastases to the skeleton (n=45; 66,2%) and bone marrow (n=34; 50%). Nearly all of patients had a stage IV disease at initial diagnosis (ID) (n=64; 94,1%) and the majority of patients (n=46; 67.6%) was negative for MYC-N proto-oncogene protein overexpression.

51/68 (75%) patients had measurable disease at study entry. The median time from stem cell transplantation to ch14.18/CHO therapy was 91 days (range 61–363 days). Performance scores were ≥ 80 (Lansky or Karnofsky)

Age at Initial Diagnosis	
Mean (years)	4,0
Median (years)	3,7
Min; Max (years)	0,4; 13,8
≤ 1,5	5 (7,6%)
>1,5 to 5	48 (72,7%)
> 5	13 (19,7%)
Sex: Female	22 (32,4%)
Male	46 (67,6%)
INSS stage at Initial Diagnosis	
1	1 (1,5%)
2	0
3	2 (2,9%)
4	64 (94,1%)
4s	1 (1,5%)
Tumor MYC-N amplification status	
Positive	19 (27,9%)
Negative	46 (67,6%)
unknown	3 (4,4%)
Tumor MIBG status	
positive	64 (94,1%)
negative	4 (5,9%)
Number of previous autologous Stem Cell Transplantations	
0	6 (8,8%)
1	58 (85,3%)
2	4 (5,9%)

Table 10-1 Demographic Profile (FAS)

10.2.3 PRIOR AND CONCOMITANT MEDICATION

All patients received concomitant medication during antibody therapy, including analgesics like morphine or hydromorphone (palladone), together with metamizole, paracetamol or ibuprofen.

The ongoing supportive medications taken since SCT were most frequently antibiotic drugs (e.g. trimethoprim, penicillin), antiviral drugs (e.g., acyclovir), antifungal medication (e.g., posaconazol), and vitamins)

As pain is a known and expected side effect with administration of ch14.18/CHO mAb, concomitant analgesic treatment was given throughout the study as needed. More than 90% of patients needed opioid analgesics; the median dose was slightly higher during the

first three cycles (0.02-0.03 mg/kg/h), but then was fairly constant throughout all treatment cycles (approx. 0.02 mg/kg/h)

10.3 STUDY MEDICATION

10.3.1 EXTENT OF EXPOSURE

A single dose of ch14.18/CHO (20 mg/m²/day, i.v. infusion) was administered for 8 hours for five consecutive days every 4 weeks. Treatment was combined with s.c. IL-2 (1x 10^6 IU/m²/day which was added at days 6, 8, 10 during cycles 4-9).

Treatment was scheduled for six cycles, but could be administered up to a maximum of 9 cycles, if patient benefit was observed.

Thirty-nine patients (57.4%) received full protocol treatment, i.e., about 600 mg/m^2 of ch14.18/CHO in cycles 1 to 6, and 21 patients (30.9%) completed six treatment cycles. Eighteen patients (26,5%) completed 9 treatment cycles.

Throughout all cycles, a planned total dose of 100 mg/m^2+/- 5% per cycle was administered. In 3,3% of the cycles more than 105% of 20mg/m^2/day and in 6,4% of the cycles less than 95% of 20mg/m^2/day were administered.

In some patients cycles were interrupted and restarted, therefore the cycle length could add up to 8 days.

10.3.2 MEASUREMENTS OF TREATMENT COMPLIANCE

Ch14.18/CHO and IL-2 were administered under controlled conditions. Hospital staff administered ch14.18/CHO intravenously in accordance with the study protocol and hospital standard procedures. Subcutaneous IL2 was administered in an outpatient setting. Tracking of the final dose received is available through source documents and CRF data but no accountability was performed.

Listing 16.2.5 provides a by-patient listing of ch14.18/CHO and IL-2 administration data.

10.4 EFFICACY RESULTS AND TABULATIONS OF INDIVIDUAL PATIENT DATA

10.4.1 ANALYSIS OF EFFICACY

10.4.1.1 “Success of Treatment”

The primary efficacy endpoint was "success of treatment" defined as a patient receiving the full protocol treatment, still alive 180 days after treatment without progression and without unacceptable toxicity and acute GvHD ≥Grade 3 or extensive chronic GvHD. Fourty of the 68 patients (58.8%) were considered to have reached the primary efficacy endpoint (Table 10-2) and achieved “success of treatment”.

Table 10-2 Success of Treatment

Parameter		Statistic	Number of Patients (N=68)
Success of treatment	Yes	N (%)	40 (58.8%)
	No	N (%)	28 (41.2%)

10.4.1.2 Overall Tumor Response and Survival Analysis

The detailed compilation of overall and event free survival is currently under evaluation and will therefore be provided at a later date.

11.4.1.5 Cytokine Levels

Cytokine levels are reported in a separate pharmacodynamics report.

10.4.2 Drug Dose, Drug Concentration, and Relationships to Response

Pharmacokinetic data are currently not available and therefore not reported.

10.4.3 Efficacy Conclusions

The primary objective of this study was to determine the safety and feasibility of treatment with ch14.18/CHO monoclonal antibody in combination with IL-2 after haploidentical stem cell transplantation in pediatric patients with relapsed neuroblastoma. Evaluation of anti-tumor response was a secondary objective only.

Because the study was not powered for this purpose and did not have a control group, efficacy results had to be interpreted with caution and no firm conclusions may be drawn from this study.

Success of treatment, which was part of the primary endpoint, was achieved in 40 patients (58.8%).

11. SAFETY EVALUATION

11.1 ADVERSE EVENTS and SEVERE ADVERSE EVENTS

Immunotherapy and stem cell transplantation were associated with several treatment-related clinical toxic effects. Effects of the stem cell transplantation were similar as for all allogenic stem cell transplantations, especially disorders of hematopoiesis, including anemia and thrombocytopenia, infections, including 3 PJP pneumonias, one fatal HHV-6 infection and two fatal e. coli blood stream infections.

Acute GvHD was experienced by 22,4 % (n=15) of the patients, mostly grade I/II acute GvHD of the skin, two patients developed grade II/III acute GvHD of the gut.

For Patients with GvHD start of the antibody treatment was delayed in a median of 17 days compared to patients without GvHD.

Induction of late onset acute GvHD (during antibody treatment) was seen in five patients, one patient developed a steroid sensitive grade III GvHD of the GI-tract, one patient grade II GvHD of the GI-tract, both resolved quickly. 3 patients developed grade I / II GvHD of the skin, without the need of systemic (steroid) therapy. No grade IV GvHD or liver GvHD occurred.

The effects of most interest related to the immunotherapy were fever, pain, tachycardia, hypotension, hypersensitivity reactions and capillary leak syndrome / fluid retention

Fever >38°C was experienced by nearly all patients, mostly grade 2 or 3, frequent during all cycles , in high fever three patients had generalized seizures.

Pain was observed in all patients and was most frequent during cycle 1, occurring in 96 % of patients, and decreasing to 68,4 % during cycle 6. The most common sites of pain were the abdomen and the legs.

95,9% of the courses were administered in the recommended dosage of 20 mg/m2 of ch14.18/CHO, in most cases lower dosages were given because of severe hypersensitivity reactions. In 11,1% of the courses a 50% decrease in the infusion rate of the ch14.18/CHO was given.

11.1.1 ANALYSIS AND DISCUSSION OF DEATHS

25 deaths were recorded the throughout the study start, relapse was a major cause of death (n=21, 84,0%)

Of the 68 patients, five patients died, resulting in a cumulative incidence of non-relapse mortality of 7,4%. Three patients died while receiving immunotherapy: Two patients because of infections, one patient with HHV-6 infection with encephalitis and pneumonitis 134 days after HSCT and one fatal bacterial infection (E.coli) 463 days after HSCT. One patient died three weeks after the second antibody cycle (189 days after HSCT)

with signs of encephalitis and/or PRES. One patient died of an acute lymphoblastic leukemia 648 days after HSCT / 230 days after the end of the antibody treatment, one patient 656 days after HSCT because of a E. coli sepsis.
The fatal outcome of this TEAE was most likely a consequence of immune suppression after myeloablative chemotherapy. The reported spectrum of fatal serious adverse events in this study is expected in patients with advanced cancer and following allogeneic SCT.

11.2 SAFETY CONCLUSION

Overall, immunotherapy with ch14.18/CHO in combination with s.c. IL-2 after haploidentical SCT was shown to be feasible in most study patients. Pain, fever, gastrointestinal AEs, effects on white and red blood cells, as well as changes in transaminases were common toxicities in this study.

The maximal toxicity during cycles was only mild or moderate toxicity (grade 1, 2) in the majority of patients. Gastrointestinal AEs (Nausea, Vomiting, Diarrhea, Constipation) were frequent and reported up to grade 3; this observation is confounded by the high usage of opioids by study patients. Skin reaction and allergy (including those of severe intensity) occurred, which is expected for chimeric antibody treatment. Bacterial infection was frequent and probably a consequence of previous SCT. IL2 therapy is also associated with impaired neutrophil function, and an increased risk of bacterial infections.

Disease progression led to death in twenty-five patients and three patients experienced fatal infections. Life-threatening central neurotoxicity and grade 4 bacterial infection was observed in some patients, and are considered likely to be consequences of disease progression and state after SCT or IL-2 treatment. This pattern of fatalities and hospitalization is expected in advanced-stage cancer patients after SCT and with concurrent use of IL-2.

Pain was the most prominent neurotoxicity observed. Pain was experienced by all patients and used opioid analgesics for symptomatic relief. Neuropathic pain requiring morphine infusions is an expected undesirable reaction with use of ch14.18/CHO mAb, especially when administered within 8 hours.

There is no apparent trend in organ toxicity over time (by treatment cycle) in this study.

Moderate effect on red blood cells (grade 2) and more severe (grade 3) on white blood cells or granulocytes were generally observed whereas effects on platelets were less common, but also included grade 4 toxicity.

Severe effects on transaminases were common in this study. No effect on the glomerular filtration rate or urinalysis was observed. It should however be noted that in patients that have received allogeneic SCT, changes in blood parameters are common, and are not considered directly related to study treatment.

Fever of moderate intensity (maximal severity throughout cycles) was commonly observed with treatment.

Expectedly, no effect on QTc was observed in the ECG. Neither there was any appreciable effect on cardiac output in the echocardiography.

Throughout nine cycles of treatment, a two cases of acute grade III or IV GvHD was observed in the 68 patients. Combined IL-2 therapy does not seem to affect the risk for GvHD.

12. DISCUSSION AND CONCLUSION

This was a Phase II, open-label, uncontrolled, multi-center safety and exploratory efficacy study of ch14.18/CHO plus IL-2 given in a single dose group after haploidentical SCT to male and female pediatric or adult patients with relapsed neuroblastoma. This preliminary report presents the results for 68 patients.

A total of 68 patients were enrolled in this study; all were evaluable for safety and efficacy. All cancer patients were in advanced stage, heavily pretreated, and had failed other treatment. Most patients (57.4%) received full protocol treatment i.e. initiated or completed 6 treatment cycles. A quarter of the patients even completed 9 cycles which was the maximum allowed number of treatment cycles. Adherence to the scheduled treatment was high. Throughout all cycles, more than 90% of the planned ch14.18/CHO and IL-2 dose per cycle was administered.

Immunotherapy does not seem to enhance the risk for GvHD after haploidentical SCT.

Overall, combination therapy with ch14.18/CHO mAb and IL-2 was tolerated in most patients. The maximal toxicity during cycles was generally only mild or moderate toxicity (grade 1, 2). Pain, fever, gastrointestinal AEs, effects on white and red blood cells, as well as changes in transaminases were common toxicities in this study.

Toxicity of aldesleukin is related to dose and route and is often severe. Capillary leak syndrome, cardiac and respiratory effects, CNS symptoms, raised liver enzymes, gastrointestinal disturbances, fever and flu-like symptoms, rashes, pruritus, hematologic effects (anemia, leucopenia, and thrombocytopenia) are common undesirable effects of IL-2 treatment. Due to impaired neutrophil function with aldesleukin therapy, there is an increased risk of bacterial infections, including sepsis. Most patients in this study experienced bacterial and/or fungal infection with IL-2 combination therapy.

Severe neuropathic pain is an expected adverse drug reaction with ch14.18/CHO mAb short-term administration that occurred in all patients (68 of 68) and remedial therapy with opioids was needed for all of them. Skin reaction and allergy (including those of severe intensity) occurred frequently with ch14.18/CHO mAb which is expected for chimeric antibody treatment. The type and frequency of undesirable effects of ch14.18/CHO mAb treatment are consistent with previous studies.

In conclusion, ch14.18/CHO mAb exposed an overall favorable human toxicity profile at the selected dose-level in pediatric patients with relapsed neuroblastoma after SCT. GD2-surface protein antibody treatment with ch14.18/CHO mAb may be safely combined with modified human recombinant IL-2 (aldesleukin).

The study design and the rather small number of exposed patients do not permit any firm conclusions with regard to efficacy of ch14.18/CHO mAb or its combination with IL-2; neither was this a primary study objective. Though ch14.18/CHO mAb demonstrated tumor response within the selected indication and the selected dosing scheme, advanced neuroblastoma is a condition difficult to treat. Between 20% and 50% of high-risk neuroblastoma patients do not respond adequately to chemotherapy and are progressive

or refractory [11] and frequently relapse. In conclusion, ch14.18/CHO mAb showed an overall favorable human toxicity profile at the selected dose-level in pediatric patients with relapsed neuroblastoma after haploidentical SCT.

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