INTERNATIONAL OPEN-LABEL PHASE I DOSE ESCALATION STUDY OF DINUTUXIMAB BETA IN COMBINATION WITH VINCRISTINE/DOXORUBICIN/CYCLOPHOSPHAMIDE AND IFOSFAMIDE/ETOPOSIDE IN PEDIATRIC, ADOLESCENT, AND ADULT PATIENTS WITH GD2-POSITIVE EWING SARCOMA

PROTOCOL TITLE:	International open-label phase I dose escalation study of dinutuximab beta in combination with vincristine/doxorubicin/cyclophosphamide and ifosfamide/etoposide in pediatric, adolescent, and adult patients with GD2-positive Ewing sarcoma
PROTOCOL CODE:	CESS-GD2
EUCT NUMBER:	2024-515245-42-00
VERSION NUMBER:	1.2
DATE OF ORIGINAL FINAL VERSION:	07. FEB. 2025
SPONSOR NAME/ADDRESS:	Gesellschaft für Pädiatrische Onkologie und Hämatologie / German Society of Pediatric Oncology and Hematology **GPOH gGmbH** Sponsor´s Office: Holsterhauser Platz 2 GERMANY – 45147 Essen

CONFIDENTIAL

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Protocol information

The trial is being conducted under the umbrella of the German Society of Pediatric Oncology and Hematology (GPOH gGmbH) acting as sponsor of the clinical trial according to the protocol, the local and European law (CTR 536/2014), and to the principles of good clinical practice.

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By my signature, I indicate I have reviewed this protocol and find its content to be acceptable.

Essen, 07.02.2025	
Place, Date	Signature Prof. Dr. Dirk Reinhardt Sponsor Representative

Compliance Statement

- I have read the current protocol version and agree to abide by all provisions set forth therein.
- I agree to conduct and personally supervise the conduct of this study at my site in accordance with the design and specific provisions of this protocol and informed consent. I agree to ensure its conduct is in compliance with the Ethics Committee (EC) procedures, instructions from GPOHs representatives, the Declaration of Helsinki, ICH Good Clinical Practices Guidelines and national regulations governing the conduct of clinical studies.
- I agree to maintain adequate and accurate records and to make those records available for audit and inspection in accordance with relevant regulatory requirements.

Investigator´s Name: ……

Site: ……

Address: ……

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Place, Date

Signature

Abbrevations

Ab	Antibody
ADCC	Antibody dependent cell-mediated cytotoxicityl
AE	Adverse event
ANC	Absolute neutrophil count
AR	Adverse reaction
ASR	Annual safety report
BSA	Body surface area
β-HCG	Beta human chorionic gonadotropin
Ccrea	Creatinin clearance
CDC	Complement dependent cytoxicity
CESS	Cooperative Ewing Sarcoma study
CHO	Chinese hamster ovary
CHMP	Committee For Medicinal Products For Human use
CNS	Central nervous system
COG	Children`s Oncology group
CR	Complete response
CRF	Case report form
CRO	Contract Research organization
CTR	Clinical trials regulation
CT	Computed tomography
CTIS	Clinical trial information system
CTCAE	Common terminology criteria of adverse events
CTV	Clinical target volume
D	Doxorubicin
DDS	Dose determing analysis set
DLT	Dose limited toxicity
DOC	Death of complications
DOR	Duration of response
DSMC	Data and safety monitoring committee
EC	Ethics committee
ECOG	Eastern Cooperative Oncology group
eCRF	Electronic case report form
EDC	Electronic data capture system
EFS	Event-free survival
EoT	End of treatment
EWS	Early warning score
FAS	Full analysis set
FCBP	Females with childbearing potential
FDA	Food and Drug administration
FDG-PET	Fluorodeoxyglucose-positron emission tomography
FPFV	First patient first visit
FISH	Fluorescence in situ hybridisation
GCP	Good clinical practice
G-CSF	Granulocyte-colony stimulating factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GD2	Disialogangliosid 2
GFR	Glomerular filtration rate
GLP	Good laboratory practice
GMP	Good manufacturing practices
GPOH	German Paediatric Oncology group
HCO3	Bicarbonate
HIV	Human immunodeficiency virus
HBV	Hepatitis B virus
HBC	Hepatitits C virus
HR	Metastatic high-risk

I	Ifosfamide
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICF	Informed consent form
ICMJE	International Committee of Medical Journal Editors
IE	Ifosfamide, etoposide
IMP	Investigational medical product
ISF	Investigator site file
ITT	Intention to treat
IV	Intravenous
LPFV	Last patient first visit
LPLV	Last patient last visit
LVEF	Left ventricular ejection fraction
MRI	Magnetic resonance imaging
MTD	Maximun tolerated dose
NCI	National cancer institute
NGS	Next generation sequencing
NCT	Neutron capture therapy
ORR	Overall response rate
OS	Overall survival
PAS	Periodic-acid-schiff
PD	Progressive disease
PDGFR	Platelet-derived growth factor receptor
PET-MRI	Positron emission tomography–magnetic resonance imaging
PIS	Patient information sheet
PK	Pharmakokinetic
PR	Partial response
QC	Quality control
QT	Measurement shown on ECG
RDE	Recommended dose of expansion
RECIST	Response evaluation criteria in solid tumours
RP2D	Reccommended phase 2 dose
RR	Respiration rate
RT-PCR	Reverse transcriptase polymerase chain reaction
SAE	Serious adverse event
SAP	Statistical analysis plan
SAR	Serious adverse reaction
SD	Stable disease
SF	Shortening fraction
SmPC	Summary of product characteristics
SoA	Schedule of activities
SOP	Standard operationg procedures
SR	Localised standard-risk
SUSAR	Suspected unexpected serious adverse reaction
TBD	To be determined
TP	Total protein
TmP	Tubular maximum reabsorption of phosphate
TV	Tumour volume
ULN	Upper limit of normal
V	Vincristine
VAI	Vincristine, actinomycin D, ifosfamide
VDC	Vincristine, doxorubicin, cyclophosphamide
WBC	White blood cell
WMA	World medical association
WOCBP	Women of child bearing potential

1. Protocol Summary

1.1. Synopsis

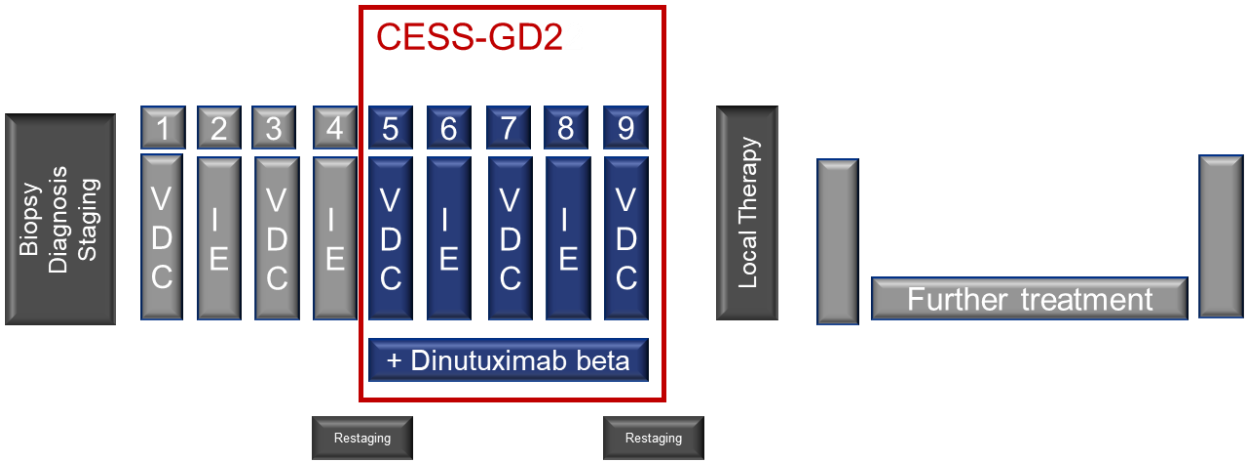
Protocol title:	International open-label phase I dose escalation study of dinutuximab beta in combination with vincristine/doxorubicin/cyclophosphamide and ifosfamide/etoposide in pediatric, adolescent, and adult patients with GD2-positive Ewing sarcoma
Short title:	International Phase I Trial of dinutuximab beta with VDC/IE in GD2-Positive Ewing Sarcoma
Protocol Code:	CESS-GD2
EU-CT Number:	2024-515245-42-00
Clinical Phase:	Phase I
Study Type:	Interventional
Sponsor:	GPOH gGmbH
International Coordinating Investigator:	Prof. Dr. med. Uta Dirksen, MD University Hospital Essen, Pediatrics III Cooperative Ewing Sarcoma Study (CESS) Group West German Cancer Centre German Cancer Consortium Hufelandstraße 55 45147 Essen, Germany uta.dirksen@uk-essen.de
Key words:	Biomarker-driven trial, GD2 expression, targeted therapy, high-risk Ewing Sarcoma trial
Rationale:	Immunotherapeutic approaches have emerged as an effective therapeutic approach in cancer. We will investigate the cell-surface ganglioside GD2 as a target since it is expressed on the cell surface of Ewing Sarcoma cells and clinical trials support the safety and efficacy of anti-GD2 antibodies in pediatric solid cancers. Dinutuximab beta is a chimeric antibody that targets the disialoganglioside GD2. GD2 is expressed on a number of solid malignancies and was clinically investigated in pediatric patients with neuroblastoma. Expression in normal tissues is restricted to cerebellar neurons, skin melanocytes and peripheral pain fibers. Due to this expression pattern, anti-GD2 antibodies have been studied as targeted immunotherapy for neuroblastoma. With the results of these clinical trials, combination therapies with dinutuximab beta became a standard component of high-risk neuroblastoma therapy. GD2 expression is found in several EWS samples with variable expression-levels, and it is expected that anti-GD2 treatment is best suited as biomarker-driven approach. The administration of vincristine, doxorubicin, cyclophosphamide, ifosfamide and etoposide (VDC/IE) has been shown to be an effective systemic therapy and is therefore currently part of the standard treatment in Ewing Sarcoma. The international newest standard induction chemotherapy in the standard- and high-risk group consists of alternating VDC/IE. High-risk patients with a

	GD2 positive tumor could benefit from an addition of dinutuximab beta to the standard chemotherapy with VDC/IE. The rationale for this trial is to investigate the feasibility, toxicity, and biological activity of dinutuximab beta in combination with standard chemotherapy in EWS.
Objective(s):	**Primary objective** Dose finding to determine the recommended phase II dose (RPD2) of dinutuximab beta in combination with vincristine, doxorubicin & cyclophosphamide and ifosfamide & etoposide. **Secondary and exploratory objectives** To assess safety, pharmacokinetic and antitumor activity of dinutuximab beta in combination with standard induction chemotherapy
Endpoints: For details, please refer to Section 3	**Primary Endpoints** Recommended dose of dinutuximab beta in combination with standard chemotherapy agents based on number of patients withs DLTs. **Secondary Endpoints** Progression Free Survival (PFS) Event Free survival (EFS) Duration of Response (DOR) **Exploratory Endpoints** • Biobanking of liquid biopsies, stool, and tumor material for ancillary studies
Investigational medicinal products (IMP): For details, please refer to Section 0	Dinutuximab beta, vincristine, cyclophosphamide, ifosfamide, etoposide, doxorubicin
Dinutuximab beta dosing: For details, please refer to Section 10.1.1	**Dose-Escalation** **Recommended dose:** 10mg/m^2/d 10-day continuous infusion The starting dose Level 1: 50% of the recommended dose; 5mg/m^2/d 10-day continuous infusion Level 2: 75% of the recommended dose; 7.5mg/m^2/d 10-day continuous infusion Level 3: 100% of the recommended dose; 10mg/m^2/d 10-day continuous infusion
Inclusion-/Exclusion-Criteria	
Inclusion criteria: For details, please refer to Section **5.1**	1. Histologically confirmed, **newly diagnosed Ewing Sarcoma** (m/f/d) or so-called Ewing-like sarcoma (i.e. translocation-positive small blue round cell sarcoma other than Rhabdomyosarcoma) of bone and / or soft tissue with evidence of EWS translocation by fluorescence in situ hybridization (FISH), real-time polymerase chain reaction (RT-PCR), or next-generation sequencing (NGS) assay 2. High risk stratification (metastatic disease)

	3. Centrally confirmed GD2-positive tumor (biopsy of original and/or residual tumor or liquid biopsy in peripheral blood) 4. Availability of fresh frozen tumor tissue for central GD2-detection 5. Age ≥12 months 6. Start of first line treatment according to standard induction treatment (Cycle 1-4: VDC – IE – VDC – IE) 7. Wash-out phase with a minimum of 14 days after the last the dose of the last chemotherapy 8. Lansky (<16 years) Performance Score ≥70% or ECOG (≥16 years) ≤ 2 9. Adequate bone marrow function as evidenced by meeting all the following requirements: a. White blood cell count > 2000/µl b. ANC ≥1000 cells/µL (G-CSF allowed) c. Platelet count 75,000 cells/µL without the use of platelet transfusion within the last 2 days d. Hemoglobin ≥9 g/dL without the use of red blood cell transfusion within the last 2 days 10. Adequate hepatic function as evidenced by meeting all the following requirements: a. Serum total bilirubin ≤1.5 x upper limit of normal (ULN) b. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 5 x ULN 11. Adequate cardiac function: confirmed by echocardiography with a left ventricular ejection fraction (LVEF) of ≥ 50% 12. Adequate renal function: creatinine clearance or glomerular filtration rate (GFR) > 60 mL/min/1.73 m^2 13. No known active HIV, HBV, or HCV infection 14. No severe neurological impairment, particularly no motor or sensory deficits, except for neurological deficits caused by Ewing sarcoma 15. Female patients of childbearing potential must present with a negative serum pregnancy test and agree to employ adequate birth control measures for the duration of the study and until 3 months after the end of treatment. Female patients who are lactating must agree to stop breast-feeding from the start of study treatment until 1 month after the end of treatment. 16. Patient or their legal representative is willing and able to comply with the requirements of the study protocol
Exclusion criteria: For details, please refer to Section 5.2	1. Relapsed or refractory Disease state 2. Patients with hypersensitivity against at least 1 component of the investigational medicinal product 3. Significant illnesses and/or any of the following: ● significant psychiatric disabilities or uncontrolled seizure disorders ● active uncontrolled peptic ulcer disease ● clinically significant neurologic deficit or objective peripheral neuropathy ● clinically significant, symptomatic fluid in a third space 4. Active and uncontrolled CNS metastases (indicated by clinical symptoms, cerebral edema, corticosteroid and/or anticonvulsant requirement, or progressive disease); for controlled CNS metastases, patient should have been off corticosteroids for at least 28 days without overt evidence of significant neurological deficits prior to enrollment

	5. Significant cardiac conduction abnormalities, including known familial prolonged QT syndrome, or screening QTc >480 msec 6. Active, uncontrolled infection or an unexplained fever >38.5°C which in the Investigator's opinion might compromise the patient's participation in the study or affect the study outcome 7. Chronic Grade ≥2 diarrhea 8. Diagnosis of any malignancy other than the disease under study 9. Any other medical or social condition deemed by the Investigator to be likely to interfere with a patient's ability to cooperate and participate in the study or interfere with the interpretation of the results.
Statistical analysis: For details, please refer to Section 15	**Design:** 3+3 design with three pre-defined dose levels (Level 1: 50%, Level 2: 75%, Level 3: 100%). At each level at least 3 at max. 6 patients will be included depending on the occurrence of DLTs. At Level 1 a toxicity-adjusted dose escalation is implemented based on the observed Common Toxicity Criteria (CTCAE Version 5) grades: • CTC grade I=100% dose escalation (=Level 3) • CTC grade II= 50% dose escalation (=Level 2)
Sample size:	**Total N=** 3 (min) - 18 (max)
Definitions:	**The evaluation of the tumor will be performed centrally according to the revised RECIST guideline (version 1.1).** **Complete response (CR):** Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm **Partial response (PR):** At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters. **Stable disease (SD):** Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study. **Progressive disease (PD):** At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

1.2. Trial Scheme



1.3. Schedule of Activities

	Screening/ Pre-Phase	1. Cycle VDC+ Dinu	2. Cycle IE+Dinu	3. Cycle VDC+Dinu	4. Cycle IE+Dinu	5. Cycle VDC+Dinu	End of Treatment (day 15+-7)	Follow up (for 11 months after EoT)	End of trial visit (at 12 months after EoT)
Timepoint	Day -14 to 0	Day 1 to 21±7	Day 22 to 42±7	Day 43 to 63±7	Day 64 to 85±7	Day 86 to 106±7	Day 107±7	every 4 weeks	
ICF	x								
Demographic and medical history	x								
Medications and Procedures	x								
Evaluation of Inclusion/ Exclusion criteria	x								
Disease state[1]								x	
Vital signs, RR[2]	x	x	x	x	x	x	x		x
Height, weight, BSA[3]	x	x	x	x	x	x	x		x
Serum Pregnancy Test[4]	x	x	x	x	x	x			
CT/MRI[5]	x						x		
[18]FDG-PET-CT/MRI[6]	x						x		
Physical Examination	x	x	x	x	x	x	x		x
Sexual development status[7]	x	(x)	(x)	(x)	(x)	(x)			
Echocardiography	x			x		x			
ECG[8]	x	x	x	x	x	x	x		
Performance status[9]	x	x	x	x	x	x	x		
Local laboratory assessment[10]	x	x	x	x	x	x	x		
Shipment Fresh Frozen Tumor sample	x								
Peripheral Blood samples for GD2 reference	x	x	x	x	x	x	x		x
AE/SAE documentation	After signing ICF	x	x	x	x	x	x		x
Concomitant Medication	x	x	x	x	x	x	x		x

[1] Patient status: dead or alive, and recording of events (progression; relapse; secondary malignancy; death, independent cause), if any.

[2] Vital signs including sitting blood pressure, heart rate, respiration rate (RR), and temperature should be made after the subject has been sitting or lying, supine or semi recumbent, for at least 5 minutes.

[3] Height in centimeters (cm) and body weight (to the nearest 0.1 kilogram (kg) in indoor clothing, but without shoes) will be measured. Height, weight and BSA may be performed within 5 days prior to the start of a cycle and ±1 day for other visits.

[4] A ß-HCG pregnancy test is required in females of childbearing potential regularly at screening until end of treatment.

[5] CT or MRI of tumor and metastases should be performed according to local practice during day -28 and start of treatment. Choice of imaging method should be same as at initial diagnosis.

[6] PET-CT/MRI of tumor and metastasis is highly recommended and may be performed according to local physicians' decision during day -28 and start of treatment. In case at initial diagnosis PET imaging was required, restaging is highly recommended.

[7] Applies to children and adolescent subjects. Patients who are staged during screening who may achieve menarche during participation of the trial, need to be evaluated regularly.

[8] ECG should be performed before each cycle and whenever clinically indicated.

[9] Lansky (<16 years) Performance Score or ECOG (≥16 years).

[10] Includes initial testing of viral serology and regular serum chemistry, hematology.

2. Introduction

2.1. Study Rationale

This study aims to investigate the cell-surface ganglioside GD2 as a target since it is expressed on the cell surface of Ewing Sarcoma cells and clinical trials support the safety and efficacy of anti-GD2 antibodies in pediatric solid cancers. Furthermore, this phase I study aims to assess the recommended phase 2 dose of dinutuximab beta.

Spasov et al. showed results, that GD2 as well-characterized tumor-associated antigen is expressed in 40% to 90% of Ewing sarcoma cells, making it a suitable target for therapeutic intervention. The group presents three cases of newly diagnosed, metastatic, GD2-positive Ewing sarcoma or Ewing-like sarcoma that were treated with the anti-GD2 antibody dinutuximab beta alongside standard chemotherapy. The treatment was well-tolerated, and all patients achieved complete remission without signs of relapse. These findings suggest that first-line anti-GD2 immunotherapy in patients with metastatic, GD2-positive Ewing sarcoma or Ewing-like sarcoma could be a promising treatment option that merits further clinical investigation. These findings form the basis to run this clinical trial.

2.1.1. Rationale for the Inclusion of Minors

Ewing Sarcoma is the second most common malignant bone tumor in children, adolescents and young adults with an age peak between 10-20 years of age.

Dinutuximab beta has been a part of treatment in childhood cancer for many years, especially in the treatment of neuroblastoma (literature). It has been shown to cause few side effects in children and young adults (Literature). Side effects include neuropathic pain, peripheral neuropathy, hypersensitivity reactions, capillary leak syndrome, photophobia, and hypotension and have demonstrated reversibility with continuous use of the antibody (Barone et al., 2021).

2.2. Background

Ewing sarcoma (ICD-10 C40-C41) is a highly malignant tumor of bone or soft tissues that occurs in children, adolescents and young adults. Current international therapeutic approaches consist of multiagent chemotherapy with anthracyclines, alkylating agents and topoisomerase inhibitors, major surgery, and radiotherapy (Grünewald et al., 2018). These therapies cure only a proportion of Ewing sarcoma patients and are associated with high acute and late toxicities (Grünewald et al., 2018; Ranft et al., 2017).

Approximately 40% of all patients suffer from recurrent disease and relapsed or refractory disease is literally incurable. Survival rates of less than 13% in this cohort imply an urgent need for innovative treatment strategies. The survival rate of primary disseminated Ewing sarcoma is less than 30% and did not improve in the past decades even with implementation of high dose chemotherapy (Rasper et al., 2014; Stahl et al., 2011).

Consequently, new treatments are required for this population of young cancer patients.

Immunotherapeutic approaches have emerged as an effective therapeutic approach in cancer and also in the treatment of sarcoma. This trial will investigate the cell-surface ganglioside GD2 as a target since it is expressed on the cell surface of Ewing sarcoma cells and clinical trials support the safety and efficacy of anti-GD2 antibodies in pediatric solid cancers.

Dinutuximab beta is a chimeric antibody that targets the disialoganglioside GD. GD2 is expressed on several solid malignancies and was clinically investigated in pediatric patients with neuroblastoma. Expression in normal tissues is restricted to cerebellar neurons, skin melanocytes and peripheral pain fibers (Hung & Yu; Marx et al., 2020). Due to this expression pattern, anti-GD2 antibodies have been

studied as targeted immunotherapy for neuroblastoma (Ladenstein et al., 2018). With the results of these clinical trials, combination therapies with dinutuximab beta became a standard component of high-risk neuroblastoma therapy. A trial conducted by the Children's Oncology Group (COG) investigated dinutuximab beta in combination with granulocyte macrophage stimulating factor in osteosarcoma patients, the trial was negative as less patients (11/39) than expected (≥16/39) remained disease free. The efficacy in combination with chemotherapy was not assessed in this group of patients (Hingorani et al., 2022). In contrast to Osteosarcoma, Ewing sarcoma cells express neuronal features similar to neuroblastoma and for some soft tissue Ewing sarcomas the term peripheral neuroectodermal tumor had been used for years (Grünewald et al., 2018).

GD2 expression is found in several Ewing sarcoma samples with variable expression-levels, and it is expected that anti-GD2 treatment is suited as a biomarker-driven approach. In the literature, case reports of patients with primary disseminated or relapsed GD2-expressing Ewing sarcomas indicate effectiveness of a treatment with dinutuximab beta. One paper describes three patients with primary high risk metastatic Ewing sarcoma with a high expression of GD2. Dinutuximab beta was given in all three patients together with VAI as a consolidation chemotherapy. Results show a positive effect, where complete response was achieved within 5-10 months and the response is ongoing over the course of at least 20 months (Spasov et al., 2022). Another manuscript showed a long-lasting response in highly pre-treated patients with relapsed Ewing sarcoma (Wingerter et al., 2021).

The administration of vincristine, doxorubicin, cyclophosphamide, ifosfamide and etoposide (VDC/IE) has been shown to be an effective systemic therapy and is therefore currently part of the standard treatment in Ewing Sarcoma (Brennan et al., 2022; Reed et al., 2023; Womer et al., 2012). Alternating VDC/IE is the new standard induction therapy in the standard- and high-risk group (Brennan et al., 2022). However, in metastatic patients the outcome remains poor (DuBois et al., 2023) and the addition of ifosfamide and etoposide to a standard regimen does not affect their outcome (Grier et al., 2003).

Patients with early relapse or progressive disease and a GD2 positive tumor could benefit from an addition of dinutuximab beta to the standard induction chemotherapy.

The rationale for this trial is to investigate the feasibility, toxicity, and biological activity of dinutuximab beta in combination with standard chemotherapy in EWS.

2.3. Risk/Benefit Assessment

2.3.1. Known Potential Risks

Extrapolation from pediatric clinical safety data with dinutuximab beta reversible side effects, such as neuropathic pain, peripheral neuropathy, hypersensitivity reactions, capillary leak syndrome, photophobia, and hypotension. All of them have demonstrated reversibility with continuous use of the antibody (Mastrangelo et al., 2021). These events are readily monitored and manageable.

More information about the known and expected benefits, risks, serious adverse events (SAE) and reasonably anticipated AE of dinutuximab beta are outlined in detail in the SmPC.

Common risks of the standard induction chemotherapy are well known and to be expected.

2.3.2. Potential Benefits

The outcome in primary metastasis remains poor without any improvement for decades. Clinical trials that tested new agents in unselected groups of patients did not improve outcome (DuBois et al., 2023). In the past decades biomarker-driven therapies proved to be game changers in many cancers.

There is an urgent need for effective combinations in primary metastatic Ewing sarcoma, as the outcome has not improved since decades. The addition of dinutuximab beta will build on a standard induction chemotherapy that has been used in several clinical trials on both sites of the Atlantic using a drug combination that is used since 1995 (Granowetter et al., 2009) and has been used in all US American

Ewing Sarcoma trials since and also on the European Continent and is the international standard for the treatment of Ewing Sarcoma (Grünewald et al., 2018).

The drug dinutuximab beta is an anti-GD2 monoclonal antibody, produced in Chinese hamster ovary (CHO) cells (ch14.18/CHO). This antibody has a better glycosylation-pattern to avoid the clearance by xeno antibodies and is more tolerated than the murine anti-GD2 antibody (Ladenstein et al., 2013). The effect relies on immune effector functions like complement-dependent cytotoxicity and antibody dependent cell-mediated cytotoxicity (ADCC). Furthermore, binding of dinutuximab beta GD2 expressing to tumor cells interferes with proliferation and invasiveness of the tumor, and induces apoptosis, which was shown also in adult type GD2-expressing malignoma (Aixinjueluo et al., 2005).

The anti-GD2 therapy improved neuroblastoma treatment outcomes. Consequently, the drug development included small children below the age of six with the youngest child included at age 1.5 years (Cheung et al., 1987). Since 2015, GD2-antibody therapy (dinutuximab) has had an FDA-approval for the initial treatment of neuroblastoma, following intensive multimodal therapy. Approval was granted based on the results from the prospective, randomized NCT00026312 trial that randomized isoretinoic acid versus dinutuximab beta and GM-CSF, interleukin-2 (IL-2), and cis-retinoic acid (isotretinoin) in pediatric children with high-risk neuroblastoma with at least a partial response to initial multimodality therapy (Yu et al., 2010).

In Europe, dinutuximab beta is approved for children aged > 12 months and in this age group for first-line treatment and at relapse. The randomized clinical trials that led to the approval showed no difference in outcome after randomization for dinutuximab beta plus subcutaneous IL2 and dinutuximab beta alone but more toxicity in the group who received IL2. The trial included patients, aged 1-20 years (Ladenstein et al., 2018). A study in patients with relapsed or refractory disease combined dinutuximab with GM-CSF, and irinotecan, and temozolomide which was effective also in patients who had received dinutuximab beta mono before with an acceptable toxicity and an overall response rate of 41.5% (Mody et al., 2017).

As described in Section 2.2 case reports of combination of dinutuximab beta and classical Ewing sarcoma chemotherapy have shown a very promising response in primary metastatic very high-risk Ewing sarcoma patients and at relapse. The immunochemotherapy approach was well tolerated in all patients (Spasov et al., 2022; Wingerter et al., 2021).

The history of dinutuximab beta shows that it is safe, with manageable side effects in very young children, adolescents and young adults with GD2 positive tumors.

2.3.3. Assessment of Potential Risks and Benefits

Given the observed safety profile of dinutuximab beta in children and young adults and the known risk profiles of the induction chemotherapy with vincristine, doxorubicin, cyclophosphamide, ifosfamide and etoposide, the risk for combination in the proposed study is expected to be manageable. Careful measures are taken to ensure participant safety; the potential risks are justified by the potential benefits for individuals with very high-risk metastatic Ewing sarcoma. The first 5-10 days of the first two cycles of dinutuximab beta following the standard chemotherapy should be applied in an inpatient setting. Special caution concerning neurotoxicity should be paid to the first cycle of dinutuximab beta following etoposide and ifosfamide, as both ifosfamide and dinutuximab beta can lead to neurotoxicity (see SmPC). In case of good tolerability of the first two cycles of dinutuximab beta treatment, an outpatient administration may be taken into consideration. Regular appointments for check-up during the cycles of dinutuximab beta administration should be made to detect any side effects at an early stage.

Based on the known toxicities of the standard chemotherapy and dinutuximab beta, the following are considered the most common and significant risks (Barone et al., 2021):

- pain
- fever
- hypersensitivity reactions/allergy
- capillary leak syndrome
- diarrhea
- elevated liver enzymes
- neurotoxicity

2.3.4. Description and Threshold of Burden

Patients treated with dinutuximab beta will, besides the known risk for side effects due to the standard chemotherapy, experience an increased risk of toxicity due to the addition of dinutuximab beta. Known toxicities are described in section 2.3.3. Special attention should be paid to the first dinutuximab beta cycle following chemotherapy with ifosfamide as both, ifosfamide and dinutuximab beta, increase the risk for neurotoxicity.

The first two cycles of dinutuximab beta following standard induction chemotherapy will be administered in an inpatient setting for at least the first 5 days of each cycle to monitor patients closely. Monitoring during the inpatient treatment should include regular assessment of vital parameters and neurological status. The administration of gabapentin and morphine during the dinutuximab beta treatment decrease the risk of side effects. However, administration of these medications also requires monitoring provided by an inpatient stay.

Patients will stay in the hospital for at least 5 days for the first two cycles of dinutuximab beta treatment, depending on the tolerability and toxicity. The following cycles can be administered in an outpatient setting depending on the side effects experienced during the first two cycles of dinutuximab beta treatment in combination with standard induction chemotherapy.

3. OBJECTIVES AND ENDPOINTS

3.1. Primary Objective

Dose finding to determine the recommended phase II dose (RPD2) of dinutuximab beta in combination with vincristine, doxorubicin & cyclophosphamide and ifosfamide & etoposide.

3.2. Secondary Objectives

The secondary objectives are to assess the safety, pharmacokinetics, and antitumor activity of dinutuximab beta when administered in combination with standard induction chemotherapy.

3.3. Exploratory Objectives

To collect and biobank liquid biopsies, stool samples, and tumor material for future ancillary studies aimed at exploring potential biomarkers and understanding the biological mechanisms associated with treatment response and resistance.

3.4. Primary Endpoint

Recommended dose of dinutuximab beta in combination with standard chemotherapy agents based on number of patients with dose limiting toxicities (DLTs).

3.5. Secondary Endpoints

The secondary endpoints of this study are designed to evaluate the clinical efficacy of the treatment regimen. These include:

1. **Progression-Free Survival (PFS):** The time from the start of treatment until the first documented evidence of disease progression or death from any cause, whichever occurs first.
2. **Event-Free Survival (EFS):** The time from the initiation of treatment to the occurrence of any treatment-related event, including disease progression, relapse, occurrence of a second malignancy, or death from any cause.
3. **Duration of Response (DOR):** The time from the first documentation of a complete or partial response to the treatment until the first occurrence of disease progression or relapse.

Progression of disease is defined as recurrent or progressive disease under active oncological therapy or at the end of treatment assessment.

Relapse of disease is defined as recurrent disease at any site in patients with complete clinical remission after completion of active oncological therapy and after the end of treatment assessments.

3.6. Exploratory Endpoints

To support the exploration of biological mechanisms and potential biomarkers, the following exploratory endpoints will be assessed:

1. **Biobanking of Liquid Biopsies and Tumor Material:** Collection and storage of liquid biopsies (e.g., blood samples), stool samples, and tumor tissue for ancillary studies. These samples will be used for future research to investigate biomarkers, treatment response, and resistance mechanisms, as well as other molecular and immunological correlates.

4. STUDY DESIGN

4.1. Overall Design

This is a multi-center, phase 1, open-label, dose escalation study enrolling high-risk Ewing sarcoma patients that have been tested with GD2-positivity in a classical 3+3 design.

4.2. Scientific Rationale for Study Design

The classical 3+3 study design, commonly utilized in phase I clinical trials, offers a structured and ethical approach to determining the safety, tolerability, and appropriate dosage levels of new treatments such as the introduction of dinutuximab beta as additional to standard chemotherapy in high-risk Ewing sarcoma patients. This design is particularly valuable in pediatric clinical trials due to the unique physiological and developmental considerations in this population. This needs to be considered even if the trial also allows the enrolment of adolescents and (young) adults.

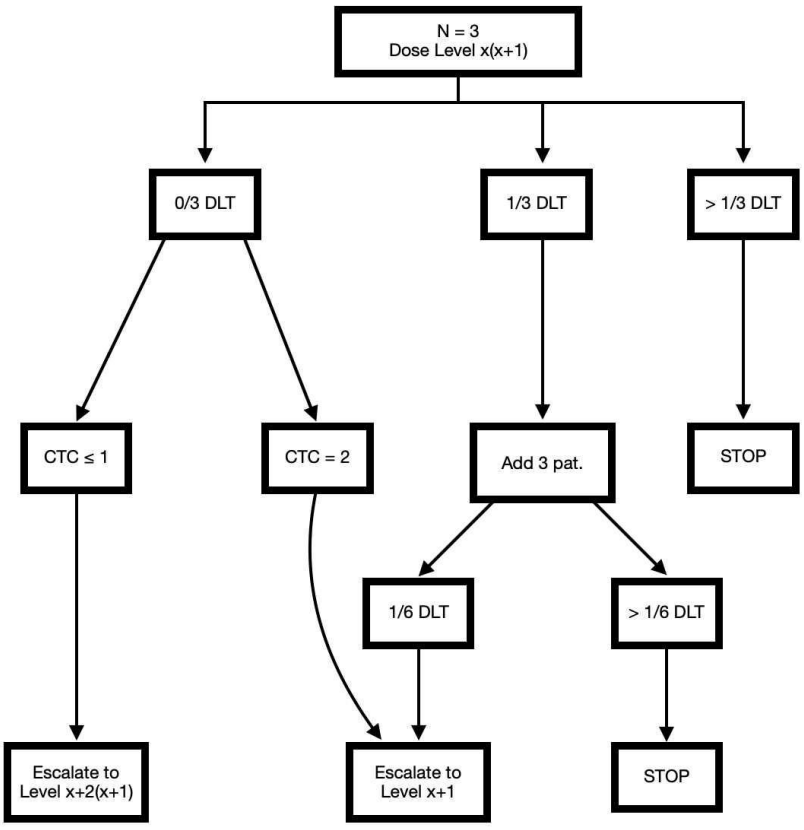
The 3+3 design helps minimize the exposure of the targeted population to potentially harmful doses. Starting at a low dose and only escalating after safety confirmation ensures a cautious and ethical approach. It allows for careful monitoring of adverse events and side effects, ensuring that any potential harm is detected early before escalating to higher doses.

The structured escalation process (3 patients per dose level, with additional cohorts if dose-limiting toxicities occur) allows for a stepwise increase in dose, providing clear data on the maximum tolerated

dose (MTD) and the recommended dose for subsequent studies. Continuous assessment of safety at each dose level ensures that CESS-GD2 can be halted or adjusted if severe adverse reactions are observed, protecting the enrolled patients from undue harm.

Additionally, the 3+3 design is straightforward, making it easier to implement and understand for clinical teams. This simplicity enhances compliance and accurate data collection. Given the limited number of newly diagnosed high-risk Ewing sarcoma patients (~40 patients/year) generally eligible, the 3+3 design optimizes the use of available participants by requiring fewer patients compared to other dose-escalation designs, especially since the frequency of GD2 positivity in high-risk Ewing's sarcoma patients is not yet well researched and therefore a more complex sample size calculation would probably jeopardize the feasibility of the clinical trial in general.

The classical 3+3 study design is well-suited for the purpose of this clinical trials due to its focus on safety, ethical considerations, feasibility, and regulatory acceptance. Its structured and cautious approach ensures that the unique needs and vulnerabilities of pediatric patients are adequately addressed, thereby fostering the safe and effective development of new pediatric treatment options.



4.3. Justification for Dosing

The decision to initiate treatment with 50% of the approved dosage for neuroblastoma in this dose-escalation study is grounded in both scientific literature and safety considerations. Starting at a lower dosage allows for the careful monitoring of patient response and minimizes the risk of adverse effects, particularly in a dose-sensitive adult population.

A risk assessment further supports this approach, indicating that starting at 50% of the approved neuroblastoma dose provides a safe baseline from which to escalate. The assessment outlines that a stepwise increase to 75% and subsequently to 100% of the approved dose allows for the identification of any dose-limiting toxicities (DLTs) in a controlled manner, thus optimizing patient safety.

The choice of 100 mg/m^2 as the maximum dosage per cycle, equivalent to the dosage used in standard neuroblastoma treatment, is justified by its established efficacy and safety profile in this specific indication. The literature supports that doses up to 100 mg/m^2 per cycle are well-tolerated in the majority of patients, with the therapeutic benefits outweighing the risks when administered within a carefully monitored clinical framework.

In conclusion, the dose-escalation schema starting at 50% of the neuroblastoma-approved dosage, followed by intermediate escalation steps to 75% and 100%, is a scientifically sound and ethically responsible approach. It balances the need for efficacy with the imperative of patient safety, as supported by both published research and thorough risk assessments.

4.4. Start and End of Trial Definition

The start of the trial is designated as the date the first investigational site is open for recruitment, that is, the date the first site received the site activation letter.

End of trial is defined as the time when the last patient completes the last follow-up visit. An interim clinical trial report will be written after all patients have completed the trial treatment or discontinued the study (independently from follow up). The final clinical trial report will be written at the end of the study (latest 6 months after database lock).

A patient is considered to have completed the trial if he or she has completed all phases of the trial including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3.

The sponsor confirms that for this trial which includes minors, Regulation (EC) No 1901/2006 Article 41, Para. 2; Guideline 2009/C 28/01 EC 4.2 as well as Ethical Considerations for Clinical Trials on Medicinal Products Conducted with the Pediatric Population, Final 2008, 19.1, apply, with possibly shorter timelines to submit the clinical study report, in case that is necessary by this legal background.

5. STUDY POPULATION

CESS-GD2 will be conducted in patients, aged at least 12 months with GD2-positive high-risk Ewing sarcoma. The investigator or a delegated designee must ensure that only patients who meet all the following inclusion and none of the exclusion criteria are offered treatment in the study.

5.1. Inclusion Criteria

1. Histologically confirmed, **newly diagnosed Ewing Sarcoma** (m/f/d) or so-called Ewing-like sarcoma (i.e. translocation-positive small blue round cell sarcoma other than Rhabdomyosarcoma) of bone and / or soft tissue with evidence of EWS translocation by fluorescence in situ hybridization (FISH), real-time polymerase chain reaction (RT-PCR), or next-generation sequencing (NGS) assay
2. High risk stratification (metastatic disease)
3. Centrally confirmed GD2-positive tumor (biopsy of original and/or residual tumor or liquid biopsy in peripheral blood)
4. Availability of fresh frozen tumor tissue for central GD2-detection
5. Age ≥12 months
6. Start of first line treatment according to standard induction treatment (Cycle 1-4: VDC – IE – VDC – IE)
7. Wash-out phase with a minimum of 14 days after the last the dose of the last chemotherapy
8. Lansky (<16 years) Performance Score ≥70% or ECOG (≥16 years) ≤ 2
9. Adequate bone marrow function as evidenced by meeting all the following requirements:
    a. White blood cell count > 2000/µl
    b. ANC ≥1000 cells/µL (G-CSF allowed)
    c. Platelet count 75,000 cells/µL without the use of platelet transfusion within the last 2 days
    d. Hemoglobin ≥9 g/dL without the use of red blood cell transfusion within the last 2 days
10. Adequate hepatic function as evidenced by meeting all the following requirements:
    a. Serum total bilirubin ≤1.5 x upper limit of normal (ULN)
    b. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 5 x ULN
11. Adequate cardiac function: confirmed by echocardiography with a left ventricular ejection fraction (LVEF) of ≥ 50%
12. Adequate renal function: creatinine clearance or glomerular filtration rate (GFR) > 60 mL/min/1.73 m^2
13. No known active HIV, HBV, or HCV infection
14. No severe neurological impairment, particularly no motor or sensory deficits, except for neurological deficits caused by Ewing sarcoma
15. Female patients of childbearing potential must present with a negative serum pregnancy test and agree to employ adequate birth control measures for the duration of the study and until 3 months after the end of treatment. Female patients who are lactating must agree to stop breast-feeding from the start of study treatment until 1 month after the end of treatment.
16. Patient or their legal representative is willing and able to comply with the requirements of the study protocol.

5.2. Exclusion Criteria

1. Relapsed or refractory Disease state
2. Patients with hypersensitivity against at least 1 component of the investigational medicinal product
3. Significant illnesses and/or any of the following:
    a. significant psychiatric disabilities or uncontrolled seizure disorders
    b. active uncontrolled peptic ulcer disease
    c. clinically significant neurologic deficit or objective peripheral neuropathy
    d. clinically significant, symptomatic fluid in a third space
4. Active and uncontrolled CNS metastases (indicated by clinical symptoms, cerebral edema, corticosteroid and/or anticonvulsant requirement, or progressive disease); for controlled CNS metastases, patient should have been off corticosteroids for at least 28 days without overt evidence of significant neurological deficits prior to enrollment
5. Significant cardiac conduction abnormalities, including known familial prolonged QT syndrome, or screening QTc >480 msec
6. Active, uncontrolled infection or an unexplained fever >38.5°C which in the Investigator's opinion might compromise the patient's participation in the study or affect the study outcome
7. Chronic Grade ≥2 diarrhea
8. Diagnosis of any malignancy other than the disease under study
9. Any other medical or social condition deemed by the Investigator to be likely to interfere with a patient's ability to cooperate and participate in the study or interfere with the interpretation of the results.

5.3. Screen Failures

Screen failures are defined as patients who consent to participate in the clinical trial but are not subsequently assigned to the trial intervention or entered in the trial. A minimal set of screen failure information is required to ensure transparent reporting of screen failure patients to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details and eligibility criteria.

Subjects who do not meet the criteria for participation in this trial (screen failure) may be rescreened within 14 business days. Rescreened participants should be assigned the same participant number as for the initial screening. See also Section 8 for details.

5.4. Definition of women of childbearing potential and of fertile men

For this protocol, a woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy.

To this protocol, a man is considered fertile after puberty unless permanently and documented sterile by bilateral orchidectomy.

All descripted requirements in this and other related sections based on the MA/CTFG "Recommendations related to contraception and pregnancy testing in clinical trials", current version, and with CPMP/ICH/286/95 ICH M3(R2).

6. INVESTIGATIONAL MEDICINAL PRODUCTS

6.1. Declaration of Investigational Medical Products and Auxiliary Medicinal Product

The following drugs are regarded as Investigational Medicinal Products (IMPs) for the purposes of this trial:

- dinutuximab beta,
- vincristine,
- cyclophosphamide,
- ifosfamide,
- etoposide,
- doxorubicin.

Regulation (EU) No 536/2014 Article 2 (5) defines an IMP as “a medicinal product which is being tested or used as a reference, including as a placebo, in a clinical trial”. Further information on IMPs can be found in “The rules governing medicinal products in the European Union” Volume 10 – Guidance documents applying to clinical trials, Clinical Trials Regulation (EU) No 536/2014 Questions and Answers (currently being updated).

The following drugs are regarded as Auxiliary Medicinal Product (AxMPs) for the purposes of this trial:

- mesna,
- G-CSF/filgrastim.

Regulation (EU) No 536/2014 Article 2 (8) defines an AxMP as “a medicinal product used for the needs of a clinical trial as described in the protocol, but not as an investigational medicinal product”.

6.2. Study Intervention Description

This phase I study is an open-label non-randomized study. Patients are enrolled into dose levels in accordance with the planned dose escalation schedule. Aim of the study is to assess safety and antitumor activity of dinutuximab beta in combination with standard induction chemotherapy. Aim of the trial is to assess the recommended phase 2 dose. The sponsor performs the dose-level allocation after the patient and/or the patient's legal representative has given their written informed consent and the pediatric patient, if appropriate, has given assent.

Furthermore, the patient must have completed the necessary screening assessments. The site staff will register the patient to the database by submission of the registration form. The sponsor will assign the patient to the trial, thereby the subject receives a patient identification number that will be used on all study-related documentation or correspondence (including fax and/or email) referencing that patient to the site. No patient will receive investigational product until the investigator or designee has received the following information in writing from the sponsor:

- Confirmation of the patient’s enrollment and;
- Specification of the dose level for that patient.

6.2.1. Dosing and Administration

Doses will be escalated in a 3+3 design with three pre-defined dose levels, namely Level 1: 50%, Level 2: 75%, Level 3: 100%. At each level at least 3 at max. 6 patients will be included depending on the occurrence of DLT. Starting dose will be 5 mg/m²/d, which equals 50% of the final dose. Dinutuximab beta will be administered as an intravenous infusion once a day over a time of 24 hours, for 10 days. At

Level 1 a toxicity-adjusted dose escalation is implemented based on the observed Common Toxicity Criteria (CTCAE Version 5) grades. Specifically, if no DLTs were observed in the initial 3 patients, CTC grades of ≤ 1 will result in a 100% dose escalation (=Level 3), equaling 10 mg/m^2/d. At least CTC grade =2 will result in a 50% dose escalation (=Level 2), which equals 7.5 mg/m²/d. The dinutuximab beta infusion starts at the end of the standard chemotherapy treatment cycle, administered sequentially after the completion of chemotherapy.

6.3. Preparation/Handling/Storage/Accountability

6.3.1. Acquisition and Accountability

Dinutuximab beta

Dinutuximab beta will be provided by the sponsor. For order and shipment details please refer to the investigator site file and/or Pharmacy File.

IMP must be received by a designated person at the study site and kept in temperature-controlled location. The IMP must be stored according to the storage conditions described on the IMP packaging label and stored in a locked, safe area to prevent unauthorized access.

The investigator or designee is responsible for taking an inventory of each shipment of IMP received and comparing it with the accompanying IMP accountability form. The investigator or designee will verify the accuracy of the information on the form, sign and date it, retain a copy in the study file, and return a copy to the sponsor.

The investigational site must maintain accurate records demonstrating dates and amounts of IMP received, to whom it was administered (subject-by-subject accounting), and accounts of any IMP accidentally or deliberately destroyed or returned. Accurate recording of all IMP administration will be made in the appropriate Section of the subject's CRF and source documents.

GPOH (or designee) will review with the investigator and relevant site personnel the process for IMP return, disposal, and/or destruction including responsibilities for the site versus GPOH (or designee).

Other IMPs

All IMPs (except dinutuximab beta) are expected to be held as routine hospital stock and should therefore be stored and handled according to local institutional policy. Treatment should be prepared and administered according to the relevant Summary of Product Characteristics (SmPC) and local practice unless the trial protocol requires otherwise. See section 6.3.4 for more information.

All IMPs shall be traceable. They shall be stored and destroyed as appropriate and proportionate to ensure the safety of the subject and the reliability and robustness of the data generated in the clinical trial. Compliance and drug accountability will be assessed by the investigator and/or study personnel at each patient visit. The information will be captured in the Drug Accountability Form.

This shall also apply to auxiliary medicinal products. Please also see the Pharmacy File for further details.

6.3.2. Formulation, Appearance, Packaging, and Labelling

Dinutuximab beta is a mouse-human chimeric monoclonal IgG1 antibody produced in a mammalian cell line (CHO) by recombinant DNA technology.

Dinutuximab beta will be supplied in vials, each contains 20 mg dinutuximab beta in 4.5 mL. 1 mL of concentrate contains 4.5 mg dinutuximab beta. Medication will be provided as multi-market pack within commercial packing (with Greek, Polish, Portuguese and English as languages available on the packaging). They will include storage conditions for the drug but no information about the patient. Supportive drug information in local languages will be provided via the trial electronic system.

The container is a clear Type I glass vial (6 mL) with a halobutyl rubber stopper and an aluminum flip-off cap, holding a minimum extractable volume of 4.5 mL of concentrate for infusion solution. Each carton contains 1 vial.

Dinutuximab beta will be provided as concentrate for infusion solution (pharmaceutical form). It appears as colourless to slightly yellow liquid.

Justification for Labelling Exemptions Based on CTR 536/2014 Annex 6, Section 8

In accordance with the provisions of CTR 536/2014, Annex 6, Section 8, the information listed in Sections A, B, and C on the label of an investigational product may be omitted if alternative mechanisms for providing this information are in place, such as the use of a centralized electronic randomization system or a central information system, provided that the safety of the trial participants and the reliability and robustness of the data are not compromised.

For this study, a centralized electronic system will be used for both the provision of dose per patient and the management of essential product information. This system ensures that all necessary data, including dosing and product identification, are securely tracked and available to the study team in real-time, thus maintaining full transparency and traceability of the investigational product.

Furthermore, the investigational product, dinutuximab beta, will not be dispensed directly to the patient. Instead, it will be handled exclusively by the study team, ensuring that administration is controlled and monitored within the clinical site. This reduces the risk of mislabelling or mishandling and ensures that all product-related information is properly recorded and accessible through the central system.

This approach ensures compliance with the regulatory requirements while safeguarding both the safety of the participants and the integrity of the clinical trial data.

All other investigational medicinal products (IMPs) should be used in their authorized formulations, with appropriate appearance, packaging, and labelling according to the legal and regulatory standards of the participating countries.

6.3.3. Product Storage and Stability

Dinutuximab beta has a shelf life of 4 years. Please check the expiry date before use.

The chemical and physical in-use stability for the diluted solution (solution for infusion) has been demonstrated for up to 48 hours at 25 °C (50 mL syringe) and for up to 7 days at 37 ºC (250 mL infusion bag), after cumulative storage in a refrigerator (2 °C – 8 °C) for 72 hours.

From a microbiological point of view, the diluted solution should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would not normally be longer than 24 hours at 2 to 8 °C, unless dilution has taken place in controlled and validated aseptic conditions.

Dinutuximab beta must be stored in a refrigerator (2 °C – 8 °C). The vial must be stored in its original outer carton to protect it from light.

For storage conditions after dilution of the medicinal product, see instructions above.

Dinutuximab beta must be received by designated personnel at the trial site, handled and stored safely and properly, and kept in a secured location to which only the investigator and designated site personnel have access. Upon receipt, Dinutuximab beta should be stored according to the instructions specified in this protocol, the pharmacy file or the shipment documentation/label.

6.3.4. Preparation and precautions for disposal and other handling instructions

The solution for infusion must be prepared under aseptic conditions. The solution must not be exposed to direct sunlight or heat.

The patient specific daily dose of dinutuximab beta is calculated based on body surface area.

Dinutuximab beta should be diluted aseptically to the patient specific concentration/dose with sodium chloride 9 mg/mL (0.9%) solution for infusion containing 1% human albumin (e.g. 5 mL of human albumin 20% per 100 mL sodium chloride solution).

For continuous infusions, the solution for infusion can be prepared freshly daily.

The daily dose depending on the respective dose level and ranges from dose level 1 to dose level 3.

Dose level 1

5 mg/m²/d 10-day continuous infusion once daily as an IV infusion over 24 hours.

Infusions starts at the end of the respective standard chemotherapy i.e. VDC day 3 and IE day 5.

Dose level 2

7.5 mg/m²/d 10-day continuous infusion once daily as an IV infusion over 24 hours.

Infusions starts at the end of the respective standard chemotherapy i.e. VDC day 3 and IE day 6.

Dose level 3

10 mg/m²/d 10-day continuous infusion once daily as an IV infusion over 24 hours.

For continuous 24-hour infusions, the daily dose is 5-10 mg/m^2 and the calculated dose should be diluted in 100 mL sodium chloride 9 mg/mL (0.9%) containing 1% human albumin.

The solution for infusion should be administered via a peripheral or central intravenous line. Other intravenously co-administered agents should be delivered via a separate infusion line. The container should be inspected visually for particulates prior to administration. It is recommended that a 0.22 micrometre in-line filter is used during infusion.

For continuous infusions, any medical device suitable for infusion at a rate of 2 mL per hour can be used, e.g. syringe infusion pumps/infusers, electronic ambulatory infusion pumps. Note that elastomeric pumps are not considered suitable in combination with in-line filters.

Any unused medicinal product or waste material should be disposed of in accordance with local requirements.

7. PRE-SCREENING FOR GD2

GD2 is a disialoganglioside, a type of glycolipid, prominently expressed on the surface of neuroectodermal tumors, such as neuroblastoma and melanoma, as well as in certain types of sarcomas. Its high expression in these cancers makes it a valuable biological marker for diagnosis and targeted therapy (Nazha et al., 2020).

GD2's limited expression in normal tissues, primarily restricted to peripheral nerves, minimizes the risk of adverse effects when targeting GD2 in cancer therapy.

Screening for GD2 expression in Ewing sarcoma can be performed using several techniques, including immunohistochemistry (IHC) (Kailayangiri et al., 2012), flow cytometry (Dobrenkov & Cheung, 2014), and molecular imaging (Theruvath et al., 2022; Wu et al., 1986)

Only patients with GD2-positive Ewing sarcoma are eligible for this study. A sample will be considered positive if GD2 is detectable within the established limit of detection. The limit of detection is determined by using known dilutions of GD2 in a tissue matrix, allowing differentiation between background signals (normal tissue, no GD2 added) and specific GD2 signals. This assessment will be performed by the central laboratory.

If the GD2 tumor status is unknown when the patient is initially considered for this study, patients/guardians will be asked to sign the molecular pre-screening ICF, if the patient is not already enrolled to the clinical trial “iEuroEwing” (Full title: International Euro Ewing (iEuroEwing) trial for treatment optimization in patients with Ewing sarcoma; EU-CT 2022-501180-40-00). The study sites will pre-screen patients for GD2 by sending a tumor or peripheral blood sample to a laboratory designated by GPOH. The tumor sample may be a previously obtained archival sample, or a sample obtained for the purpose of pre-screening for this study (fresh-frozen tumor). Additionally, in case of local GD2 determination, the corresponding report should be submitted along with the patient’s archival tumor sample to the central laboratory, which will perform GD2 detection as a part of the determination of patient’s eligibility for the trial. Provision of the tumor sample to the central laboratory for the GD2 detection is mandatory. The GD2 analysis results will be communicated to the study center within approximately 2 weeks of receipt of the sample.

7.1 Central GD2 determination

The assay used to detect and quantify GD2 in any frozen tissue uses liquid chromatography-coupled tandem mass spectrometry (LC-MS/MS). Upon receipt of frozen tumor tissue samples, these are dried using a SpeedVac and pulverized using a tissue homogenizer. To ensure the accuracy of GD2 positivity determination, a calibration and linearity assay was conducted. The levels of GD2 at known concentrations were evaluated in relation to an internal standard.

The relationship between signal intensity and GD2 concentration was analyzed and demonstrated to be linear within the tested range of concentrations.

- The GD2 assay demonstrated excellent linearity, with a coefficient of determination (R^2) of 0.9996, indicating a strong correlation between the measured responses and the GD2 concentrations.
- The linearity was assessed using a weighted regression approach, specifically employing a weighting factor of $1/y^2$.
- The assay's limit of quantification (LOQ) was determined to be 2.4 pmol in 200 µL, and the limit of detection (LOD) was 0.8 pmol in 200 µl, confirming its sensitivity for low-concentration measurements.

These values were calculated using the three lowest concentrations of the regression line, following one of the standard methods for determining LOQ and LOD: $LOQ = 10\sigma / S$ and $LOD = 3.3\sigma / S$, were σ represents the standard deviation of the response, and S is the slope of the regression line. This method confirms the assay's high sensitivity and reliability for low-concentration GD2 measurements.

Because the amount of GD2 required to answer to anti-GD2 based treatments is not known, the cut-off for GD2 positivity was set at the LOD (0.8 pmol) and the cut-off for GD2 quantification at the LOQ (2.4 pmol). Based on published data assessing GD2 concentration via LC-MS/MS, the GD2 levels in Neuroblastoma range from 40 pmol to 2000 pmol (Paret et al., 2022). In Ewing sarcoma (ES), the GD2 concentration is heterogeneous and in most samples is expected to be at the lower end of the Neuroblastoma range based on methods such as immunohistochemistry (Kailayangiri et al., 2012). Due to the high sensitivity of our assay, also ES samples with low GD2 expression will be detected.

8. SCREENING AND INFORMED CONSENT

The investigators will recruit patients from their existing clientele or from newly referred patients. Additional recruitment measures are not planned and are not ethically justifiable.

Whether a patient is offered participation in the clinical trial is a decision of the respective investigator and is based solely on the inclusion and exclusion criteria. If there are competing studies, each trial site has its own comprehensible rules as to which clinical trials are discussed with the patient. The sponsor will not influence the trial sites regarding expected recruitment or a decision to include a specific patient.

8.1. Informed Consent

Investigators must ensure that they adequately explain the aim, trial treatment, anticipated benefits, and potential risks of taking part in the trial to the patient and/or parent/legal guardian as appropriate. The Investigator should also explain that the patient and/or parent/legal guardian is completely free to refuse to take part or withdraw from the trial at any time. The patient and/or parent/legal guardian should be given sufficient time (e.g. 24 hours, whenever clinically possible) to read the patient information sheets (PIS) and to discuss the patient’s participation with others outside of the site research team if they wish to. The patient and/or parent/legal guardian must be given an opportunity to ask questions which should be answered to their satisfaction. The right of the patient and/or parent/legal guardian to refuse to participate in the trial without giving a reason must be respected.

As the trial includes children and adolescents, written consent/assent will be obtained from the patient wherever it is possible to do so (as appropriate according to age and national legislation). For those children who are not able to read, write or understand regarding assent, the clinician will explain the study and obtain verbal assent which will be documented in the patient's medical records.

Patients should be re-consented at the age of majority in accordance with national guidance/legislation. Minor patients who reach legal age during trial participation will only be allowed to continue participating, if their prior assent is superseded by a legal informed consent.

If the patient and/or parent/legal guardian agrees to participate in the trial, they should be asked to sign and date the latest version of the Informed Consent Form (ICF). The Investigator must then sign and date the form on the same day or later, but before any study-related procedure started. A copy of the ICF should be given to the patient and/or parent/legal guardian, a copy should be filed in the patient's medical records, and the original placed in the Investigator Site File (ISF) or country specific equivalent.

It is strongly recommended that details of the informed consent discussions should be recorded in the patient's medical records; this should include date of, and information regarding, the initial discussion, the date consent was given, with the name of the trial and the version number of the PIS and ICF. Throughout the trial, the patient and/or parent/legal guardian should have the opportunity to ask questions about the trial and any new information that may be relevant to the patient's continued participation should be shared with them in a timely manner. On occasion, it may be necessary to reconsent the patient, in which case the process above should be followed and the patient's right to withdraw from the trial respected.

Investigators will be expected to maintain a screening log of all potential study participants. This log will contain limited information about the potential participant and will include the date and outcome of the screening process.

8.2. Patient numbering

Each patient is identified in the trial by a Patient Number (Patient No.), assigned when the patient is first pre-screened and is retained as the primary identifier for the patient throughout his/her entire participation in the trial. The Patient No. consists of a 4-digit Site Number (Center No.) (as assigned by GPOH to the study site) with a sequential 5-digit patient number suffixed to it, so that each patient is numbered uniquely across the entire database.

Upon signing the informed consent form (ICF), the patient is assigned to the next sequential Patient No. available to the investigator.

8.3. Treatment assignment

The assignment of a patient to a particular cohort will be coordinated by the sponsor.

8.4. Information to be collected on screening failures

Patients who sign the pre-screening ICF or main ICF but do not meet the screening requirements, the inclusion/exclusion criteria or cannot be assigned to a treatment cohort (because the slots are already fully recruited) will be considered a screen failure. The reason will be entered on the Screening Log, and each patient's screening information will be added to the clinical database. No other data will be entered into the clinical database for patients who are screening failures.

Patients who are tested for GD2 but are negative are not documented as screening failures because they do not meet the biological prerequisite for study participation. Screening failure only refers to GD2-positive patients who still do not meet the inclusion criteria or for whom an exclusion criterion is identified during the screening assessments.

9. STUDY PROCEDURES AND ASSESSMENTS

9.1. Prior Medication/Procedures

All prior medications/procedures within 14 days of ICF signing should be recorded. All treatment-related prior medications/procedures should be recorded regardless of time.

9.2. Demographics and Medical history

Demographics including gender and month of birth will be documented at the time of the Screening Visit. A complete medical history including evaluation for past (up to 5 years) or present cardiovascular, respiratory, gastrointestinal, renal, hepatic, neurological, endocrine, lymphatic, haematological, immunologic, dermatological, psychiatric, genitourinary, obstetrical, surgical history, including those symptoms related to the Ewing sarcoma, or any other diseases or disorders will be performed at screening.

9.3. Vital signs

Vital signs (including sitting blood pressure, heart rate, respiration, and temperature) will be measured at each visit from the time of signing of the ICF until last follow-up visit, including any unscheduled visits. Recordings of systolic and diastolic blood pressures (by standard sphygmomanometer reading), heart rate, and temperature should be made after the subject has been sitting or lying, supine or semi recumbent, for at least 5 minutes. Close monitoring of the cardiac function should be implemented. ECG should be performed prior each cycle and whenever clinically indicated.

9.4. Body Surface Area

Height in centimetres (cm) and body weight (to the nearest 0.1 kilogram (kg) in indoor clothing, but without shoes) will be measured. Body surface area (BSA) will be calculated using the patient's weight and height, in accordance with local site procedure. The baseline BSA will be used to determine the dose at C1D1 latest. Height, weight and BSA may be performed within 5 days prior to the start of a cycle and ±1 day for other visits.

9.5. Physical examination

All subjects will have a complete physical examination at screening until last follow-up and at any unscheduled visits as clinically indicated. Subjects, who discontinue treatment should have a physical examination done at the 28-day Safety Follow-Up Visit.

9.6. Performance status

Performance status will be assessed using the ECOG or Lansky performance scales, depending on the patient's age (Appendix I). The assessment can be performed within 5 days prior to the start of a cycle and with a ±1-day window for other visits. For patients turning 13 during the study, the Lansky scale will continue to be used throughout the study period to maintain consistency in assessment.

9.7. Laboratory evaluations

Laboratory tests will be conducted and analysed by the trial site's local laboratory, even if the study medication is temporarily halted. More frequent examinations can be performed at the investigator's discretion if medically necessary.

Efforts should be made to minimize the volume of blood drawn throughout the study. Use the smallest tube required by the local laboratory for each test and combine blood draws for routine safety labs to minimize wastage and reduce the number of venepunctures. If a patient has an indwelling venous catheter suitable for blood draws, it should be utilized whenever possible. The maximum volume of blood that may be drawn within a 24-hour period is 0.8 mL/kg, and within a 4-week period is 2.4 mL/kg according to the "Ethical considerations for clinical trials on medicinal products conducted with the paediatric population." Eur J Health Law. 2008 Jul;15 (2):223-50. The maximum blood volumes required by this protocol over 24-hour and 4-week periods are provided in the table below. If the volume

requested exceeds these limits for a particular patient, the additional blood samples should not be taken unless necessary for the patient's medical care. If it is anticipated that the volume limit will be exceeded, prioritize haematology, chemistry, and pregnancy test samples.

Assessment	Volume per Assessment	Screening	1. Cycle	2. Cycle	3. Cycle	4. Cycle	5. Cycle	EoT	FU
Weeks		2	2-4	2-4	2-4	2-4	2-4	2-4	4
Serum Pregnancy Testing[1]	2 ml	x	x	x	x	x	x		
Blood Count	0.2 ml	x	x	x	x	x	x		
Serum Chemistry	2.4 ml	x	x	x	x	x	x		
Virus diagnostics	2ml	x							
PK	2 ml*		x*	x*	x*	x*	x*		
CDC	2 ml**		x*	x*	x*	x*	x**		
GD2 liquid biopsy	7 ml	x	x	x	x	x	x	x	x
Max.	**21.6 ml**	**13.6 ml**	**19.6 ml**	**19.6 ml**	**19.6 ml**	**19.6 ml**	**21.6 ml**	**7 ml**	**7ml**

[1] Not applicable to infants and male participants and can be replaced by a urine pregnancy test
* samples collected twice
** samples collected three times

At any point during the study, any clinically relevant abnormal laboratory parameters (such as those requiring dose modification, interruption of a cycle, leading to clinical signs or symptoms, or necessitating therapeutic intervention) must be documented in the database, regardless of whether they are specifically requested in the protocol.

GPOH will receive a copy of the laboratory certification and a tabulation of normal ranges for each required parameter. Additionally, if a patient has laboratory tests conducted by a different external laboratory, GPOH must also be provided with the certification and normal range tabulation from that laboratory.

9.8. Pharmacokinetic (PK) and Complement-Dependent Cytotoxicity (CDC) Sampling

The collection of blood samples for the specified purposes is part of the exploratory endpoint.

PK samples will be collected during all cycles at the following time points:

- Prior to the antibody (Ab) infusion (pre-dose)
- Day 10, immediately after the end of the infusion

Complement-Dependent Cytotoxicity (CDC) will be collected at the following time points:

- during the cycle 1-4:
  - Prior to the antibody infusion
  - Day 10 immediately after the end of the infusion
- during the cycle 5:
  - Prior to the antibody infusion
  - Day 10 immediately after the end of the infusion
  - After the end of the antibody infusion on Day 21

Details are given in the respective lab manual.

In case that the decision is taken to discontinue the study treatment before cycle 5, within the window given in table above Section 9.7, for CDC please take an additional sample after the end of the antibody infusion on Day 21 of this respective last cycle.

9.9. Pregnancy Testing

A ß-HCG pregnancy test will be obtained in females of childbearing potential (FCBP) (see Inclusion Criteria for definition) regularly at screening until end of treatment in serum.

If a subject achieves menarche during the study period, the subject must start having pregnancy testing performed as per protocol. The subject may not receive the protocol treatment until the investigator has verified that the result of the pregnancy test is negative. Any pregnancies that occur in women, who have received study treatment must be reported.

In addition, FCBP and male subjects that have reached puberty and are less than 18 years of age must agree to undergo physician-approved reproductive education and discuss the side effects of the chemotherapy on reproduction with parent/parents and/or guardians/guardians at screening according to local practice.

9.10. Imaging

During the screening phase (within 28 days prior to the start of the first protocol cycle), all patients will undergo either a CT scan with intravenous contrast or an MRI. For patients who cannot tolerate iodine-based contrast agents, CTs may be performed without contrast, or an MRI may be utilized. Visible skin lesions and palpable subcutaneous tumours will be measured through physical examination using a ruler or callipers. Ultrasound should not be used for measuring disease sites.

Local imaging during screening and at the end of treatment (EOT) should be performed in line with initial imaging method of tumor and metastases site(s). Where FDG-PET-CT/MRI was already indicated to detected burden of disease during initial diagnosis, it is highly recommended to perform same method during screening and at EOT within the trial. All imaging methods are expected to be performed according to local practice and cover all known tumour sites.

Subsequent disease evaluations will be conducted only if regular patient care necessitates imaging. After the last cycle, a disease evaluation must be performed on Day 15 (±7 days), and before initiating any further treatment (including additional chemotherapy, local therapy, or radiation therapy), or sooner if there is clinical evidence of disease progression. If local protocol includes a disease evaluation at EOT, these data can be additionally recorded. If the last disease evaluation was within 28 days of EOT or if there is already documented evidence of disease progression, repeat evaluations at EOT are not necessary.

Disease evaluations following the screening will include all disease sites identified at baseline, using the same methods as at the screening. Regions without evidence of disease at baseline do not need to be imaged in subsequent assessments unless there is clinical concern for a new lesion in that region.

Reference Evaluation of Images:

All images obtained during the clinical trial and follow-up will be reviewed by a radiology reference panel appointed by the sponsor. Disease will be assessed according to RECIST v1.1 at a central review for CT and MRI and will determine disease response and progression.

10. TREATMENT

10.1. Study Treatment

All dosages of the treatment cycles prescribed and dispensed to the patient and all dose changes during the trial must be recorded on the Dosage Administration Records within the eCRF.

Alternating cycles of VDC/IE + dinutuximab beta should be given at 21-day intervals +/- 7 days and upon haematological recovery to absolute neutrophil count (ANC) ≥ $0.75*10^9$/L and platelets ≥ $75*10^9$/L.

Average treatment time of 21 days is assumed and begin of next cycle should be made by local investigator if starting criteria of haematological recovery are met and no other laboratory findings are present, which could in investigators view increase patients' risk or require any intervention.

Time between day1 of two cycles should not be less than 14 days and not longer than 28 days. Therapy should be continued as soon as starting criteria for the next cycle are fulfilled.

Treatment Plan for VDC + Dinutuximab beta

DRUG	DAILY DOSE	APPLICATION DAY IN CYCLE	CUMULATIVE DOSE PER CYCLE	ROUTE AND DURATION OF ADMINISTRATION
Vincristine	2 mg/m^2/d, max. 2 mg/single dose	d1	2 mg/m^2/cycle	IV push or short infusion
Doxorubicin	37.5 mg/m^2/d	d1, d2	75 mg/m^2/cycle	IV infusion, 24 h
Cyclophosphamide	1200 mg/m^2/d	d1	1200 mg/m^2/cycle	IV infusion, 1 h
Dinutuximab beta	See Section 10.1.1	d3-12	See Section 10.1.1	IV infusion, 24 h

Continuation with IE upon haematological recovery to white blood cell count (WBC) ≥ 2.0*10^9/L with ANC ≥ 1.0*10^9/L and platelets ≥ 80*10^9/L.

Treatment Plan for IE + Dinutuximab beta

DRUG	DAILY DOSE	APPLICATION DAY IN CYCLE	CUMULATIVE DOSE PER CYCLE	ROUTE AND DURATION OF ADMINISTRATION
Ifosfamide	1800 mg/m^2/d	d1-d5	9 g/m^2/cycle	IV infusion, 4 h
Etoposide	100 mg/m^2/d	d1-d5	500 mg/m^2/cycle	IV infusion, 2 h
Dinutuximab beta	See Section 10.1.1	d6-d15	See Section 10.1.1	IV infusion, 24 h

10.1.1. Dosing Regimen

Doses will be escalated in a 3+3 design with three pre-defined dose levels, namely Level 1: 50%, Level 2: 75%, Level 3: 100%.

Dinutuximab beta dose will be assessed in a 3+3 design.

Dose level 1

5 mg/m²/d 10-day continuous infusion once daily as an IV infusion over 24 hours

Infusions starts at the end of the respective standard chemotherapy i.e. VDC day 3 and IE day 6.

Dose level 2

7,5 mg/m²/d 10-day continuous infusion once daily as an IV infusion over 24 hours.

Infusions starts at the end of the respective standard chemotherapy i.e. VDC day 3 and IE day 6.

Dose level 3

10 mg/m²/d 10-day continuous infusion once daily as an IV infusion over 24 hours.

Infusions start at the end of the respective standard chemotherapy VDC day 3 and IE day 6.

Starting dose will be 5 mg/m²/d, which equals 50% of the final dose. At each level at least 3 at max. 6 patients will be included depending on the occurrence of DLT. At Level 1 a toxicity-adjusted dose escalation is implemented based on the observed Common Toxicity Criteria (CTCAE Version 5) grades. Specifically, a CTC grades of $\leq$ 1 will result in a 100% dose escalation (=Level 3). At least one CTC grade=2 will result in a 50% dose escalation (=Level 2).

10.1.2. Treatment Duration of Dinutuximab Beta

Dinutuximab beta will be administered as a continuous intravenous infusion over a period of 10 days. Each infusion will be administered over 24 hours following the last day of the standard induction chemotherapy cycle. Dinutuximab beta will be administered in addition to the last 5 cycles of induction chemotherapy, consisting of alternating cycles of Vincristine, Doxorubicin and Cyclophosphamide and Ifosfamide and Etoposide.

10.2. Dose Escalation Guidelines

10.2.1. Starting Dose Rationale

Doses will be escalated in a 3+3 design with three pre-defined dose levels. Starting dose will be 5 mg/m²/d, which equals 50% of the final dose.

10.2.2. Provisional Dose Levels

The dose escalation will continue until the MTD and/or the RDE dose is reached, based on the schedule given in Section 10.1.1. Following completion of the dose escalation in this clinical trial, a dose can be chosen for the expansion part within a phase II clinical trial. The dose for a potential expansion will be opened at either the MTD, or a lower dose that is determined to be the RDE.

10.2.3. Guidelines for dose escalation and determination of Maximum Tolerated Dose (MTD)

For the purposes of dose escalation decisions, each cohort will consist of 3 to 6 newly enrolled patients who will be treated at the specified dose level. The first cohort will be treated with the starting dose of 5 mg/m²/d.

Patients must complete a minimum of 1 cycle of treatment with the minimum safety evaluation and drug exposure or have had a DLT within the first cycle of treatment to be considered evaluable for dose escalation decisions. Dose escalation decisions will occur when the cohort of patients has met these criteria.

Dose escalation decisions will be made by DSMC and GPOH trial team. Decisions will be based on a synthesis of all relevant data available from all dose levels evaluated in the ongoing study, including safety information, DLTs, all CTCAE Grade ≥ 2 toxicity data during Cycle 1.

Within CESS-GD2 the dose escalation involves treating small cohorts of patients with increasing doses of the dinutuximab beta, monitoring for dose-limiting toxicities (DLTs), and deciding whether to escalate, de-escalate, or stop dose escalation based on observed toxicities. The trial will be conducted according to the following dose escalation schedule to determine the MTD and RDE:

Initial Cohort at Dose Level 1: 5 mg/m²/day

Cohort 1 (3 patients)	
Observation:	Treatment of 3 patients at the starting dose of 5 mg/m²/day.

Outcome:	• If 0 out of 3 patients experience a DLT:
	  ○ CTC ≤ 1 Escalate to 100% dose level (10 mg/m²/day)
	  ○ CTC ≥ 2 Escalate to 75% dose level (7.5 mg/m²/day)
	• If 1 out of 3 patients experience a DLT: Enroll 3 more patients at the same dose level
	• If 2 or more out of 3 patients experience a DLT: stop the trial

Expansion Cohort 1 (if needed)	
Observation:	Treatment of 3 **additional** patients at the dose level of 5 mg/m²/day.
Outcome:	• If 1 or fewer out of these 6 patients experience a DLT in total: Escalate to the next dose level (7,5 mg/m²/day) • If 2 or more out of these 6 patients experience a DLT: stop the trial

Second Cohort at 7.5 mg/m²/day

Cohort 2 (3 patients)	
Observation:	Treatment of 3 patients at the second dose level of 7.5 mg/m²/day.
Outcome:	• If 0 out of 3 patients experience a DLT • If 1 out of 3 patients experience a DLT: Enroll 3 more patients at the same dose level • If 2 or more out of 3 patients experience a DLT: stop the trial
Expansion Cohort 2 (if needed)	
Observation:	Treatment of 3 **additional** patients at the dose level of 7,5 mg/m²/day.
Outcome:	• If 1 or fewer out of these 6 patients experience a DLT in total: Escalate to the next dose level (10 mg/m²/day) • If 2 or more out of these 6 patients experience a DLT: stop the trial

Third Cohort at 10 mg/m²/day

Cohort 3 (3 patients)	
Observation:	Treatment of 3 patients at the second dose level of 10 mg/m²/day.
Outcome:	• If 0 out of 3 patients experience a DLT: This dose level may be considered safe, and potentially, no further escalation is possible • If 1 out of 3 patients experience a DLT: Enroll 3 more patients at the same dose level • If 2 or more out of 3 patients experience a DLT: stop the trial
Expansion Cohort 3 (if needed)	
Observation:	Treatment of 3 **additional** patients at the dose level of 10 mg/m²/day.
Outcome:	• If 1 or fewer out of these 6 patients experienced a DLT, 10 mg/m²/day can be regarded as MTD • If 2 or more out of these 6 patients experience a DLT: stop the trial

Summary of Decisions Making

Observation	**Decision**
No DLTs	Escalate to the next dose level
1 DLT in 3 patients	Expand cohort to 6 patients
2 or more DLTs per cohort	stop the trial

The aim is to determine the highest dose at which fewer than 2 out of 6 patients experience a DLT, which will be considered the MTD.

The dosage will start at 5 mg/m² per day, which is 50% of the target final dose. In Level 1, a toxicity-adjusted dose escalation will be applied based on the severity of side effects observed according to the Common Toxicity Criteria (CTCAE Version 5). If the observed toxicity is Grade 1 or lower, the dose will be escalated directly to the full final dose (Level 3), skipping the intermediate dose level. However, if a Grade 2 toxicity is observed, the dose will only be increased to 75% of the final dose (Level 2), without skipping any levels.

10.2.4. Definitions of dose limiting toxicities (DLTs)

All dose levels of dinutuximab beta will be evaluated for the occurrence of dose limiting toxicities (DLT).

A DLT is defined as an adverse event or abnormal laboratory value assessed as unrelated to disease, disease progression, inter-current illness, or concomitant therapies that occurs within the first 21 days (Cycle 1) of treatment with the protocol compliant treatment and meets any of the criteria included in listing below.

A study participant will be considered evaluable for a DLT if at least one cycle of dinutuximab beta in combination with chemotherapy (VDC/IE) is administered.

Participants who discontinue treatment in the first cycle for reasons unrelated to the IMPs toxicity, are not evaluable for DLT and will be replaced in enrolment (maximum number of replacement subjects will be 3 per dose level). In case of doubt, the DSMC will determine whether toxicity qualifies as DLT.

DLTs will be assessed within the Common Toxicity Criteria for Adverse Events (CTCAE Version 5.0).

The following are considered drug-related DLTs:

- allergic reaction grade ≥3,
- any immune mediated adverse event, regardless of grade, which requires discontinuation of study treatment,
- serum sickness grade ≥3,
- severe, unrelenting neuropathic pain (grade ≥3) unresponsive to continuous infusion of morphine and gabapentin,
- any of the following neurotoxicities:
    - sensory changes grade ≥3 interfering with daily activities >2 weeks after completing dinutuximab beta therapy,
    - objective motor weaknesses grade ≥3,
    - vision toxicity grade ≥3,
    - prolonged grade ≥3 peripheral motor neuropathy;
- hyponatremia grade ≥3 (125-129 mmol/L symptomatic; 120-124 mmol/L regardless of symptoms) lasting >24 hours despite appropriate fluid management,
- capillary leak syndrome grade ≥3,
- cardiac toxicity grade ≥3,
- any grade ≥4 toxicity requiring any mechanical ventilation/inotropic support/hemofiltration and therefore, intensive care unit hospitalization, except for sepsis as an expected side effect of chemotherapy,
- any toxic death (grade 5 toxicity).

10.3. Dose Modifications

For vincristine, doxorubicin, cyclophosphamide, ifosfamide and etoposide: In patients < 10 kg, the calculation of dosage is converted from m² BSA to kg BW divided by 30.

For dinutuximab beta: No dose modifications are allowed, beside those described in section 10.2.3 .

NOTE: The maximum single dose for vincristine is 2 mg.

Symptom specific dose modifications are outlined below.

10.3.1. Hematological Toxicity

Dose / time intensity is regarded as an essential aspect of induction part. In case of significant bone marrow toxicity, preference is given to early G-CSF support rather than dose reduction in order to maintain dose intensity.

If significant toxicity continues as defined by hematological recovery delayed > 6 days:

- if hematological recovery to ANC ³ 0.75*109/L and platelets ³ 75*109/L is delayed > 6 days before VDC: Reduce doxorubicin and cyclophosphamide dose by 20% in the next cycle. If ANC recovers in the next cycle until day 18, increase doses by 20%,
- if hematological recovery to white blood cell count (WBC) ³ 2.0*109/L with ANC ³ 1.0*109/L and platelets ³ 80*109/L is delayed > 6 days before IE: Reduce isocyanides and etoposide dose by 20% in the next cycle. If ANC recovers in the next cycle until day 18, increase doses by 20%.

If neutropenic sepsis CTCAE grade ≥ 3 occurs:

- if neutropenic sepsis CTCAE grade ≥ 3 occurs before VDC: Reduce doxorubicin and cyclophosphamide dose by 20% in the next cycle. If ANC recovers in the next cycle until day 18, increase doses by 20%,
- if neutropenic sepsis CTCAE grade ≥ 3 occurs before IE: Reduce ifosfamide and etoposide dose by 20% in the next cycle. If ANC recovers in the next cycle until day 18, increase doses by 20%.

In the event of further episodes of toxicity, the dose is to be reduced by an additional 20%. If necessary, it is advised to omit etoposide completely rather than reducing the doses of the other four drugs.

10.3.2. Mucositis / Gastrointestinal Toxicity CTCAE Grade ≥ 3

If mucositis / gastrointestinal toxicity CTCAE grade ≥ 3 occurs:

- If mucositis / gastrointestinal toxicity CTCAE grade ≥ 3 occurs and persists beyond day 18 after start of VDC: Reduce doxorubicin dose by 20% in the next cycle.
- If mucositis / gastrointestinal toxicity CTCAE grade ≥ 3 occurs and persists beyond day 21 after start IE: Reduce ifosfamide and etoposide dose by 20% in the next cycle.

10.3.3. Nephrotoxicity / Renal Function Monitoring

It is recommended to monitor serum creatinine prior to each cycle of ifosfamide. Glomerular function is to be assessed according to national group guidelines, applying either isotope clearance or calculated creatinine clearance.

Schwartz's Formula (1-18 years)

According to Schwartz's formula (Schwartz et al., 1987), creatinine clearance (Ccrea) can be calculated from single serum samples:

$$C_{crea} = \frac{F \times Height\ [cm]}{Crea_{serum}[mg/dL]}\ [mL/min/1.73 m^2]$$

where **F** is proportional to body muscle mass, hence depending on age and sex:

Infants (< 1 year of age)	**F** = 0.45
Males, 1-16 years	**F** = 0.55
Females, 1-21 years	**F** = 0.55
Males, > 16-21 years	**F** = 0.70

Normal values [ml/min/1.73 m²]:

Infants (7 days):	45
Infants (6 months):	60-80
> 1 year:	120

Cockroft-Gault Formula (> 18 years)

Females $\frac{1.05\,(140 - age\ (yrs))\ wt(kg)}{Crea_{serum}[\mu mol/L]}$ or $\frac{0.85\ (140 - age\ (yrs))\ wt(kg)}{72 \times Crea_{serum}[mg/dL]}$

Males $\frac{1.25\,(140 - \text{age (yrs)})\ \text{wt(kg)}}{\text{Crea}_{\text{serum}}[\mu\text{mol/L}]}$ or $\frac{(140 - \text{age (yrs)})\ \text{wt(kg)}}{72 \times \text{Crea}_{\text{serum}}[\text{mg/dL}]}$

NOTE: These equations are not confirmed in patients receiving repeated cycles of intensive chemotherapy or in adolescents. Renal function may be overestimated by these methods.

Tubular Function (Tp/Ccrea or Tmp/GFR)

Tubular function may be assessed using serum electrolyte and bicarbonate levels and the calculation of fractionated phosphate reabsorption, relative amino acid reabsorption and / or fractionated sodium excretion from single urine samples (Rossi et al., 1994; Rossi et al., 1995)

Fractionated phosphate reabsorption:

Reference values in three age groups. "limit" refers to mean – 2 SD for T_P/Ccrea

	< 1 month		1-12 months		> 1 year	
	mean	limit	mean	limit	mean	limit
T_P/Ccrea [µmol/ml]	2.13	1.90	2.10	1.00	1.50	1.07

Ifosfamide Adjustment to Renal Function:

If either glomerular filtration rate (GFR) or Tp/Ccrea (Tm_p/GFR) or HCO_3 is reduced, classify toxicity as grade 0/1, 2, or 3/4 and adjust ifosfamide treatment as indicated:

Toxicity grade*	GFR (ml/min/1.73 m^2)	Tp/Ccrea (Tm_p/GFR) (mmol/L)	HCO_3# (mmol/L)	Action (apply worst grade)
0 / 1	≥ 60	≥ 1.00	≥ 17.0	Continue ifosfamide dose at 100%
2	40-59	0.80 - 0.99	14.0 - 16.9	Reduce ifosfamide dose by 30%
3 / 4	≤ 40	≤ 0.80	≤ 1 4.0	Use cyclophosphamide instead, 1500 mg/m²/d, d1

* Toxicity is graded from 0 to 4, analogous to the CTCAE system; however, for the purpose of modifying treatment, grades 0 and 1 and grades 3 and 4 are considered together.

Low values of HCO_3 may be re-checked when the patient is clinically stable (to rule out infection as a cause, etc.) before modifying ifosfamide dose / treatment.

Etoposide Adjustment to Renal Function:

If GFR < 60 ml/min/1.73 m^2, reduce etoposide dose by 30%.

10.3.4. Cardiac Toxicity

If SF < 28% or left ventricular ejection fraction < 40% or parameters decrease by an absolute value of ³ 10 percentile points from previous tests, delay chemotherapy cycle for seven days and repeat echocardiography:

- if SF has recovered to ≥ 29%, proceed to next cycle,
- if SF remains < 29%, omit doxorubicin and substitute actinomycin D of 0.75 mg/m^2/d (d1, d2) (IV push) (1.5 mg/m^2/cycle) (max. single dose/d: 1.5 mg/d).

Repeat cardiac tests prior to next doxorubicin-containing cycle. If results have normalised, apply doxorubicin at normal dosage. If SF remains abnormal, please see paragraph above.

10.3.5. Neurotoxicity CTCAE Grade ≥ 3

Ifosfamide-induced Encephalopathy:

If ifosfamide-induced encephalopathy occurs supportive care may be given according to institutional guidelines. Ifosfamide infusion may be prolonged to 4-8 hours according to institutional guidelines.

If repetitive CTCAE grade ≥ 3 central neurotoxicity occurs in association with ifosfamide, consider withholding ifosfamide and substitute cyclophosphamide 1500 mg/m² BSA/cycle on day 1. Substitution with cyclophosphamide is only on day 1.

10.4. Follow up for toxicities

Beside the regular trial follow up of 3 months after the last dose of an IMP, patients whose treatment is interrupted or permanently discontinued due to an adverse event (AE) or an abnormal laboratory value deemed treatment-related must be followed until the toxicity resolves or stabilizes. The follow-up procedures for patients experiencing specific dose-limiting toxicities are detailed in the table below. In addition to the parameters listed in the table, other relevant clinical and laboratory metrics should be monitored as clinically appropriate.

In general, toxicities should be followed until they resolve to grade 1 or the patient's baseline value. However, frequent follow-up as outlined in table may be discontinued if the toxicity has stabilized at a higher level and is considered irreversible.

Toxicity	Follow-up assessment
Hematology	If CTCAE grade 4 neutropenia, thrombocytopenia, or anaemia, or grade 3 or 4 febrile neutropenia occurs, white blood cell count with differential (including neutrophil count), platelet count, and haemoglobin must be measured after 3 days, and then at least once a week until the condition resolves to ≤ CTCAE grade 2 or returns to baseline.
Hepatic/Liver Toxicitiy	If a hepatic DLT occurs, ALT, AST, and total bilirubin levels must be measured at least once a week until they resolve to ≤ CTCAE grade 1 or return to baseline. For patients with total bilirubin levels ≥ grade 2 (regardless of duration), bilirubin should be fractionated into total/direct or indirect/direct components, and any additional evaluations indicated by these results should be conducted.
Gastrointestinal	If vomiting or diarrhoea DLTs occur, blood electrolytes must be measured at least once a week until the symptoms resolve to ≤ CTCAE grade 1 or return to baseline. If a nausea DLT occurs without clinically relevant vomiting or diarrhoea, the patient must be monitored weekly, as clinically appropriate, until the condition resolves to ≤ CTCAE grade 2 or baseline.

Lung	Monitor patients for pulmonary symptoms indicative of pneumonitis and hold treatment during diagnostic evaluation. Exclude infectious causes and follow the dose modification guidelines outlined in Section 10.3.
Cardiac	If QTc exceeds 500 ms, blood electrolytes, including calcium, magnesium, phosphorus, and potassium, should be measured and any abnormalities corrected. Concomitant medications should be reviewed for other potential causes of QTc prolongation. An ECG should be repeated daily until the QTc prolongation resolves to grade 1 or less, and the patient should be evaluated by a qualified physician before leaving the trial site.
Other Toxicities	Patients who experience other DLTs must be evaluated at least once a week, including appropriate laboratory assessments, until the condition resolves to CTCAE grade 1 or the patient's baseline.

Please note: Follow-up for toxicities ends either when the toxicities decrease as described in the table, when the patient receives further therapy outside the trial (whether chemotherapy, local therapy, or radiation), or when the regular follow-up period has concluded. Whichever occurs first.

11. CONCOMITANT THERAPY

11.1. Required Concomitant Therapy as part of supportive care

An anticipated side effect of the treatment with dinutuximab beta is neuropathic pain. Therefore, in the first cycles patients will be treated with gabapentin p.o. and morphine i.v. (Barone et al., 2021). The treatment in the following cycles can be adapted.

Gabapentin

Gabapentin should be administered before every infusion cycle of dinutuximab beta. Patients should begin with an oral gabapentin medication three days prior to the dinutuximab beta cycle. Recommended oral dose of gabapentin is 1x10 mg/kg/d three days prior to the dinutuximab beta administration and should be increased to 2x10 mg/kg/d 2 days before the start of dinutuximab beta administration and, if required by the patient, further increased to 3x10 mg/kg/d during the antibody infusion. The treatment with gabapentin should be decreased and finally stopped in the reverse order starting on the first day after the end of dinutuximab beta infusion (i.e. day 1 after dinutuximab beta treatment: 2 × 10 mg/kg p.o.; day 2 after dinutuximab beta treatment: 1 × 10 mg/kg p.o.; day 3 after dinutuximab beta treatment: stop).

Morphine

As a prevention of severe visceral and neuropathic pain opioids are the standard medications given with dinutuximab beta. Doses of morphine should be started and then gradually increase. Side effects include: respiratory depression and sedation, nausea, vomiting, constipation, pruritus, urinary retention, lowered seizure threshold.

During the first cycle i.v. morphine should be given as follows:

- morphine loading infusion (30 µg/kg/h) over 60 minutes before the start of the continuous infusion of dinutuximab beta.

- afterwards, administration of morphine sulfate as a continuous infusion on the first day with a rate of 30 µg/kg/h. Morphine boluses can be given as required. Depending on the individual patient's pain tolerance, treatment with morphine can then be decreased and doses can be adapted in the following cycles.

Other opioids are allowed in the decreasing phase and after continuous morphine infusion, i.e.:

- oral morphine; administered at a dose of 0.2 to 0.4 mg/kg every 4-6 hours,
- oral tramadol, if pain is well-controlled on low doses of p.o. morphine.

11.2. Permitted concomitant Therapy

Expected inflammatory side effects may require a treatment with antipyretic drugs. An antihistaminergic prophylaxis and prevention of gastritis is recommended. All concomitant therapy is permitted according to institutional guidelines as not stated otherwise in this protocol.

11.3. Prohibited concomitant therapy

Below prohibited medications are listed. The use of these medications is a protocol violation and must be recorded in the electronic case report form (eCRF).

Intravenous Immunoglobulins

Due to the risk of interference with dinutuximab beta-dependent cellular cytotoxicity, the treatment with intravenous immunoglobulins is not allowed.

Vaccination

Vaccinations are not allowed during administration of dinutuximab beta until 10 weeks after the last treatment course, due to the risk for neurological toxicities due to immune stimulation through dinutuximab beta.

Other medications

Furthermore, treatment with any other systemic anticancer treatments not described in this protocol are not allowed.

11.4. Contraceptive Requirements

To this protocol, methods that can achieve a failure rate of less than 1% per year when used consistently and correctly are considered as highly effective birth control methods. Such methods include:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation[1]:
    - oral
    - intravaginal
    - transdermal
- progestogen-only hormonal contraception associated with inhibition of ovulation[1]:
    - oral
    - injectable
    - implantable[2]
- intrauterine device (IUD) [2]
- intrauterine hormone-releasing system (IUS) [2]
- bilateral tubal occlusion [2]
- vasectomised partner [2,3]
- sexual abstinence[4]

[1] Hormonal contraception may be susceptible to interaction with the IMP, which may reduce the efficacy of the contraception method

[2] Contraception methods that in the context of this guidance are considered to have low user dependency.

[3] Vasectomised partner is a highly effective birth control method if partner is the sole sexual partner of the WOCBP trial participant and that the vasectomised partner has received medical assessment of the surgical success.

[4] In the context of this guidance sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.

For male subjects with WOCBP the following methods must be considered:

- condom,
- consider contraception for non-pregnant WOCBP partner,
- contraception during treatment and until the end of relevant systemic exposure in the male subject.

Duration of Contraconception/Contraception methods in subjects should be at least 6 months.

Duration might be extended depending on further treatment or according to local procedure. Due to the potential impact on fertility, patients and care givers should seek advice on fertility preservation, prior to treatment given by the investigator.

12. OTHER SUPPORTIVE CARE

The outlined therapeutic regimen within this trial protocol is intensive and aggressive and will be followed by severe bone marrow depression. Hence, treatment according to this protocol should be restricted to institutions with knowledge in both the administration of intensive, aggressive combinational chemotherapy and the handling and feasibility of full supportive care.

In paediatric patients only those preparations should be used that are either approved for use in children (according to the SmPC) or whose dose-precise and safe use complies with current guidelines.

13. STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

If a patient stops CESS-GD2 protocol treatment, the reason should be recorded in the patient's medical records and be reported on the appropriate CRF whether it is due to either the patient's, parent/legal guardian's or clinician's decision. Reasons for stopping protocol treatment include, but are not limited to:

- the patient and/or patient's parent/guardian does not wish to continue with further trial treatment,
- unacceptable toxicity/DLT,
- disease progression whilst on therapy or other important medical events,
- pregnancy.

In case of the listed criteria treatment must be discontinued.

CESS-GD2 will be analysed on an intention-to-treat (ITT) basis and all patients who stop trial treatment will remain in the trial for follow-up unless the patient and/or parent/legal guardian explicitly withdraws consent for data collection.

13.1. Patient replacement

Patients who have not received dinutuximab beta will be replaced. If a patient is deemed as non-evaluable for the Dose-determining set, enrolment of a new patient to the current cohort will be considered if there is less than the required number of evaluable patients. Enrolment of new patients may be considered until at least the minimum number (3) or at most the maximum number (6) of evaluable patients is achieved within the cohort. Minimum and maximum numbers of evaluable patients per cohort are defined in Section 15.2.

13.2. Withdrawal of consent to data collection

The patient and/or parent/legal guardian may withdraw consent at any time during the study. For the purposes of this trial, withdrawal is defined as:

The patient would like to withdraw from trial medication and is not willing to be followed up for the purposes of the trial at any further visits (i.e. only data collected prior to the withdrawal of consent can be used in the trial analysis).

The details of withdrawal should be clearly documented in the patient's medical records. A patient's wishes with respect to their data must be respected.

13.3. Lost to follow-up

If a patient is lost to follow-up, every effort should be made to contact the patient's primary physician to obtain information on the patient's status. Similarly, if a patient's care is transferred to another clinician, the Sponsor should be informed, and follow-up information be obtained.

14. SAFETY MONITORING AND REPORTING

14.1. Definition and Reporting of Adverse Events (AE)

An adverse event is defined as the onset of, or an increase in, any unwanted sign(s), symptom(s), or medical condition(s) that occur after the patient and/or guardian has signed the informed consent form (ICF).

AEs will be assessed continuously and graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

Abnormal laboratory values or test results that arise after informed consent has been given are considered adverse events only if they cause clinical signs or symptoms, are deemed clinically significant, require medical intervention (such as a transfusion or haematological stem cell support), or necessitate changes to the study medication(s). See for details section 14.1.1.

Except for screening failures, adverse events that begin or worsen after informed consent should be recorded in the Adverse Events CRF. Pre-existing conditions at the time of informed consent should be documented in the Relevant Medical History/Current Medical Conditions section of the patient's CRF. Adverse event monitoring should continue for at least 30 days following the final dose of the last cycle within the protocol. Adverse events, including lab abnormalities constituting AEs, should be described using a diagnosis whenever possible, rather than listing individual signs and symptoms. If a clear diagnosis cannot be determined, each sign or symptom should be reported as a separate adverse event.

If CTCAE grading is unavailable for an adverse event, the severity will be classified as mild, moderate, severe, or life-threatening, corresponding to Grades 1 through 4. CTCAE Grade 5 (death) requires detailed information about the event and will be recorded on the End of Treatment (EOT) or death form.

Adverse events should be identified through non-directive questioning of the patient or guardian during the screening process after informed consent has been obtained and at each subsequent visit during the study. Adverse events may also be identified if reported voluntarily by the patient during the screening process or between visits, or through physical examination, laboratory tests, or other assessments.

Adverse events will be systematically recorded and presented in the form of a classic line listing for thorough and clear analysis. The line listing will contain the following information for each reported AE:

- **Event Term/Description** – According to NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0
- **Duration** – The time period over which the adverse event persisted, including both onset and resolution dates or categorized as "Ongoing" at EoT,
- **Severity** – According to NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (grading from 1-5)
- **Relationship to Trial Treatment** – The investigator's assessment of the relationship to trial treatment; reasonable possibility that AE is related: "yes" or "no".
- **Action Taken** – Documentation of any action taken with respect to investigational treatment: "none", "dose decreased", "dose increased", "temporarily interrupted", "permanently discontinued", "unknown", "not applicable"
- **Seriousness** – Classification of the event as serious or non-serious, in accordance with meet a seriousness criterion as defined in Section 14.2
- **Concomitant Medications** – A list whether medication or therapy was given:" No concomitant medication/non-drug therapy", "concomitant medication/non-drug therapy"

All adverse events should be managed appropriately. If concomitant therapy is administered, this should be documented on the Adverse Event CRF. Once an adverse event is identified, it must be monitored until resolution or deemed permanent. At each visit (or more frequently, if necessary), the severity, suspected relationship to trial treatment, required interventions, and outcome should be assessed.

Progression of the underlying Ewing sarcoma, including fatal outcomes, documented using appropriate methods (RECIST 1.1 criteria) should not be reported as a serious adverse event. However, adverse events separate from the malignancy progression should be reported according to standard guidelines, with proper attribution regarding their relation to the trial treatment.

14.1.1. Handling of Laboratory test abnormalities

Laboratory abnormalities that are considered adverse events (i.e., those that are clinically significant, induce clinical signs or symptoms, require concomitant therapy, or necessitate changes in protocol compliant treatment) should be documented in the Adverse Events CRF. Whenever possible, provide a diagnosis rather than a symptom (e.g., anaemia instead of low haemoglobin). Such laboratory abnormalities should be monitored until they return to normal, or an adequate explanation is found. If an abnormal laboratory or test result corresponds to a sign/symptom of an already reported adverse event, it is not necessary to record the lab/test result as an additional event.

Laboratory abnormalities that do not meet the criteria for an adverse event should not be reported as such. A Grade 3 or 4 event (severe) according to CTCAE 5.0 does not automatically qualify as a serious adverse event (SAE) unless it meets the definition of serious as described below or as determined by the investigator. If the protocol requires a dose hold or medication for the lab abnormality, the lab abnormality must be reported as an adverse event.

14.2. Definition and Reporting of Serious Adverse Events (SAE)

A Serious Adverse Event (SAE) is defined as regarding to the "Detailed guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use – 2011/C 172/01 CT-3" and Article 2(33) (EU) No 536/2014 as:

A Serious Adverse Event (SAE) is any untoward medical occurrence or effect that at any dose

- results in death,
- is life-threatening,
- requires hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability or incapacity, or
- is a congenital anomaly or birth defect.

Any important medical event (defined as a medical event(s) that may not be immediately life threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [e.g. medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) should also be considered as an SAE.

Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)

Suspected transmission of an infectious agent (e.g. pathogenic or non-pathogenic) via the trial treatment should be also considered as an SAE.

Although pregnancy, overdose and cancer are not always serious by regulatory definition, but these events should be handled as SAEs.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the Pregnancy Surveillance Form. An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as an SAE.

The following reasons for hospitalizations should be not considered as SAEs due to clinical situation the regular patient care:

- a visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event),
- elective surgery, planned prior to signing consent,
- admissions as per protocol for a planned medical/surgical procedure,
- routine health assessment requiring admission for baseline/trending of health status,
- medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases,
- admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g. lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason),
- admission for administration of anticancer therapy in the absence of any other SAEs (applies to the nature of this oncology protocol)

To ensure patient safety, every SAE, regardless of suspected causality, occurring from the timing given in section 14.2.1 until at least 30 days after last dose of protocol therapy must be reported within 24 hours of becoming aware of its occurrence. For further details please see section 14.2.1.

Recurring episodes, complications, or progression of the initial SAE must be reported as follow-up to the original incident within 24 hours of receiving the follow-up information. An SAE occurring at a different time interval or otherwise deemed completely unrelated to a previously reported one should be reported separately as a new event.

14.2.1. Timing of SAE Reporting

SAE reporting starts after the patient and/or guardian has signed the informed consent form (ICF), but no earlier than the administration of the first protocol-compliant therapy in the 1st cycle. Adverse Events that occur before this time point and meet a seriousness criterion will not be reported as SAEs to clearly distinguish them from pre-treatment events.

This also applies to events that already exist when the patient enters the study, and the SAE is already present. If an existing AE worsens after the first administration in the first cycle, this worsening must be reported as an SAE.

The SAE reporting ends either 30 days after last dose of protocol therapy, when the patient receives further therapy outside the trial (whether chemotherapy, local therapy, or radiation), or when the regular follow-up period has concluded. Whichever occurs first.

Any SAEs experienced beyond this period should only be reported if the investigator suspects a causal relationship to dinutuximab beta.

14.2.2. Serious Adverse Event Reporting

Information on all SAEs must be collected and documented on the Serious Adverse Event Report Form within the trial database. All relevant sections of the form should be completed to ensure a comprehensive clinical report. The investigator is responsible for assessing and recording the relationship of each SAE to the protocol treatment. If the completed SAE Report cannot be done in the clinical trial database, a signed fax form can be sent to the safety desk within 24 hours.

Safety Desk

Holsterhauser Platz 2, 45147 Essen Germany

Phone: +49 201 7494 96-0

Fax: +49 201 8777 5484

E-Mail: safety@gpoh-trials.org

The FAX needs to include the patient´s pseudonymized patient ID and the main information regarding the SAE. The original SAE Report Form and fax confirmation sheet must be kept with the case report form documentation at the study site. In case of using fax, trial staff must enter the SAE report into the database as soon as possible.

Follow-up information should be sent to the same contact(s) who received the original SAE Report Form, using a new form indicating it is a follow-up and referencing the date of the original report. Each recurrence, complication, or progression of the original event should be reported as a follow-up, regardless of when it occurs. The follow-up should detail whether the event has resolved or is ongoing, how it was treated, and whether the patient continued or withdrew from the study.

The assessment of causality (relationship with the study medication: related/not related) and expectedness (expected/not expected) is essential to identify SUSARs (Suspected Unexpected Serious Adverse Reactions). This causality assessment must be performed by the reporting investigator and will be re-evaluated by the sponsor's delegate's representative. If the reporting investigator determines a potential causality, the sponsor's delegate's representative cannot downgrade this assessment; any discrepancies will be documented. All other events categorized as SUSARs will be reported within the legal timeframe.

The sponsor will report individual events categorized as SUSARs to the EudraVigilance database. Timelines for SUSAR reporting are as follows:

- initial fatal or life-threatening SUSARs will be reported to the competent authorities as soon as

possible but no later than 7 calendar days from initial receipt of SAE by Sponsor. A completed

follow-up will be submitted within an additional 8 calendar days.

- all other SUSARs will be reported to the competent authorities as soon as possible but no later

than 15 calendar days from initial receipt of SAE by Sponsor.

All safety related information collected within the trial will be summarized in an Annual Safety Report (ASR) annually from the date of receipt of the first Clinical Trial Authorization for the trial to the submission of the End of Trial Declaration. This ASR will cover all IMPs, as due to consecutive administration of the IMPs, adverse events cannot be identified as related to a single IMP.

14.3. Serious Breach Reporting

According to CTR 536/2014 any deviation of the approved protocol version or the clinical trial regulation that is likely to affect the safety, rights of trial participants and/or data reliability and robustness to a significant degree in a clinical trial needs to be reported as serious breach.

The sponsor will perform the assessment of a (suspected) serious breach in a timely manner from the moment they have received this information.

Reporting will be done by the sponsor or its delegated parties within seven calendar days after becoming aware via the clinical trial information system (CTIS). Sponsor will also report (suspected) serious breaches as defined above which occur outside the EU/EEA.

14.4. Reporting of Pregnancy

For subjects who receive protocol compliant treatment, pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject occurring while the subject is on protocol compliant treatment or until EoT visit of the subject are considered immediately reportable events. In case that a pregnant patient is on protocol compliant treatment, the treatment is to be discontinued immediately.

The pregnancy, suspected pregnancy, or positive pregnancy test must be reported to Safety Desk immediately using the Pregnancy Initial Report Form.

The female subject may be referred to an obstetrician-gynaecologist or another appropriate healthcare professional for further evaluation.

The investigator will follow the female subject until completion of the pregnancy and must notify Safety Desk immediately about the outcome of the pregnancy (either normal or abnormal outcome) using the Pregnancy Follow-up Report Form.

If the outcome of the pregnancy was abnormal (e.g., spontaneous abortion), the investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE in accordance with the details given in section 14.2.

All neonatal deaths that occur within 28 days of birth should be reported, without regard to causality, as SAEs (“Other important medical event”). In addition, any infant death after 28 days that the investigator suspects is related to the in utero exposure to the protocol compliant treatment should also be reported to Safety Desk within 24 hours of the investigator’s knowledge of the event using the SAE Report Form.

If a female partner of a male subject taking part in this trial becomes pregnant, the male being treated should notify the investigator, and the pregnant female partner should be advised to call their healthcare provider immediately.

15. STATISTICAL CONSIDERATIONS

The sections below describe the statistical methods as planned at time of protocol finalization. Statistical analyses will however be described in detail in a statistical analysis plan (SAP) which will be finalized before the enrolment of the first patient.

15.1. Statistical Hypotheses

No formal hypothesis will be stated and statistically tested. All parameters will be descriptively analysed using standard statistical methods.

15.2. Sample Size Determination

No formal sample size calculation has been undertaken for this phase I trial. In this dose-finding trial using a 3+3 design to determine the recommended phase II dose (RPD2) of dinutuximab beta in combination with vincristine, doxorubicin, cyclophosphamide, ifosfamide, and etoposide, the sample size will be determined by the following steps:

Initial Dose Cohorts:

- *each dose level will start with 3 patients.*

The trial will follow the following Dose Escalation Rules:

- *if 0 out of 3 patients of the initial dose cohort at dose level 1 experience a dose-limiting toxicity (DLT), the trial will escalate to the next higher or highest dose level, depending on when CTC levels ≤1 or at least one CTC level =2 is present, respectively.*
- *If 1 out of 3 patients experience a DLT, an additional 3 patients will be enrolled at the same dose level into an expansion cohort.*
- *If 1 out of 6 patients experience a DLT, the dose escalation will proceed to the next level, if a higher level available.*
- *If 2 or more out of 3 or 6 patients experience a DLT, the current dose will be considered above the MTD (maximum tolerated dose), and dose escalation will stop. The previous dose will be considered the RPD2.*

The trial will follow the following Stopping Rules:

- *The trial will continue to enrol patients up to the maximum number of 6 patents per dose level and escalate doses until the MTD or RP2D is identified.*

Based on the guidelines for dose escalation and determination in section 10.2.3, the trial will enroll between n=3 and n=18 patients.

15.3. Populations for Analyses

15.3.1. Full Analysis Set (FAS)

The Full Analysis Set (FAS) comprises all patients who have received at least one dose of dinutuximab beta. Patients will be categorized according to the treatment they were assigned to receive. The FAS will be used for all raw data listings.

15.3.2. Safety Set (SAF)

The safety set consists of all patients who have received at least one dose of any IMP and have at least one valid safety assessment after baseline. A statement indicating no adverse events (AEs) on the Adverse Event CRF qualifies as a valid safety assessment.

For patients on the specified regimen, classification by dose level received will be as follows:

1. the assigned dose level, if received at least once.

2. The initial dose level received in the study, if the assigned dose level was never administered.

As it is unlikely that a treated patient would provide no safety data at all; therefore, the Safety Set (SAF) should be equal to the Full Analysis Set (FAS). Typically, the FAS is equal to or a subset of the SAF, but it should never exceed the SAF.

The FAS will be used for efficacy analyses, while the SAF will be used for safety analyses.

15.3.3. Per-protocol set

Not applicable.

15.3.4. Dose-Determining Analysis Set (DDS)

The Dose Determining Set (DDS) includes all patients from the safety set who either meet the minimum exposure criterion with sufficient safety evaluations or discontinue early due to a dose-limiting toxicity (DLT). A patient is considered to have met the minimum exposure criterion if they have received at least 1 full cycle out of the 5 planned treatment cycles.

Patients who do not experience a DLT during the first cycle are considered to have sufficient safety evaluations if they have been observed for at least 21 days following the first dose and are deemed by both the Sponsor and Investigators to have enough safety data to conclude that a DLT did not occur.

Patients failing to meet these minimum safety evaluation requirements will be regarded as ineligible for the DDS. DDS is to be considered as subset of SAF.

15.4. General Approach

The sponsor or a designated collaborator will analyse the data from this study. Any independent data analysis by the investigator must be submitted to GPOH prior to publication or presentation. The data will be summarized using descriptive statistics for quantitative data and contingency tables for qualitative data, covering demographic and baseline characteristics, efficacy observations and measurements, and safety observations and measurements. The analysis and reporting will include data from all patients up to the point when all have either completed the five treatment cycles or discontinued the study.

Patients treated with the MTD, or RDE if different, during the dose escalation phase will be pooled with those receiving the same dose and regimen in the expansion cohort. A treatment group is defined by the dose level.

15.5. Statistical Analyses

Detailed statistics will be described in the statistical analysis plan (SAP).

15.5.1. Study Treatment

The clinical study report will detail the actual dose of dinutuximab beta and number of cycles of all protocol compliant treatment. Additionally, it will include the dose intensity, calculated as the ratio of the actual dose received to the actual duration, and the relative dose intensity, calculated as the ratio of dose intensity to the planned dose received and planned duration. These metrics will be listed and summarized using descriptive statistics. The summary data will be presented separately for each treatment cycle and collectively for all study days. The Full Analysis Set (FAS) will be utilized for these analyses. Definitions of actual dose and actual duration will be given in the SAP.

15.5.2. Protocol Compliance

Compliance with the protocol will be evaluated by counting and calculating the proportion of patients with protocol deviations. These deviations will be identified before the database lock, listed, and summarized accordingly.

15.6. Analysis of the Primary Endpoint(s)

The primary objective of this study is to determine the recommended dose of dinutuximab beta in combination with standard chemotherapy agents, based on the incidence of dose-limiting toxicities (DLTs) observed during the trial. The primary endpoint is the incidence of dose limiting toxicities (DLTs).

Listing/summary of DLTs

DLTs will be listed, and their incidence summarized by primary system organ class, worst grade based on the CTCAE version 5.0, type of adverse event (serious or non-serious), and by treatment. The DDS will be used for these summaries.

15.6.1. Handling of Missing Values, Censoring, and Discontinuations

During dose-escalation, patients who are deemed ineligible for the dose-determining set may be replaced if necessary. However, patients in the safety expansion phase will not be replaced.

Missing data will not be replaced.

As of the data cut-off date for the primary Clinical Study Report (CSR) for reporting purposes:

- patients continuing treatment: Time-to-event data related to response (e.g., progression-free survival) will be censored at the date of the last radiological disease assessment before the cut-off date.
- ongoing Events (e.g., Adverse Events): These will be summarized using the cut-off date as the date of completion, with an indication in the listings that the event is ongoing.

For patients who discontinue the study while experiencing ongoing events related to secondary efficacy endpoints, these events will be censored at the discontinuation date.

The reasons for study discontinuation will be summarized and listed, along with the dates of first and last dinutuximab beta administration, duration of exposure to dinutuximab beta, and the date of discontinuation for each patient.

15.7. Analysis of the Secondary Endpoint(s)

15.7.1. Early Efficacy signals

The anti-tumor activity of dinutuximab beta in combination with standard chemotherapy agents will be assessed through centralized tumor response, using RECIST 1.1 criteria. This assessment will be summarized in terms of duration of response (DOR), event-free survival (EFS) and progression-free survival (PFS).

Kaplan-Meier analyses of progression-free survival (PFS), event-free survival (EFS) and duration of response (DOR) will be conducted both overall and by dose level, provided there is a sufficient number of patients.

15.8. Safety Analyses

For all safety analyses, the safety set will be utilized. Unless specified otherwise, all listings and tables in the clinical study report will be presented by dose level, with patients classified according to the treatment received, as described in Section15.3. Data from patients in the expansion cohort will be combined with data from patients in the initial cohort who received the same dose.

A minimum of safety analyses, at least SAE reporting, will be done for all patients, even if they were replaced and do not qualify for the safety set. This will be described in detail in the SAP.

15.8.1. Analysis of Adverse Events (AEs)

All adverse events (AEs) reported during the course of the study will be documented, assessed, and managed according to regulatory guidelines and institutional policies. An AE is defined as stated above.

Adverse events will be systematically recorded and presented in the form of a line listing for thorough and clear analysis. The cumulative data will be analyzed periodically to identify any safety concerns or trends, and appropriate actions will be taken to ensure patient safety.

Deaths reportable as SAEs and non-fatal SAEs will be listed by patient and categorized by primary system organ class, type of adverse event, and treatment group. Additional collected information, such as start/end dates, duration of the adverse event, severity, or relation to the study medication, will be listed as appropriate.

15.8.2. Analysis of Laboratory Abnormalities

All laboratory values will be converted into SI units where applicable, and their severity will be graded using CTCAE version 5.0. Parameters without existing CTCAE grading will be categorized as low, normal, or high based on laboratory normal ranges.

For each laboratory test (e.g., hematology, biochemistry), a detailed listing of values will be provided, organized by laboratory parameter, patient, and dose level. The occurrence of significant lab abnormalities (newly occurring CTCAE grade 3 or 4 laboratory toxicities) will be reported by parameter, cycle, and dose level. Additionally, the frequency of all laboratory abnormalities will be tabulated by parameter, the worst CTCAE 5.0 grade experienced, and dose level. Laboratory data will be summarized using grade shift tables for parameters that can be classified according to CTCAE version 5.0. Remaining data will be summarized using shift tables based on normal ranges.

Laboratory data will also be presented through summary statistics of raw data and changes from baseline values, including means, medians, standard deviations, and ranges.

15.8.3. Analysis of Tolerability

The tolerability of dinutuximab beta in combination with standard chemotherapy agents will be evaluated by summarizing the frequency of tolerated cycles, dose interruptions and dose modifications. The reasons for these dose adjustments will be documented for each patient and then summarized. Additionally, cumulative dose, dose intensity, and relative dose intensity of dinutuximab beta will be listed and summarized for each patient.

15.9. Planned Interim Analyses

No formal interim analyses are planned. However, the dose-escalation design requires decisions to be made based on the current data before the study concludes. Specifically, after each cohort within the dose escalation levels, the decision is contingent on the observed data and needs to be taken and confirmed by the DSMC.

The analyses necessary to discuss dose escalation with the DSMC do not constitute a temporary halt of the study in the regulatory sense and therefore will not be reported as such, even if recruitment has to be temporarily suspended for this reason.

Exploratory analyses, including the analysis of exploratory endpoints, as well as any additional analyses, will be detailed in the Statistical Analysis Plan (SAP).

16. DATA HANDLING AND RECORD KEEPING

16.1. Data Management

Data will be collected via case report forms (CRF) and entered into the clinical database. This information will be electronically verified using programmed edit checks defined by the clinical team. Any discrepancies identified in the data will be communicated to the clinical team and, if necessary, to the investigational site personnel. Resolutions to these issues will be documented and reflected in the database.

As this trial is utilizing Electronic Data Capture (EDC), designated investigator staff will enter the protocol-required data usually directly into the Electronic Case Report Forms (eCRF). These eCRFs are constructed using fully validated, secure web-enabled software that adheres to 21 CFR Part 11 requirements. Investigator site staff will receive training before gaining access to the EDC system.

The Principal Investigator is responsible for ensuring that the data entered into the eCRF is complete, accurate, and updated in a timely manner. Besides the data entered into the eCRFs, requisition forms may also need to be completed for biomarker sample collection or imaging.

Source Data Documentation

In accordance with ICH E6 (R2) guidelines, all data entered into the electronic Case Report Form (eCRF) must be supported by corresponding source documentation recorded in the patient's medical records.

No data will be entered directly into the eCRF without prior written or electronic record in the source documents.

This includes all clinical, laboratory, and procedural data collected during the study. Investigators are required to ensure that all data points reflected in the eCRF are substantiated by appropriate and contemporaneous entries in the patient's medical record or equivalent source documents, in line with the Good Clinical Practice (GCP) guidelines.

The study does not foresee any instances where data would be recorded directly onto the eCRF without an associated source document. This policy applies uniformly across all study sites and for all data collection processes.

16.2. Record Retention

The investigator must retain all essential documents in accordance with local laws or requirements. Essential documents include, but are not limited to:

- signed informed consent/assent documents for all subjects,
- subject identification code list, screening log (if applicable), and enrolment log,
- approval letters of all local/national competent authorities and ECs,
- record of all communications between the investigator, GPOH, and their authorized representative(s),
- list of sub-investigators and other appropriately qualified persons to whom the investigator has delegated significant study-related duties, along with their roles in the study, curriculum vitae, and signatures,
- copies of CRFs and documentation of corrections for all subjects,
- investigational medical product (IMP) accountability records,
- record of any retained body fluids or tissue samples,
- all other source documents (subject records, hospital records, laboratory records, etc.),
- all other documents as listed in Section 8 of the ICH (International Conference on Harmonization) consolidated guideline on GCP (Essential Documents for the Conduct of a Clinical Trial).

The investigator must notify GPOH if they wish to assign the essential documents to someone else, relocate them, or are unable to retain them for a specified period. Written approval from GPOH must be obtained before destroying any records. If the investigator cannot meet this obligation, they must request permission from GPOH to make alternative arrangements and document the details of these arrangements.

All study documents should be made available if required by relevant health authorities. Measures should be taken to prevent accidental or premature destruction of these documents.

16.3. Archiving

It is the responsibility of the Principal Investigator at each site to ensure all essential trial documentation and source records (e.g. signed Informed Consent Forms, Investigator Site Files, Pharmacy Files, patients' hospital notes, copies of (e)CRFs etc.) at their site are securely retained the legally outlined period after the end of the trial.

16.4. End of Trial Definition

The trial will remain open until the date of the last patient's last visit. The sponsor will notify the relevant Competent Authority and Ethics Committee that the trial has ended at the appropriate time and will provide them with a summary of the clinical trial report within 12 months of the end of trial.

The sponsor confirms that for this trial which include minors, Regulation (EC) No 1901/2006 Article 41, Para. 2; Guideline 2009/C 28/01 EC 4.2 as well as Ethical Considerations for Clinical Trials on Medicinal

Products Conducted with the Paediatric Population, Final 2008, 19.1, apply, with possibly shorter timelines to submit the clinical study report, in case that is necessary by this legal background.

17. OPERATIONAL CONSIDERATIONS

17.1. Regulatory, Ethical, and Study Oversight Considerations

The accepted basis for the conduct of clinical trials in humans is founded on the protection of human rights and the dignity of human beings regarding the application of biology and medicine and requires compliance with the principles of GCP and detailed guidelines in line with those principles. GCP is a set of internationally recognized ethical and scientific quality requirements which must be observed for designing, conducting, recording and reporting clinical trials that involve the participation of human subjects. Compliance with GCP provides assurance that the rights, safety and well-being of trial subjects are protected, and that the results of the clinical trials are credible. All involved parties agreed on GCP principles, especially in line with Art. 47 Eu No. 536/2014.

All involved parties shall consider all relevant guidance and applicable laws with respect to commencing and conducting a clinical trial.

The conduct of the trial shall be based on the following international ethical and statutory sources:

- the WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects,
- if the region has adopted the Convention for the Protection of Human Rights and Dignity of the Human Being regarding the Application of Biology and Medicine: Convention on Human Rights and Biomedicine (CETS No.: 164),
- regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014 on clinical trials on medicinal products for human use, and repealing Directive 2001/20/EC,
- directive 2005/28/EC of 8 April 2005 laying down principles and detailed guidelines for good clinical practice as regards investigational medicinal products for human use, as well as the requirements for authorization of the manufacturing or importation of such products (Official Journal L 91, 09/04/2005 P. 0013 – 0019),
- directive 95/46/EC of the European Parliament and of the Council of 24 October 1995 on the protection of individuals with regard to the processing of personal data and on the free movement of such data (Official Journal L 281, 23/11/1995 P. 0031 – 0050),
- scientific guidelines relating to the quality, safety and efficacy of medicinal products for human use, as agreed upon by the CHMP and published by the Agency, as well as the other pharmaceutical Community guidelines published by the Commission in the different volumes of the rules governing medicinal products in the European Community (Directive 2005/28/EC (9)).

17.1.1. Statement on the future use of biological samples

The compliance with the applicable regulations for the future use of biological samples from clinical trial subjects will be made overall according to

- regulation [EU] No 536/2014 Annex I, section D, No. 17, lit. s
- the WMA Declaration of Taipei on ethical considerations regarding health databases and biobanks

As the future use of biological samples from clinical trial subjects will be regulated according to the given ICFs, the patient information sheet covers national legal requirements, which may apply.

17.2. Study Discontinuation and Closure

Additionally, to the criteria described in section 13 of this protocol, the following events, if applicable, will/must cause premature termination of the clinical trial:

a) unjustifiable risk and/or toxicity in risk-benefit analysis (decision taken by sponsor), e.g. when adverse events occur, unknown to date in respect of their nature, severity, duration or frequency in relation to the current established safety profile (substantial changes in risk-benefit considerations), and therefore medical and/or ethical reasons affect the continued performance of the trial;

b) new scientific evidence becomes available during the study that could affect the patient's safety (benefit-risk analysis no longer positive), e.g. new insights from other clinical trials;

c) request of the sponsor or regulatory agency, e.g. as a consequence of monitoring or inspection; favourable opinion withdrawn by ethics commission the clinical trial as a whole, either in all participating countries or also in a specific participating country;

d) in case of difficulties in the recruitment of the planned number of subjects in the indicated time (insufficient recruitment rate);

e) permanently unavailability of an IMP or withdrawal of the license to manufacture or of the permission to import an IMP.

The decision will be made by the sponsor or its representatives in consultation with the DSMC. If there is an imminent risk of harm to the enrolled patients, the sponsor will immediately discontinue the enrolment and verify the applicability of the above listed criteria.

17.2.1. Temporary Halt

According to Expert Group on Clinical Trials: “Regulation (EU) No 536/2014 Questions & Answers”, May 2022, Nos. 427-429

A temporary halt implies that the sponsor makes unforeseen stops of this clinical trial activity of a sub study described in the protocol (i.e. recruitment only or recruitment and treatment) or its sub studies (cohorts) or a cohort (arm), due to unexpected circumstances that could affect the benefit/risk ratio or not. In case of safety issues subjects need to be monitored/followed up, please refer to the respective protocol sections.

During the temporary halt the issues of concern are assessed together with the need for possible changes in the clinical trial. After this analysis is completed, and reassurance that any potential problem may be solved or mitigated, the sponsor could either restart or end the clinical trial or its sub studies (cohorts) or a cohort (arm).

In case the reasons for the temporary halt have the potential to affect the benefit/risk balance (i.e. concern related to safety, lack of efficacy or IMP quality defect), the sponsor will request a restart of the clinical trial or its sub studies (cohorts) or a cohort (arm) through a substantial modification subject to authorization, providing the justification for the restart, including conclusions of the analysis, the mitigation measures if applicable and an updated benefit/risk assessment.

A restart will be considered as substantial modification.

The protocol compliant conduct of this clinical trial as it is described in section 15.9 is not affected by this section.

17.3. Confidentiality and Privacy

GPOH affirms the subject's right to protection against invasion of privacy and to be in compliance with ICH and other local regulations (whichever is most stringent). GPOH requires the investigator to permit GPOH´s representatives and, when necessary, representatives from regulatory authorities, to review and/or copy any medical records relevant to the study in accordance with local laws.

Should direct access to medical records require a waiver or authorization separate from the subject's signed informed consent/assent document, it is the responsibility of the investigator to obtain such permission in writing from the appropriate individual.

Personal data of all subjects i.e. date of birth and data regarding the disease, treatment and follow-up will be collected. All investigational materials and data will be pseudonymized in accordance with data protection legislation before scientific processing. The investigators and all members of a site or other persons involved in the study are obliged to keep study data and information confidential. Access to data is strictly limited to authorized persons. All legal requirements concerning the safety and confidentiality of data and prevention of data loss will be respected. All involved individuals are sworn to secrecy. Subjects will be informed that their pseudonymized data will be passed on in accordance with the documentation and notification obligations pursuant to GCP requirements to the recipients described there. Subjects who do not agree that the information may be passed on in this way will not be enrolled into the trial. All international and national data protection laws will be considered in the respective countries.

Details will be described in the data protection concept.

A description of measures that will be implemented in case of data security breach to mitigate the possible adverse effects required by Regulation [EU] No 536/2014 Annex I, section D, No. 7, lit. am. outlined in Appendix 3.

17.4. Key Roles and Study Governance

17.4.1. Sponsor

The German Society of Pediatric Oncology and Hematology (GPOH) is the Sponsor for this clinical trial. In addition, the GPOH will transfer different responsibilities to a qualified CRO.

17.4.2. Data Safety Monitoring Committee (DSMC)

Such a committee has been installed. Members of the DSMC are experienced researchers not involved in the trial who will be responsible for providing the principal investigators with guidance on the trial conduction and, in case of problems, on whether the trial should be stopped, modified or continued (for details see the DSMC Charter in its actual version).

The DSMC closed meetings will take place at least once a year through e-mail communications and possibly conference calls. Additional meetings will be possible and can be scheduled in case the DSMC judges that these are needed.

The DSMC will receive from the trial statistician a report at time intervals defined in the charter, starting from the international birthday of the clinical trial. All reports will be focused on recruitment/trial integrity and safety, as well as patient safety and quality of trial..

Before reporting on the safety and results of the trial the DSMC will consider not only the interim results of the study but also any major new information from other sources considered to be relevant to the trial. In this perspective, the DSMC will be guided by the pre-assigned statistical rules for dose modification stated in this protocol but will not apply them automatically.

The DSMC will be set up according to EMEA/CHMP/EWP/5872/03 Corr.

17.4.3. Quality Management

All aspects of the study will be carefully monitored by GPOH or its authorized representative for compliance with applicable government regulations with respect to current GCP and standard operating procedures.

17.4.4. Clinical Monitoring

All aspects of the study will be carefully monitored by GPOH or its authorized representative for compliance with applicable government regulations with respect to current GCP and standard operating procedures.

GPOH ensures that appropriate monitoring procedures are performed before, during and after the study. All aspects of the study are reviewed with the investigator and the staff at a study initiation visit, conference call and/or at an investigator meeting.

Monitoring will include on-site visits with the investigator and his/her staff as well as any appropriate communications by mail, email, fax, or telephone. During monitoring visits, the facilities, investigational product storage area, CRFs, subject's source documents, and all other study documentation will be inspected/reviewed by the GPOH representative in accordance with the Study Monitoring Plan.

Accuracy will be checked by performing source data verification that is a direct comparison of the entries made onto the CRFs against the appropriate source documentation. Any resulting discrepancies will be reviewed with the investigator and/or his/her staff. Any necessary corrections will be made directly to the CRFs or via queries by the investigator and/or his/her staff. Monitoring procedures require that informed consent/assents, adherence to inclusion/exclusion criteria and documentation of SAEs and their proper recording be verified. Additional monitoring activities may be outlined in a study-specific monitoring plan.

17.4.5. Quality Assurance and Quality Control

Each clinical site will conduct internal quality management for conduct, data collection, biological specimen collection, documentation, and completion. A tailored quality management plan will be developed to outline the trials specific quality management procedures.

Quality control (QC) procedures will be implemented, starting with the data entry system, and data QC checks will be run on the generated database. Any missing data or anomalies will be communicated to the respective site(s) for clarification and resolution.

Monitors will follow written Standard Operating Procedures (SOPs) to verify that the clinical trial is conducted, data is generated, and biological specimens are collected, documented, and reported in compliance with the protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), and applicable regulatory requirements such as Good Laboratory Practices (GLP) and Good Manufacturing Practices (GMP).

The investigational site will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

17.5. Publication and Data Sharing Policy

The definition and scope of work for a new manuscript, abstract, poster, oral presentation, or other publication should be determined by the sponsor, the lead author, with input from the Study statistician(s) and DSMC and further team members as appropriate. All proposed publications must fulfil ethics and GCP criteria.

Guidelines for multi-centre publication of a study under the terms of this protocol:

The lead author shall determine the appropriate co-authors of the Study in accordance with ICMJE guidelines for authorship (http://www.icmje.org/icmje-recommendations.pdf). The author/co-author must:

- demonstrate substantial contributions to the conception or design of the proposed publication; or the acquisition, analysis, or interpretation of data for the proposed publication; and
- participate in drafting the proposed publication or revising it critically for important intellectual content; and
- give final approval of the version to be published; and
- agree to be accountable for all aspects of the proposed publication in ensuring that questions related to the accuracy or integrity of any part of the proposed publication are appropriately investigated and resolved.

Each author is expected to comply with their own institutional authorship guidelines and collaborative group guidelines.

Authorship of multi-centre publication of Study under the terms of this protocol:

Shared first authorship for Investigators participating in the study should be the goal of each manuscript. Listing of other authors should be based on contribution and confirmed by the Sponsor. Senior authorship will be shared by the lead Investigators overseeing the study and should be confirmed at the completion of enrolment. The study statistician and anyone responsible for ancillary studies (pharmacology, biology) whose results are reported in the publication should be considered for authorship. All participating sites who register at least one subject on study should be listed as an annex of the publication. If third parties have played a substantial role in the concept of the study, having one representative author is to be discussed.

I. APPENDIX 1 LANSKY AND ECOG SCALE

LANSKY SCALE FOR PATIENTS <16 YEARS OF AGE (LANSKY ET AL., 1987)

Performance status	Definition
100	Fully active, normal
90	Minor restrictions in strenuous physical activity
80	Active, but gets tired more quickly
70	Greater restriction of play and less time spent in play activity
60	Up and around, but active play minimal; keeps busy by being involved in quieter activities
50	Lying around much of the day, but gets dressed; no active playing participates in all quiet play and activities
40	Mainly in bed; participates in quiet activities
30	Bedbound; needing assistance even for quiet play
20	Sleeping often; play entirely limited to very passive activities
10	Doesn't play; does not get out of bed
0	Unresponsive

EASTERN COOPERATIVE ONCOLOGY GROUP (ECOG) PERFORMANCE STATUS FOR PATIENTS ≥16 YEARS OF AGE (ZUBROD ET AL., 1960)

Performance status	Definition
0	Fully active; no performance restrictions.
1	Strenuous physical activity restricted; fully ambulatory and able to carry out light work.
2	Capable of all self-care but unable to carry out any work activities. Up and about >50% of waking hours.
3	Capable of only limited self-care; confined to bed or chair >50% of waking hours.
4	Completely disabled; cannot carry out any self-care; totally confined to bed or chair.
5	Dead

II. APPENDIX 2 PROTOCOL CONTENT ACCORDING TO ANNEX 1 (EU) NO 536/2014

	Protocol section/ Comment
The protocol shall be identified by:	
• the title of the clinical trial; • the EU trial number; • the sponsor's protocol code number specific for all versions of it (if relevant); • the date and number of the version, to be updated when it is amended; • a short title or name assigned to the protocol; and	Title page; protocol information; synopsis
• the name and address of the sponsor, as well as the name and function of the representative or representatives of the sponsor authorised to sign the protocol or any substantial modification to the protocol.	Protocol information
The protocol shall, when possible, be written in an easily accessible and searchable format, rather than scanned images.	Protocol is a converted document and OCR compliant
The protocol shall at least include:	
• a statement that the clinical trial is to be conducted in compliance with the protocol, with this Regulation and with the principles of good clinical practice;	Regulatory, ethical and study oversight
• a comprehensive list of all investigational medicinal products and of all auxiliary medicinal products	Investigational medicinal product
• a summary of findings from non-clinical studies that potentially have clinical significance and from other clinical trials that are relevant to the clinical trial	Background
• a summary of the known and potential risks and benefits including an evaluation of the anticipated benefits and risks to allow assessment in accordance with Article 6; for subjects in a clinical trial in an emergency situation, the scientific grounds for expecting that the participation of the subjects has the potential to produce a direct clinically relevant benefit shall be documented	Assessment of potential risks and burden
• where patients were involved in the design of the clinical trial, a description of their involvement	Not applicable
• a description of, and justification for, the dosage, the dosage regime, the route and mode of administration, and the treatment period for all investigational medicinal products and auxiliary medicinal products	Justification for dosing
• a statement of whether the investigational medicinal products and auxiliary medicinal products used in the clinical trial are authorised; if authorised, whether they are to be used in the clinical trial in accordance with the terms of their marketing authorisations, and, if not authorised, a justification for the use of non-authorised auxiliary medicinal products in the clinical trial	Investigational medicinal product

• a description of the groups and subgroups of the subjects participating in the clinical trial, including, where relevant, groups of subjects with specific needs, for example. age, gender, participation of healthy volunteers, subjects with rare and ultra-rare diseases	Study design; Rationale for the inclusion of minors
• references to literature and data that are relevant to the clinical trial, and that provide background for the clinical trial	References
• a discussion of the relevance of the clinical trial to allow assessment in accordance with Article 6;	Study rationale; Background; Risk/Benefit Assessment
• a description of the type of clinical trial to be conducted and a discussion of the trial design (including a schematic diagram of trial design, procedures and stages, if relevant)	Overall Design
• a specification of the primary endpoints and the secondary endpoints, if any, to be measured during the clinical trial	Objectives and endpoints
• a description of the measures taken to minimise bias, including, if applicable, randomisation and blinding	TBD
• a description of the expected duration of subject participation and a description of the sequence and duration of all clinical trial periods, including follow-up, if relevant	Synopsis (trial duration)
• a clear and unambiguous definition of the end of the clinical trial in question and, if it is not the date of the last visit of the last subject, a specification of the estimated end date and a justification thereof;	Synopsis; End of trial definition
• a description of the criteria for discontinuing parts of the clinical trial or the entire clinical trial;	Study intervention discontinuation and participant discontinuation
• arrangements for the maintenance of clinical trial treatment randomisation codes and procedures for breaking codes, if relevant;	Not applicable
• a description of procedures for the identification of data to be recorded directly on the Case Report Forms considered as source data;	Data handling and record keeping
• a description of the arrangements to comply with the applicable rules for the collection, storage and future use of biological samples from clinical trial subjects, where applicable, unless contained in a separate document	Statement on the future use of biological samples
• a description of the arrangements for tracing, storing, destroying and returning the investigational medicinal product and unauthorised auxiliary medicinal product in accordance with Article 51	Preparation and precautions for disposal
A description of the statistical methods to be employed, including, if relevant:	
• timing of any planned interim analysis and the number of subjects planned to be enrolled;	Planned interim analyses
• reasons for choice of sample size;	Sample size determination
• calculations of the power of the clinical trial and clinical relevance;	Statistical considerations
• the level of significance to be used;	

• criteria for the termination of the clinical trial;	Dose-limiting toxicities
• procedures for accounting for missing, unused, and spurious data and for reporting any deviation from the original statistical plan; and	Handling of missing values
• the selection of subjects to be included in the analyses;	Populations for analyses
A description of the subject inclusion and exclusion criteria, including criteria for withdrawing individual subjects from treatment or from the clinical trial.	Study population
A description of procedures relating to the withdrawal of subjects from treatment or from the clinical trial including procedures for the collection of data regarding withdrawn subjects, procedures for replacement of subjects and the follow-up of subjects that have withdrawn from treatment or from the clinical trial.	Study intervention discontinuation and participant discontinuation
A justification for including subjects who are incapable of giving informed consent or other special populations, such as minors.	Rationale for the inclusion of minors
A justification for the gender and age allocation of subjects and, if a specific gender or age group is excluded from or underrepresented in the clinical trials, an explanation of the reasons and justification for these exclusion criteria.	Only age below 12 months excluded
A detailed description of the recruitment and informed consent procedure, especially when subjects are incapable of giving informed consent.	Screening and informed consent
A description of the treatments, including medicinal products, which are permitted or not permitted, before or during the clinical trial.	Concomitant therapy
A description of the accountability procedures for the supply and administration of medicinal products to subjects including the maintenance of blinding, if applicable.	Not applicable
A description of procedures for monitoring subject compliance, if applicable.	Not applicable
A description of arrangements for monitoring the conduct of the clinical trial.	Clinical monitoring
A description of the arrangements for taking care of the subjects after their participation in the clinical trial has ended, where such additional care is necessary because of the subjects' participation in the clinical trial and where it differs from that normally expected for the medical condition in question.	Follow-up for toxicities
A specification of the efficacy and safety parameters as well as the methods and timing for assessing, recording, and analysing these parameters.	Safety monitoring and reporting
A description of ethical considerations relating to the clinical trial if those have not been described elsewhere.	Regulatory, ethical and study oversight
A statement from the sponsor (either in the protocol or in a separate document) confirming that the investigators and institutions	Separate document: financial arragement

involved in the clinical trial are to permit clinical trial-related monitoring, audits and regulatory inspections, including provision of direct access to source data and documents.	(within site agreements)
A description of the publication policy.	Publication and data sharing policy
Duly substantiated reasons for the submission of the summary of the results of the clinical trials after more than one year.	Not applicable.
A description of the arrangements to comply with the applicable rules on the protection of personal data; in particular organisational and technical arrangements that will be implemented to avoid unauthorised access, disclosure, dissemination, alteration or loss of information and personal data processed	Appendix: Description of measures in case of data security breach
A description of measures that will be implemented to ensure confidentiality of records and personal data of subjects	
A description of measures that will be implemented in case of data security breach in order to mitigate the possible adverse effects	
If a clinical trial is conducted with an active substance available in the Union under different trade names in a number of authorised medicinal products, the protocol may define the treatment in terms of the active substance or Anatomical Therapeutic Chemical (ATC) code (level 3-5) only and not specify the trade name of each product.	Declaration of IMP and AxMP
With regard to the notification of adverse events, the protocol shall identify the categories of:	
• adverse events or laboratory anomalies that are critical to safety evaluations and must be reported by the investigator to the sponsor, and	Definition and reporting of adverse events
• serious adverse events which do not require immediate reporting by the investigator to the sponsor.	Definition and reporting of serious adverse events
The protocol shall describe the procedures for:	
• eliciting and recording adverse events by the investigator, and the reporting of relevant adverse events by the investigator to the sponsor;	Definition and reporting of adverse events
• reporting by the investigator to the sponsor of those serious adverse events which have been identified in the protocol as not requiring immediate reporting;	Definition and reporting of serious adverse events
• reporting of suspected unexpected serious adverse reactions by the sponsor to the Eudravigilance database; and	Serious adverse event reporting
• follow-up of subjects after adverse reactions including the type and duration of follow-up.	Definition and reporting of adverse events
In case the sponsor intends to submit a single safety report on all investigational medicinal products used in the clinical trial in accordance with Article 43(2), the protocol shall indicate the reasons	Reporting of serious adverse events

thereof.	
Issues regarding labelling and the unblinding of investigational medicinal products shall be addressed in the protocol, where necessary.	Formulation, Appearance, Packaging, and Labelling
The protocol shall be accompanied by the Charter of the Data Safety Monitoring Committee, if applicable.	DSMC Charter submitted as separate document
The protocol shall be accompanied by a synopsis of the protocol	Synopsis

III. APPENDIX 3 - DESCRIPTION OF MEASURES IN CASE OF DATA SECURITY BREACH

The sponsor and/or its representatives provide organizational, contractual, and technical security measures to guarantee the protection of patient's privacy. Therefore, to provide safeguards for organizational risks, a set of dataflow rules and processes have been established. Those include:

Organizational measures

- Internal guidelines and policies, that define what data use is allowed and prohibited. The supervision of queries to patient related data sets will be always performed by the sponsor or its representatives.

- Processes ensuring controlled environment of data handling, which guarantees data's traceability. The quality of the trial data, appropriate to the proposed objectives, and the absence of identifiers will be checked by the sponsor or its representatives.

Contractual measures

- Agreements with data providers are regulated by means of a contract. The contractual relationship established between data providers and the sponsor, or its representatives sets out the conditions for the use of the data and exploitation by the third parties in comply with the contracts minimum content established by the GDPR.

Technical measures

To prevent access to data, sets of measures implementing several restrictions are established. These effective means guarantee that datasets within all trial related data bases will not be linked or combined to the original datasets within the data provider. Technical controls include safeguards and countermeasures, for an information system that are implemented and executed primarily by the information system through mechanisms contained in the system's software, hardware or firmware components.

- Access control includes access control policy, procedures and enforcement, account management, information flow enforcement, separation of duties, least privilege, unsuccessful logon attempts, system use notification, concurrent session control, session lock, session termination, permitted actions without identification or authentication, remote access, wireless access, access control for mobile devices, use of external information systems, information sharing, publicly accessible content.

- Physical (access keys) and electronic (personal tag) – established policy, procedures and controlled mechanisms to access the security perimeters where the trial-related data bases or data sets are set. Employees must go through the registration process, which includes specifying identity (name, employer organization), dates and duration of access (start and end time) to grant a specific access authorization.

- Network and host access controls - only authorized employees and registered users are granted access to the network and servers with the absolute minimum permissions necessary to perform their duties. The information system enforces a limit of consecutive invalid log in attempts by a user and automatically locks the account/node until released by an administrator; delays next log in, when the maximum number of unsuccessful attempts is exceeded. Additionally, all access to high-security servers is monitored and logged, with access logs stored on a dedicated log server. Access to servers and program source code is controlled and granted only to

authorized entities. Special procedures are in place for assigning passwords and creating and managing users during operations.

- Network communication protection - the information security system has only the most important ports open for incoming and outgoing communications, with all traffic filtered by a switch firewall.

- Security monitoring - will be assessed using software and new information from reliable sources. Any newly discovered security vulnerability will be logged on the management site, assigned to a responsible person and resolved as soon as possible. Proper risk assessment will be performed in accordance with risk assessment procedures. Continuous monitoring program facilitate ongoing awareness of vulnerabilities, threats and information security to support risk management decisions. The security controls and information security-related risks are analyzed at a frequency sufficient to support organizational risk-based decisions. The results of continuous monitoring programs generate appropriate risk response actions and allow to maintain the security authorizations over time with changing program needs, threats, vulnerabilities, and technologies. Having access to security-related information on a continuing basis through reports and frequent updates to security authorization packages, hardware, software, firmware inventories, and other system information gives the capability to make more effective and timely risk management decisions, including ongoing security authorization decisions.

In case of data security breaches all organizational, contractual, and technical security measures to guarantee the protection of patient's privacy will be reassessed to mitigate the possible adverse effects.

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