23 March 2017
EMA/263814/2017
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Dinutuximab beta Apeiron

International non-proprietary name: dinutuximab beta

Procedure No. EMEA/H/C/003918/0000

Note

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

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

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List of abbreviations

13-cis-RA	13-cis-retinoic acid
α-gal	A-galactose
ABMT	Autologous bone marrow transplantation
ADCC	Antibody-dependent cell-mediated cytotoxicity
ADR	Adverse drug reaction
AE	Adverse event
ALT	Alanine transaminase
ANR	Advances in Neuroblastoma Research
ASCO	American Society of Clinical Oncology
ASCR/T	autologous stem cell rescue/transplantation
AST	Aspartate transaminase
BuMel	busulfan and melphalan
CD	Circular dichroism
CD16	Fcγ receptor
CDC	complement-dependent cytotoxicity
CDR	Complementarity determining regions
CEM	carboplatin, etoposide and melphalan
CFU	Colony forming unit
CHMP	Committee for Medicinal Products for Human Use
CHO	Chinese hamster ovary
CNS	Central nervous system
COG	Children's Oncology Group
COJEC	cisplatin, vincristine, carboplatin, etoposide, and cyclophosphamide
CPE	Cytopathic effect
CR	Complete response
CRF	Case report form
CRP	C-reactive protein
CSR	Clinical study report
CT	computed tomography
Da	Dalton
DF	Diafiltration
DHFR	Dihydrofolate reductase
DLT	Dose limiting toxicity
DNA	Deoxyribonucleic Acid
DoE	Design of Experiment
DP	Drug product
DS	Drug substance
DSP	Downstream processing
ECG	Electrocardiogram
ECHO	Echocardiogram
EEG	Electroencephalogram
EFS	event-free survival
ELISA	Enzyme-linked immunosorbent assay
EM(E)A	European Medicines Agency
EOT	End of treatment
EPC	End of Production Cells

ESI-TOF-MS	Electrospray ionisation time-of-flight mass spectrometry
FAS	Full analysis set
FCS	Fetal calf serum
FcγR	FC gamma receptor
FDA	Food and Drug Administration
G0	Glycan without core fucose and no galactose (also called Gn2)
G0F	Glycan with core fucose and no galactose (also called F1Gn2)
G1	Glycan without core fucose and one galactose (also called Gn2G1)
G1F	Glycan with core fucose and one galactose (also called F1Gn2G1)
G2F	Glycan with core fucose and two terminal galactose (also called F1Gn2G2)
GCP	Good Clinical Practice
GD2	Disialoganglioside
GGT	Gamma glutamyltransferase
GM-CSF	granulocyte macrophage colony-stimulating factor
GMP	Good Manufacturing Practice
GvHD	Graft vs. host disease
HACA	human anti-chimeric antibody response
HAMA	Human anti-mouse antibody
Haplo-SCT	Haploidentical stem cell transplantation
HC	Heavy chain
HCP	Host Cell Protein
HHL	2 heavy chains and 1 light chain
HMWS	High molecular weight species
HPAEC-PAD	High performance anion-exchange chromatography with pulsed amperometric detection
HPLC	High performance liquid chromatography
ICH	International Conference on Harmonisation
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IEF	Isoelectric focussing
IgG1	Immunoglobulin G, subtype 1
IL-2	interleukin 2
INSS	International Neuroblastoma Staging System
IPC	In-process control
ITT	Intention to treat
LC	Light chain
LMWS	Low molecular weight species
LN	lymph nodes
LRV	Log_{10} reduction value
MAA	Marketing Authorization Application
mAb	monoclonal antibody
MALDI-TOF MS	Matrix-assisted laser desorption time-of-flight mass spectrometry
Man 5	Mannose 5
MAT	myeloablative therapy
max	Maximum
MCB	Master Cell Bank
MedDRA	Medical Dictionary for Regulatory Activities
MHC	Major histocompatibility complex
MIBG	123/131iodine-meta-iodobenzylguanidine

min	Minimum
MKI	mitosis-karyorrhexis index
mM	Millimolar
MR	Minimal response
MRD	minimal residual disease
MRI	magnetic resonance imaging
MS	Mass spectrometry
MTD	Maximum Tolerated Dose
MVM	Minute virus of mouse
MW	Molecular weight
MYCN	v-myc myelocytomatosis viral related oncogene
N/A	Not applicable
NCI CTC	National Cancer Institute Common Toxicity Criteria
NK cells	Natural killer cells
NLT	Not less than
NMT	Not more than
ORR	Overall response rate
OS	overall survival
PAR	Proven acceptable range
PBS	Phosphate-buffered saline
PBSCR	Peripheral blood stem cell rescue
PD	Progressive disease
PDL	Population doubling limit
Ph. Eur.	European Pharmacopoeia
pI	Isoelectric point
PK	pharmacokinetics
pO_2	Partial pressure of oxygen
PP	Product pool
PP	Per protocol
PPQ	Process performance qualification
PR	Partial response
PT	Preferred term
QC	Quality Control
qPCR	Quantitative Polymerase Chain Reaction
R/R	relapsed/refractory
RBC	Red blood cell
RCB	Research Cell Bank
RECIST	response evaluation criteria in solid tumours
Rt	Retention time
SAE	Serious adverse event
SAF	Safety Analysis Set
SAP	Statistical analysis plan
SD	Standard deviation
SD	Stable disease
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SE-HPLC	Size-exclusion HPLC
sIL2r	Soluble IL-2 receptor
SIOPEN	International Society of Pediatric Oncology Europe Neuroblastoma Group
SmPC	Summary of Product Characteristics

SOP	Standard operating procedure
SPR	Surface plasmon resonance
TEAE	Treatment emergent adverse event
TEM	Transmission electron microscopy
TFF	Tangential flow filtration
TVD	topotecan, vincristine, doxorubicin
UF	Ultrafiltration
USP	United States Pharmacopoeia
UV	Ultraviolet
VGPR	Very good partial response
VLP	Virus-like particles
WBT	Whole blood cytolytic activity test
WCB	Working Cell Bank
WFI	Water for injection

1. Background information on the procedure

1.1. Submission of the dossier

The applicant APEIRON Biologics AG submitted on 6 May 2015 an application for marketing authorisation to the European Medicines Agency (EMA) for Dinutuximab beta Apeiron, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 19 December 2013.

Dinutuximab beta Apeiron was designated as an orphan medicinal product EU/3/12/1062 on 8 November 2012. Dinutuximab beta Apeiron was designated as an orphan medicinal product in the following indication: treatment of neuroblastoma.

The applicant applied for the following indication:

"Dinutuximab beta Apeiron is indicated for the treatment of patients with neuroblastoma.

90 % of patients diagnosed with neuroblastoma are below 5 years of age, with a median age at diagnosis of 22 months. Less than 10 % of the cases occur in patients being older than 10 years."

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Dinutuximab beta Apeiron as an orphan medicinal product in the approved indication. The outcome of the COMP review can be found on the Agency's website: ema.europa.eu/Find medicine/Human medicines/Rare disease designation.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that dinutuximab beta was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0094/2014 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0094/2014 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No. 847/2000, the applicant did submit a critical report addressing the possible similarity with the authorised orphan medicinal product Unituxin. However, following withdrawal of the marketing authorisation for Unituxin on 22 March 2017, it was considered unnecessary for the CHMP to conclude on possible similarity with Unituxin.

Derogation(s) from market exclusivity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No. 847/2000, the applicant submitted claims addressing the following derogations laid down in Article 8.3 of Regulation (EC) No 141/2000; the holder of the marketing authorisation for the original orphan medicinal product is unable to supply sufficient quantities of the medicinal product and the applicant can establish in the application that the medicinal product, although similar to the already authorised orphan medicinal product Unituxin, is safer, more effective or otherwise clinically superior. However, following withdrawal of the marketing authorisation for Unituxin on 22 March 2017, it was considered unnecessary for the CHMP to conclude on the derogations claimed.

Applicant's request(s) for consideration

New active Substance status

The applicant requested the active substance dinutuximab beta contained in the above medicinal product to be considered as a new active substance in comparison to dinutuximab previously authorised in the European Union as Unituxin, as the applicant claimed that dinutuximab beta is a biological substance that has a different manufacturing process and differs significantly in properties with regard to safety and/or efficacy from the already authorised active substance.

Protocol Assistance

The applicant received Protocol Assistance from the CHMP on 17 February 2011. The Protocol Assistance pertained to quality, non-clinical and clinical aspects of the dossier.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Robert James Hemmings Co-Rapporteur: Paula Boudewina van Hennik

- The application was received by the EMA on 6 May 2015.
- The procedure started on 28 May 2015.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 17 August 2015. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 19 August 2015. The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on 28 August 2015.
- During the meeting on 24 September 2015, the CHMP agreed on the consolidated List of Questions to be sent to the applicant, including a request for a critical report addressing the possible similarity with an authorised orphan medicinal product (Unituxin) and, if applicable, a derogation report.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 23 March 2016, including a similarity report and a derogation report claiming clinical superiority to Unituxin.
- The following GCP inspection(s) was requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:
  - A GCP inspection at one clinical investigator site in Germany and one CRO in Croatia was conducted between October and November 2015. The integrated inspection report was issued on 09 March 2016.

- The PRAC Rapporteur's Assessment Report was circulated to all PRAC members on 4 May 2016.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 6 May 2016.
- During the PRAC meeting on 13 May 2017, the PRAC agreed on the PRAC Assessment Overview and Advice to CHMP.
- During the CHMP meeting on 26 May 2016, the CHMP agreed on a list of outstanding issues to be addressed in writing and/or in an oral explanation by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 13 September 2016.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CHMP members on 28 September 2016.
- During the CHMP meeting on 13 October 2016, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- During the CHMP meeting on 10-13 October 2016, the CHMP agreed on a 2nd list of outstanding issues to be addressed in writing and/or in an oral explanation by the applicant. The 2nd list of outstanding issues was adopted via written procedure on 19 October 2016.
- The applicant submitted the responses to the CHMP 2nd List of Outstanding Issues on 24 January 2017 and complemented it on 6 February 2017 with a derogation report claiming that the holder of the marketing authorisation for the original orphan medicinal product (Unituxin) is unable to supply sufficient quantities of the medicinal product.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the 2nd List of Outstanding Issues to all CHMP members on 10 February 2017.
- The CHMP adopted via written procedure on 14 February 2017 a List of Questions for Dinutixumab beta Apeiron on the derogation claim that the holder of the marketing authorisation for the original orphan medicinal product (Unituxin) is unable to supply sufficient quantities of the medicinal product.
- The consultation procedure with the concerned stakeholders (patients´ organisations, healthcare professionals, Heads of Medicines Agencies and the MAH of Unituxin) regarding the inability to supply derogation claim was started on 14 February 2017.
- During the CHMP meeting on 23 February 2017, the CHMP agreed on a 3rd list of outstanding issues to be addressed in writing and/or in an oral explanation by the applicant.
- The applicant submitted the responses to the CHMP 3rd List of Outstanding Issues on 28 February 2017.
- The Rapporteurs circulated the Updated Joint Assessment Report on the applicant's responses to the 3rd List of Outstanding Issues to all CHMP members on 16 March 2017.
- During the CHMP meeting on 21 March 2017, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- During the meeting on 20-23 March 2017, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Dinutuximab beta Apeiron on 23 March 2017.

- Following withdrawal of the marketing authorisation for Unituxin on 22 March 2017, it was considered unnecessary for CHMP to conclude on possible similarity with Unituxin and on the derogations claims submitted by the applicant.

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Neuroblastoma is an embryonal tumour of the autonomic nervous system, meaning that the cell of origin is thought to be a developing and incompletely committed precursor cell derived from neural-crest tissues. Neuroblastomas generally occur in very young children; the median age at diagnosis is 17 months.

2.1.2. Epidemiology

Neuroblastoma is the most common extracranial solid tumour of childhood. Its incidence is 10.2 cases per million children under 15 years of age and accounts for approximately 7% of paediatric malignancies in this age category; it is the most common cancer diagnosed during the first year of life (Modak 2007).

2.1.3. Biologic features

The tumours arise in tissues of the sympathetic nervous system, typically in the adrenal medulla or paraspinal ganglia, and thus can present as mass lesions in the neck, chest, abdomen, or pelvis.

2.1.4. Clinical presentation, diagnosis and stage / prognosis

The clinical presentation is highly variable, ranging from a mass that causes no symptoms to a primary tumour that causes critical illness as a result of local invasion, widely disseminated disease, or both.

The diagnosis of neuroblastoma is based on the presence of characteristic histopathological features of tumour tissue or the presence of tumour cells in a bone marrow aspirate or biopsy accompanied by raised concentrations of urine catecholamines. Computed tomography and magnetic resonance imaging are the preferred methods for the assessment of tumor in the abdomen, pelvis, mediastinum, or in paraspinal lesions, respectively. For enhanced detection of tumor, radiolabeled-metaiodobenzylguanidine (MIBG) scintigraphy is used. Other methods are used to detect minimal residual disease such as bone marrow aspirates and biopsy, pathological evaluation and polymerase-chain reaction-based techniques to identify GD2 synthase, tyrosine hydroxylase and protein gene product 9.5.

For over a century, researchers have noted that neuroblastomas exhibit diverse and often dramatic clinical behaviours. On the one hand, neuroblastoma accounts for disproportionate morbidity and mortality among the cancers of childhood; on the other hand, it is associated with one of the highest proportions of spontaneous and complete regression of all human cancers.

There have been substantial efforts to develop a risk-classification algorithm for patients with newly diagnosed neuroblastoma. Most cooperative groups use a system that combines the assessment of easily measured clinical variables, such as the patient's age and the tumour stage, with specific biologic variables. The age at diagnosis is considered a surrogate for underlying biologic characteristics, in that younger patients are more likely to have tumours with biologic features that are associated with a benign

clinical course. To address this issue of classification, a new International Neuroblastoma Risk Group (INRG) classification system has been proposed in 2009 with four broad categories –very low risk, low risk, intermediate risk, and high risk – based on the assessment of the following prognostic factors: age at diagnosis (2 cut-offs, 12 and 18 months), INRG tumour stage (L1, L2, M, MS), histologic category, grade of tumour differentiation, DNA ploidy (hyperploidy/diploidy), *MYCN* oncogene status (amplified or not), aberrations at chromosome 11q (presence/absence) (Cohn 2009).

2.1.5. Management

First-line setting

The treatment of choice is risk category adapted.

For low risk neuroblastomas, the treatment encompasses surgery followed by observation; chemotherapy with or without surgery (for symptomatic disease or unresectable progressive disease after surgery); observation (considered an option in case of perinatal neuroblastoma with small adrenal tumours).

For intermediate risk neuroblastomas, the treatment encompasses chemotherapy (e.g. carboplatin, cyclophosphamide, doxorubicin, and etoposide) with or without surgery; surgery and observation (in infants); radiation therapy (only for emergency treatments, like progressive disease or life-threatening events related to the disease that does respond to treatment otherwise).

For high-risk neuroblastomas, the current treatment can be divided into three distinct phases (Maris 2010):

- induction of remission with intensive chemotherapy. The backbone of the most commonly used induction therapy includes dose-intensive cycles of cisplatin and etoposide alternating with vincristine, cyclophosphamide, and doxorubicin (Kushner 2014). Topotecan was added to this regimen based on the anti-neuroblastoma activity seen in relapsed patients (Park 2011). After a response to chemotherapy, resection of the primary tumour is usually attempted.

- consolidation of the remission with myeloablative chemotherapy which attempts to eradicate minimal residual disease using lethal doses of chemotherapy followed rapidly by rescue with autologous hematopoietic progenitor cells to repopulate the bone marrow.

- and finally a maintenance phase used to treat potential minimal residual disease (MRD) following HSCT to reduce the risk of relapse (Matthay 2009), e.g. with ch14.18 (dinutuximab) and isotretinoin, a molecule that induces terminal differentiation of neuroblastoma cell lines.

On 14 August 2015, a marketing authorisation was granted to Unituxin (dinutuximab) with the following indication: *treatment of high-risk neuroblastoma in patients aged 12 months to 17 years, who have previously received induction chemotherapy and achieved at least a partial response, followed by myeloablative therapy and autologous stem cell transplantation (ASCT). It is administered in combination with granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-2 (IL-2), and isotretinoin*.

- GD2 targeting is associated with acute pain, which is related to GD2 expression on peripheral nerve fibres and requires use of analgesia. The antibodies are also unable to treat bulky disease.
- The use of IL-2 is associated with substantial toxicity, including infections, pancytopaenia, capillary leak syndrome, aggravation of auto-immune disease, renal and/or hepatic impairment. Fatal complications after use of IL-2 have been observed.
- Recognised adverse reactions of isotretinoin (13-cis RA) include teratogenicity, myelotoxicity, headache, as well as laboratory abnormalities.

Recurrent setting

Patients with low risk neuroblastoma (ca 37% of all neuroblastoma patients) and intermediate risk (ca 18%) reach survival in approximately 90 and 70-90% respectively. Most favourable clinical results are usually obtained in infants and good outcomes may be the result of restraint (spontaneous regression after observation) or surgery.

Despite intensive multimodal therapy, over 50% of patients with high-risk neuroblastoma relapse with a dismal long-term outcome. Historically, recurrent neuroblastoma has been treated with a combination of chemotherapy and radiotherapy for the purposes of palliation only. In more recent times, treatment has evolved comprising salvage chemotherapy, radiotherapy and surgery, and ^{131}I-MIBG therapy, and ch14.18 monoclonal antibody therapy with aldesleukin-2 (IL-2) and oral isotretinoin (13-cis RA).

- Salvage regimens in the recent era have altered the natural disease course and prolonged post-relapse survival. Second line chemotherapies with mild to modest toxicities that have not been included in frontline treatment are often considered for salvage. Frequently used combinations are topotecan, vincristine, and doxorubicin (TVD), temozolomide and irinotecan (TEM/IRN), or topotecan and cyclophosphamide. Up to 60% of response or arrest of disease progression can be achieved.

- Depending on the type of relapse (localised vs metastatic), location of tumour, previous treatment history, etc. it may be that surgery or external beam radiotherapy are part of the treatment strategy.

- ^{131}I-MIBG (metaiodobenzylguanidine) therapy may be an appropriate treatment option for children having MIBG avid (or MIBG positive) disease and has formed a core part of the treatment of relapsed neuroblastoma over the last few years. It may be expected to improve or consolidate the response to the intense chemotherapy.

- A new aggressive treatment approach is being explored but further research is needed to show whether it can produce long lasting remissions. Haploidentical stem cell transplantation (Haplo-HSCT) involves taking stem cells from a parent and transplanting them into the patient following myeloablative chemotherapy to completely destroy the existing bone marrow system. The transplant process effectively equips the patient with a new immune system, which is hoped to be able to target any remaining cancer cells in a way the patient's own immune system was unable to. The graft of donor stem cells is engineered in such a way that T and B cells are depleted, but large numbers of NK cells are infused.

Salvage regimens in the recent era have altered the natural disease course and prolonged post-relapse survival. Many of the factors at diagnosis that are prognostic of survival also influence survival after disease progression or relapse. Factors identified as most highly prognostic of poor survival were age≥18 months, use of intensive multi-modality treatment at diagnosis, stage 4, elevated serum ferritin, elevated LDH, unfavourable histology, high MKI, and MYCN amplification; in addition, shorter time to first relapse was a significant adverse factor for survival.

Up to half of these patients achieve some response or stable disease, and survival after relapse is longer in patients who have received salvage therapy. In an INRG analysis of 2,266 patients who experienced first progression/relapse, the median time to relapse was 13 months, and 5-year OS from the time of first relapse was 20% (London & Castel et al, 2011). The longer survival after relapse is also likely due to early detection of disease recurrence as a result of employing more sophisticated surveillance studies in recent years.

There is rationale for a child who has responded to second-line chemotherapy and/or MIBG therapy, and now has only MRD, to receive immunotherapy with ch14.18 and isotretinoin (13-cis RA) in order to try and achieve long-term remission.

About the product

Dinutuximab beta is a chimeric monoclonal IgG1 antibody that is specifically directed against the carbohydrate moiety of disialoganglioside 2 (GD2), which is overexpressed on neuroblastoma cells.

Dinutuximab beta has been shown in vitro to bind to neuroblastoma cell lines known to express GD2 and to induce both complement dependent cytotoxicity (CDC) and antibody dependent cell-mediated cytotoxicity (ADCC). In the presence of human effector cells, including peripheral blood nuclear cells and granulocytes from normal human donors, dinutuximab beta was found to mediate the lysis of human neuroblastoma and melanoma cell lines in a dose-dependent manner. Additionally, in vivo studies demonstrated that dinutuximab beta could suppress liver metastasis in a syngeneic liver metastasis mouse model.

Neurotoxicity associated to dinutuximab beta is likely due to the induction of mechanical allodynia that may be mediated by the reactivity of dinutuximab beta with the GD2 antigen located on the surface of peripheral nerve fibres and myelin (see section 5.1 of the SmPC).

The claimed indication was:

“Isqette is indicated for the treatment of patients with neuroblastoma.

90 % of patients diagnosed with neuroblastoma are below 5 years of age, with a median age at diagnosis of 22 months. Less than 10 % of the cases occur in patients being older than 10 years.”

The CHMP recommended the following indication for Dinutuximab beta Apeiron: treatment of high-risk neuroblastoma in patients aged 12 months and above, who have previously received induction chemotherapy and achieved at least a partial response, followed by myeloablative therapy and stem cell transplantation, as well as patients with history of relapsed or refractory neuroblastoma, with or without residual disease. Prior to the treatment of relapsed neuroblastoma, any actively progressing disease should be stabilised by other suitable measures.

In patients with a history of relapsed/refractory disease and in patients who have not achieved a complete response after first line therapy, Dinutuximab beta Apeiron should be combined with interleukin 2 (IL 2).

The recommended posology consists of 5 consecutive courses, each course comprising 35 days. The individual dose is determined based on the body surface area and should be a total of 100 mg/m^2 per course.

Two modes of administration are possible:

- a continuous infusion over the first 10 days of each course (a total of 240 hours) at the daily dose of 10 mg/m^2
- or five daily infusions of 20 mg/m^2 administered over 8 hours, on the first 5 days of each course.

When IL-2 is combined with Dinutuximab beta Apeiron, it should be administered as subcutaneous injections of 6×10^6 IU/m^2/day, for 2 periods of 5 consecutive days, resulting in an overall dose of 60×10^6 IU/m^2 per course. The first 5-day course should start 7 days prior to the first infusion of dinutuximab beta and the second 5-day course should start concurrently with dinutuximab beta infusion (days 1 to 5 of each dinutuximab beta course).

Prior to starting each treatment course, the following clinical parameters should be evaluated and treatment should be delayed until these values are reached:

- pulse oximetry > 94% on room air

- adequate bone marrow function: absolute neutrophil count ≥ 500/µL, platelet count ≥ 20.000/µL, haemoglobin > 8.0 g/dL
- adequate liver function: alanine aminotransferase (ALT)/ aspartate amino transferase (AST) < 5 times upper limit of normal (ULN)

adequate renal function: creatinine clearance or glomerular filtration rate (GRF) > 60 mL/min/1.73 m^2.

Type of Application and aspects on development

This application has been submitted under Article 8.3 of Directive 2001/83/EC - complete and independent application. During the evaluation of the application, the CHMP considered that the data provided in support of this application is not comprehensive and proposed to consider marketing authorisation under exceptional circumstances, and consulted with the applicant. The applicant supported proposal for a marketing authorisation under exceptional circumstances and provided the following justification:

- The benefit-risk balance is positive.
- The applicant is unable to provide comprehensive data. Since 2009, when the results of NCI/COG study ANBL0032 (supporting the MA for Unituxin) were reported at ASCO and later on also published (Yu, Gilman et al. 2010) immunotherapy with ch14.18 antibodies has become the "standard of care" for patients suffering from high-risk neuroblastoma, worldwide within the scientific community of paediatric oncologists and patient associations/parents. For this reason, it is considered unethical not to provide patients with GD2 specific immunotherapy excluding the possibility to have a control arm in the context of a randomised clinical study.
- The approval would ensure the availability of an effective treatment modality to patients with neuroblastoma patients in the European Union (which is considered as standard of care) and thus represent a significant improvement to patient care.
- The uncertainties related to the efficacy and safety of the product will be further studied with the conduct of a drug exposure registry (SAFARY). In addition to long-term safety information, data will be specifically generated on pain intensity and need for pain medications, effect on peripheral and central nervous system, including visual impairment; data on short-term anti-tumour response and long-term survival will also be collected.

2.2. Quality aspects

2.2.1. Introduction

Dinutuximab beta (also called ch14.18/CHO or APN311) is a monoclonal antibody that is specifically directed against carbohydrate disialoganglioside GD2, which is overexpressed by cells of neuroectodermal origin such as neuroblastoma cells. APN311 triggers complement-dependent cytotoxicity (CDC) resulting in activation of the complement cascade and lysis of target cells, as well as antibody-dependent cell-mediated cytotoxicity (ADCC), mediated by recruitment of natural killer (NK) cells via the Fc-receptor (CD16, FcγR) interaction of the constant region of the heavy chains, finally leading to target cell lysis.

Dinutuximab beta Apeiron is formulated as a concentrate for solution for infusion that comprises 4.5 mg/mL dinutuximab beta formulated with histidine, sucrose, polysorbate 20, hydrochloric acid for adjustment to pH 6.0 and water for injections. To ensure an extractable volume of 4.5 mL the vials are

filled with 4.9 mL finished product. A solution of 0.9% sodium chloride containing 1% human albumin for dilution (provided separately) is used for the preparation of the final solution for infusion.

2.2.2. Active Substance

General Information

Dinutuximab beta is a monoclonal, chimeric (murine/human) antibody of IgG1 subclass consisting of 2 light chains (220 amino acids) and 2 heavy chains (443 amino acids). The monoclonal antibody incorporates human constant regions for the heavy chain IgG1 and the kappa light chain, along with the mouse variable regions targeted specifically against human disialoganglioside (GD2).

The relative molecular mass of the intact antibody is approximately 150,000 Dalton. There is a single N-linked glycosylation site (Asn 293) and mass spectrometry analysis revealed that the heavy chain contains the typical IgG diantennary fucosylated N-glycans with 0, 1 or 2 galactose residues, with a smaller fraction of glycans with sialic acid and oligomannose residues. No Gal-α-1,3 Gal is present in these glycans, typical for IgG expression in CHO cells.

Manufacture, characterisation and process controls

The active substance is manufactured at Rentschler Biotechnologie GmbH, Erwin-Rentschler-Str. 21, Laupheim 88471, Germany.

Manufacturing process and controls

The monoclonal antibody ch14.18/CHO is expressed in a recombinant CHO cell line. The production of one batch APN311 active substance commences with thawing of one vial APN311 working cell bank (WCB). During a series of four sub-cultivations, the cells are propagated in shake flasks and subsequently in 20 L, 100 L and 500 L bioreactors to achieve cell expansion for subsequent inoculation of the production bioreactor. Cultivation of the cells in the production bioreactor is performed in fed-batch mode, using a fixed feeding strategy. The cultivation is terminated when a viability is reached, followed by harvesting and clarification of the supernatant. The biomass is separated by centrifugation and the supernatant is depth filtered followed by a 0.2 µm filtration.

The harvest of one production bioreactor is ultra-filtered for concentration of the harvest and capture of dinutuximab beta is performed in up to three Protein A chromatography runs, depending on product concentration of the ultrafiltration harvest. After washing, the bound antibody is eluted from the column using a pH-shift and is collected as a single fraction per cycle. Every Protein A capture elution is followed immediately by a low pH virus inactivation step. The virus-inactivated intermediates are pH adjusted then filtered, pooled and further purified in one anion exchange flow-through chromatography cycle (Q Sepharose Fast Flow), to reduce endotoxins, host cell proteins (HCP), DNA and Protein A leachables. The anion exchange chromatography is also defined as a virus removal step. The flow-through fraction is ultrafiltered and diafiltered, followed by a mixed mode flow-through chromatography step (Capto Adhere), to reduce product-related impurities. The last steps are the third ultrafiltration/diafiltration step, performed to adjust the product concentration and to exchange the matrix in the final formulation buffer. This is followed by the virus nanofiltration step for retention and removal of potential viral particles, with filtration through a cascade of a 0.2/0.1 µm filter and finally a virus reduction filter. The final formulation step is to adjust the final active substance excipient composition and protein concentration. A final 0.2 µm filtration is performed before aliquoting the APN311 active substance in single use containers (bags), which results in a single batch of APN311 active substance. The labelled active substance bags are stored at for up to 9 months until further processing, at Rentschler Biotechnologie.

Reprocessing is not allowed nor anticipated for any of the steps described.

5 GMP batches were manufactured using the proposed commercial process v.2.1: GMP1, GMP2, GMP3, GMP4 and GMP5.

Control of materials

The quality of raw materials is controlled in accordance with the principles of ICH Q6B. All raw materials (except for the cell line), used in the upstream and downstream manufacturing process of APN311 active substance are animal component free. All media and supplements are serum and protein free; the media do not contain any material of animal origin and are manufactured without the use of raw materials containing animal origin components. The buffers, reagents and raw materials are suitably documented.

Source, history and generation of the cell substrate

DHFR-deficient CHO cells were transfected with the ch14.18 plasmid, pdHL7, containing coding sequences for mouse-human chimeric dinutuximab beta (GD2) monoclonal antibody. This is the same plasmid as previously used for generation of ch14.18/SP2/0 and ch14.18/NSO in early development.

Vector sequencing was performed and the nucleotide sequences of the heavy chains and light chains were confirmed. Following transfection, high producing clones were isolated using selection medium and two rounds of limited dilution sub-cloning, to isolate a suitable clone for production of dinutuximab beta (ch14.18/23B11/20D2/6A8). These cells were adapted to serum-free culture conditions and also protein-free culture, before a third sub-cloning step for selection of the serum-free sub-clone which was used to generate the research cell bank (CHO/ch14.18/6A8/6F12/SF). Six ampoules of the research cell bank (RCB) were stored in liquid nitrogen.

The Master Cell Bank (MCB) was prepared in August 2004 by expansion of cells from one ampoule of the primary RCB and stored in protein-free freezing medium in a liquid nitrogen tank. The MCB was shown to be stable during long term cultivation over 6 months and in the presence of methotrexate (total cell number and viability, specific growth rate and specific production rate), showing that the MCB is suitable for large scale production. Genetic characterisation confirmed vector integration in a single, specific site and the mean copy number was 6 copies / cell for antibody light chain and 3 copies / cell for antibody heavy chain. Adventitious agent testing was performed on the initial MCB, with additional testing on transfer of the MCB. The MCB was negative for the presence of microbial contaminations, mycoplasma, adventitious viruses and various specific viruses (murine, bovine and porcine viruses) and retroviruses. In addition, quantitative polymerase chain reaction (qPCR) assays were employed for specific detection of minute virus of mice (MVM)-DNA sequences. Although one original *in vivo* assay showed a positive result on embryonated cells and cytopathic effect on CHO cells, this finding could not be repeated in three further replicates. No evidence was found for presence of adventitious viruses, in compliance with ICH Q5D and Q5A.

The WCB was prepared in May 2011 by expansion of cells from one ampoule of the MCB and subsequently stored in protein-free freezing medium in a liquid nitrogen tank. Phenotypical characterisation and microbiological safety testing were performed, but no further viral safety testing was done. This is acceptable since the parental MCB was fully tested for viral safety and *in vitro*/*in vivo* tests are performed at the level of the end-of-production cells (EPC), in accordance with ICH Q5A. In 2013, genetic characterisation and further tests for microbial contamination were performed, which were satisfactory.

Two EPC banks were prepared: EPC1 comprised cells obtained from the harvest at the end of a production run for GMP2 and EPC2 was generated using an aliquot of cells obtained from the same bioreactor run at day 6 and cultivated in shake flasks up to day 19, to maintain the viability of cells in order to perform safety assays. Microbial safety testing showed EPC1 was negative for microbial contamination and

mycoplasma. EPC2 was tested for adventitious viruses and retroviruses, with qPCR assays used for specific detection of MVM-DNA sequences. These were all negative, although intracytoplasmic A-type particles were detected by quantitative transmission electron microscopy (TEM) in 6 of 200 cells; no virus-like particles (VLP) were detected in the supernatant and the possible viral load was calculated as < 4.2×10^4 VLPs/mL. No evidence was found for reverse transcriptase activity or replication competent retroviruses, suggesting that there are no infectious retroviruses present in EPC2.

Comparative genetic characterisation included sequence analysis of APN311-specific cDNA derived from MCB, WCB and EPC (RT-PCR and cDNA sequencing), showing heavy chain and light chain is identical in MCB, WCB and EPC (0, 5 and 10 days, also 19 days at small scale) and corresponds to the reference sequence, confirming genetic stability of the cell line. Integration of the recombinant expression vector into the host cell genome at the chromosomal level was determined by fluorescence in-situ hybridisation on MCB cells, although the results showed a high level of chromosome rearrangements compared with the hybridisation negative control (CHO $dhfr^-$ host cells), which is likely to be induced by methotrexate. Based on this analysis it was not possible to confirm clonal status of MCB, although one single specific and stable vector integration site was detected for the MCB cells in 95% of all metaphase chromosomes, which suggests stable integration of the vector.

Determination of the transgene copy number showed 6 copies per cell for light chain and 2 - 3 copies per cell for heavy chain (MCB and WCB), with a slightly lower copy number for the day 19 extended culture samples (5 copies for light chain and 2 copies for heavy chain). While these results might indicate some instability over extended production, no reduction in productivity was detected up to 10 days in the production bioreactor. Differences observed in the SDS-PAGE band pattern at the expected molecular mass for IgG under non-reducing conditions, particularly after 45 passages for the MCB, have been explained. Genetic stability of the WCB and EPCs at mRNA level (in comparison to the MCB) for the intended period of use was confirmed. The potential impact of different copy numbers for light and heavy chain on product quality has been discussed; although there are twice as many gene copies for the light chain in the production cell line, if excess light chain fragments were present these would be removed during the purification process. This is confirmed by the level of low-molecular weight species (LMWS) detected in GMP production runs.

Safety testing for the cell bank is in accordance with ICH Q5A. The source of the foetal calf serum (FCS) is considered satisfactory.

No protocol with description of manufacture and testing acceptance criteria for replacement of the WCB was provided. The Applicant confirmed they will file a variation in case a new working cell bank is introduced.

Control of critical steps and intermediates

Suitable in-process controls (IPC) have been identified in the dossier. Acceptance criteria and ranges for cell culture / fermentation (upstream process, USP) and purification (downstream process, DSP) were based on process characterisation studies and process performance qualification (PPQ), which was performed in combination with risk analysis exercises for all process parameters and steps.

For cell culture/fermentation, minimum viable cell number and/or concentration were defined to ensure proper growth of cells. It is noted that the PPQ batches showed harvest cell viability of 27 - 40%. Controls were defined to ensure absence of contamination by adventitious agents. Bulk harvest testing by *in-vitro* virus assay as well as for absence of mycoplasma is performed routinely. Bioburden and endotoxins are controlled at multiple steps with limits throughout the entire manufacturing process, i.e. from harvest to bulk active substance (and finished product) to guarantee that the process is under control.

Once the production bioreactor is harvested, supernatant is clarified and APN311 purified using a number of concentration, capture and flow-through purification steps. The Protein A capture elution is followed immediately by a low pH virus inactivation step. The anion exchange flow-through chromatography cycle (Q Sepharose Fast Flow) reduces impurities such as endotoxins, HCP, DNA and Protein A leachables and a mixed mode flow-through chromatography step (Capto Adhere column) reduces levels of high and low molecular weight species, which are controlled as critical quality attributes (CQAs) of APN311. Defined minimum yield levels ensure proper performance of all steps.

A systematic overview of all critical process parameters (CPPs) and IPCs related to the chromatographic steps has been given with an associated justification. This approach is considered as a traditional process description, which is substantiated with enhanced development and characterisation studies. For a few parameters the ranges have been defined, which are supported by 2^2 full-factorial design of experiments (DoE) studies for each step; while for other parameters, only set-points are defined and no ranges are attached, therefore validation only at set-point is performed. The data are sufficient to conclude that the proposed CPPs and IPCs are justified and able to control the manufacturing process appropriately.

There are no isolated intermediates with extended storage time during manufacture of APN311 active substance. The clarified harvest holding time is either ≤ 24 hours at 18 - 26°C, or ≤ 72 hours at 2 - 8°C. Short-term holding times (depending on the process step) have been defined based on data obtained from the accumulative hold time study. The maximum duration between successive process steps at room temperature (18 - 26°C) is ≤ 24 hours.

Process validation

Process characterisation studies performed for USP and DSP Proven Acceptable Range (PAR) studies investigated the influence of varying process parameters during APN311 manufacturing process on cell/process performance and product quality. For USP, representative small-scale bioreactors (5 - 10 L scale) were used in PAR experiments for the evaluation of cultivation parameters considered a high risk or with potential impact on quality, such as pH, temperature, seed cell concentration and low oxygen pressure ($pO_{2)}$. A total of 23 bioreactor runs was performed, followed by further processing in DSP and subsequent product analysis. The DoE studies showed that for the production bioreactor, pH/ temperature/seed concentration all influence the content of the major N-glycans and pH affects the activity of APN311. Yield was also affected, but was above the level required for further purification at harvest. The process parameter pO_2 is a non-key operational parameter. The proposed operating ranges are relatively tight and data from proven acceptable ranges (PAR) studies support the IPC ranges for upstream processing of APN311.

For downstream processing, a 2^2-full factorial DoE approach was performed for the three chromatographic steps to systematically test the influence of two different parameters on each other, e.g. column load and load material pH. The centre point runs of the DoE were used as scale-down verification runs (for virus validation studies), as well as column lifetime studies and to determine the maximum processing times for individual steps. Elevated aggregate levels were observed after thawing various starting materials from GMP1, although it is noted that this is not an issue in production since the intermediate storage temperature is 5 ± 3°C. The main observed differences between the runs were the step/overall yield, which ranged from 33.4% to 77.1%, as well as slight variations in high molecular weight species (HMWS) content and HCP level for various intermediates, although the HMWS was reduced to 0.1% by downstream processing in all cases. The GD2 activity and CD16 affinity was comparable across best case, worst case and centre point runs, compared with GMP1 and GMP2 values. The majority of critical and key operational parameters for the downstream process are acceptable, with the minimum and maximum loading density for MabSelect SuRe, Q Sepharose-FF and CaptoAdhere chromatography steps defined during process development and process characterization studies in a DoE-based approach.

Although these are wider than the range observed in the four PPQ runs, the loading ranges have been justified. For the viral reduction steps, maximum loading density for Protein A and Q Sepharose chromatography in the viral validation studies is in line with the claimed maximum load for these steps.

A clarified description of the worst case ultra-/diafiltration (UF/DF) runs was submitted, which demonstrates the validity and robustness of these UF/DF steps. The suggested tightening of the relevant CPPs/PPs for step I30 has been implemented; the retentate flow rate is updated from 380 - 580 to 480 - 580 L/(hxm^2), because the data demonstrated a decrease in the amount of monomers under these conditions.

Results of the column lifetime studies using the representative scale-down models supported use up to 20 cycles (Protein A column), 10 cycles (Q Sepharose FF) and 11 cycles (Capto Adhere column). Column lifetimes will be evaluated in ongoing GMP manufacturing batches at production scale. Column cleaning, regeneration and sanitization are addressed both in PPQ and in large-scale column lifetime studies (ongoing) and corresponding small-scale studies. For all columns, blank runs are used to evaluate efficiency of cleaning procedures and potential bioburden contamination or carryover of impurities such as HCP, residual DNA and protein.

Process validation at manufacturing scale was performed on four consecutive production runs (PPQ). Given the low number of overall production batches required for the orphan indication, the 2 first consecutive GMP runs were included and retrospectively analysed, whereas GMP runs 3 and 4 were executed according to pre-defined process validation protocols. Process parameters and product quality of all 4 GMP runs were in compliance with defined acceptance ranges, criteria and specifications. Additional criteria showed that the cell culture process is well within the maximum allowed population doubling levels (PDLs) covered by genetic stability testing, that the acceptance criteria for the monitoring of potential contaminants in the production bioreactor and clarified harvest were fulfilled, including bioburden, mycoplasma, viruses and endotoxins. For DSP, evaluation of additional parameters during process validation showed reliable removal of product-related impurities and process-related impurities down to acceptable levels. Removal of process-related impurities has been assured by additional in-process testing at step 13 (V10-PP) and step 16 (C30-PP), with these impurities below LOQ at the tested intermediates. Acceptance criteria for these process-related impurities have been included in the active substance specification. Microbiological control strategy showed that the process is under control with respect to potential contaminants. Data from the four PPQ batches confirmed that the active substance manufacturing process is well controlled and results in APN311 of consistent quality. The step yield limits for I20 and I30 product pools for ultrafiltration / diafiltration, or I50 product pool for formulation have been revised in line with the large scale batch data (GMP1 - GMP4). Additional parameters will also be monitored as part of continuous process verification with concurrent analysis of data at commercial scale. All tested parameters as defined in the revised control strategy will be evaluated on an ongoing basis covering all lots (current and future batches), including statistical evaluation as part of continuous process verification.

Manufacturing process development

Changes were made between APN311 manufacturing campaigns v.1.0 in 2004 (batch T65) and 2009/2010 (batch T90), and manufacturing campaign v.2.0 in 2012 (batch T110), with an intermediate engineering run (batch T99) using manufacturing process v.2.0. Further minor changes were made following transfer of APN311 in 2013 - 2014, with 5 GMP batches manufactured using the proposed commercial process v.2.1: GMP1, GMP2, GMP3, GMP4 and GMP5.

Upstream changes from process v.1.0 to process v.2.0 included introduction of a WCB (engineering batch T99), minor changes in culture medium, concentration of clarified supernatant by ultrafiltration (storage of concentrated 0.2 µm-filtered retentate instead of clarified supernatant), change in bioreactors at new

facility (FR02, also FR03 and FR04 which can be run in tandem), scale up with a slightly different fill and feed strategy (batch T110) and more efficient clarification and increase in ultrafiltration membrane area. The storage vessels used for media and production harvest at the new facility are all disposable bags. Significant changes are the facility changeand introduction of the ultrafiltration step and different bioreactors. Manufacturing steps were essentially unchanged on transfer (process v.2.1). Upstream fermentation steps were comparable, with a few minor improvements made in thawing, pre-culture, seed culture and production steps at RB. None of the changes were expected to have a negative impact on quality.

Changes in downstream processing included introduction of a nanofiltration step (T90), change of Protein A chromatography from expanded bed (Protein A resin) to fixed bed mode (MabSelect SuRe) with 2 - 3 cycles per harvest (T99 / T110), introduction of an additional purification step to reduce fragments and dimers (Capto Adhere mixed mode chromatography) and change in formulation buffer from phosphate-buffered saline (PBS) to sucrose, histidine and Tween 20, pH 6.0 ± 0.5, in order to reduce precipitation observed in PBS. The changes are expected to improve the quality of the product, in terms of safety (virus nanofiltration) and quality (reduction in impurities). Other changes in downstream processing on transfer to RB include holding the cooled/ 0.2µm filtered harvest at ambient temperature overnight, change in the supplier of UF/DF filters and the virus nanofiltration followed by a separate formulation step. Changes in chromatography column peak cuts and wash steps were also included, which improved product yields.

Comparability exercise to support manufacturing process development

A comparability study evaluated an extensive range of quality, purity, identity, activity and safety attributes and compared these for T65 and T90 (process v.1.0) and T110 (process v.2.0) GMP manufactured material (including T99 engineering run material), with analytical results generated during batch release testing and additional comparative characterization. Further comparability studies were performed for active substance manufactured in 2013/2014 using the proposed commercial process (v.2.1) with batch T110 (process v.2.0), in line with ICH Q5E. No significant differences could be observed between processes v.1.0, v.2.0 and v.2.1 according to release testing results, other than in the pH value and osmolality (due to the change in active substance formulation), or in purity where 100% monomer was detected following optimisation of the purification process for T99/T110 batches. Comparability was demonstrated in batch release testing for T65, T90 and T110, and also for T110-finished product, GMP1-active substance, GMP2-active substance and GMP1-finished product. No significant differences in potency were found between the v.1.0 and v.2.0 batches, or v.2.0 and v.2.1 batches; with specific GD2-target binding by ELISA and biological activity. SE-HPLC analysis showed that monomer (area%) was also consistent between T110, GMP1 and GMP2. Although glycans, HMWS/LMWS impurities by SDS-PAGE, CIEX HPLC main/acidic/basic peaks or potency were not determined for T110-finished product at release testing, some of these were determined in the extended characterisation studies.

Extended characterisation data showed similar structural features for APN311 manufactured by these different processes, by far-UV and near-UV circular dichroism spectra, similar light and heavy chain masses (including major glycosylation forms of the non-reduced antibody) by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) and electrospray ionisation time of flight mass spectrometry (ESI-TOF-MS), with comparable results by peptide mapping (sequence coverage of 100% light chain and 96 - 98% by MALDI-TOF MS or >90% by ESI-TOF MS for heavy chain). Confirmation of C- and N-terminal sequences was provided, with C-terminal lysine missing for all the heavy chain samples (T110 and GMP1/GMP2). Correct positions of the disulfide bridges were shown and quantitation of free SH groups was demonstrated to be very low for all samples. Deamidation rates were all below 1% by liquid chromatography electrospray ionisation tandem mass spectrometry (LC-ESI-MS/MS). Analysis by weak cation-exchange chromatography (WCX-IEC) indicated higher acidic

peaks in batches T65 and T90 at the time of analysis, but this was attributed to the effect of storage (up to 8 years), since sample characteristics were similar at the time of release. The WCX main peak and acidic peaks were comparable for T110 and GMP 1 and 2, with no basic peaks detected in any samples. Slight differences were attributed to the age of T110 at the time of testing (12 months at 5°C).

Slightly higher levels of G0F were noted in batches T90/T99/T110, compared with batch T65, with a corresponding reduction in G1F and G2F; sialylated glycans were also slightly reduced in these later batches. Similar results were obtained for the glycan analysis by HPAEC-PAD for T110 (process v.2.0) and GMP1/GMP2 (process v.2.1).

Based on the comparison of the relevant quality attributes, both from release testing and formal side-by-side testing, pre-change (process v.1.0: T65, T90) and post-change active substance (process v.2.0: T110) and the proposed commercial active substance (process v.2.1: GMP1 and GMP2) are considered comparable.

A head-to-head comparative stress testing stability study (3 months at 40°C) between T110 finished product (process v.2.0) and GMP2 (process v.2.1) was performed to evaluate potential differences in the degradation profiles. It is noted that active substance and finished product have the same formulation and concentration, thus comparison of data from either is acceptable. Although the T110-finished product was stored in glass vials for up to 16 months at 5°C prior to pooling into 30mL PETG bottles before starting the comparability stress testing, the values at the beginning were similar and would not explain the large change seen in GD2 binding activity and particularly the CDC assay results for T110 finished product. Comparable results were obtained for stress testing for CD16 binding activity, changes in the charge variants by CIEX-HPLC over 3 months at 40°C (reduction in main peak and increase in acidic peaks and slight increase in basic peaks), monomer and LMWS.

Direct comparison between finished products was made for T110-finished product and the first GMP finished product batch (1018750) derived from GMP1 active substance, in a second comparative stress testing study performed at 40°C. In this study, GD2 binding activity and CDC assay showed similar profile for T110 finished product and GMP1 finished product in glass vials, with comparable results for CD16 binding activity, CIEX-HPLC, monomer (and LMWS), purity (by reducing and non-reducing SDS-PAGE) and N-glycosylation profiles, over 3 months at 40°C. This second study provides some reassurance following discrepancies noted in the first stress stability study.

Additional data from the second comparative stress study performed at 40°C was provided to support comparability of clinical and commercial material. Analysis of the charge heterogeneity showed that differences are mainly due to changes during storage, and that the basic (intrinsic) pattern is similar. C1q binding and CD16 binding do not appear to be stability-indicating; however, GD2 binding, and concomitantly CDC and ADCC are clearly stability-indicating and the comparable data support that potency of the batches is similar. Changes during storage and the extent of oxidation at M34/W36 and M248 (HC Fc-region), or isomerisation at D52 (HC CDR2) and D261/266 (HC Fc-region), or deamidation at N33/35 (HC CDR1), as well as N33 (LC CDR1), was investigated by storage under stress conditions (40°C, over 1 – 3 months). Data clearly indicate a relevant effect on binding (GD2 and CD16) and potency (CDC and ADCC), which were correlated to the formation of charge variants by CIEX-HPLC. The content of acidic and basic variants is controlled as CQAs in the routine release testing. These represent suitable specification limits to ensure control of oxidation and deamidation in APN311.

Characterisation

Most lots of APN311 produced were analysed and submitted to extended characterisation, including early lots T65, T90, T99 and T110, as well as recent batches, GMP1 and GMP2. Analysis of primary reference standard SR-951PI.1 (derived from GMP1) has also been included as a representative sample.

The amino acid sequence of APN311 was confirmed, with sequence coverage of up to 98% for the heavy chain and 100% for the light chain. The C-terminal lysine of the heavy chain was missing in the majority of samples; APN311 with intact C-terminus was detected among the basic charge variants, typically present at less than 10%. All peaks in the peptide map were identified, with potential areas for modification analysed further by mass spectrometry following storage under stress conditions. Secondary structure was determined as typical for an antibody, by circular dichroism. The results are in the expected range for mass determination for light chain and heavy chain, by MALDI-TOF MS and ESI-TOF-MS, with the mass of reduced light chain of 24071 ±1 Da and masses of the reduced heavy chains (without C-terminal lysine) comprising the three major glycosylation forms G0F, G1F and G2F, with two major signals at 49753 ±1 (G0F), 49915 ±1 (G1F) and two minor peaks of 49525 Da (Man5) and 50077 ±1 Da (G2F). Glycosylation analysis was further evaluated using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) showing neutral oligosaccharide structures as the main glycan component consisting of predominantly 1,6-fucosylated diantennary glycans without one and two ß-galactose residues. Diantennary glycans with a-1,6-linked fucose, truncated glycans lacking ß-galactose and N-acetylglucosamine residues and oligomannosidic Man4-9GlcNAc2 glycan chains were found in all antibody samples. Sialylated glycans (N-acetylneuraminic acid) were detected in ≤ 3.6% of total N-glycans. Further information supports site occupancy for glycans of >99%, and confirms that the glycan pattern is consistent between batches.

A theoretical extinction coefficient is used for determination of ch14.18/CHO concentration, which was subsequently confirmed by determination of the experimental extinction coefficient. Purity of APN311 was determined using a number of different methods, with reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE); non-reducing SDS-PAGE or by SE-HPLC. Identity by non-reducing SDS-PAGE shows the expected band of about 150 kDa corresponding to the expected size of an intact IgG1, but smaller faint bands were also visible at about 100 kDa, 70kDa and 26kDa on the gels. The identity of these bands has been clarified as product-related variants of ch14.18/CHO, with only traces of HCP. Bands at 50kDa appear to contain heavy chain and two light chains, with the band at 25 kDa appearing to be mainly free light chain with low levels of HCP. Since these are all at levels well below the main band (by densitometry), these product-related impurities are not expected to have an adverse impact on the dinutuximab product.

CIEX-HPLC of ch14.18/CHO shows main, acidic and basic peaks. Different charge variants are part of the normal distribution, correlated to presence or absence of C-terminal lysine and mono-, di- and tri-sialylated variants of glycans, which are regarded as product-related substances. Increase/decrease in these groups may change due to degradation, and other modifications such as oxidation or deamidation/isomerization of amino acid side chains.

Potency was determined by specific GD2-binding activity and CD16 binding activity, which is used as a surrogate for ADCC activity, as well as CDC activity. Additional information was provided to support the use of relative binding for both CD16 and GD2 binding. Measurement of CD16 receptor binding instead of ADCC activity was also justified, using data from production batches, stressed samples and different charge forms. Under accelerated conditions (25°C) or stress testing (40°C), an increase of acidic charge variants was observed by CIEX-HPLC, which was correlated to a reduction of GD2 target binding.

Process-related impurities have been shown to be removed to sufficiently low levels during the purification process.

Specification

The active substance specification for dinutuximab beta is presented in and includes identity, purity and impurities, potency and other general tests. Potency is controlled by 3 methods:

- GD2-binding activity: This test method is designed and validated for quantitative evaluation of the potency of APN311 by measuring the specific binding activity to the antigen GD2 *in vitro*.

- CD16-binding activity: CD16 Binding activity as a surrogate for Antibody Dependent Cell-Mediated Cytotoxicity (ADCC) activity. Specific binding to Fc receptor CD 16 determines the efficacy of the antibody to bind to natural killer (NK) cells.

- Complement Dependent Cytotoxicity (CDC): This test method is designed and validated for the quantitative evaluation of the potency by measuring CDC for specific and dose-dependent target cell lysis induced by APN311.

Analytical methods for release and stability testing of the active substance were described.

Methods used for testing of active substance and additional impurity testing include SDS-PAGE for the quantitative determination of purity/impurities under non-reducing conditions (method APA-QC-025, Protein A-HPLC for ch14.18/CHO content (APA-QC-002).

Contaminants such as endotoxins, bioburden and mycoplasma are tested by the relevant Ph. Eur. methods (2.6.14, 2.6.12 and 2.6.7, respectively), with adventitious virus testing (14 days *in vitro* assay) by SOP APC-0271. Osmolality and pH are also tested using Ph. Eur. methods (2.2.35 and 2.2.3, respectively).

Data has been provided to substantiate that separate control of oxidation and deamidation is not necessary, because changes to the ion-exchange HPLC (IEX-HPLC) pattern are sufficient to pick up these changes and the specifications for IEX-HPLC are sufficiently well-defined. The limit for residual HCP of should be reviewed/revised after manufacture of 20 batches, since current batch data is limited. The specification limit for the Man5 glycans has been tightened based on the small-scale transfer and process characterisation runs, as well as the 5 GMP batches to date. In order to align acceptance limits for all afucosylated structures quantified, the proposed limits for cGN2 have been tightened.

A commercial assay has been used for detection of host cell protein and this assay is not specific to the manufacturing process. Further data has been provided to demonstrate that theHCP assay provides sufficient coverage of the HCPs present in the APN311 upstream samples (harvest after filtration), confirming that the HCP assay is sufficiently representative for quantitative detection of HCPs in APN311 in-process control samples to enable this to be used in place of a process-specific HCP assay. Information on the samples used for determination of linearity in the HCP assay has also been provided.

The validation of analytical procedures was performed. In addition, matrix validation was performed for compendial tests for bioburden and endotoxin.

The 4 GMP batches of APN311 show comparable results and all results are within specification. Identity was confirmed and comparable purity/impurity profiles were obtained, with only low levels of HMWS and LMWS observed in these batches. Protein characterisation data show comparable results for all batches, with similar CIEX profiles for all 4 batches. N-glycan analysis profiles were similar, although some batch-to-batch variability was observed. Robustness of the sample workup (specifically N-glycosidase F lots and incubation time) for N-glycan analysis has now been validated.

Potency was consistent across batches, with GD2 levels relative to reference standard, CD16 relative to reference standard and CDC levels. Both product-related and process-related impurities were cleared to acceptably low levels, in many cases well below the set acceptance criteria. Endotoxin was well below the specification limits (and below LOQ) and bioburden levels were 0 cfu/10mL.

Reference standards

The primary reference standard SR-951PI.1 has been used for analyses of active substance / finished product since April 2014 (GMP3 and GMP4), as well as for stability testing. This primary reference standard (SR-951PI.1) was derived from GMP1 and characterised using the previous interim reference standard SR-950KU.1 (derived from T110 in 2012), with the exception of the complement-dependent cytotoxicity (CDC assay). Reference standard SR-950KU.1 was previously used for validation of the different analytical methods and release of active substance batches GMP1 and GMP2.

Reference material L03/CH1418/UDF was derived from non-GMP production in 2009 using process v.1.0 and used for release of batches T90 and T110, as well as method validation and stability testing. Prior to this, early development reference material (48P8-1PS/01) was manufactured in 1997.

Results presented demonstrate that the interim reference standard SR-950KU.1 and primary reference standard SR-951PI.1 are comparable. Additional data, including reducing and non-reducing SDS-PAGE gels and Western blots, IEF gels; peptide map fingerprint; chromatograms for N-glycan analysis, SE-HPLC and CIEX-HPLC were given for primary reference standard SR-951PI.1. The primary reference standard SR-951PI.1 has been used for active substance and finished product release since April 2014. This reference standard was shown to be suitable for its intended purpose by comprehensive analysis, including GD2 binding activity, CD16 binding activity and CDC analysis. A potency value of 1.28 (relative to previous reference standard L03/ch14.18/UDF) was assigned to SR-951PI.1 for the CDC assay. Aliquots of the reference standard (200 µL) are stored at -70°C ± 10°C and stability testing will continue for 60 months. Stability has been demonstrated for 24 months at -70°C to date and also at 5°C storage for 2 weeks, with no changes observed within this period.

Further internal reference standards will be qualified in comparison to SR-951PI.1 using an appropriate set of methods and reviewed via a post-approval variation.

Container closure system

Details have been provided for the primary container for the active substance, comprising pre-sterilised single-use storage bags (Flexboy containers). Certification has been provided to demonstrate that these meet appropriate standards for containers for biopharmaceutical products, including an EC certificate. The Flexboy bags are sterilised by gamma irradiation in a validated sterilisation process, with the efficiency of the minimum dose of 25 kGy validated to obtain 10^{-6} sterility assurance level (SAL).

Stability

Stability studies confirm the stability of APN311 under long term storage conditions, with no changes observed for appearance, identity, protein content or glycan profile. For potency under long term stability conditions, the data shows some variations, but all remain within the acceptance criteria given for these parameters. All GD2 binding was between 98 - 111% up to 24 months (for GMP1 and GMP2) and up to 18 months (for GMP3). For CD16 binding, values were between 87 and 123%, while CDC activity was determined as 84 - 110% up to 24 months (GMP1 and GMP2) and up to 18 months (GMP3). No significant changes were observed in the other parameters for these batches over 24 or 18 months.

Supportive data under accelerated conditions (5 ± 3°C) confirmed that no major changes could be observed for GMP1 - GMP3 (and T99 in the new formulation buffer), although there were variations in the CDC activity, with an increase from 107% to 122% for GMP1, reduction from 105 to 85% for GMP2 and 108 to 98.4% for GMP3. Supportive data from T99 under these conditions (up to 16 months) did not show any distinct trends. No changes were observed in the other potency parameters, GD2 binding and CD16 binding, although there were fluctuations in the values across the time-points (0 to 6 months) for these analytical results. A minor trend was observed with slightly elevated quantities of acidic and basic forms, with a corresponding reduction in main peak, determined by CIEX-HPLC for GMP1 - GMP3 under these conditions.

The freeze/thaw stability program was extended to include two additional batches (GMP2 and GMP3). Data from these batches confirm stability of the active substance over three freeze/thaw cycles and stability-indicating parameters (size-exclusion chromatography (SEC), CIEX) do not indicate changes/degradation for all three batches.

2.2.3. Finished Medicinal Product

Description of the product and Pharmaceutical Development

The finished product is presented as concentrate for solution for infusion in a clear Type I glass vial (6 mL) with a halobutyl rubber stopper and aluminium flip-off cap, containing a minimum extractable volume of 4.5 mL concentrate for solution for infusion.

One mL of concentrate contains 4.5 mg/ml dinutuximab beta. Each vial contains 20 mg dinutuximab beta in 4.5 mL.

The active substance is formulated with sucrose, polysorbate 20, histidine, water for injections and hydrochloric acid (for pH adjustment). All the excipients are of Ph. Eur. standard and a slight overfill (4.9 mL) is given to ensure that 4.5 mL can be extracted from the vials.

Pharmaceutical development

Formulation occurs at the last step of the active substance manufacturing, prior to immediate sterile filtration and aseptic fill or storage at -70°C. In accordance with ICH Q5E, an evaluation of the relevant quality attributes has been performed following formulation change and other active substance manufacturing process changes (see active substance section). Comparison of APN311 finished product in the two different formulations showed similar degradation curves under short-term stress conditions. Batches formulated in PBS were T65 and T90, used in the Phase I clinical trials and clinical trials 301 (HR), 201 (Haplo), 202 (LTI) and 303 (LTI-Pilot). Batch T110 was formulated in histidine/sucrose/polysorbate 80 and was also used in clinical trials 301 (HR), 201 (Haplo), 202 (LTI), as well as clinical trials 102 (Japan) and 304 (Single Agent). Both formulations (concentrate for solution for infusion) were/are used for the preparation of the final solution for infusion by dilution in 0.9% sodium chloride and 1% human albumin (HSA). The commercial saline/1% HSA as diluent of the solution for infusion remained unchanged throughout all clinical trials and is the proposed commercial diluent.

Since the finished product manufacturing process consists of thawing of active substance, sterile filtration and aseptic filling into glass vials, this process has not been changed over the course of development (other than the filter material and rubber stopper) and complete process validation has been performed.

Finished product is filled into type I glass vials, closed with coated bromobutyl rubber stoppers and aluminium crimping caps, with a blue flip-off seal. Container closure system integrity was demonstrated to be acceptable for finished product, shown using methylene blue dye ingress testing. Long-term stability studies are ongoing for the finished product in the new formulation, under long-term and accelerated conditions, including sterility testing.

The finished product is a sterile concentrate intended for the preparation of a solution for infusion. The concentrate for solution for infusion is diluted into commercial 0.9% sodium chloride and 1% human albumin, which results in physiological conditions for the final solution infusion, i.e. pH 6.6 and osmolality of about 283 mOsmol/kg, while maintaining product stability at ambient temperature for the duration of infusion.

Manufacture of the product and process controls

One finished product batch is produced using aliquots from one active substance batch (20 - 30L), but it is noted that the finished product pooling consisted of three active substance aliquots (GMP1), four active substance aliquots (GMP2 and GMP4) or five active substance aliquots (GMP3) in the PPQ batches.

Active substance in bags is thawed and held up to 24 hours before homogenisation, filtered and sterile-filtered (with two in-line 0.2 µm filters) into a sterile glass vessel in a Class A area. Filters are washed with buffer and conditioned with bulk solution prior to filtration, with integrity testing before and after filtration. Samples taken before both or after the first filter are tested for bioburden and endotoxin, respectively. The solution is filled into pre-sterilised/depyrogenated vials and stoppers applied immediately after filling. Capping and crimping are followed by 100% visual inspection of the vials before storage at 5°C ± 3°C. There are no isolated intermediates during manufacture of APN311 finished product.

Process validation

Process validation was performed retrospectively for GMP1 and GMP2, with prospective validation of GMP3 and GMP4. Process validation included filter validation (retention of microbes, adsorption/dilution of active substance, extractables and leachables), container closure integrity and microbial safety of the manufacturing process (in-process control and holding times, media fills, sterilisation and depyrogenation of containers/closures and equipment, environmental monitoring).

A mixing study in the 50 L glass bottle examined stirring speed and time, which were tested at or near the limits for GMP3 (maximum limits) and GMP4 (minimum limits). These confirmed homogeneity of the formulated bulk, without any impact on quality (determined by protein content, excipients, SE-HPLC for formation of HMWS/LMWS and GD2/CD16 binding activity). This process characterisation study was used to define a control strategy for this mixing step and CPPs with appropriate limits (shaking frequency and time, stirring speed and time) will ensure homogeneity of the formulated bulk during routine production. Filtration flux was not within the operating range for GMP3, but all other operating parameters were within the defined operating range. The filter flush volume was increased prior to GMP4, to prevent dilution of the filtered bulk. The performance parameters for thawing, pooling, filtration, filling, capping/crimping and visual inspection were all within the acceptance criteria, except for an increased number of rejects for GMP3 vials, which was attributed to inaccurately seated stoppers. Adaptation of the filling needles and pump parameters resulted in a reproducible filling process.

Control of excipients

The composition of APN311 finished product is identical to that of APN311 active substance. All excipients used in the formulation of active substance/finished product are of pharmacopoeial grade quality according to the respective monographs in the current editions of Ph. Eur., and are tested accordingly by the supplier. Since no additions or changes to the formulation from active substance to finished product are applied, the excipients specifications are identical. No further excipients are used in the manufacture of APN311 finished product.

Product specification

The specification for the finished product is presented.

The majority of analytical methods are the same as for active substance, which reflects the fact that the finished product manufacturing process is limited to sterile filtration and aseptic filling.

Acceptance criteria for the additional tests required for finished product (visible particles, sterility, uniformity of dosage units, sub-visible particles and extractable volume) are justified based on Ph. Eur. requirements. Appropriate limits for polysorbate, histidine and sucrose have also been added.

Container closure system

Finished product is filled in 6 ml sterile type I (DIN6R) glass vials, 4.5 mL with a 0.4mL overfill to comply with Ph. Eur. 3.2.1 for extractable volume. The vials are closed with 20 mM Flurotec-coated grey bromobutyl rubber stoppers, which comply with Ph. Eur. 3.2.9. Crimping seals are aluminium caps with a blue polypropylene flip-off seal. These have been described in sufficient detail and do not lead to any concerns. Suitable testing is described and the glass vials and rubber injection stopper are sterilised using dry heat and steam sterilisation, respectively. The sterilisation procedures are validated. APN311 finished product is transported in the sealed glass vials inserted into card board boxes as secondary packaging.

Stability of the product

The Applicant claimed a shelf life of 24 months when stored at 5 ± 3°C, which is supported by the stability data and is therefore considered acceptable. Stability studies are in progress for 3 batches of finished product (GMP1, GMP2 and GMP3) manufactured using the proposed commercial process (v.2.1), under long term (5 ± 3°C), accelerated (25 ± 2°C) and stress conditions (40 ± 2°C). Vials from GMP1 were stored in both upright and inverted positions at 5 ± 3°C. Stability data generated under these long term conditions is available for 30 months (GMP1), 18 months (GMP2) or 12 months (GMP3), confirming that there was no changes or obvious trends compared with the day 0 data for these batches. No changes are observed in GD2 binding, CD16 binding or CDC activity under long term storage conditions. Photostability studies were also performed for up to 3 months under long term conditions, showing that APN311 was reasonably stable on exposure to light, although reduction in CDC activity and increase in acidic forms was observed after 4 cycles of light exposure. Section 6.4 of the SmPC includes the statement '*Keep the vial in the outer carton in order to protect from light'* to address this.

Supportive stability data has been generated using batches T99 and T110 (process v.2.0), formulated in the same 20 mM histidine, 5% sucrose, 0.01% polysorbate 20 buffer, pH 6.0 and with the same primary packaging. Data up to 12 months (batch T99, an engineering batch which was not used in clinical studies) and 24 months shows that this material was stable under long term conditions (5 ± 3°C) up to 24 months, although clinical batch T110 results for CDC activity were out-of-specification (OOS) for CDC activity at 30 and 36 months (60% and 59% relative activity, respectively). All the other results remained within specification limits. This stability study for clinical batch T110 will continue for a further 2 years under long term conditions.

Additional data has been given for batches T65 and T90 (process v.1.0), although these were formulated in phosphate buffered saline, pH 7.0. Stability data up to 96 months (T65) and 48 months (T90) showed an increase in HMWS and LMWS (close to the limits), but GD2-binding activity and CDC activity were comparable to time 0 values for T65, with slightly reduced CDC activity but not GD2-binding activity for T90.

Accelerated stability studies with GMP1, GMP2 and GMP3 (up to 6 months at 25 ± 2°C) shows that CDC activity is out-of-specification at 3 months under these conditions. GD2-binding activity was also OOS after 3 or 6 months in these GMP batches. Degradation of APN311 under these conditions is confirmed by changes in the IEF pattern and a significant increase in acidic forms after 6 months, with a smaller increase in basic forms, determined by CIEF-HPLC. Higher ratios of LMWS were also observed. No degradation was observed up to 1 month at 25°C, confirming that handling for short periods at room temperature is acceptable. Data from GMP batches was supported by data generated under similar conditions for batches T99 and T110. Data generated under stress conditions (40 ± 2°C) showed a similar

pattern in a shorter timeframe, with OOS results for CDC activity and GD2 binding after 2 weeks, and formation of acidic forms leading to OOS results after 4 weeks. Increase in LMWS was also observed in these stress studies up to the 2 month time-point.

In-use stability

Details of the two infusion devices used for administration of dinutuximab beta (provided separately) and the product contact material have been given for the syringe and infusion bag. The Applicant has provided the in-use stability study report for dinutuximab beta finished product to support the clinical phase III studies and commercial use using these two devices. Finished product is diluted to 0.2 mg/mL in a physiological sodium chloride solution (0.9% NaCl) with 1% human albumin, to be administered by continuous intravenous infusion via a 50 mL syringe in an infusor device, or via a peristaltic mini-pump from a 250 mL medication cassette reservoir. Stability data in the 50 mL syringe were comparable to the initial values after 72 hours at 5°C and 24 / 48 hours at 37°C, except for the pH which decreased from 6.55 to 6.42 over the storage time at 37°C. For the infusion solution in the 250 mL bag, analytical results were comparable to the initial values after 72 hours at 5°C and 7 days at 37°C. This confirms that the dilution matrix and materials used does not impact the dinutuximab beta; both strategies for continuous infusion are acceptable.

Results from a more comprehensive in-use stability study have been submitted to demonstrate that different containers / devices (50mL syringe and 250mL infusion bags) in combination with an infusion line and 0.22 µm in-line filter, representing the typically-used devices in hospitals, are suitable for the administration of APN311. The in-use study was performed at minimum and maximum worst case concentrations of 0.1125 mg/mL to 0.4420 mg/mL APN311 in 0.9% NaCl with 1% human albumin, which represents the range of concentrations expected to be infused via the intended route of administration, based on the patient body surface area. In-use stability was demonstrated for 48 hours at 25°C (with the 50mL syringe) or 7 days at 37°C (with the 250mL infusion bag) and no impact on potency or other tested parameters was identified. It is noted that the human albumin and sodium chloride solution (0.9%) used for dilution of the APN311 are provided by the hospital and are expected to be approved for human use.

The SmPC Section 6.6 includes a note that '*Elastomeric pumps are not considered suitable in combination with in-line filters*', as recommended.

From a microbiological point of view, the product 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.

The absence of formal extractable/leachable studies with dinutuximab beta and the proposed infusion bags and syringes is based on compliance of these devices with Ph. Eur. and the use of physiological saline as diluent.

Adventitious agents

All raw materials, except for the cells themselves, used in the upstream and downstream manufacturing process of APN311 active substance are animal component free. Raw materials, including media and excipients are tested for bioburden (Ph. Eur. 2.6.12) and endotoxins (Ph. Eur. 2.6.14), except sodium hydroxide and hydrochloric acid due to methodical reasons. In addition, buffers used for final manufacturing steps are tested for bioburden and endotoxins.

The manufacturing process does not contain any animal-derived starting materials, which were only used in the development of the cell line: FCS (cattle, Australia), thymidine (salmon) and trypsin (porcine) were used. Corresponding certificates of analysis and conformity are provided in 3.2.A.2. Trypsin and FCS were

negative for the presence of porcine parvovirus and selected bovine viruses (bovine viral diarrhea virus (BVDV), Bovine herpesvirus 1 (BHV-1), Parainfluenza-3). The cells were adapted to serum-free medium during the manufacture of the RCB prior to laying down the MCB.

The MCB was extensively tested *in vitro* and *in vivo* for the presence of adventitious viruses and for various specific viruses (murine, bovine and porcine viruses) and retroviruses. In addition, qPCR assays were employed for specific detection of MVM-DNA sequences. No viruses were detected and all acceptance criteria were fulfilled. TEM analysis showed no intra-cytoplasmic A-type virus-like particles or extracellular C-type VLPs, or other virus-like particles or viruses, detected on 200 evaluated MCB cells. When analyzing test article supernatant, no virus-like particles were detected. Reverse transcriptase activity was not detectable by product-enhanced reverse transcriptase (PERT) assay and replication-competent retroviruses were not detectable by the S+L- assay, confirming lack of infectious retroviruses in the MCB.

An EPC bank (EPC2APA) was established and tested for latent or inducible viruses/retroviruses, as given for the MCB. For the EPC, intracytoplasmic A-type particles were detected in about 3% (6 cells) of 200 evaluated cells by TEM analysis and the positive cells exhibited 1-15 VLPs. No C-type particles, or other VLPs or viruses were detected. When analyzing test article supernatant, no virus-like particles were detected. As a result, the calculated possible load is $< 4.2 \times 10^4$ VLPs/mL. Reverse transcriptase activity was not detectable by PERT assay and replication competent retroviruses were not detectable by the S+L- assay or by co-cultivation of cells with *Mus dunni* cells and S+L- assay, confirming lack of infectious retroviruses in the EPC (extended culture).

The WCB was not tested for viral safety. As the parental MCB1APA was fully characterised according to ICH Q5A, no further viral safety testing was required. Unprocessed bulk harvest produced from the WCB tested negative for adventitious viruses and infectious retroviruses and no virus-like particles were detected in test article supernatant from three representative batches of bulk harvest. A potential load of 6.3×10^4 was used as basis for calculation of the overall viral safety margin of the manufacturing process.

Virus validation studies

The process steps C10 (Benzonase / Triton X-100 inactivation wash step), V10 (low pH inactivation), C20 (chromatographic step) and I20 (filtration step) were tested for their potential to clear viral contaminants. Only low clearance of Reo3 and MVM were observed in the initial study report for Protein A, therefore further analysis was performed separately for low pH inactivation and the Benzonase/ Triton X-100 wash step, evaluated by incubation with these reagents for 30 minutes at 18°C. This resulted in acceptable clearance of pseudorabies virus (PRV) (≥ 5.36 $\log_{10}$), but lower viral reduction of xenotropic murine leukemia virus (X-MuLV) (≥ 2.99 $\log_{10}$) under these conditions. Similar results were obtained for low pH, with clearance of PRV (≥ 5.03 $\log_{10}$), but lower clearance of X-MuLV (≥ 2.86 $\log_{10}$). Good clearance was shown for all viruses with the virus filtration step (I20) and the Q-Sepharose chromatography (flow-through).

The combination of virus safety data from the cell banks (both MCB and EPC), data from TEM analysis of crude bulk harvest and the current safety margin obtained for X-MuLV during virus validation shows that the production process of APN311 is suitable for clearance of potential viral contamination. Overall $\log_{10}$ reduction factors were ≥ 17.09 for X-MuLV, ≥ 13.65 for MVM, ≥ 23.89 for PRV and ≥ 13.32 for Reo3. The safety margin (based on X-MuLV) is ≥ 9.14 $\log_{10}$.

TSE Issues

FCS was obtained from cattle in Australia. An EDQM certificate has been provided for this FCS (R0-CEP 2002-167-Rev 01) which was used during generation of the RCB. Cells were subsequently adapted to serum-free medium during manufacture of the RCB, prior to manufacture of the MCB.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Active substance

Generation of the cell line and cell banks for manufacture of APN311 is sufficiently described. Cell line stability was demonstrated. The apparent discrepancies in the cell bank data were subsequently explained.

The description of the active substance manufacturing process and related information was updated, with CTD sections S.2.2-4 completely revised. Limits for the IPCs were included in the flow charts for upstream and downstream processing. IPCs were added for the production bioreactor at harvest (titre and product yield) and a minimum limit set for cell viability at harvest, to provide conditions for effective processing with no impact on the subsequent filtration step. For downstream processing, the IPC limits are acceptable, with the minimum and maximum loading density for the MabSelect SuRe, Q Sepharose and CaptoAdhere chromatography steps defined during process development and process characterisation studies in a DoE-based approach. The Applicant has defined terminology used in the dossier; these are broadly in line with ICH principles.

The process characterisation studies are of acceptable quality and can be deemed suitable for a manufacturing process where no design space or similar flexibility is claimed. Additional information has been provided to link the process characterisation studies with the control strategy. The Applicant provided a systematic overview of all CPPs and IPCs related to the chromatographic steps, with an associated justification. This approach considered as comprising a traditional process description, which is substantiated with enhanced development and characterisation studies. For a few parameters the ranges have been defined, which are supported by 2^2 full-factorial DoE studies for each step, while for other parameters, only set-points are defined and no ranges are attached; therefore it is sufficient that only validation at set-point is performed. The data are considered sufficient to conclude that the proposed CPPs and IPCs are justified and able to appropriately control the manufacturing process.

Process validation was supported by DoE studies and confirmed at the commercial scale in 4 GMP runs. The provided PPQ data (both prospective and retrospective) confirm that the Applicant is able to manufacture an active substance which meets its specifications and that the individual steps are under control. Impurity removal and process robustness were more fully addressed in response to the questions, including appropriate data that small (non-proteinaceous) impurities will be removed by the UF/DF steps and by other process steps. Worst case UF/DF runs were performed to demonstrate the validity and robustness of these steps.

Characterisation studies investigated the physicochemical and biological attributes of dinutuximab beta, which are as expected for a monoclonal antibody, including predominantly diantennary glycosylation and very little C-terminal lysine. Potency was determined by specific GD2-binding activity and CD16 binding activity, as well as CDC activity. Further information was provided to justify the measurement of CD16-binding as a surrogate for ADCC activity, since ADCC is part of the mechanism of action for APN311.

Additional physicochemical and biological data was provided for the characterisation exercise for active substance. This pertains to physicochemical identification of CQAs, and the link to biological characterisation of GD2 and CD16 (FcRIII) binding, as well as CDC activity. Measurement of CD16 (FcγRIII) receptor binding instead of ADCC activity was also justified, using data from production batches, stressed samples and different charge forms. Under accelerated conditions (25°C) or stress testing (40°C), an increase of acidic charge variants was observed by CIEX-HPLC, which was correlated to a reduction of GD2 target binding. The extent of oxidation / deamidation / isomerisation has been evaluated in the context of product characterisation and is controlled via specification limits for acidic and basic species in APN311.

While some changes were made between the early manufacturing process and the proposed commercial process, these did not have a significant impact on the quality of active substance and the three manufacturing processes (v.1.0, v.2.0 and v.2.1) yielded comparable dinutuximab beta. The comparability exercise between clinical and commercial batches has demonstrated that material from these manufacturing processes are comparable, with additional data from the second comparative stress study performed at 40°C provided to support comparability of clinical and commercial material.

The reference standard is satisfactory and can also be used for the finished product, since no changes are made in the concentration of dinutuximab beta or excipients compared with active substance. Additional stability data has been provided for this reference standard.

The proposed acceptance criteria for many of the active substance and finished product specifications, including purity and potency, have been revised based on clinical batch data and process capability. The same specification limits apply to release and shelf life for all analytical methods. The proposed specification limit for the afucosylated Man5 structure has been tightened based on the small-scale transfer and process characterisation runs, as well as the 5 GMP batches to date. In order to align acceptance limits for all afucosylated structures quantified, the proposed limits for cGN2 have been tightened.

For the HCP assay, initial comparison of the HCP-profiles of an upstream sample APN311 (harvest after filtration) against the HCP standard (silver-stained 2D SDS-PAGE) showed acceptable spot coverage of 73%. In an additional experiment, the coverage of kit antibodies for HCPs present in a representative APN311 upstream sample (filtered and concentrated harvest) was shown using 2D PAGE and Western blot, demonstrating detection of 71% of spots over the entire pH and molecular weight range. This confirms the ability of the antibody in the kit to detect the majority of HCPs in the APN311 harvest fraction.

The number of batches of APN 311 produced so far is limited, since this is an orphan medicine indication; therefore the Applicant proposes to tighten the HCP specification limit. While this is still high compared with the measured HCP, this can be accepted at present, but the Applicant is recommended to review the HCP levels and revise the specification limits (if appropriate) once a total of 20 batches have been manufactured.

Stability studies were conducted to support the claimed long-term storage of active substance for up to 12 months at -70°C. There is no clear trend over time and stability data has been provided up to 24 months (GMP1 and GMP2) and 18 months (GMP3), to further support the proposed shelf life for APN311 active substance of 12 months under long term storage conditions.

It has been established which physicochemical changes in CQAs during storage result in changes to biological activity. Supportive data generated under accelerated or stress conditions suggest that the stability-indicating methods are CDC activity, GD2 binding and CD16 binding, in addition to CIEX-HPLC (with an increase in acidic and basic forms). Additional stability data have also been provided for the reference standard SR-951PI.1 (up to 24 months).

Finished product

The finished product is manufactured by thawing the active substance, followed by a sterile filtration and aseptic filling into sterile glass vials. A slight overfill (4.9 mL) is made to allow extraction of 4.5 mL finished product. The finished product is filled into type I glass vials, sealed with a bromobutyl stopper and aluminium crimp. The container closure system is demonstrated to be suitable for dinutuximab finished product using methylene blue challenge studies and stability studies. Finished product manufacturing and process control are acceptable.

Process validation for the 4 commercial scale batches was satisfactory, although it is noted that two of these were retrospective and two were prospective process validation batches.

The initial formulation in early clinical studies was phosphate-buffered saline, but this has been changed to 20 mM histidine, 5% sucrose and 0.01% polysorbate 20, pH 6.0. Excipients are all Ph. Eur. quality. The finished product is a sterile concentrate for solution for infusion, diluted into commercial 0.9% sodium chloride and 1% human albumin.

The proposed product specification is satisfactory, following revision of the acceptance criteria for potency and purity.

Stability studies were provided to support the long term storage conditions at 5 ± 3°C. This includes 3 batches of finished product (GMP1, GMP2 and GMP3) manufactured using the proposed commercial process (v.2.1) with stability data up to 30 months and supportive data from one batch of finished product (T110) manufactured using process v.2.0, up to 36 months. This is sufficient to support the claimed shelf life of 24 months at 5 ± 3°C. Supportive data has also been provided under accelerated and stress conditions for finished product. Batches manufactured using process v.1.0 in the previous formulation (PBS) also demonstrated good stability under long term storage conditions, although an increase in HMWS and LMWS was found with this original formulation.

In relation to adventitious agents, microbial/non-viral adventitious agents are appropriately controlled.

No animal-derived raw materials are used during production. The MCB is adapted to serum-free medium; sufficient information is given regarding the animal materials used before establishment of the MCB. Testing for adventitious agents and viral safety was satisfactory for the MCB, WCB and end-of-production cells (EPC).

Virus validation studies were conducted to demonstrate that the manufacturing process is capable of inactivating/removing any potential viruses and adventitious agents with an acceptable safety margin. The virus validation reports provide sufficient details of the process parameters, limits and validity of the small scale models used in the studies. Virus validation has not been performed using aged resin for Q-Sepharose chromatography, but this will be limited to a maximum of 10 runs based on the small scale column lifetime studies, or until the chromatography resin expiry date.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

Overall, the quality of Dinutuximab beta Apeiron is considered to be in line with the quality of other approved monoclonal antibodies. The different aspects of the chemical, pharmaceutical and biological documentation comply with existing guidelines. The fermentation and purification of the active substance are adequately described, controlled and validated. The active substance is well characterised with regard to its physicochemical and biological characteristics, using state-of-the-art methods, and appropriate specifications are set. The manufacturing process of the finished product has been satisfactorily described and validated. The quality of the finished product is controlled by adequate test methods and specifications.

Viral safety and the safety concerning other adventitious agents including TSE have been sufficiently assured.

The overall quality of Dinutuximab beta Apeiron is considered acceptable when used in accordance with the conditions defined in the SmPC.

2.2.6. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommended a point for investigation:

2.3. Non-clinical aspects

2.3.1. Introduction

The non-clinical development programme of ch14.18/CHO did not follow the pre-specified, ICH-dependent path. Various non-clinical data were generated within academic studies. In addition to these available non-clinical data from the literature, APEIRON performed pharmacodynamic studies to further elucidate the mode of action as well as studies to define a relevant animal species in order to further access pharmacokinetics and toxicity of ch14.18/CHO.

The majority of data which are available from published papers have been generated with older development branches of GD2-specific antibodies, namely the monoclonal mouse antibody 14G.2a and the chimeric mouse-human antibody ch14.18/SP2/0. Therefore, comparability of ch14.18/SP2/0 and ch14.18/CHO in regards to *in vitro* binding affinity, specificity, effector functions like complement-dependent cytotoxicity (CDC) and antibody dependent cellular cytotoxicity (ADCC) and pharmacokinetics in mice has been investigated.

2.3.2. Pharmacology

Primary pharmacodynamic studies

Table 1: The primary pharmacology data submitted in support of this application are summarised in the table below.

Overview			Test Article: APN311		
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**GLP Compl.**	**Study No. / Reference**
Primary Pharmacodynamics					
GD2 binding data	ELISA/SPR technology	In vitro	APEIRON Biologics GmbH; Vienna, Austria	No	01-2014
GD2 binding data	ELISA	In vitro	Charite Children's Hospital, University Medicine, Berlin, Germany	No	(Zeng *et al.*, 2005)
GD2 expression on LAN-1 and M21 cells	ELISA/SPR technology	In vitro	APEIRON Biologics GmbH; Vienna, Austria	No	01-2014
Effector function data (CDC, ADCC, WBT)	Serum, PBMC, blood	In vitro	APEIRON Biologics GmbH; Vienna, Austria	No	01-2014
Effector function data	Serum, PBMC	In vitro	Charite Children's Hospital, University Medicine, Berlin, Germany	No	(Zeng *et al.*, 2005)
Efficacy (syngeneic tumor mouse model)	A/J mouse	i.v. injection (4 and 12 mg/kg/day for consecutive 5 days)	Charite Children's Hospital, University Medicine, Berlin, Germany	No	(Zeng *et al.*, 2005)
Secondary Pharmacodynamics	**No secondary pharmacodynamics studies have been conducted been conducted**				
Safety Pharmacology	**No safety pharmacology studies have been conducted**				
Pharmacodynamic Drug Interaction	**No pharmacodynamic drug interaction studies have been conducted**				

In vitro studies

Binding constants of ch14.18/CHO as well as 14G.2a (a fully murine version of ch14.18/CHO) to its antigen GD2 (Study No. 01-2014).

Three experiments were conducted. In the first experiment, binding of ch14.18/CHO (batch T651204), 14G.2a, rituximab (as unspecific control) and an isotyope control were compared at concentrations of 25.0, 12.5, 6.25, 3.13, 1.5, 0.75, 0.37 and 0.0 µg/ml.

Binding curves for ch14.18/CHO and 14G.2a were similar. They showed mostly the same shape while affinity of ch14.18/CHO apparently was slightly lower when compared with 14G.2a (EC50: 4.5 and 3.1 µg/ml for ch14.18/CHO and 14G.2a respectively). Specificity of the assay was confirmed with a murine IgG2a isotype control as well as with the chimeric antibody rituximab which both did not show binding activity.

In the second experiment, 10 µg/ml of antibody were exposed to decreasing amounts of the anti-idiotypic antibody gangliodiomab, starting at 43 µg/mL down to 0.33 µg/mL. Binding of ch14.18/CHO and 14G.2a to their target antigen GD2 was, as expected, inhibited by increasing amounts of the anti-idiotypic antibody gangliodiomab. Curve shapes were very similar, confirming that despite several differences between the constant domains of ch14.18/CHO and 14G.2a, both antibodies showed similar inhibition of GD2-binding by Gangliodiomab.

In the third experiment, it was determined that KD, ka and kd values were very similar for the two ch14.18/CHO batches (T65 and T90). Moreover, the measured values are within the expected ranges for mid to high affinity, monoclonal antibodies targeting cell surface antigens for tumour therapies.

Binding curves of ch14.18 prepared from CHO, NS0 and SP2/0 cells to its antigen GD2 (Zeng et al.2005).

Bibliographic data is cited where the binding of ch14.18 preparations from CHO, NS0 and SP2/0 cells and Mabthera/Rituximab as a negative control to gangliosides was determined in solid phase ELISAs. Results indicate a highly specific binding of all ch14.18 antibody preparations (with similar affinities) to ganglioside GD2 in contrast to the anti-CD20 mAb controls.

Binding of APN311 to GD2-expressing tumour cell lines LAN-1 and M21 (Study No. 01-2014)

The aim of this study was to confirm GD2 expression on LAN-1 (human neuroblastoma cell line) and M21 (human melanoma cell line) cell lines via flow cytometry. Results indicated that APN311 strongly interacted with both cell lines in comparison to the isotype control indicating that both cell lines express GD2. This would enable subsequent use of these cells lines to be used to measure APN311-mediated effector function.

Effector Function Studies (all reported within Study No. 01-2014)

An experiment was conducted to determine the ch14.18/CHO-triggered CDC activity against the GD2-expressing cell lines LAN-1 and M21 (human melanoma cell line) in vitro. In this study, ^{51}Cr-labeled LAN-1 or M21 cells were used as target cells. Human serum (1:5 final dilution) derived from healthy donor served as the effector source. Following treatment, radioactivity which is proportional to the release of ^{51}Cr after cell lysis was measured in all samples and expressed in counts per minute (cpm). Results were calculated as % lysis subtracting the cpms of spontaneous lysis (background) from all sample values and relating to the cpm of the maximal achievable lysis (with a surfactant) which is set to 100%. APN311 showed strong lysis of the GD2-positive cell lines LAN-1 and M21 via complement-mediated cytotoxicity. Calculated EC50 values were 157 and 138 ng/ml, respectively.

Antibody-dependent cell-mediated cytotoxicity (ADCC)

In the same study, it was determined if there was ch14.18/CHO-triggered ADCC activity against the GD2-expressing cell lines LAN-1 and M21. Here, human peripheral blood mononuclear cells (PBMC; effector to target cell ratio 30:1 for M21 and 5:1 for LAN-1 cells) derived from healthy donor, heparinized whole blood served as the effector source. The concentration range of ch14.18/CHO (batch T110) tested was

1-400 ng/mL and 20-1200 ng/mL on LAN-1 and M21 cells, respectively. APN311 had EC50 values of 2.0 and 4.1 ng/mL for ADCC on LAN-1 and M21 cells, respectively.

Whole blood cytolytic activity (WBT)

Finally, in addition to the two standard effector function assays, CDC and ADCC, the whole blood cytolytic activity test (WBT) was established to better mimic the in vivo physiology by using naïve healthy volunteer- (in vitro) or treated patient-derived (ex vivo) whole blood samples. APN311 had EC50 values of 65 and 140 ng/mL in a whole blood mediated cytolytic assay performed on LAN-1 and M21 cells, respectively.

Effector Function Studies (reported within (Zeng et al. 2005)

Zeng et al. reported that CDC mediated by the different ch14.18 preparations were mAb concentration dependent and specific, since chimeric anti-CD20 mAb (rituximab) used as a non-specific control was ineffective. Identical CDC activity of ch14.18/CHO compared to ch14.18/SP2/0 was evident. However, results suggested a lower CDC activity of ch14.18/NS0. In the same paper, it was reported that the ADCC, effect was dependent on time and correlated with the mAb concentration. The ADCC effect mediated by ch14.18/CHO was superior to that of ch14.18/SP2/0 and ch14.18/NS0 by a factor of 2 at low mAb concentrations (0.001–0.0003 µg/ml) ($p < 0.01$). The ch14.18/NSO mAb preparation was less effective in antibody-dependent cellular cytotoxicity (ADCC) than ch14.18/CHO or ch14.18 SP2/0 at lower mAb concentrations (<1.0 µg/ml). Results suggest that there may be an improved ADCC effect with ch14.18/CHO at low antibody titres.

A reduction of fucose content within the glycosylation site of antibody Fc domains is reported (Suzuki et al. 2007) to result in an increase of ADCC activity due to higher affinity binding of the fucose-reduced antibody to the FcgammaRIIIa receptor on natural killer cells.

In vivo studies

Anti-Tumour Activity of ch14.18/CHO and ch14.18/NS0 in a Syngeneic Tumour Mouse Model (Zeng et al. 2005)

The purpose of this study was to evaluate and compare the anti-tumour effect of ch14.18/CHO and ch14.18/NS0 in vivo. Therefore, a syngeneic, neuroblastoma cell, liver metastasis mouse model was used. Liver tumour burden was a measure for the therapeutic effect 28 days after tumour inoculation.

In this study 1 x 10^5 murine NXS2 neuroblastoma (NB) tumour cells were i.v. injected into the tail vein of female A/J mice (n = 6 per group). One group was untreated, the other groups were treated from Day 3 post-tumour inoculation for 5 consecutive days with i.v. injected PBS (vehicle control), 100 or 300 µg/day ch14.18/CHO (4 or 12mg/kg/day) or ch14.18/NS0. Another group was treated identically at 300 µg/day ch14.18/CHO but received 50 µl i.p. anti-Asialo GM1 antiserum on Days -2, 4 and 10 before/after tumour cell inoculation in order to deplete NK-cells. Mice were sacrificed on Day 28 or when they became moribund. The therapeutic effect was determined by analysis of hepatic tumour burden measured as wet liver weights.

Dose-dependent and antigen specific suppression of metastases was demonstrated in mice treated with ch14.18. The treatment at the dosage of 300 µg ch14.18/day reduced neuroblastoma liver metastasis, as indicated by a decrease of the liver weight from 2.3 g±0.3 g (PBS controls) to 1.1 g±0.1 g (ch14.18/CHO treated mice) and 1.3 g±0.3 g (ch14.18/NS0) ($p > 0.05$). The latter two values are superimposable to those found in healthy control animals. As expected, the effect of ch14.18/CHO treatment was not statistically different from that obtained upon treatment with ch14.18/NSO ($p > 0.1$). The specificity of ch14.18 therapy was demonstrated, since treatment with an equivalent amount of non-specific anti-CD20 antibody was completely ineffective. Notably, this control antibody binds to the human B-cell

associated CD20 molecule with no cross-reactivity to murine CD20. A reduction of liver metastasis was also achieved at 100 µg ch14.18/day but to a lower extent compared to 300 µg/day. The liver weight decreased from 2.2 g±0.4 g (PBS) to only 1.5 g±0.4 g (ch14.18/CHO) and 1.9±0.2 g (ch14.18/NS0), respectively, but the differences were statistically not significant ($p > 0.05$). Finally, the depletion of NK-cells resulted in complete abrogation of the therapeutic effect of ch14.18/CHO, indicating the critical role of NK-cells, likely mediating ADCC in the ch14.18/CHO induced anti-neuroblastoma immune response. Comparable efficacies of ch14.18/CHO and ch14.18/NS0 were expected since the advantage of the increased ADCC activity of APN311 is not relevant in mice. Furthermore, there was no evidence of toxicity in mice treated with ch14.18/CHO as indicated by a stable body weight after treatment with ch14.18/CHO.

Secondary pharmacodynamic studies

No secondary pharmacodynamic studies have been performed.

Safety pharmacology programme

No stand-alone safety pharmacology studies have been performed.

Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies were submitted.

2.3.3. Pharmacokinetics

No classical absorption/distribution/metabolism/excretion (ADME) studies have been performed with APN311. The pharmacokinetic (PK) profile of APN311 was investigated in a GLP absorption study after single intravenous (i.v.) 24-hr infusion of APN311 to male Guinea pigs (Study No. 29338). Furthermore, the toxicokinetic (TK) profile of APN311 was characterised as part of a GLP repeat-dose toxicity study in Guinea pigs (Study No. 29339).

Following a single administration of the DP to male guinea pigs, APN311 Cmax and exposure exhibited dose-dependent, linear pharmacokinetics over the administered dose range of 1.25 (= 10 mg/m^2), 6.25 (= 50 mg/m^2) and 12.5 mg/kg (=100 mg/m^2). The terminal half-lives were similar (ca. 40 h) between doses with a slight trend towards increasing values with increased dose.

Table 2: PK parameter calculated via non-compartmental analysis after 24-hr APN311 i.v. infusion to male Guinea pigs (Study No. 29338)

Group	Dose# [mg/kg b.w.]	C_{max}## [µg/mL]	t_{max}## [h]	$t_{1/2}$ [h]	K_{el} [1/h]	$AUC_{0-144\ h}$ [µg·h/mL]	$AUC_{0-\infty}$ [µg·h/mL]	$AUC_{0-\infty}$/Dose [µg·h/L]	CL [mL/(h·kg)]	Vz [mL/kg]
1	1.25	18.1	24.0	36.7	0.019	926.8	1001.7	801.4	1.25	66.0
2	6.25	82.4	24.0	38.4	0.018	4529.8	4933.0	789.3	1.27	70.2
3	12.5	173.4	24.0	43.4	0.016	10035.7	11142.2	891.4	1.12	70.2

‘: administered as intravenous infusion over a period of 24 hours
“:values obtained from analytical results, all other values calculated by pharmacokinetic analysis (in part, values were rounded compared to the result tables)

In the repeated dose toxicology study; the 10-day infusion of APN311 to male and female guinea pigs had to be terminated prematurely at the end of Cycle 2 dosing because of a high number of unscheduled death/moribund animals seen in all dose groups (see toxicology section below). The causes of death/preliminary termination were reportedly due to valvular/thrombotic endocarditis (as a result of the long-term infusion induced injection site changes and/or post-surgical trauma/infection) as well as rupture of infusion catheter. Therefore only TK data could be generated for the first dosing cycle: APN311 Cmax and exposure exhibited dose-dependent, near to linear pharmacokinetics over the administered dose range of 1.25, 6.25 and 12.5 mg/kg/day after 10 consecutive days of dosing.

No clear sex differences in exposure levels were observed. Comparison of single versus repeated dose PK parameters in males revealed similar Cmax levels but ca. 3-4-fold lower exposure (AUC) levels after the 10-day infusion regimen counteracting the anticipated accumulative effects of repeat infusions. This was likely due to the potential early onset of an anti-ch14.18/CHO antibody response. Indeed the occurrence of anti-drug antibodies was confirmed for Day 34 samples of the study, but no early samples (e.g. Day 10) have been measured.

A GLP, 10-day repeat-dose toxicity in Cynomolgus monkey with daily, 4-hr continuous i.v. infusions of ch14.17/CHO has been conducted and reported under Study No. 31743. Cmax levels of ~ 20 fold the levels observed in humans are reported. Serum levels and AUC values revealed a dose-related, linear exposure with no sex differences.

Table 3: Pharmacokinetic parameters of ch14.18/CHO during a 10-day, daily 4-hr i.v. infusion to Cynomolgus monkeys (Study No. 31743) (Days 1-15)

Group*	Dose[a] [mg/kg/day]	Dose[a] [mg/m^2/day]	Cmax[b] [µg/mL]	tmax[b] [h]	AUC0-∞ [µg*h/mL]	t1/2 [h]
Males (Days 1-15)						
2	2.5	30	149	220	20473	22.4
3	8.33	100	375	180	60115	32.8
Females (Days 1-15)						
2	2.5	30	103	179	14733	17.8
3	8.33	100	339	118	50311	23.4
Males and females combined (Days 1-15)						
2	2.5	30	126	199	17603	20.1
3	8.33	100	357	149	55213	28.1

* Group 1 was the vehicle control group and showed no exposure.
[a] This dose was administered daily (4-hr i.v. infusion) for 10 consecutive days (Days 1-10).
[b] Values obtained from analytical results, all other values are calculated by PK analysis.

No studies on metabolism have been performed.

Following a pilot study (Study No. 29355), in vitro cross-reactivity studies in Guinea pig (Study No. 30666) and human tissues (Study No. 2014-AP-001) were conducted. A further tissue cross-reactivity study was conducted with a panel of tissues derived from the cynomolgus monkey (Study No. 31882). The staining pattern in both species followed the expected expression pattern of GD2 on central and peripheral nerves and organ innervations structures.

2.3.4. Toxicology

Single dose toxicity

No stand alone, acute toxicity studies have been conducted.

Repeat dose toxicity

The toxicology program conducted with APN311 consists of one GLP intravenous (continuous i.v. infusion) repeat-dose toxicity study with APN311 (0, 1.25, 6.25 and 12.5 mg/kg/day = 10, 50, 100 mg/m2/day) conducted in juvenile Guinea pigs (7 wks at start of first dose).

Table 4: Toxicology studies conducted to date

Study ID	Species/ Sex/ Number/ Group	Dose/Route	NOAEL (mg/kg/day)	Test-item related findings
29339	Guinea pig, Dunkin-Hartley, Crl: HA	1.25, 6.25, 12.5 mg/kg/day = 10, 50, 100 mg/m2/day continuous i.v. infusion for 10 consecutive days	1.25	-Body weight reduction (up to 30%) -Statistically significant decreases in numbers of white blood cells, reticulocytes and platelets -APTT was increased on Day 12 -increased plasma levels of cholesterol and urea and an increase of alpha-amylase and glutamate dehydrogenase (GLDH) activity -decreased plasma creatinine and glucose - increase in urine volume (up to 82%) - Myeloid hyperplasia of the bone marrow

The administration scheme (cyclic administration) mimicked exactly the clinical administration scheme; 10 consecutive days of continuous i.v. infusion followed by a 25-day off-period before the next 10 consecutive days of dosing started. Dosing was stopped at the end of Cycle 2 because a high number of animals were terminated prematurely/found dead, with comparable numbers of deaths amongst control and test item groups. The causes of death/preliminary termination were valvular/thrombotic endocarditis (as a result of the long-term infusion induced injection site changes and/or post-surgical trauma/infection) as well as rupture of infusion catheter. Due to the high mortality during the study, the amount of animals in satellite and recovery groups is sometimes limited to one. Drug-related toxicities comprising of clinical signs, body weight and food consumption decrease, changes in haematology and bone marrow findings are said to be well in accordance with the expected toxicities of ch14.18/CHO, i.e. induction of pain by binding to peripheral nerve tissue structures (resulting in the observed, dose-dependent clinical signs, food and body weight decreases) and interference with the hematopoietic system/bone marrow. However, repeated administration of APN311 resulted in the induction of anti-drug antibodies interfering with the pharmacokinetics of ch14.18/CHO as well as possibly compensating ch14.18/CHO-induced clinical signs and haematologic changes. At the end of the recovery period all changes noted during the treatment period had subsided beside a reduction in absolute and relative liver organ weight and a still increased myeloid:erythroid ratio in high dose (100 mg/m^2/day) males. Under the present test conditions the NOAEL is judged to be 1.25 mg/kg/day (10 mg/m^2/day) as the observed changes at that dose level are associated with the pharmacodynamic mode of action of a GD2-targeting, ADCC/CDC-triggering antibody. The MTD is 12.5 mg/kg/day (100 mg/m^2/day).

The results of a GLP-compliant, repeat-dose toxicity in Cynomolgus monkey with daily, 4-hr continuous i.v. infusions of ch14.17/CHO has been submitted (Study 31743): 0, 30 (3x human dose) and 100 mg/m2/day (10x human dose) APN311 were administered to groups of 6 Cynomolgus monkeys

(n=3/sex/group main study, n=2/sex/group recovery) for 10 consecutive days and termination of animals on Day 15 (i.e. 5 days after start of final dosing on Day 10; main study animals) and Day 36 (recovery group). Cmax levels of ~ 20 fold the levels observed in humans are reported. Serum levels and AUC values revealed a dose-related, linear exposure with no sex differences. The dosing schedule was in line with FDA advice received in 2014 and reflects the proposed 10-day clinical dosing regimen with adequate human safety margins. Two unscheduled deaths occurred in the high dose group on Day 14 and the cause remains inconclusive, although it is stated that both animals had shown moderate to severe reduction in overall activity for period of 7 or 12 days before death. In addition reduction in thymus sizes and reddening of the caecum mucosa were noted. Underactivity and reduction of thymus size was also observed in all other high-dose animals. Other drug-related toxicities included reduction in body weight and food consumption; bone marrow changes including atrophy of myeloid and erythroid precursor cell lineages were also observed. These findings were expected toxicities of ch14.18/CHO, i.e. induction of pain and interference with the hematopoietic system. Beside reduction of thymus size observed in one high dose female, animals recovered from all findings during the 4-week recovery period. The no-observed-adverse effect level (NOAEL) was determined to be 30 mg/m2/day, as was the maximum tolerated dose. Results from in vitro cross-reactivity studies using cynomolgus monkey tissue (as well as guinea pig and human tissue) served to validate the cynomolgus monkey as a relevant toxicology species; with tissue binding of the antibody 14.G2a (predictive for APN311) limited to some neuronal tissues, including cerebellum, cerebrum, and peripheral nerves, as well as external and internal innervation structures of non-neuronal tissue types.

Genotoxicity

No genotoxicity studies have been conducted.

Carcinogenicity

No carcinogenicity studies have been conducted.

Reproduction Toxicity

No reproductive and developmental toxicity studies have been conducted.

The applicant has included the histopathological results of the analysis of the reproductive organs in Guinea Pigs and in Cynomolgus monkey. No APN311 related effects on reproduction organs in guinea pig and cynomolgus monkey were reported.

The mammary gland in female animals was missing in 6/11 animals compared to 1/11 in controls. Mammary gland in guinea pig seemed to be present according to the gross pathological examination, but during histopathological examinations it was absent.

Toxicokinetic data

APN311 C_{max} and exposure exhibited dose-dependent, but not fully linear pharmacokinetics over the administered dose range after 10 consecutive days of dosing. No clear sex differences in exposure levels were observed. Comparison of pharmacokinetic parameters measured in the PK study after a single 24-hour i.v. infusion of ch14.18/CHO to male Guinea pigs with PK parameters obtained after the 10-day continuous i.v. infusion in Cycle 1 revealed similar C_{max} levels but ca. 3-4-fold lower exposure (AUC) levels after the 10-day infusion regimen.

Local Tolerance

No local tolerance studies have been conducted.

2.3.5. Ecotoxicity / environmental risk assessment

No environmental risk assessment studies were submitted for dinutuximab beta.

2.3.6. Discussion on non-clinical aspects

The non-clinical package presented is based on repeat dose studies in the guinea pig and the cynomolgus monkey as relevant species, supported by toxicokinetic data in both species as well as *in vitro* tissue cross-reactivity studies in guinea pig, cynomolgus monkey and human tissues which served to validate the toxicology species.

The *in vitro* pharmacology studies are generally supportive of the proposed indication. Binding constants of ch14.18/CHO as well as 14G.2a (a fully murine version of ch14.18/CHO) to its antigen GD2 were determined in a series of experiments. Binding curves for ch14.18/CHO and 14G.2a were similar. Binding curves of ch14.18 prepared from CHO, NS0 and SP2/0 cells were also shown to be comparable. Further *in vitro* results demonstrated that APN311 showed strong lysis of the GD2-positive cell lines LAN-1 and M21 via complement-mediated cytotoxicity.

The majority of data which are available from published literature data have been generated with the older development branches of GD2-specific antibodies. Therefore the comparability of ch14.18/SP2/0 and ch14.18/CHO in regards to *in vitro* binding affinity, specificity, effector functions like complement-dependent cytotoxicity (CDC) and antibody dependent cellular cytotoxicity (ADCC) is accepted based on the *in vitro* data presented thus far.

In vivo biodistribution data has been generated with ^{125}I-labeled ch14.18/CHO in neuroblastoma-bearing, female A/J mice (Ladenstein, R., et al., 2013). This *in vivo* data, together with *in vitro* tissue cross reactivity data in non-human primates supports the therapeutic profile of the proposed product.

In the guinea pig 10-day repeated dose toxicology study, toxicokinetic analyses are hampered by several factors: early formation of ADA resulting in rapid decline of plasma levels during infusion and possibly interference in the assay, unexplained higher plasma levels at the end of cycle 2 as compared to end of cycle 1, limited measurements after cycle 2 due to mortalities in the satellite group of the repeated dose toxicity study, and detection of ADA in control animals using a validated method but not using a non-validated method.

Not only toxicokinetic satellite animals died during the study, also main animals in all groups did not last longer than 2 cycles. This was probably due to the method of administration, which was not suitable for Guinea pigs. As a consequence of mortalities and formation of ADA and therefore lack of exposure, only the first cycle is taken into account for the safety assessment of APN311.

During the procedure, the applicant submitted a repeat dose toxicity study in cynomolgus monkeys. In contrast to the expectations of 3 cycles as per ICH guidance ICH S9, only one cycle of repeated dosing in the monkey was conducted. This has been justified based on clinical signs (underactivity) and body weight loss observed during the 10 day dosing period. It is stated that the signs most probably relate to acute pain induction due to the test item targeting GD2 within the peripheral nerve system. It is accepted that continued dosing would not be justified based on the above toxicities and furthermore it is stated that no additional toxicities would be expected to arise due to repeated cycles other than that related to pain

as histopathological changes in the peripheral nervous system or innervations structures of non-neuronal tissues were observed. In addition literature data support the view that pain (also observed in humans) is acute rather than chronic. Prolonged dosing in the guinea pig resulted in an immune response and it is theorised by the applicant that this too is likely to have occurred in the non-human primate. Further reassurance is given as the clinical database does not appear to indicate long term neurotoxic effects following ch14.18-based neuroblastoma therapy. It is concluded that no further non-clinical dosing can be expected.

Findings of note in the repeat-dose toxicity regimens included changes (decrease) in thymus weight as well as bone marrow changes (atrophy affecting myeloid and erythroid precursor cell lines). The bone marrow changes were slight to severe and recovered after cessation of dosing. No effects on cardiovascular functions (ECG, blood pressure) were observed in monkeys (see section 5.3 of the SmPC).

The absence of genotoxicity and carcinogenicity studies is agreed since APN311 is an antibody not expected to interact with DNA or to be carcinogenic. No reproductive and developmental toxicity studies have been performed with APN311. In the repeat-dose toxicity studies in Guinea pigs and cynomolgus monkeys, no adverse effects of dinutuximab beta were observed on reproductive organs at exposure levels above clinical levels (see section 5.3 of the SmPC).

The target of APN311, GD2, is expressed on neuronal tissues especially during embryofetal development and taking into account the potential of placental transfer of antibodies; APN311 may cause foetal harm when administered to pregnant women. Moreover, retinoic acid, a concomitant drug administered during APN311 treatment, is a well-known, potential teratogen. In addition, considering the type of disease and overall clinical conditions/treatment of the patients as well as the fact that the main patient population (≥ 90%) intended to receive treatment with APN311 is below an age of 10 years, pregnancy seems to be an unlikely event to occur during treatment with APN311. Considering the above arguments, the lack of specific reproduction toxicity studies with APN311 is accepted.

The finding on the mammary gland in female animals is of possible concern. The applicant noted, that it is possible that although during processing it seems to be present, but it turns out not to be mammary gland tissue upon histopathological examination. Furthermore, the applicant argued that in 10-40% of small and juvenile animal's mammary gland tissue cannot be found. In this case, it is 60% of the animals, which exceeds the 40%. However, since the development of the mammary gland in cynomolgus monkeys exposed to higher levels of APN311 is similar to control animals, the effect on mammary gland in guinea pig, is likely not relevant for human.

A lower exposure (AUC) was observed after 10 day-continuous infusion regimen compared to a single 24-hour i.v. infusion of ch14.18/CHO. In contrast, one would have been expected higher values due to accumulation effects comparing same daily dose levels. However, this observation can be explained by a potential early onset of an anti-ch14.18/CHO antibody response, compensating for ch14.18/CHO accumulation effects and resulting in an increased clearance or interfering with the ELISA method. The occurrence of anti-drug antibodies was confirmed for Day 34 samples of the study, but no early samples (e.g. Day 10) have been measured.

It can be, therefore, expected that the formation of anti-ch14.18/CHO antibody rendered the toxicokinetics and extent of the toxicities reported for ch14.18/CHO in the Guinea pigs largely underestimated and hence the reported clinical safety margins not representative.

In accordance with the Guideline on Environmental Risk Assessment of Medicinal Products for Human use [CHMP/SWP/4447/00], proteins are exempt from the need to conduct environmental risk assessment studies. Therefore, no environmental risk assessment studies were provided.

2.3.7. Conclusion on the non-clinical aspects

There are no objections to the approval of the proposed product from a non-clinical perspective. The long term dosing schedule in the toxicology species was not in line with the requirements of ICH S9; however for the reasons discussed above – no further dosing in the toxicology species can be justified.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Table 5: Tabular overview of clinical studies

Study code Phase	NB Setting	Design	APN Scheme Dose(s) (mg/m^2/ cycle) No. of cycles	Co-treatment	Patients Treated/planned Age	Assessments
Main study						
APN311-303 (Comp. Use)	R/R [a]	OL, uncontrolled, single-centre	24h / 10d 100 Up to 6 (each 35 days)	IL-2, 13-cis RA	54/54 >1 y to ≤45 y	Safety, Efficacy, Pharmacology ***Completed***
Supportive studies						
APN311-101 Phase I	R/R	OL, uncontrolled, multi-centre, dose-escalation	8h / 5d 50, 100, 150 1-3 (each 28 days)	none	15/12 [b] 15 in dossier >1 y to ≤21 y	Safety, Efficacy, Pharmacology ***Completed***
APN311-201 Phase II	R/R	OL, uncontrolled, multi-centre	8h / 5d 100 Up to 9 (each 28 days)	none (cycles 1-3), IL-2 (cycles 4-9)	35/35 [c] ≤21 y Amended to include a total 60 patients	*Safety, Efficacy, Pharmacodynamics* ***Ongoing***
APN311-202 Phase I/II	R/R	OL, uncontrolled, multi-centre, dose-escalation, dose-schedule finding	24h / 10d 100, 150, 210 5 (each 35 days)	IL-2, 13-cis RA	44/140 [d] >1 y to ≤21 y Recruitment extended	Safety, Efficacy, Pharmacology ***Ongoing***
APN311-301/302 Phase III	High risk (first line therapy)	OL, randomized, controlled, multi-centre	8h / 5d 100 5 (each 28 days)	301: 13-cis RA 302: IL-2, 13-cis RA	A: 34/34 <21 y B: 406/400 [f] <21 y Recruitment extended	Safety, Efficacy ***Ongoing***

[a] Also first line patients have been accrued to a limited extent.
[b] A total of 16 patients were treated in the study. However, since the signed ICF for one patient could not be found at the time of data collection and analysis, only data from 15 patients were collected and are reported.
[c] Data cut-off date 28 Feb 2015 – last update 05 September 2016
[d] As of 17 Feb 2015. In amendment 1 to the protocol an expansion cohort of 100 patients was determined.
[e] Data cut-off date for manuscript: 03 July 2014 – CSR: 22 January 2016; updated addendum: 05 September 2016

NA = not applicable, OL = open label, R/R = relapsed/refractory, TBD = to be determined.

The main study is a retrospective data collection from the compassionate use of the product in a single centre where a heterogeneous population of patients with neuroblastoma was treated. The supportive studies are investigator-sponsored Phase I/II trials to evaluate primarily the safety, pharmacokinetics, and pharmacodynamics of the product. All these trials have enrolled patients with relapsed/refractory neuroblastoma.

The only controlled trial is the ongoing first-line trial in high risk neuroblastoma conducted by the International Society of Paediatric Oncology Europe Neuroblastoma (SIOPEN), which evaluates the effects of adding IL-2 to ch14.18/CHO and 13-cis RA. During the procedure, interim data have been submitted in the form of a CSR following a retrospective SAP (cut-off date 22 January 2016). An updated analysis of the same study has also been submitted during the procedure (cut-off date 05 September 2016).

Results from study APN311-301 have also been submitted by the Applicant. This study, which aimed at comparing 13-cis-RA treatment to 13-cis-RA treatment plus ch14.18/CHO therapy in high risk neuroblastoma, was stopped after the publication of the US COG trial and had enrolled 34 patients only. No conclusion can be drawn from the provided study results.

All studies included mainly paediatric and also some adolescent patients. Most received ch14.18/CHO treatment in combination with IL-2 and 13-cis-RA except for the control arm of the first-line trial (APN311-302) and the trial after haploidentical stem cell transplantation (APN311-201). Finally, two studies investigated a continuous infusion paradigm (APN311-202 and -303).

PK and PD assessments were part of the general study design of the clinical APN311 studies in patients with refractory/relapsed (R/R) neuroblastoma (APN311-101, 201, 202, and 303). PD assessments included determination of complement-dependent and antibody-dependent cell-mediated cytotoxicity (CDC and ADCC), analysis of cytolytic activity in whole blood (whole blood cytolytic activity test [WBT]), and analysis of activated white blood cells. In addition, the occurrence of human anti-chimeric antibodies (HACA) was determined.

2.4.2. Pharmacokinetics

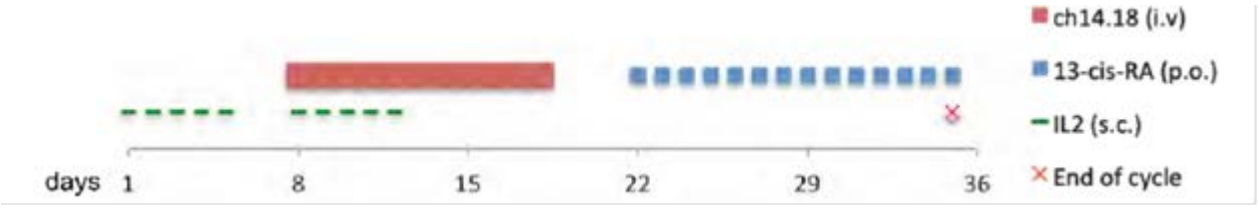
Data are available from three studies described below.

APN311-101

Patients received three 28-day treatment cycles, each consisting of 5 consecutive days of 8-hr intravenous infusions with ch14.18/CHO (monotherapy). Although three doses were tested (10, 20 and 30 mg/m^2/day, data were only provided for the medium dose of 20 mg/m^2/day, i.e. a cumulative dose of 100 mg/m2 per cycle.

APN311-202

Ch14.18/CHO was applied from Day 8 to 18 of each 35-day cycle as a 10-day continuous infusion of 100 mg/m^2 per cycle. Concomitant treatment with interleukin 2 (IL-2) at the dose of 6x10^6 IU/m^2/day was given on Days 1-5 and Days 8-12 of each cycle. Cis-retinoic acid (cis-RA) was applied for 14 days starting at cycle Day 22 at the dose of 160 mg/m^2/day.



The treatment was applied for 5 cycles but PK data are available for the first cycle only. This study started with a dose schedule finding phase and the first patient cohort was allocated to receive 100 mg/m² over 10 days (the established dose already applied in previous studies based on an 8h-short infusion scheme). Only this dosing schedule was evaluated because it was considered the suitable ch14.18/CHO infusion dose based on pre-defined PD and PK endpoints. Therefore, it was also used for the confirmatory expansion cohort.

APN311-303

The same dosing regimen as in the previous trial was applied (the initial 4 patients received a slightly different treatment regimen). A total of up to 6 cycles was given but PK data were provided for 5 treatment cycles.

The PK parameters for the first treatment cycle in the three studies are summarised below.

Table 6: Summary statistics of PK parameters for Cycle 1 (studies APN311-101, -202, 303)

	C_{max} (µg/mL)	t_{max} (h)	AUC_{last} (h·ug/mL)	AUC_{0-xxx} (h·ug/mL)	$t_{1/2}$ (h)
APN311-303 (time 0-504 h)					**(time 0-768 h)**
N	46	46	46	30	15
Mean	12.86	216.00	3066.94	3758.72	180.90
SD	4.62	87.34	1555.90	1422.50	65.35
Min	3.40	48.00	696.00	1184.40	70.13
Median	12.35	240.00	3057.00	3550.20	186.52
Max	22.20	504.00	7137.60	7137.60	295.37
APN311-202 (time 0-840 h)					**(time 0-840 h)**
N	39	39	39	39	19
Mean	11.91	236.92	3602.65	3053.83	197.71
SD	4.25	104.06	1436.63	1155.52	79.03
Min	0.20	168.00	58.80	18.90	77.14
Median	11.80	240.00	3682.80	3031.20	178.01
Max	19.70	840.00	7531.20	6150.80	396.23
APN311-101 (time 0-672 h)					**(time 0-672 h)**
N	7	7	7	6	6
Mean	16.47	77.93	2349.84	2596.91	96.56
SD	6.48	47.15	1035.90	1014.52	63.71
Min	7.42	8.50	1222.49	1287.17	25.62
Median	15.02	104.50	2145.63	2522.10	90.58
Max	26.58	112.00	4293.42	4293.42	196.64

The PK characteristics of ch14.18/CHO are summarised hereafter.

Absorption

Dinutuximab beta is dosed via the IV route and therefore is completely bioavailable.

Bioequivalence

Ch14.18/CHO was manufactured at 3 different sites. No bioequivalence studies were conducted but pharmacokinetic parameters of ch14.18/CHO were in general comparable between studies APN311-202 and -303.

Distribution

Peak levels were generally reached at the end of the 10-day infusion; the mean C_{max} was 13 µg/mL in Cycle 1.

The volume of distribution is stated to be 5- 6.5 L/m^2.

Elimination

No excretion or metabolism studies have been submitted.

The half-life observed in studies 1 and 2 was in the range of 190 hours, i.e. 8 days (see section 5.2 of the SmPC).

Dose proportionality

In the publication of study APN311-202 (Ladenstein, 2013), pharmacokinetics appeared roughly proportional over the dose range of 10 to 30 mg/m^2/day.

There are limited data to assess dose proportionality. Following the 5 x 8-hour infusion regimen and the 10-day continuous infusion, C_{max} was roughly similar (only slightly lower with the 10-day infusion compared to the same total dose administered as 5 short infusions).

Time dependency

There appears to be possible time dependency in the data presented with an increase in half-life with a longer duration of dosing.

Intra- and inter-individual variability

Inter-individual variability is high with a CV of 46.5% on clearance from the study utilising an 8-hour infusion. This is surprising for a drug given intravenously. A non-compartmental re-analysis of the data from studies APN311-202 and -303 is required and should include an assessment of inter-individual variability. Intra-individual variability should also be assessed in a PopPK analysis.

Special populations

A population pharmacokinetic analysis conducted on the data obtained with the initial non validated assays was used to investigate the important covariates on PK. The relationship for clearance and volume of distribution (Vd) with weight is with the expected exponents of 1.0 and 0.75, respectively. Gender did not have a significant impact on the pharmacokinetics. The assay for anti-drug antibody is also not considered robust; however, the suggestion is an effect on Vd, but not clearance.

There are no data in patients with renal or hepatic impairment. This would not be expected for a monoclonal antibody. In the PopPK analysis markers of renal and hepatic clearance (eGFR and bilirubin) did not show a relationship with exposure (C_{max} and AUC_{24h} on day 1 and day 10 during a 10-day infusion).

The exposure (Cmax and AUC24h on day 1 and day 10 during a 10 day infusion) is predicted to be similar in subjects with ages less than or equal to 12 years and decreases slightly for older, heavier subjects. Effects of age was not found to influence the pharmacokinetics of dinutuximab beta.

The posology of APN311 is based on mg/m^2 which suggests clearance increases linearly with BSA, this is consistent with the relationship of clearance on weight.

There are very limited data in children less than 2 years of age.

An effect of ADA formation on the volume of distribution was found (increase of 37% in volume). Therefore, ADA formation would be predicted to have a slight impact (less than 10% decrease) on exposure within 24 hours after administration, under non steady state conditions. After reaching steady state, no difference in exposure is predicted, with and without ADA formation.

Pharmacokinetic interaction studies

No interaction studies have been performed. Ch14.18/CHO has been co-administered with IL-2 in the two PK studies with continuous infusions.

It has been shown in study APN311-201 that APN311 induced the release of cytokines, in particularly IL-6 and TNFα.

2.4.3. Pharmacodynamics

Mechanism of action

Ch14.18/CHO was shown *in vitro* to specifically bind to GD2 with similar binding affinities compared to ch14.18/SP2/0 or ch14.18/NS0. This was expected as the variable regions of the antibodies are identical.

Ganglioside GD2 is a glycolipid antigen devoid of an intracellular signal transduction domain. Therefore, the mechanism of action of anti-GD2 mAbs mostly relies on immune effector functions like CDC and ADCC. CDC is induced through binding of a serine protease complex C1 to the Fc domains of two or more mAb molecules binding to antigens expressed on tumour cells. This classical complement pathway results in an activation cascade resulting in a membrane attack complex disrupting the target cell. ADCC is a result of Fc-gamma receptor (FcγR) mediated interaction with effector immune cells such as NK cells, macrophages and granulocytes. The binding of the FcγR to the Fc domain induces both release of granzymes and perforin from effector cells leading to a target cell lysis and Fc-dependent tumour cell phagocytosis.

The reason for combining IL-2 to ch14.18 was because it stimulates the proliferation, growth and activation of natural killer cells, generates lymphokine-activated killer (LAK) cells, and therefore augments ADCC. In the literature, *in vitro* studies with anti-GD2 antibodies and IL-2 have demonstrated that the addition of IL-2 enhanced the lysis of GD2-expressing cells as compared to anti-GD2 antibody alone. IL-2 is also known to increase regulatory T cell function.

PD marker results

In the clinical APN311 studies, effects on pharmacodynamics parameters were investigated as secondary endpoints. These included ADCC, CDC, and whole blood cytolytic activity, absolute counts of immune cells, NK and T cell activation, and soluble IL-2 receptor as well as HACA analyses. Time points with most available data were D1 (before IL-2), D8 (3 days after the end of the first IL-2 course and before the second IL-2 course combined with ch14.18/CHO treatment), and Day 15 (3 days after the end of the second IL-2 course during ch14.18 treatment). Sufficient data at subsequent time points (from the end of ch14.18 (Day 18) until the end of the cycle) were only available in one trial. Therefore, the respective effects of IL-2 and CH14.18/CHO are difficult to disentangle.

Effect on immune cells

IL-2 induced an increase in the absolute count of leukocytes and lymphocytes, activated NK cells and their subsets (cytotoxic and cytokine producing NK cells), T cells and their subsets (regulatory, helper and cytotoxic); the increase in activated NK cells and T lymphocytes was prolonged so that the response to IL-2 was enhanced throughout the cycles. IL-2 also increased the proportion of regulatory T-cells at the expense of cytotoxic T-cells.

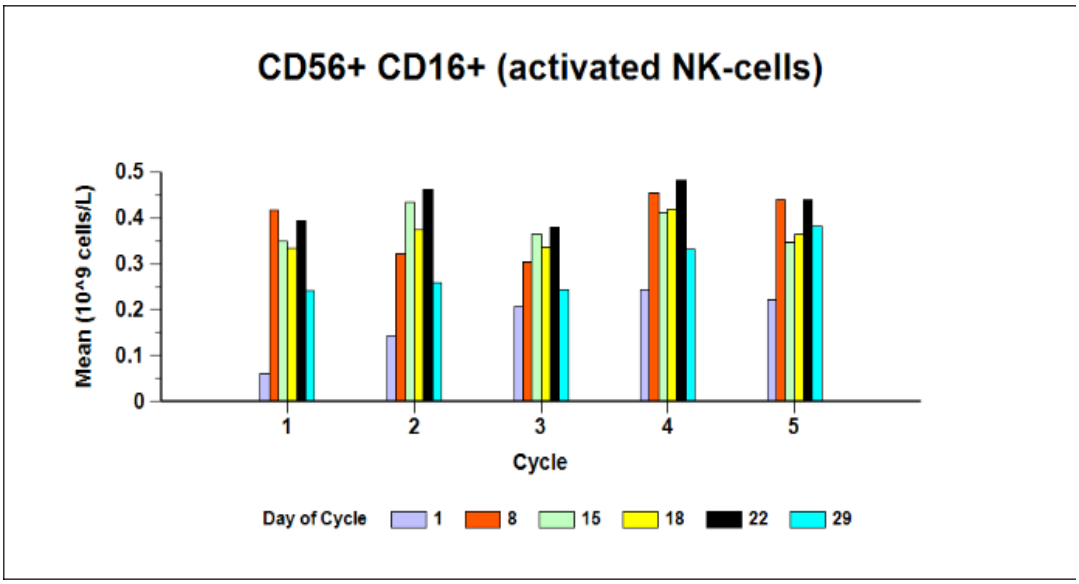


Figure 1 Mean values of activated NK cells as result of ch14.18/CHO treatment in combination with IL-2 and RA (study 202)

In contrast to lymphocytes, granulocytes, especially neutrophils, seemed essentially unaffected by IL-2 but increased during Ch14.18/CHO treatment although this effect seemed to diminish over the cycles. Finally, monocytes seemed marginally influenced by the two products. However, IL-2 appeared to activate granulocytes and monocytes although this effect also seemed to diminish over the cycles.

The effect of ch14.18 on its own could be evaluated in study APN311-201 where it was administered alone (or followed by low doses of IL-2). Lymphocyte counts and activated CD56+CD16+ (cytokine-secreting and cytolytic) NK cells were substantially reduced after ch14.18/CHO administration and returned to pre-treatment levels afterwards. In line with these results, when ch14.18/CHO was administered after IL-2, lymphocyte counts decreased whereas activated NK cells plateaued or decreased before a slight rebound was observed; it might be speculated that activated NK cells migrate to the tumour site and are then replenished after a few days.

Effector functions

As expected, IL-2 on its own did not impact on CDC and ADCC activities, which were increased by ch14.18/CHO. With the assays used, the magnitude of the CDC effect was high compared to that of ADCC, which appeared to vary greatly amongst patients and tended to decrease after the first cycle to be almost abolished in the last cycle (cycle 5). It is noteworthy that this was observed in parallel with a decrease in neutrophils and activated granulocytes/monocytes, which are also involved in ADCC.

As expected given the limited samples available, no strong correlation has been shown between FCγR or KIR/HLA KIR ligand and ADCC. Nevertheless, trends consistent with the literature on anti-GD2 were found, i.e. ADCC activity tended to be higher in patients with mismatched KIR/HLA-KIR compared with patients with matched KIR/HLA-KIR and in patients with high-affinity FcγR2A 131-H/H genotype compared to those with FcγR2A 131-R/R genotype. High-affinity FcγR2A 131-H/H genotype reflects neutrophil and macrophage ADCC and IL-2 treatment is known to induce release of GM-CSF by IL-2 responsive cells, in turn stimulating these ADCC effector FcγR2A-bearing cells. The Applicant's hypothesis that the decrease in ADCC over time, especially in patients with high affinity receptors, may be correlated with the decrease in tumour burden is plausible.

To further assess the lysis capacity of ch14.18/CHO, a whole blood cytolytic activity test (WBT), mimicking *in vivo* physiology by using whole blood as the effector source and a GD2-positive LAN-1

human neuroblastoma cell line as target cells. Cytolytic activity was increased after the administration of ch14.18/CHO (mean ~ 70%) and this response was abolished in the presence of ganglidiomab (anti-idiotypic antibody that neutralizes the binding of ch14.18/CHO to GD2), confirming that this response is mediated by ch14.18/CHO. In contrast with the ADCC activity, the WBT cytolytic activity was maintained throughout the five treatment cycles. It is usually postulated that ADCC is the main mechanism of action for anti-GD2 therapies, but these data would suggest that CDC may be an important contributor *in vivo*.

Based on these data it cannot be concluded that IL-2 does not enhance the response to ch14.18/CHO as this should require comparing ch14.18/CHO alone and in combination with IL-2. The design of study APN311-201 where IL-2 was added after the administration of ch14.18/CHO at cycles 4 to 6 to minimize the risk of GvHD and at a much lower dose than that used in the other trials cannot address this question.

Soluble IL-2 receptors are expected to be upregulated following IL-2 mediated stimulation of cells, resulting in a positive feedback loop. Indeed, the mean concentration of sIL2r peaked after IL-2 application but the peak decreased over the cycles. A slight increase after ch14.18 alone was observed, which is not unexpected given that this is a marker for overall immune activation.

HACAs

Antibody generation against the GD2 antibody ch14.18/CHO may interfere with the functioning of the antibody and thus limit the efficacy of ch14.18/CHO treatment. Human anti-chimeric antibodies to ch14.18/CHO were determined; a new antibody binding assay was developed using electrochemiluminescence on the Meso Scale Discovery platform.

Using this new assay, 65/105 (62%) of the patients developed ADAs; 11 out of 15 (73%) patients with data available were ADA positive in APN311-101, 27 out of 42 (64%) patients in APN311-202, and 27 out of 48 (56%) patients in APN311-303. No patient (out of the 32 analysed) was ADA positive in study APN311-201, because these patients were B-cell depleted following a CD3/CD19-depleted, hematopoietic stem cell transplantation.

Overall, there was a trend towards a slight reduction of CDC, ADCC and WBT lysis capacity correlating with formation of an ADA response measured in samples from study APN311-303. This result is in alignment with predicted correlations between impact of ADA formation on serum APN311 levels (i.e. reduction in Cmax and AUC). There were no consistent relationships between ADA positivity and efficacy measure response, EFS and OS, across studies. There was no correlation between ADA positivity and TEAE.

IL-2 is also known to be immunogenic and no data on anti-IL-2 antibodies have been provided. However, there was no apparent indication of a decrease in its main PD effects over the treatment cycles.

2.4.4. Discussion on clinical pharmacology

Ch14.18/CHO is a mouse-human chimeric monoclonal anti-GD2 IgG1 antibody produced in a mammalian CHO cell line. In the US, clinical studies have been conducted with ch14.18 produced in SP2/0 murine cells. The glycosylation pattern varies between ch14.18/CHO and ch14.18/SP2/0 due to the different production cell lines, and this may influence pharmacokinetics, efficacy and safety of the antibody in the treatment of neuroblastoma patients.

No direct comparison between the two products has been conducted. Pharmacokinetics of ch14.18/CHO and ch14.18/SP2/0 were only compared across studies based on literature data for ch14.18/SP2/0 (Uttenreuther-Fischer et al, 1995). It should be noted that there are differences in study design (e.g. administration of IgG before treatment with ch14.18/CHO, administration with GM-CSF for

ch14.18/SP2/0, different doses) and ch14.18 analyses in serum were performed with different assays. Further, for both products inconsistencies in pharmacokinetic parameters between studies were reported. Therefore, this comparison between ch14.18/CHO and ch14.18/SP2/0 is of limited value with regard to bridging purposes. Since the efficacy of ch14.18/CHO was evaluated in comparison with historical controls that did not receive immunotherapy, the lack of adequate bridging between ch14.18/CHO and ch14.18/SP2/0 is no concern.

Ch14.18/CHO has been manufactured at three different sites. The commercial product has not been used in any clinical study but quality data do support the comparability of these products.

Pharmacokinetics

Pharmacokinetics of ch14.18/CHO has only been evaluated in paediatric patients with neuroblastoma, which is acceptable given the toxicity of the product.

Dinutuximab beta is a protein for which the expected metabolic pathway is degradation to small peptides and individual amino acids by ubiquitous proteolytic enzymes. Classical biotransformation studies have not been performed. As the target for APN311 is a membrane-bound receptor, target-mediated degradation is also expected.

The terminal half-life of APN311 could only be estimated in a fraction of subjects (less than half the subjects) as the elimination phase was not captured by the end of the cycle and the blood sampling times were not appropriate. Even for those individuals where a half-life was estimated, it is not considered that the fit for the data allowed for the calculation of a half-life. In the continuous infusion studies, a mean half-life of 8 days in Cycle 1 (vs. 4 days in the short-infusion study) is likely to be underestimated. It increased to 12 days in Cycle 4. In addition, exposure increased by approximately 50% by Cycle 5 and this would suggest a longer half-life than calculated by the Applicant.

The volume of distribution is stated to be 5- 6.5 L/m2, which is consistent with what would be expected for a monoclonal antibody. However, in the PopPK analysis, it was estimated at 2L, which is unexpectedly low.

Due to the issues encountered with the bioanalytical assays, PK data cannot be considered to be robust and therefore the conclusions from the data are limited. This has to be taken into consideration when interpreting PK parameters (C_{max}, exposure, half-life) listed in section 5.2 of the SmPC.

A further investigation of PK has been requested in the context of a specific obligation (see Annex II).

The objective of the first trial (APN311-101) was to bridge the PK profile of ch14.18/CHO to that of the previous ch14.18 produced in SP2/0 cells by comparison with literature data. The PK profile was considered sufficiently similar so that it was decided to pursue the clinical development of APN311 using the same daily dose as the other product, i.e. 20 mg/m^2 (Ladenstein, 2013). In the publication, PK data were also provided for patients treated at 10 and 30 mg/m^2/day (3 subjects each). No clear dose toxicity relation was observed, therefore, no firm conclusions on the optimal doses of ch14.18/CHO can be drawn.

In the second dose-finding study (APN311-202), the starting dose was 100 mg/m² over 10 days, which was the established cumulative dose applied in previous studies with ch14.18 produced in other cells based on an 8h-short infusion scheme. Eventually, only this dosing schedule was evaluated because it was considered suitable based on pre-defined PD and PK endpoints, i.e. ch14.18/CHO concentration >1µg/ml at Day 15 of cycle 1 in ≥ 80 % of patients AND an increase of 500% and/or an absolute minimum increase to ≥100 cells/µL of the CD16/CD56 positive activated NK cells compared to baseline in ≥ 80 % of patients. It is noteworthy that all these PD criteria were based on literature data for IL-2.

A population pharmacokinetic analysis was conducted on the data obtained from studies APN311-101, -202 and -303 with the initial non validated assays. A number of covariates have been investigated, i.e. markers of renal and liver function, gender, age, body weight, BSA, ADA, infusion length and cycle number. Gender does not appear to have a significant effect. ADA seems a significant covariate on Vd but not clearance, suggesting a difference in exposure in the presence of ADA at earlier doses but not at steady state. The relationship for weight is consistent with dosing on an mg/m^2 basis. Markers of renal and hepatic function, eGFR and BIL1 do not have a significant effect on exposure. There are insufficient data to support dosing of children less than 2 years.

Ch14.18/CHO is considered to have a low potential for drug-drug interactions given its target and elimination profile. However, in study APN311-201, APN311 induced the release of cytokines, in particularly IL-6 and TNFα. Cytokines can affect expression levels of cytochrome P450s and IL-6 is known to decrease the activity of cytochrome P450. A risk for indirect reduction of CYP activity due to higher TNF-α and IL-6 levels and, therefore, interactions with concomitantly used medicinal products, cannot be excluded (see section 4.5 of the SmPC).

Pharmacodynamics

The mechanism of action of anti-GD2 mAbs and rationale for adding IL-2 is widely documented in the literature. A significant battery of tests has been deployed to evaluate the activities of the combination as reflected by the proliferation and activation of immune cells and their cytolytic effects on tumour cells. The results showed that cytolytic activity was durably elevated by ch14.18. It is noteworthy that this activity was abolished in a patient who developed HACAs.

No data or discussion has been provided on the relationship between drug concentration and PD effects since the exposure data are not robust. *In vitro* studies indicate that ch14.18 can induce tumour cell lysis via CDC and ADCC at concentrations <1 µg/ml. For that reason a limit of measurable ch14.18/CHO level of at least 1 µg/mL could be understood although at day 15 the infusion with ch14.18/CHO is still ongoing and patients have considerably higher ch14.18/CHO serum concentrations at this time point than during days 20-35 of the cycle and up to day 8 of the next cycle. In study APN311-202, only 11/35 patients (31%) having received more than 40 mg/m² had trough concentrations (before the next infusion) at that level. Therefore, the validity of this target concentration for ch14.18/CHO at day 15 of cycle 1 is doubtful.

The relationship between CD16/CD56 positive activated NK cells, which are involved in ADCC-mediated lysis of tumour cells, and the efficacy of ch14.18/CHO has been investigated. There was no clear correlation between CD16/CD56+ activated NK cell expression and tumour response rate but a trend towards longer overall survival in patients with high expression of CD16/CD56+ activated NK cells.

In addition, the possible impact of genetic polymorphism of FcγR and KIR/KIR-Ligand on the PD response has been investigated. Trends consistent with the literature on anti-GD2 were found, i.e. ADCC activity tended to be higher in patients with mismatched KIR/HLA-KIR compared with patients with matched KIR/HLA-KIR and in patients with high-affinity FcγR2A 131-H/H genotype compared to those with FcγR2A 131-R/R genotype.

Compared to the *in vitro* CDC and whole blood cytolytic activity of ch14.18/CHO, higher ch14.18/CHO serum concentrations seem to be needed for maximal effect in the *ex vivo* CDC and whole blood cytolytic activity assay. The cytolytic activity was higher at day 15 than at day 1 of the next cycle while ch14.18/CHO serum concentrations were still > 1 µg/ml for most patients. Based on the *in vitro* results (maximal lysis at 1 µg/ml ch14.18 concentration) a maximal cytolytic effect would have been expected at Day 1 of cycles 2-5. However, no definite conclusion can be drawn since the exposure data are not robust.

Immunogenicity

The validated HACA assay appears to have poor drug tolerance and its results are not considered reliable. No neutralising assay is currently available. Nevertheless, it is noted that the majority of patients developed HACAs (56% to 73% depending on the studies) except in one study (APN311-201) because the patients were B-cell depleted following a CD3/CD19-depleted, hematopoietic stem cell transplantation.

Upon CHMP request, the Applicant developed new analytical methods for drug and HACA measurement and will develop a neutralising assay. The validation reports showed major issues with drug–HACA interaction, and therefore, both drug and antibody measurements are not considered robust at the time being.

The current analyses are considered exploratory only and further research post-authorisation will be needed to enable conclusive interpretations (see Annex II).

The Applicant has provided theoretical arguments as to why the ch14.18/CHO molecule is unlikely to affect cardiac repolarisation. Furthermore, non-clinical and clinical data (ECG) collected so far did not enable to detect any abnormalities that could suggest QT prolongation.

2.4.5. Conclusions on clinical pharmacology

The PK profile of ch14.18 has been poorly characterised due to major issues with bioanalytical assays. At the moment, the uncertainties regarding the pharmacokinetic parameters because of limitations of the bioanalytical assays have been addressed in the SmPC.

Although the evaluation of the PD response to ch14.18 and IL-2 has been extensively studied, these data do not enable to determine the contribution of IL-2 to the response to the anti-GD2 antibody and it is not known whether the blood cytolytic activity is similar with or without IL-2. Further data from study APN311-202v3 will be provided as a post authorisation measure (see Annex II).

Due to poor drug tolerance of the new validated HACA assay, the immunogenicity data are not considered reliable. The Applicant's analyses of the impact of HACAS on PD, efficacy and safety can only be considered exploratory (See Annex II).

No firm conclusions on the optimal dose of ch14.18/CHO can be drawn because there was no clear dose toxicity response (see Annex II).

The CHMP considers the following measures necessary to address the issues related to pharmacology:

- The applicant will perform an evaluation of PK/PD and immunogenicity profile based on data collected from completed and ongoing trials (APN311-202v1-2-3 and -304) in order to better characterise the PK/PD profile and the impact of HACAs on PD, efficacy and safety.
- The applicant will submit the results of study APN311-202v3, in order to evaluate the add-on effect of IL-2 in patients with relapsed refractory neuroblastoma.

2.5. Clinical efficacy

2.5.1. Dose response studies and main clinical studies

Two dose response trials were submitted, but no dose response assessment was actually conducted. In both trials, all data relate to a 100 mg/m^2 treatment course, administered either as 5 daily infusions of 20 mg/m^2 (APN311-101) or one continuous 10-day infusion at 10 mg/m^2/day (APN311-202). The former regimen corresponds to the regimen administered in previous German trials (NB90/NB97) using ch14.18

produced in NS0 cell lines (Simon 2005). In the second trial, PK and PD criteria were pre-defined and therefore, this regimen was pursued.

Study APN311-202

A Phase I/II Dose Schedule Finding Study of ch14.18/CHO Continuous Infusion Combined with Subcutaneous Aldesleukin (IL-2) in Patients with Primary Refractory or Relapsed Neuroblastoma

Stage 1: dose schedule finding phase

Planned daily doses to be investigated in cohorts of 10 patients: 7, 10, or 15 mg/m² with infusion durations varying between 10 to 21 days resulting in total doses of 100, 150 or 210 mg/m²

The dose schedule finding phase was rule-based accounting for both the pain-toxicity profile and immunomodulatory (efficacy) capacity. Results on both endpoints were to be evaluated after each cohort of 10 patients. On the basis of the results, the next rule-based infusion schedule was assigned for the next cohort until one was identified that met all primary endpoint criteria in at least 80% of patients.

Stage2: confirmatory phase to treat an expansion cohort of 100 patients for five 35-day treatment cycles (ongoing)

Methods

Study Participants

Main inclusion criteria

- Patients aged 1 to 21 years
- with neuroblastoma diagnosed according to INSS
- having received at least 1 previous high-dose treatment followed by stem cell rescue after conventional therapy to reduce tumour burden
- fulfilling one of the following criteria:
    - Primary refractory patients with stage 4 disease with at least 2 lines of treatment prior to high-dose therapy/autologous stem cell transplantation (ASCT), causing a delay from diagnosis to ASCT of over 9 months
    - Treated and responding relapse after primary stage 4 disease
    - Treated and responding disseminated relapsed neuroblastoma having received ASCT
- Patients may have had prior central nervous system (CNS) metastasis providing the following criteria were all met:
    - the CNS disease had been previously treated;
    - the CNS disease had been clinically stable for 4 weeks prior to starting this study;
    - the patient was off steroids for CNS disease for 4 weeks prior to starting on study and during the course of the study

Main exclusion criteria

- progressive disease
- previous treatment with ch14.18/murine, non-secreting myeloma cells (SP2/0) or ch14.18/CHO

- likely to require, corticosteroid or other immunosuppressive drugs

Treatments

- Ch14.18/CHO was administered as a 10-day continuous infusion in cycles of 35 to 49 days (depending on infusion duration) starting on Day 8 of each cycle, for a total of 5 cycles; however, only the first cycle was assessed for the dose schedule finding

- Ch14.18/CHO was combined with IL-2 and 13-cis RA (see details of the regimen in the PK section).

Concomitant treatment

Pain management

Prior to each cycle of ch14.18/CHO the patient was to be primed with oral gabapentin, which was then continued during ch14.18/CHO administration.

Prior to each infusion of ch14.18/CHO, intravenous morphine was to be given first as a bolus, then as a continuous infusion and ideally weaned off over the first 5 days depending on the individual patient pain tolerance.

Allergic reactions

- oral or i.v. diphenhydramine before ch14.18/CHO treatment and every 4 hours as needed.
- cetirizin 5 mg every afternoon (<30 kg) or 5 mg twice daily (>30 kg).

Prohibited treatment

- chemotherapy, hormonal anticancer therapy, or experimental anticancer medications other than those that were study-related

- glucocorticoids, or other drugs with known immunosuppressive activity, during and for 2 weeks prior to entry onto the trial except for life threatening symptoms

- radiotherapy

- i.v. immunoglobulin (because they could interfere with the antibody dependent cellular toxicity) within 2 weeks of starting ch14.18/CHO and 1 week after completing ch14.18/CHO

Objectives

- Primary: to find a tolerable treatment schedule which reduces the pain-toxicity profile of ch14.18/CHO whilst maintaining immunomodulatory efficacy

- Secondary: assessment of pain intensity and relief, pharmacokinetics, pharmacodynamics, immunogenicity, anti-tumour response in patients with measurable disease

Outcomes/endpoints

Primary endpoints

Only the first cycle was taken into account.

• Pain-toxicity endpoint: i.v. morphine free ch14.18/CHO infusion schedule after the first 5 days during the first cycle in ≥ 80% of patients

• Efficacy endpoint: on Day 15 of the first cycle in ≥ 80% of patients:

a) an increase of 500% and/or an absolute minimum increase to ≥100 cells/µL of the CD16/CD56 positive activated NK cells, AND

b) a measurable ch14.18/CHO level of at least 1 µg/mL.

Secondary endpoints

- ADCC and activated NK cell concentrations above baseline levels in≥80% of patients;
- Appearance of soluble IL-2 receptor and CDC;
- Detection of anti-idiotype response by appearance of HAMA and HACA;
- Increase of absolute lymphocyte counts by 50% over baseline;
- Increase of absolute NK cell numbers >1000 cells/µL in ≥80% of patients;
- Ch14.18/CHO concentrations;
- Anti-tumour response in patients with measureable disease (bone marrow [BM], skeletal lesions, soft tissue lesions, lymph nodes and/or primary tumour site) as measured by immunocytology, meta-iodobenzylguanidine (mIBG), computed tomography (CT) and/or magnetic resonance imaging (MRI). Assessments were to be done at screening, mid evaluation and end of treatment evaluation

Sample size

N/A

Randomisation

N/A

Blinding (masking)

N/A

Statistical methods

N/A

Results

Patient population

Forty four (44) patients with relapsed/refractory (R/R) neuroblastoma were enrolled at the cut-off date (February 2015).

Recruitment

Patients were enrolled from January 2012 in 10 centres in Spain (10), France (9), Italy (9), UK (6), Germany (5), Israel (4), and Austria (1).

Baseline data

Table 7: Demographics and disease history

Parameter		Number of Patients (N=44)
Gender, n (%)	Male	28 (63.6%)
	Female	16 (36.4%)
Ethnicity, n (%)	White / Caucasian	36 (87.8%)
	Black	-
	Asian	1 (2.4%)
	Unknown	4 (9.8%)
	Missing*	3
Age at initial diagnosis [years]	n	44
	Mean (SD)	3.2 (2.0)
	Median	3.0
	Min, Max	0, 9
Age at start of treatment [years]	n	44
	Mean (SD)	6.1 (3.4)
	Median	5.0
	Min, Max	1, 17
MYCN amplification	no	39 (92.9%)
	yes	3 (7.1%)
	missing	2
INSS stage at initial diagnosis	1	1 (2.3%)
	4	41 (93.2%)
	4s	2 (4.5%)
Patients with refractory disease, n (%)		25 (56.8%)
Patients with relapsed disease, n (%)		19 (43.2%)

BM = bone marrow, CT = computed tomography, INSS = International Neuroblastoma Staging System, Max = maximum, mIBG = meta-iodobenzylguanidine, Min = minimum, MRI = magnetic resonance imaging, MYCN = v-myc myelocytomatosis viral related oncogene, SD = standard deviation

For a total of 23 patients the overall number of relapses/progressions and the date of the most recent relapse/progression was documented. Sixteen out of the 23 patients experienced only 1 relapse/progression. Most patients (56.5%) experienced relapse/progression of the combined type, i.e. not limited to one location. For 38 patients the performance status before study treatment was evaluated. The minimum performance score was 80 and the maximum was 100 (mean 98.4 ± 4.4) at baseline.

Table 8: Relapse / Progression prior to immunotherapy

Parameter		Number of Patients (N=44)
Number of relapses/progressions	n	23
	Mean (SD)	1.5 (1.1)
	Median	1.0
	Min, Max	1, 6
Number of relapses/ progressions (categories)	1	16 (69.6%)
	2	6 (26.1%)
	6	1 (4.3%)
Time from initial diagnosis to most recent relapse/ progression [days]	n	16
	Mean (SD)	1099 (1091)
	Median	618.0
	Min, Max	253, 4123
Most recent relapse/progression type	Bone marrow alone	3 (13.0%)
	Combined	13 (56.5%)
	Other metastatic sites alone	1 (4.3%)
	Primary tumour site alone	2 (8.7%)
	Skeleton alone	4 (17.4%)

BM = bone marrow, CT = computed tomography, Max = maximum, mIBG = meta-iodobenzylguanidine, Min = minimum, MRI = magnetic resonance imaging, SD = standard deviation
*Percentages were based on all evaluable patients, excluding missing values

First-line treatment consisted of single courses or combinations of the following treatments: surgery, radiotherapy, chemotherapy, intensive chemotherapy and maintenance therapy with 13-cis-retinoic acid

(RA). Most frequently patients received rapid COJEC followed by high-dose BuMel (busulfan and melphalan) + autologous stem cell transplantation (ASCT) treatment. About 55% of the patients received radiotherapy and 43% received 13-cis-RA maintenance therapy prior to immunotherapy.

As treatment of R/R disease, 14 patients received another intensive chemotherapy regimen followed by ASCT. Nine patients (20.5%) received radiotherapy as local therapy and 8 patients (18 %) underwent surgery. About 20% of patients received 13-cis-RA maintenance therapy.

Although the response to the most recent therapy was not recorded, all patients had to have responded adequately to their previous treatment and no patient had signs of progression at study entry. Most patients had evidence of disease at baseline before immunotherapy, either detected by $^{123/131}$iodine-meta-iodobenzylguanidine (mIBG) and/or bone marrow (BM) histology or measured by magnetic resonance imaging (MRI) and/or computed tomography (CT) (see table below).

Following CHMP request, the Applicant indicated that five patients could be considered first-line patients.

Table 9: Disease Status at Baseline

Disease Status		Number of Patients (N=44) n (%)
Relapsed patients	Measurable by MRI and/or CT	4 (21.1%)
	Evaluable only by mIBG and/or BM histology	8 (42.1%)
	No evidence of disease	7 (36.8%)
Refractory patients	Measurable by MRI and/or CT	8 (32.0%)
	Evaluable only by mIBG and/or BM histology	13 (52.0%)
	No evidence of disease	4 (16.0%)

BM = bone marrow, CT = computed tomography, Max = maximum, mIBG = meta-iodobenzylguanidine, Min = minimum, MRI = magnetic resonance imaging, SD = standard deviation

Outcomes and estimation

Anti-tumour response

Table 10: Study APN311-202: Treatment response in patients with detectable disease at baseline

	Statistics	End of 2nd cycle n (%) of patients (N=33)	End of treatment n (%) of patients (N=33)	Best response n (%) of patients (N=33)
No evidence of disease	N(%)	6 (19.4%)	6 (19.4%)	8 (25.8%)
Improved disease	N(%)	9 (29.0%)	8 (25.8%)	9 (29.0%)
Stable disease	N(%)	9 (29.0%)	5 (16.1%)	7 (22.6%)
Progressive disease	N(%)	6 (19.4%)	12 (38.7%)	7 (22.6%)
Mixed response	N(%)	1 (3.2%)	-	
Missing	N	2	2	2

At the end of treatment (i.e. approximately 6 to 8 months after treatment initiation or earlier in case of progressive disease), a response was observed in 14/33 patients (42%) with evidence of disease at baseline. Two patients were non evaluable.

The treatment response was the same in patients with disease evaluable by mIBG/BM only (43%; 9/21) and in patients with disease measurable by MRI/CT (42%; 5/12). It was higher in refractory disease (48%; 10/21) than in relapsed disease (33%; 4/12). The range for the duration of response was very broad (5 weeks to 3 years); the median was about 2.3 years regardless of baseline status and disease type. No other factors were investigated.

Overall survival

See section “Analysis performed across trials”

Study APN311-303

Retrospective analysis of data collected during the administration of ch14.18/CHO continuous infusion combined with subcutaneous aldesleukin (IL-2) in patients with high risk neuroblastoma under a compassionate use program

Methods

Study Participants

Main inclusion criteria

a) ≥ 1 year and ≤ 45 years of age at treatment start

b) Diagnosis of high risk neuroblastoma according to the INSS criteria, i.e. INSS stage 2, 3, 4, or 4s with MYCN amplification, or INSS stage 4 without MYCN amplification or relapsed or refractory neuroblastoma of any stage

c) Off any standard or experimental treatments for at least two weeks prior to treatment start and fully recovered from the short term major toxic effects

d) No immediate requirements for palliative chemotherapy, radiotherapy or surgery

e) ≥ 4 weeks after major surgery (e.g. laparotomy or thoracotomy) and fully recovered from any post-surgical complications

f) Patients with seizure disorders were enrolled if on anticonvulsants and if seizure disorders were well controlled

g) No dyspnoea at rest and a pulse oximetry >94% on room air

h) Adequate bone marrow. liver renal function

Main exclusion criteria

a) Progressive disease

b) Previous treatment with ch14.18/SP2/0 and/or ch14.18/CHO, if positive for HACA

c) Significant intercurrent illnesses and/or any of the following:

- Symptoms of congestive heart failure or uncontrolled cardiac rhythm disturbance.
- Significant psychiatric disabilities or uncontrolled seizure disorders
- Active infections.
- Clinically significant neurologic deficit or objective peripheral neuropathy (Grade >2)

d) Clinically significant, symptomatic, pleural effusions

e) Requirement or likely requirement for corticosteroid or other immunosuppressive drugs

f) Concurrent treatment with any other anticancer therapies

Treatments

Ch14.18/CHO was given in combination with fixed doses of s.c. aldesleukin (IL-2) and oral isotretinoin (13-cis-RA). Patients initially received i.v. ch14.18/CHO in combination with s.c. IL-2.

Aldesleukin (IL-2)

Subcutaneous IL-2 was usually given at a dose of 6 x 10^6 IU/m^2/day. The majority of patient received it in two 5-day blocks (days 1-5 and 8-12). In these patients, IL-2 was given concurrently with ch14.18/CHO on days 8-12. Initial patients, however, received IL-2 on days 1-5 only as they started with the combination of IL-2 and ch14.18/CHO. Patients ≤ 12 kg were dosed according to body weight: 0.2 x 10^6 IU/kg/day.

ch13.18/CHO

The dose level of ch14.18/CHO was limited by tolerability although a target daily dose of 10 mg/m^2, which relates to a total dose of 100 mg/m^2/cycle was aimed for. Patients initially received 50 mg/m^2 in their first treatment cycle in order to assess feasibility and tolerability of the treatment regimen. The majority of patients started with s.c. IL-2 in the first week, followed by a combination of ch14.18/CHO and s.c. IL-2 in the second week. The total duration of a cycle varied between 28 and 35 days. In each cycle treatment ended with oral isotretinoin (13-cis-RA) after the completion of the ch14.18/CHO infusion. A total of up to 6 cycles was given.

Table 11: Treatment schedules - Study APN311-303

Treatment schedule	Length of cycle	Ch14.18/CHO i.v.		Aldesleukin (IL-2) s.c.		Isotretinoin (13-cis-RA) p.o.	
		Dose (mg/m^2) * day = total dose (mg/m^2)	Days	Dose (IU/m^2/day)	Days	Dose (mg/m^2/ day)	Days
1.	28 days (4 weeks)	5 mg/m^2 * 10 days = 50 mg/m^2	1-11	6 x 10^6 IU/m^2/day	1-5	160 mg/m^2/day	15-28
2.	28 days (4 weeks)	10 mg/m^2 * 10 days = 100 mg/m^2	1-11		1-5		15-28
3.	35 days (5 weeks)	10 mg/m^2 * 10 days = 100 mg/m^2	8-18		1-5, 8-12		22-35

In order to explore the safety and tolerability of the combination (ch14.18/CHO, IL-2 and 13-cis-RA) the first 4 patients were treated according to less intensive treatment schedule 1 in their first treatment cycle. As the safety profile of the administered treatment was acceptable all 4 patients continued with higher dose of ch14.18/CHO from their 2nd cycle onwards (treatment schedule 2 in the Table 1 above).

Tolerability and safety of both 4-week treatment schedules were acceptable and therefore the following 50 patients were treated according to intensified treatment schedule 3. A maximum of 6 cycles was allowed. Some patients received reduced doses of IL-2 and/or ch14.18/CHO for safety/tolerability reasons.

13-cis RA

Patients received isotretinoin (13-cis-RA) at a total daily dose of 160 mg/m²/day administered in two equal oral doses twice a day for 14 days after the completion of the ch14.18/CHO infusion. Doses were rounded to the nearest 10 mg. The starting day was either day 14 or day 21. Patients ≤12 kg were given 5.33 mg/kg/day divided into two equal doses given orally twice a day for 14 days.

Objectives

The primary objective of study APN311-303 was to evaluate retrospectively the safety and pain-toxicity profile of prolonged continuous infusion of ch14.18/CHO in combination with s.c. (IL-2) and oral

13-cis-RA in patients with high risk neuroblastoma treated under a compassionate use programme in one single centre in Germany. Secondary objectives included the retrospective evaluation of anti-tumour response through clinical assessment (CT/MRI, ^{123}I mIBG, bone marrow examination) in patients with measurable disease, Overall Survival (OS) and Event-Free Survival (EFS), pharmacodynamics and pharmacokinetics of ch14.18/CHO.

Outcomes/endpoints

Primary Endpoints

Safety and tolerability were evaluated by:

- Pain intensity/morphine use
- Incidence, grade and type of adverse events, vital signs and changes in clinical laboratory tests

Secondary Endpoints

- Response rate in patients with measurable/evaluable disease (skeletal lesions, soft tissue lesions, lymph nodes and/or primary tumour site, bone marrow) as measured by mIBG, CT/MRI and/or bone marrow examination at the end of cycle 3 and at the end of treatment (after 5th or 6th cycle)
- Durability of the response
- Overall survival (OS)
- Event-free survival (EFS), calculated as number of days from starting the compassionate use programme treatment until relapse or disease progression observed and detected by any of the 3 methods, CT/MRI, mIBG or bone marrow examination
- Pharmacodynamic parameters
- Correlation between activated NK cells and ch14.18/CHO level with ADCC
- Pharmacokinetic parameters

Response Criteria

The definition of overall response was based on modified International Neuroblastoma Response Criteria (INRC). The response criteria integrated response at all sites, including CT/MRI soft tissue lesions, which met RECIST criteria version 1.1, mIBG positive lesions, and bone marrow disease.

Table 12: Response criteria - Study APN311-303

Response by individual site				Overall response
CT/MRI lesions (soft tissue)	mIBG lesions	Bone marrow	Catechols	
PD	Any	Any	Any	PD
Any	PD	Any	Any	PD
Any	Any	PD	Any	PD
CR	CR	CR	Normal	CR
PR	PR/CR in bone lesions, may have SD/PR/CR in soft tissue sites corresponding to lesions on CT/MRI	CR	Any	PR
CR	PR	CR	Any	PR
SD	SD/PR/CR	SD/CR	Any	SD
SD/PR/CR	SD	SD/CR	Any	SD
SD/PR/CR	SD/PR/CR	SD	Any	SD

Sample size

N/A

Randomisation

N/A

Blinding (masking)

N/A

Statistical methods

Descriptive summaries and Kaplan-Meier curves for survival analyses were presented.

The safety population (SAF) included all patients who were enrolled in this retrospective analysis.

The full analysis set (FAS, intention-to-treat population, ITT) included all patients who were enrolled, and from whom a screening mIBG or CT/MRI was available.

Two per protocol (PP) populations were defined, PP-RESP for the overall response evaluation and PP-SURV for event free and overall survival.

For the PP-RESP, the following patients were considered:

- with evidence of disease at screening assessment and
- with MRI/CT at baseline and at MID/EOT evaluation or mIBG at baseline and at MID/EOT assessment and
- receiving ch14.18/CHO and IL-2.

For the PP-SURV all patients receiving ch14.18/CHO and IL-2 were considered.

Efficacy data were analysed overall and separately for patients having received first-line therapy who had evidence or no evidence of disease at baseline, and patients with relapsed or refractory neuroblastoma (separately and together).

Results

Participant flow

Fifty-four (54) patients were treated in this compassionate use programme. About half of the patients (56%) had relapsed neuroblastoma, i.e. the patients had experienced at least one relapse after previous neuroblastoma treatment, although they reacted adequately to the most recent treatment prior to immunotherapy. Fifteen patients (28%) had a refractory disease status and 9 patients (17%) had only received first-line neuroblastoma treatment with either a complete response or with minimal residual disease (see table 16).

Recruitment

Patients were enrolled and treated between November 2009 and August 2013.

Conduct of the study

This was a retrospective data analysis which started after all patients were enrolled and treated.

Baseline data

Table 13: Status at study entry by baseline disease status - Study APN311-303

Baseline disease status		Number of Patients (N=54)
Relapsed patients (N = 30)	Disease measurable by MRI and/or CT, n (%)	7 (23.3%)
	Disease evaluable only by mIBG and/or BM histology, n (%)	16 (53.3%)
	No evidence of disease, n (%)	7 (23.3%)
Refractory patients (N = 15)	Disease measurable by MRI and/or CT, n (%)	6 (40.0%)
	Disease evaluable only by mIBG and/or BM histology, n (%)	7 (46.7%)
	No evidence of disease, n (%)	2 (13.3%)
Patients with frontline therapy only (N = 9)	Disease measurable by MRI and/or CT, n (%)	2 (22.2%)
	Disease evaluable only by mIBG and/or BM histology, n (%)	1 (11.1%)
	No evidence of disease, n (%)	6 (66.7%)

BM = bone marrow, CT = computed tomography, Max = maximum, mIBG = meta-iodobenzylguanidine, Min = minimum, MRI = magnetic resonance imaging

Table 14: Demographics and disease history by disease type - Study APN311-303

Parameter		Relapsed Patients [1] (N=30)	Refractory Patients [1] (N=15)	Frontline Patients [1] (N=9)
Time since first diagnosis to SCR visit [months]	n	30	15	9
	Mean (SD)	44.6 (27.3)	21.3 (11.7)	14.2 (4.7)
	Median	35.5	16.0	14.0
	Min, Max	21, 116	10, 55	9, 23
Age at first diagnosis, n (%)	<547 days	4 (13.3%)	6 (40.0%)	1 (11.1%)
	≥547 days	26 (86.7%)	9 (60.0%)	8 (88.9%)
INSS stage	1	1 (3.4%)	-	-
	2a	1 (3.4%)	-	-
	3	2 (6.9%)	1 (6.7%)	1 (11.1%)
	4	25 (86.2%)	14 (93.3%)	8 (88.9%)
MYCN amplification	no	17 (81.0%)	9 (69.2%)	3 (37.5%)
	yes	4 (19.0%)	4 (30.8%)	5 (62.5%)
Grade NB differentiation, n (%)	Differentiated	6 (46.2%)	8 (72.7%)	1 (50.0%)
	Undifferentiated	7 (53.8%)	3 (27.3%)	1 (50.0%)
MKI, n (%)	Low	1 (33.3%)	2 (66.7%)	-
	Intermediate	1 (33.3%)	-	-
	High	1 (33.3%)	1 (33.3%)	-
LDH [µkat/L]	n	8	6	1
	Mean (SD)	7.32 (3.72)	7.21 (6.81)	0.09 (.)
	Median	6.95	4.64	0.09
	Min, Max	2.9, 13.6	3.0, 21.0	0.1, 0.1
Serum ferritin [µg/L]	n	6	5	1
	Mean (SD)	1237.85 (1746.77)	1270.00 (712.55)	159.00 (.)
	Median	341.85	1287.0	159.00
	Min, Max	79.1, 4458.0	606.0, 2369.0	159.0, 159.0
Initial treatment, n (%)	Observation, surgery or standard chemotherapy	4 (13.3%)	-	-
	Intensive multimodality	26 (86.7%)	15 (100.0%)	9 (100.0%)

[1] Note: Missing values are not displayed.
LDH = lactate dehydrogenase, Max = maximum, Min = minimum, MRI = magnetic resonance imaging, SD = standard deviation

Six patients (11%) had INSS stage < 4 at diagnosis but suffered from disseminated or combined relapse, and therefore, are considered to have similar prognosis as stage 4 patients. Information on MYCN amplification status is missing for 12 patients (22%); it was positive in 13 patients (24%).

For 31 patients (30 with relapsed disease and 1 with refractory disease) the dates of previous relapses/progressions were documented. Most of the patients had experienced only 1 relapse/progression prior to enrolment to immunotherapy. The median time since the first relapse/progression to the start of immunotherapy was 12 months, the median time since the most recent relapse/progression was 10 months. The average time from the initial diagnosis to the first relapse/progression was 708 days (± 311) days, which would suggest a population with a relatively good survival prognosis.

First-line treatment included in most patients intensive combined chemotherapy followed by ASCT: 24 had BuMel+ASCT and 24 had CEM+ASCT. Salvage therapies of the recurrence included amongst others second-line therapy with irinotecan/temozolomide or topotecan/temozolomide, radiotherapy, and radionuclide therapy with MIBG.

Numbers analysed

Table 15: **Data sets analysed - Study APN311-303**

	Number of Patients (N=54)
Enrollment	54 (100.0%)
Safety Set (SAF)	54 (100.0%)
Full Analysis Set (FAS/ITT)	54 (100.0%)
Per Protocol Set - Response analysis (PP_RESP)	37 (68.5%)
Per Protocol Set - Survival analysis (PP_SURV)	53 (98.1%)

Outcomes and estimation

Anti-tumour response

This was evaluated in patients with evidence of disease at baseline and at least one assessment post-baseline.

Table 16: Overall response in patients with evidence of disease at baseline - Study APN311-303

			Response at end of cycle			
Category		Statistics	1 to 3 (N=35)	5 to 6 (N=26)	Best Response (N=37)	End of treatment (N=37)
Overall	Evaluable	N (%)	35 (100.0%)	26 (100.0%)	37 (100.0%)	37 (100.0%)
	CR	N (%)	5 (14.3%)	3 (11.5%)	5 (13.5%)	3 (8.1%)
	PR	N (%)	7 (20.0%)	8 (30.8%)	10 (27.0%)	9 (24.3%)
	SD/no response	N (%)	15 (42.9%)	8 (30.8%)	12 (32.4%)	8 (21.6%)
	PD	N (%)	8 (22.9%)	7 (26.9%)	10 (27.0%)	17 (45.9%)
	Not evaluable	N	-	-	-	2

At the end of treatment (i.e. 5-6 cycles or earlier in case of progressive disease), a response (CR+PR) was observed in 12/39 patients (31%) with evidence of disease at baseline while progression occurred in 17/39 patients (44%). Two patients were not evaluable. The response rate was the same regardless of baseline status (MRI/CTI or MIBG/BM) although CR (3 cases) was only reported in patients with detectable disease by mIBG and/or BM histology. However, the duration of response (overall: median 313 days; range 71 – 847) was longer in patients with disease only detectable by MIBG/BM (median of 338 days; range: 97 - 659) compared to measurable disease with MRI/CT (median of 183 days; range: 71 - 847) as could be expected.

In patients with R/R disease, the response rate was only 10/36 (28%). Amongst the 15 patients without detectable disease at baseline, one was non-evaluable (no control) and two progressed under treatment.

Overall survival and event-free survival

Table 17: Event-free survival (EFS) and overall survival (OS) rates in relapsed and refractory patients

		Relapsed patients N=29	Refractory patients N=15
EFS	1 year	45%	58%
	2 years	31%	29%
OS	1 year	90%	93%
	2 years	69%	70%

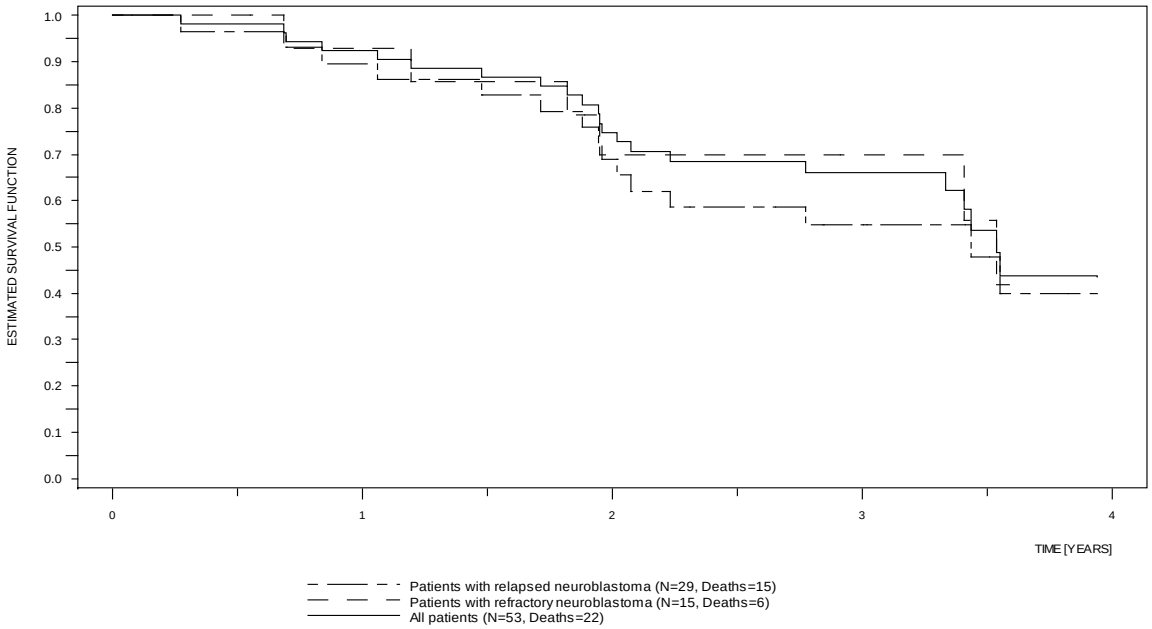


Figure 2: Overall survival Kaplan Meier curve by disease type - Study APN311-303

Ancillary analyses

Comparison to historical controls

The overall survival in study APN311-303 was compared with that of historical controls. In a retrospective study, Garaventa and colleagues investigated the outcome of neuroblastoma children with relapse or progression documented in the Italian Neuroblastoma Registry from 1979 to 2006 (Garaventa et al, 2009). These patients had received treatment according to the protocols of the Associazione Italiana di Ematologia e Oncologia Pediatrica (AIEOP), which included tumour resection, chemotherapy, radiotherapy, and myeloablation followed by ASCR, but no immunotherapy. Treatment was thus comparable to the treatment used prior to immunotherapy in patients included in study APN311-303. Since neuroblastoma treatment regimens had changed over the decades, only data from historic control patients with a date of initial diagnosis ≥1999 were included in the historic control group.

To further ensure comparability of data, historic control analyses were restricted to patients with relapsed neuroblastoma, patients who were ≥1 year of age at initial diagnosis/relapse and who presented with INSS stage 4 at initial diagnosis or nonlocal type of first relapse. The cut-off date for these patients follow-up was 22 July 2014.

Since the historic control patients had not been treated with ch14.18/CHO, an auxiliary starting point had to be defined; this was equal to the date of first relapse plus the median time between first relapse/progression and start of antibody therapy for the APN311-303 patients (~ 1 year).

The historical cohort included fewer females and more patients with stage 4 disease and MYCN amplification (i.e. less favourable prognosis) than the immunotherapy cohort but the time between diagnosis and first relapse was comparable.

Table 18: Patient characteristics - APN311-303 vs. historical controls

Patients' characteristics		Historic Control (N=29) 303	APN311-303 (N=30)
Period of diagnosis		1999-2004	2000-2010
Gender, n (%)	Male	20 (69.0%)	15 (50.0%)
	Female	9 (31.0%)	15 (50.0%)
Age (years) at initial diagnosis[1]	N	29	30
	Mean (SD)	4.3 (2.4)	4.8 (4.1)
	Median	4.0	3.5
	Min, Max	1, 13	1, 17
Age category at nitial diagnosis[1], n (%)	≤ 5 years	21 (72.4%)	22 (73.3%)
	> 5 years	8 (27.6%)	8 (26.7%)
INSS stage	1	0 (0%)	1 (3.3%)
	2A	0 (0%)	1 (3.3%)
	3	1 (3.4%)	2 (6.7%)
	4	28 (96.6%)	25 (83.3%)
	Missing	0 (0%)	1 (3.3%)
MYCN status	Amplified	8 (27.6%)	4 (13.3%)
	Not amplified	21 (72.4%)	17 (56.7%)
	Missing	0 (0%)	9 (30.0%)
Time between diagnosis and first relapse	N	29	30
	Mean (SD)	1.87 (1.00)	1.96 (0.85)
	95% CI	1.70	1.60
	Median	0.3, 5.8	1.0, 4.3
	Min, max	29	30

INSS = International Neuroblastoma Staging System; MYCN = v-myc myelocytomatosis viral related oncogene; SD = standard deviation.
[1] Age was calculated as year of initial diagnosis – year of birth

The difference in OS between the two cohorts was highly significant (p = 0.0009) in favour of APN311-303. When adding prognostic factors for OS (ie, age at diagnosis, gender, MYCN amplification, and INSS stage) in a Cox model, the difference in OS time was still statistically significant (p = 0.002).

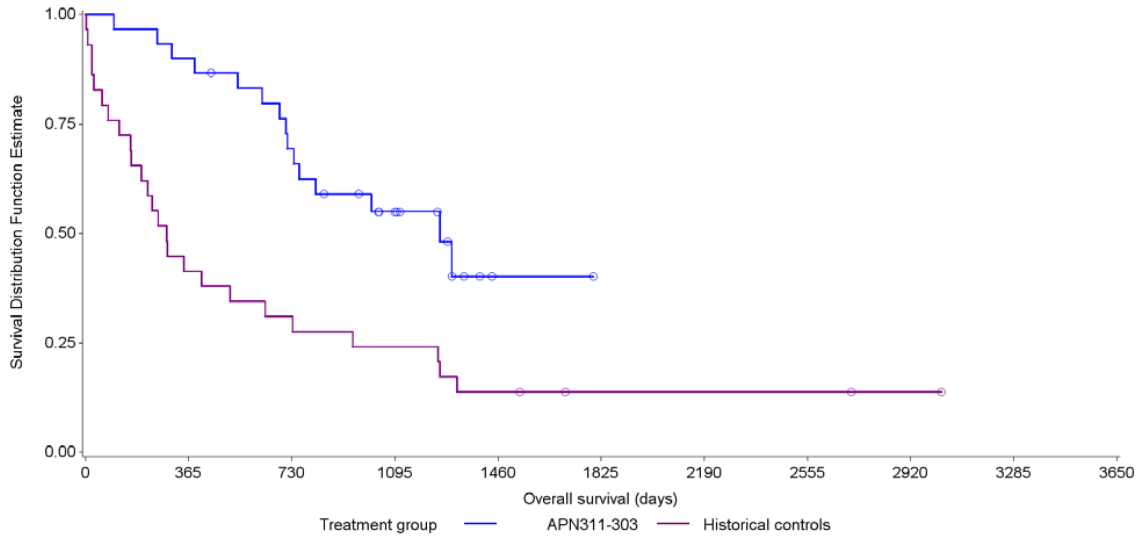


Figure 3: APN311-303 vs. historical controls: Overall survival Kaplan Meier curve

Study APN311-302 (HR-NBL-1/SIOPEN)

Safety and efficacy of ch14.18/CHO from data collected in the high risk neuroblastoma (HRNBL1) study 1.5 of SIOP-Europe (SIOPEN)

Methods

This data set came from a multinational, open-label, randomised, controlled Phase III trial in high-risk neuroblastoma patients conducted by SIOPEN to control minimal residual disease after intensive combination chemotherapy, myeloablative therapy (MAT) with stem-cell rescue, and radiation therapy. Study APN311-302 evaluated the add-on effect of IL-2 to the ch14.18/CHO + 13-cis RA regimen. Patients were randomised to ch14.18/CHO (100 mg/m^2 per cycle in 5 daily 8-hour infusions) with or without IL-2 (60 x 10^6 IU by cycle) and all received 13-cis RA.
The study included three main study phases: an induction phase, a consolidation (MAT) phase, and a maintenance phase. During the latter patients receive immunotherapy.
The study protocol was recently amended and is now recruiting additional patients treated with continuous infusions instead of short infusions of ch14.18/CHO. A total of 406 patients were enrolled in study APN311-302 by August 2013 in 10 European countries, Australia and Israel. The Applicant has provided a CSR of the maintenance phase on 385 randomised patients, for whom Case Report Forms have become available so far (last cut-off date 05 September 2016).

Study Participants

Main inclusion criteria

- Established diagnosis of neuroblastoma according to the International Neuroblastoma Staging System (INSS).
- Age below 21 years.
- High risk neuroblastoma, defined as either:
    - INSS stages 2, 3, 4 or 4s with MYCN amplification of any age below 21 years
    - INSS stage 4 without MYCN amplification aged ≥12 months at diagnosis and in patients aged 12-18 months only in the presence of segmental chromosomal alterations (SCA)
- No previous chemotherapy except for 1 cycle of etoposide and carboplatin (VP/Carbo).
- Tumour cell material available for determination of biological prognostic factors.

Main exclusion criteria

Patients aged 12-18 months at diagnosis with stage 4 neuroblastoma, no MYCN amplification, and without SCA were not eligible for R2. These patients stopped treatment after induction therapy and surgery to the primary tumour because these patients present a special disease entity considered intermediate risk only.

Treatments

13-cis-RA was administered orally, at a dose of 160 mg/m²/day over 14 days, every 4 weeks over 6 courses, and was started after completion of local irradiation, no later than Day 120 post PBSCR. 13-cis-RA was provided in 5 mg and 20 mg capsules depending on the total daily dose required.

Ch14.18/CHO was administered as an 8-hour i.v. infusion, at a dose of 20 mg/m²/day over 5 days, every 4 weeks over 5 courses. The first course started 3 weeks after the initiation of 13-cis-RA.

Patients randomized to receive ch14.18/CHO and subcutaneous IL-2 started their immunotherapy with IL-2 at Week 3. IL-2 was given according to the following administration schedule:

- During Weeks 3, 7, 11, 15 and 19 IL-2 was given at a dose of 6 MIU/m^2/day over 5 days subcutaneously (Monday-Friday).
- During Weeks 4, 8, 12, 16 and 20 IL-2 was given 2 hours after the stop of the anti-body infusion at a dose of 6 MIU/m^2/day over 5 days subcutaneously.

Objectives

Primary objective:

To test the hypothesis that the addition of subcutaneous aldesleukin (IL-2) to immunotherapy with ch14.18/CHO in addition to differentiation therapy with isotretinoin (13-cis-RA) following myeloablative therapy (MAT) and autologous stem cell rescue (ASCR), will improve 3-year event-free survival (EFS) in patients with high risk neuroblastoma.

Secondary objectives:

- To determine the tolerance of immunotherapy with ch14.18/CHO with or without subcutaneous (s.c.) IL-2 in addition to 13-cis-RA following MAT.
- To collect data on selected, validated biological features, and to determine the effect of these on EFS and overall survival (OS).

Outcomes/endpoints

Primary endpoint:

3-year EFS, calculated from the date of randomisation. Disease progression or relapse, death from any cause and second neoplasm were considered as events.

Secondary Endpoints:

- overall survival, calculated from date of randomisation to death from any cause
- cumulative incidence of relapse/progression
- cumulative incidence of death by disease progression, infection and other reason
- overall response based on the investigator's assessment

- relationship of response rates, survival, EFS, and the cumulative incidence of relapse or progressions with disease status before immunotherapy.

Sample size

The 3-year EFS in the group without IL-2, i.e. 13-cis-RA and ch14.18/CHO) was anticipated to be 55%. This trial aimed to demonstrate an improvement of 12.5% by the addition of aldesleukin (IL-2). With a sample size of 400 patients, a recruitment period of 4 years, a minimum follow up of 2 years, and two-sided α = 5%, the study had a power of 80%.

Randomisation

Patients were randomized to 13-cis-RA and ch14.18/CHO, with or without IL-2.

Blinding (masking)

The study was open label.

Statistical methods

Efficacy was analysed based on the full analysis set (FAS) and the per-protocol set (PPS). The primary endpoint, the 3-year EFS, was analysed using Kaplan-Meier methods.

Overall survival was calculated as the number of days until death from any cause using Kaplan-Meier methods. Patients lost to follow-up without event were censored at the date of their last follow-up evaluation.

For the analysis of EFS and OS, treatment groups were compared using the log-rank test adjusted by previous treatment group (BuMel or CEM).

Response evaluations were summarized descriptively at baseline, after the immunotherapy phase and at the 1-year follow-up. Additionally, a cross-tabulation of the response evaluations at baseline vs after the immunotherapy phase was prepared. The number of patients with relapses and/or progression after immunotherapy phase was summarized with frequency tables.

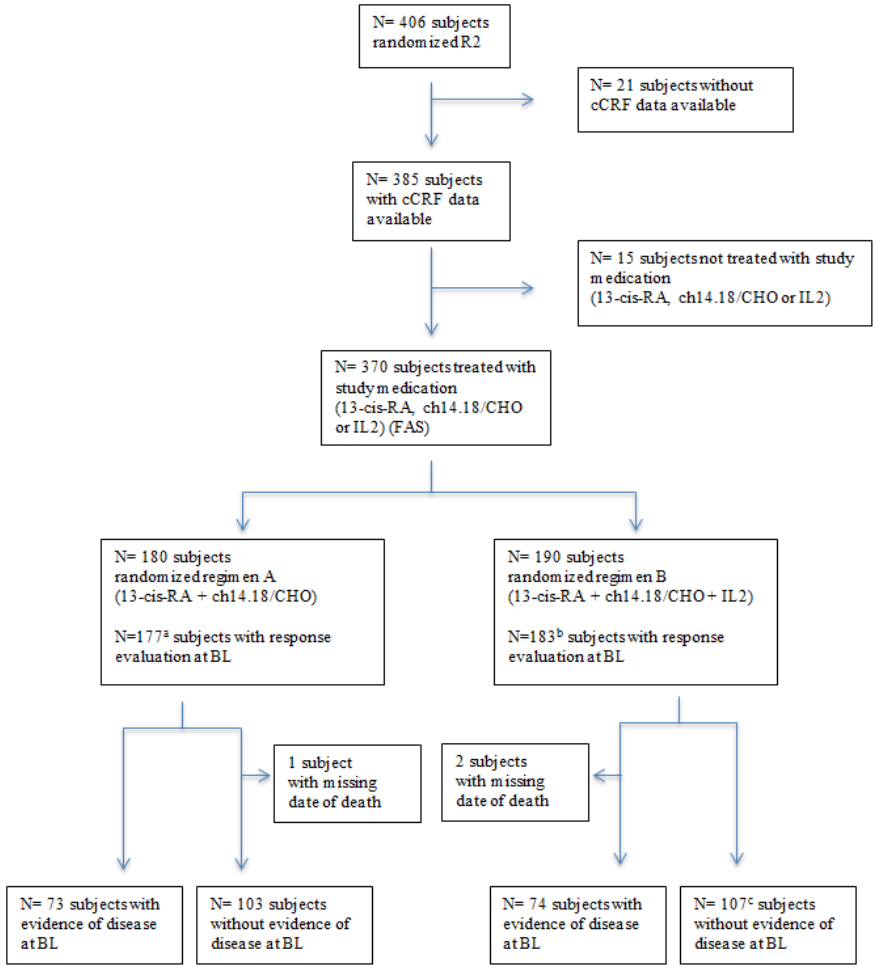
A sub-group analysis was performed for EFS and OS (FAS and PPS).

The sub-groups consisted of:

- patients without evidence of disease at baseline (CR) and
- patients with evidence of disease at baseline (VGPR, PR, MR, NR, PD).

Results

Participant flow



BL = baseline, EoD = evidence of disease

Recruitment

A total of 406 patients were enrolled in study APN311-302 by August 2013 in 10 European countries, Australia and Israel. The first patient was enrolled on R2 (immunotherapy randomisation) on 30 November 2009 and the last patient was enrolled on R2 on 12 August 2013. A confirmation CRF was available from 385 patients. Data from these patients were used in the analysis submitted as part of this application.

Baseline data

Table 19: Demography and Baseline Characteristics of Patients (FAS)

		ch14.18/CHO + 13-cis-RA (N = 180)	ch14.18/CHO + 13-cis-RA + IL-2 (N = 190)	All (N = 370)
Sex n (%)	Male	116 (64.4)	120 (63.2)	236 (63.8)
	Female	64 (35.6)	70 (36.8)	134 (36.2)
Age [years]	n	180	189	369
at randomization	Mean (SD)	3.55 (2.23)	3.79 (2.97)	3.68 (2.63)
	Median	3.00	3.00	3.00
	Min, Max	0.6, 19.0	0.7, 20.0	0.6, 20.0
Age n (%)	<1 year	5 (2.8)	5 (2.6)	10 (2.7)
	1 to 1.5 years	8 (4.4)	6 (3.2)	14 (3.8)
	>1.5 years to 5 years	123 (68.3)	132 (69.8)	255 (69.1)
	>5 years	44 (24.4)	46 (24.3)	90 (24.4)
	Missing	-	1	1
Weight [kg]	n	179	189	369
	Mean (SD)	15.33 (5.24)	16.18 (7.51)	15.77 (6.51)
	Median	14.00	14.30	14.20
	Min, Max	6.4, 55.5	7.0, 54.4	6.4, 55.5
Height [cm]	n	134	152	286
	Mean (SD)	100.46 (16.03)	102.37 (18.80)	101.47 (17.55)
	Median	100.00	98.00	99.00
	Min, Max	71.0, 179.0	70.0, 172.0	70.0, 179.0
Time from diagnosis to randomization [months]	n	180	190	370
	Mean (SD)	8.36 (1.93)	8.61 (3.23)	8.48 (2.68)
	Median	8.00	8.00	8.00
	Min, Max	6.0, 25.0	6.0, 48.0	6.0, 48.0

13-cis-RA = 13-cis retinoic acid, FAS = full analysis set, IL-2 = aldesleukin, Min = minimum, Max = maximum, N = number of patients, n = number of patients with observations, SD = standard deviation.

Table 20: Tumour Characteristics (FAS)

		Number (%) of Patients		
		ch14.18/CHO + 13-cis-RA (N = 180)	ch14.18/CHO + 13-cis-RA + IL-2 (N = 190)	All (N = 370)
Tumor stage	2 [a]	1 (0.6)	-	1 (0.3)
	3 [a]	16 (8.9)	18 (9.5)	34 (9.2)
	4	159 (88.3)	169 (88.9)	328 (88.6)
	4s [a]	4 (2.2)	3 (1.6)	7 (1.9)
MYCN amplification	yes	69 (41.6)	83 (46.4)	152 (44.1)
	no	87 (52.4)	94 (52.5)	181 (52.5)
	Not available	10 (6.0)	2 (1.1)	12 (3.5)
	Missing	14	11	25

13-cis-RA = 13-cis retinoic acid, FAS = full analysis set, IL-2 = aldesleukin, N = number of patients, [a] MYCN amplified.

Numbers analysed

A total of 23 patients were not treated as randomized: 15 patients received neither 13-cis-RA nor ch14.18/CHO and IL-2; 4 patients received only treatment with 13-cis-RA and 4 patients randomised to concomitant treatment with IL-2 were not treated with IL-2.

Table 21: Numbers analysed

	Number of Patients		
	ch14.18/CHO + 13-cis-RA	ch14.18/CHO + 13-cis-RA + IL-2	All
Randomized	187	198	385
FAS (as randomized)	180	190	370
SAF (as treated)	183	183	366
PPS (as treated)	167	166	333

13-cis-RA = 13-cis retinoic acid, FAS = full analysis set, IL-2 = aldesleukin, PPS = per-protocol set, SAF = safety set.
Source table: 14.1.1.1.

Outcomes and estimation

Primary endpoint

Table 22: 3-Year Event-Free Survival

		All Patients		Patients with Evidence of Disease at Baseline		Patients without Evidence of Disease at Baseline	
		ch14.18/CHO 13-cis-RA	ch14.18/CHO 13-cis-RA + IL-2	ch14.18/CHO 13-cis-RA	ch14.18/CHO 13-cis-RA + IL-2	ch14.18/CHO 13-cis-RA	ch14.18/CHO 13-cis-RA + IL-2
FAS	N	180[1]	190[2]	73	76[2]	104[1]	107
Events	n (%)	79 (44.1)	69 (36.5)	36 (49.3)	31 (41.3)	41 (39.8)	36 (33.6)
Censored	n (%)	100 (55.9)	120 (63.5)	37 (50.7)	44 (58.7)	62 (60.2)	71 (66.4)
EFS	KM estimate	55.4%	61.2%	45.9%	53.8%	61.7%	66.2%
Log-Rank test[3]	p-value[4]	0.3202		0.4944		0.5648	
PPS	N	167[1]	172[2]	68	71[2]	99[1]	101
Events	n (%)	71 (42.8)	63 (36.8)	32 (47.1)	29 (41.4)	39 (39.8)	34 (33.7)
Censored	n (%)	95 (57.2)	108 (63.2)	36 (52.9)	41 (58.6)	59 (60.2)	67 (66.3)
EFS	KM estimate	56.5%	60.6%	48.3%	53.2%	62.1%	66.0%
Log-Rank test[3]	p-value[4]	0.5005		0.6957		0.5831	

13-cis-RA = 13-cis retinoic acid, EFS = event-free survival, FAS = full analysis set, IL-2 = aldesleukin, KM = Kaplan-Meier, N = number of patients, n = number of patients with observations, PPS = per-protocol set.
1 1 patient [redacted] with missing date of death and without progression was excluded from the analysis.
2 1 patient [redacted] with missing date of death and without progression was excluded from the analysis.
3 Adjusted for previous treatment (busulfan and melphalan, carboplatin, etoposide and melphalan).
4 Note that the p-value refers to the overall EFS analysis and not only to the 3-year analysis.

Secondary endpoint

Table 23: EFS at 1 and 2 years

			All Patients		Patients with Evidence of Disease at Baseline		Patients without Evidence of Disease at Baseline	
			ch14.18/CHO 13-cis-RA	ch14.18/CHO 13-cis-RA + IL-2	ch14.18/CHO 13-cis-RA	ch14.18/CHO 13-cis-RA + IL-2	ch14.18/CHO 13-cis-RA	ch14.18/CHO 13-cis-RA + IL-2
FAS		N	180[1]	190[2]	73	76[2]	104[1]	107
EFS	1 yr	KM estimate	72.3%	72.3%	66.6%	72.3%	76.5%	72.6%
	2 yr	KM estimate	63.2%	66.3%	58.1%	61.6%	66.7%	69.5%
Log-Rank test[3]		p-value[4]	0.3202		0.4944		0.5648	
PPS		N	167[1]	172[2]	68	71[2]	99[1]	101
EFS	1 yr	KM estimate	72.6%	73.0%	68.6%	73.2%	75.3%	72.9%
	2 yr	KM estimate	63.4%	66.3%	61.1%	61.7%	64.9%	69.7%
Log-Rank test[3]		p-value[4]	0.5005		0.6957		0.5831	

Table 24: Overall Survival

			All Patients		Patients with Evidence of Disease at Baseline		Patients without Evidence of Disease at Baseline	
			ch14.18/CHO 13-cis-RA	ch14.18/CHO 13-cis-RA + IL-2	ch14.18/CHO 13-cis-RA	ch14.18/CHO 13-cis-RA + IL-2	ch14.18/CHO 13-cis-RA	ch14.18/CHO 13-cis-RA + IL-2
FAS		N	180[1]	190[2]	73	76[2]	104[1]	107
Events		n (%)	60 (33.5)	56 (29.8)	29 (39.7)	26 (35.1)	30 (29.1)	29 (27.1)
Censored		n (%)	119 (66.5)	132 (70.2)	44 (60.3)	48 (64.9)	73 (70.9)	78 (72.9)
OS	1 yr	KM estimate	86.3%	87.9%	82.9%	86.0%	89.2%	88.5%
	2 yr	KM estimate	76.0%	75.4%	73.1%	71.2%	78.2%	77.8%
	3 yr	KM estimate	64.1%	69.1%	54.2%	63.3%	71.0%	72.2%
Log-Rank test[3]		p-value	0.6114		0.5710		0.9571	
PPS			167[1]	172[2]	68	71[2]	99[1]	101
Events		n (%)	56 (33.7)	51 (30.0)	26 (38.2)	24 (34.8)	30 (30.6)	27 (26.7)
Censored		n (%)	110 (66.3)	119 (70.0)	42 (61.8)	45 (65.2)	68 (69.4)	74 (73.3)
OS	1 yr	KM estimate	87.1%	87.8%	84.7%	86.5%	88.7%	88.8%
	2 yr	KM estimate	76.8%	74.5%	76.1%	70.3%	77.2%	77.3%
	3 yr	KM estimate	64.3%	68.2%	56.4%	61.8%	69.7%	72.7%
Log-Rank test[3]		p-value	0.7556		0.8095		0.8187	

13-cis-RA = 13-cis retinoic acid, FAS = full analysis set, IL-2 = aldesleukin, KM = Kaplan-Meier, N = number of patients, n = number of patients with observations, OS = overall survival, PPS = per-protocol set, yr = year.

[1] 1 patient [redacted] with missing date of death were excluded from the analysis

[2] 2 patient [redacted] and [redacted] with missing date of death were excluded from the analysis.

[3] Adjusted for previous treatment (busulfan and melphalan, carboplatin, etoposide and melphalan).

Treatment compliance (Safety set)

In the IL-2 arm, a proportion of patients not only did not receive the full IL-2 treatment but had also lower exposure to ch14.18/CHO than patients in the ch14.18/CHO alone arm 39% (with IL-2) vs 78% (without IL-2) of patients received at least 50% of the planned doses of ch14.18/CHO or IL-2. Of the 183 patients receiving IL-2, 70 had an IL-2 dose reduction and 29 patients discontinued IL-2 treatment. Moreover in the IL-2 arm, there were 64 dose reductions and 34 discontinuations of ch14.18/CHO compared to 29 dose reductions and 25 discontinuations in the 183 patients of the ch14.18 alone arm. The mean cumulative ch14.18 dose was 289 mg (ch14.18 alone) vs 234 mg (with IL-2).

Comparison to historical controls

As requested by the CHMP, the Applicant has provided an appropriate historical comparison for the use of Dinutuximab beta Apeiron in the first line treatment of high risk neuroblastoma by identifying patients enrolled in the same SIOPEN protocol but in an earlier phase (R1; 2002-2010); these patients did not receive immunotherapy but only 13-cis retinoic acid (RA).

Table 25: Main patient demographics and disease characteristics

Parameter		MAT N=450	MAT and immunotherapy N=370	Total N=820
Gender, n (%)	Male	275 (61.1)	236 (63.8)	511 (62.3)
	Female	175 (38.9)	134 (36.2)	309 (37.7)
Age at initial diagnosis (years)[a]	Mean (SD)	3.24 (2.18)	2.46 (2.60)	3.34 (2.38)
	Median	2.65	2.90	2.70
	Min, Max	0.1, 16.8	0.0, 19.5	0.0, 19.5
	Missing	0	1	1
Age category (years)[a], n (%)	< 1	5 (1.1)	28 (7.6)	33 (4.0)
	≥ 1.5 - < 1.5	56 (12.4)	25 (6.8)	81 (9.9)
	> 1.5 - ≤ 5	322 (71.6)	249 (67.3)	571 (69.6)
	> 5	67 (14.9)	67 (18.1)	134 (16.3)
	Missing	0	1 (0.3)	1 (0.1)
MYCN status n (%)	Amplified	215 (47.8)	152 (41.1)	367 (44.8)
	Not amplified	204 (45.3)	181 (48.9)	385 (47.0)
	Missing	31 (6.9)	37 (10.0)	68 (8.3)
INSS stage at initial diagnosis	local	59 (13.1)	35 (9.5)	94 (11.5)
	4	391 (86.9)	328 (88.6)	719 (87.7)
	4S	0	7 (1.9)	7 (0.9)

INSS = International Neuroblastoma Staging System; MAT = myeloablative therapy; MYCN = N-myc proto-oncogene protein; SD = standard deviation.
[a] Age at initial diagnosis was calculated as (date of initial diagnosis – date of birth)/365.25. Half a year was defined as 183 days and a whole year as 365.25 days.
[b] local includes INSS stage 2, 2/3, 2A, 2B and 3

The survival analyses are shown below.

Table 26: Kaplan Meier results of overall survival

		MAT (N=450)	MAT and immunotherapy (N=367)	Total (N=817)
Deaths	n (%)	238 (52.9)	115 (31.3)	353 (43.2)
Censored[b]	n (%)	212 (47.1)	252 (68.7)	464 (56.8)
Overall survival[a] (days)	Mean[c]	2447.1	1359.4	2680.6
	Standard error	90.3	31.4	70.7
	Median	1869	-[d]	4448
	95% CI	1304-3302	-[e]	2221[f]
Overall survival rate[a] at: 1 year	KM estimate	0.83	0.89	0.86
2 years	KM estimate	0.69	0.78	0.73
3 years	KM estimate	0.59	0.71	0.64
5 years	KM estimate	0.5	0.65	0.56
Log-rank test	p-value (two-tailed)	<0.0001		

CI = confidence interval; KM = Kaplan Meier; MAT = myeloablative therapy
[a] Overall survival was defined as time from the auxiliary starting point (see Statistical Analysis Plan in Appendix 8.2 and Listing 2.1) to death from any cause.
[b] Patients without an event were censored at the date of their last follow-up evaluation.
[c] The mean survival time and its standard error were underestimated for both groups and total because the largest observation was censored and the estimation was restricted to the largest event time.
[d] Estimation of the median survival time was not possible.
[e] Estimation of the upper and lower limits was not possible.
[f] Estimation of the upper limit was not possible.

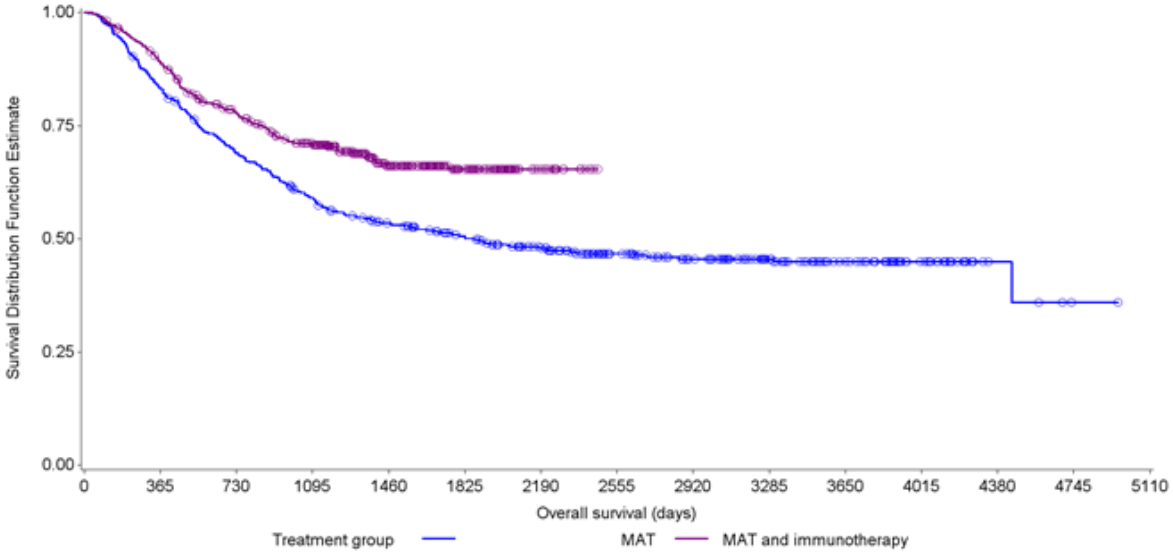


Figure 4: Overall survival Kaplan Meier curves

Summary of main studies

The following tables summarise the efficacy results from the main studies supporting the present application. It was considered more appropriate to reflect in these tables the comparison vs historical controls. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 27: Summary of efficacy for trial APN311-303 vs historical control

Title: Retrospective analysis of data collected during the administration of ch14.18/CHO continuous infusion combined with subcutaneous aldesleukin (IL-2) in patients with high risk neuroblastoma under a compassionate use program				
Study identifier	APN311-303			
Design	Retrospective data collection with comparison to historical controls from the Italian Neuroblastoma Registry (Garaventa 2009) Analysis in the subgroup of patients with relapse			
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		not applicable not applicable not applicable	
Hypothesis	Superiority			
Treatments groups	APN311		APN311 + IL-2 + 13-cis RA for 5-6 cycles (N=30)	
	Control		No treatment (N=29)	
Endpoints and definitions	Overall survival	OS	Time from the starting point to the date of death from any cause. Starting point: APN311: first day of first immunotherapy cycle Control: date of first relapse plus the median time between first relapse/progression and start of antibody therapy for the APN311-303 patients (~ 1 year)	
Database lock	December 2015: APN311 July 2014: historical control (initial diagnosis from 1999 to 2004)			
Results and Analysis				
Analysis description	**Primary Analysis**			
Analysis population and time point description	retrospective			
Descriptive statistics and estimate variability	Treatment group	APN311	Control	Log-rank test
	Number of subject	30	29	
	1-year OS KM estimate	0.90	0.41	P = 0.0009
	2-year OS KM estimate	0.69	0.31	
	3-year OS KM estimate	0.55	0.24	
	Median (days)	1254	287	
	95% CI	715 - NA	160 - 636	

Table 28: Summary of efficacy for trial APN311-302

Title: Retrospective analysis of data collected during the administration of ch14.18/CHO continuous infusion combined with subcutaneous aldesleukin (IL-2) in patients with high risk neuroblastoma under a compassionate use program				
Study identifier	APN311-302			
Design	Historical control data analysis of patients with high-risk neuroblastoma			
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		not applicable not applicable not applicable	
Hypothesis	Superiority			
Treatments groups	APN311		APN311 +/- IL-2 + 13-cis RA for 5 cycles (N=370)	
	Control		13-cis RA (N=450)	
Endpoints and definitions	Overall survival	OS	Time from the starting point to the date of death from any cause Starting point: APN311: randomisation date Control: date of ASCT + the median time between ASCT and randomisation in APN311-302	
Database lock	Not provided APN311-302: patients randomised 2009-2013 Historical controls: patients randomised 2002-2010 (SIOPEN R1)			
Results and Analysis				
Analysis description	**Primary Analysis**			
Analysis population and time point description	retrospective			
Descriptive statistics and estimate variability	Treatment group	APN311	Control	Log-rank test
	Number of subject	367	450	
	1-year OS KM estimate	0.89	0.83	P < 0.0001
	2-year OS KM estimate	0.78	0.69	
	3-year OS KM estimate	0.71	0.59	
	Median (days)	Not possible	1869	
	95% CI		1304 - 3302	
Comment	A sensitivity analysis was conducted excluding 23 historic controls who experienced relapse/progression or died within the first two months (60 days) after the auxiliary starting point; it showed very similar results			

Analysis performed across trials (pooled analyses and meta-analysis)

Anti-tumour response analyses

A pooled analysis of studies APN311-202 and -303 is presented.

Table 29: Treatment response at end of treatment – (% and 95% Confidence Interval)

Study	CR + PR [95% CI]	SD (95% CI)	PD (95% CI)	Not evaluable
APN311-202 (N = 33)	14 (42.4%) [25.48 ; 60.78]	5 (15.2%) [5.11 ; 31.90]	12 (36.4%) [20.40 ; 54.88]	2[a]
APN311-303 (N = 39)	12 (30.8%) [17.02 ; 47.57]	8 (20.5%) [9.30 ; 36.46]	17 (43.6%) [27.81 ; 60.38]	2[b]
Pooled (N = 72)	26 (36.1%) [25.12 ; 48.29]	13 (18.1%) [9.98 ; 28.89]	29 (40.3%) [28.88 ; 52.50]	4*

** non evaluable patients: no response assessments post-treatment (3); no IL-2 treatment (1)*

Due to the small sample sizes, the ORR confidence intervals of the two trials overlapped.

Survival analyses

Event-free survival was defined as time between first day of study medication administration to date of relapse/progression or death. EFS for patients without event was censored at their last date of being known to be alive or at their last visit date or at the database cut-off date, whatever came first.

Table 30: Survival rates in R/R patients – Studies APN311-202 and -303

Analysis Populations			APN311-303 N=29	APN311-202 N=19	APN311-303 N=15	APN311-202 N=25
			Relapsed patients		Refractory patients	
	EFS	1 year	44.8%	42.1%	58.2%	60.0%
		2 years	31.0%	36.8%	29.1%	55.7%
		3 years	24.1%	36.8%	29.1%	44.6%
	OS	1 year	89.7%	73.7%	92.9%	100.0%
		2 years	69.0%	42.1%	69.8%	78.3%
		3 years	54.7%	42.1%	69.8%	62.5%

Comparison to historical controls

The overall survival of the pooled relapsed patients from studies APN311-303 and -202 was compared with that of two cohorts of historical controls.

In a retrospective study, Garaventa and colleagues investigated the outcome of neuroblastoma children with relapse or progression documented in the Italian Neuroblastoma Registry from 1979 to 2006 (Garaventa et al, 2009). These patients had received treatment according to the protocols of the Associazione Italiana di Ematologia e Oncologia Pediatrica (AIEOP), which included tumour resection, chemotherapy, radiotherapy, and myeloablation followed by ASCR, but no immunotherapy. Treatment was thus comparable to the treatment used prior to immunotherapy in patients included in APN311 studies. Since neuroblastoma treatment regimens had changed over the decades, only data from historic control patients with a date of initial diagnosis ≥1999 were included in the historic control group.

Another historical cohort was extracted from patients enrolled in the SIOPEN high risk neuroblastoma study (HRNBL1) in an earlier phase (R1; 2002-2010) than study APN311-302 (R2; 2009-2013); these patients did not receive immunotherapy but only 13-cis retinoic acid (RA), and had experienced a relapse during their follow-up.

To further ensure comparability of data, historic control analyses were restricted to patients with relapsed neuroblastoma, patients who were ≥1 year of age at initial diagnosis/relapse and who presented with INSS stage 4 at initial diagnosis or nonlocal type of first relapse.

Since the historic control patients had not been treated with ch14.18/CHO, an auxiliary starting point had to be defined; this was equal to the date of first relapse plus the median time between first relapse/progression and start of antibody therapy for the APN311 patients.

The description of the patient and relapse characteristics for the pooled APN311 population and historical controls is shown below.

Table 31: Demographics and baseline characteristics

	APN311-202 + APN311-303 N=48	Historic controls Garaventa N=29	Historic controls R1 N=52
Gender, [N (%)]			
Male	25 (52.1)	20 (69.0)	33 (63.5)
Female	23 (47.9)	9 (31.0)	19 (36.5)
Age			
Mean; years (SD)	4.4 (3.6)	4.3 (2.4)	4.2 (2.4)
Median; years	4.0	4.0	4.0
Min, max; years	0, 17	1, 13	1, 15
MYCN status [N (%)]			
Amplified	5 (10.4)	8 (27.6)	14 (26.9)
Not amplified	32 (66.7)	21 (72.4)	32 (61.5)
Missing	11 (22.9)	0	6 (11.5)
INSS stage at initial diagnosis, [N (%)]			
1	2 (4.2)	0	0 (0)
2A	1 (2.1)	0	0 (0)
3	2 (4.2)	1 (3.4)	1 (1.9)
4	42 (87.5)	28 (96.6)	51 (98.1)
Missing	1 (2.1)	0	0 (0)
1p deletion, [N (%)]			
No loss or aberration	6 (12.5)	11 (37.9)	-
Deletion and imbalance	-	1 (3.4)	-
Deletion	2 (4.2)	6 (20.7)	-
Imbalance	-	6 (20.7)	-
Missing	40 (83.3)	5 (17.2)	-
Number of relapses, [N (%)]			
1	36 (75.0)	20 (69.0%)	-
2	9 (18.8)	7 (24.1%)	-
3	-	2 (6.9%)	-
5	1 (2.1)	-	-
6	1 (2.1)	-	-
8	1 (2.1)	-	-
Type of first relapse, [N (%)]			
Combined	28 (58.3)	10 (34.5%)	-
Disseminated	16 (33.3)	17 (58.6%)	-
Local	4 (8.3)	2 (6.9%)	-
Time between diagnosis and first relapse			
Mean; years (SD)	2.34 (1.94)	1.87 (1.00)	2.26 (1.42)
Median; years	1.65	1.70	1.80
Min, max; years	1.0, 11.3	0.3, 5.8	1.0, 7.4
Missing; N (%)	6	0	0
Response to treatment of last relapse prior to starting point, [N (%)]			
CR	14 (29.2%)2	7 (24.1%)	-
VGPR/PR/SD	34 (70.8)3	8 (27.6%)	-
PD	04	7 (24.1%)	-
Missing	-	7 (24.1%)	-

The survival results are shown hereafter.

Sensitivity analyses were conducted excluding historic control patients who died within the first two months (60 days) after the auxiliary starting point, i.e. patients who might not have been fit enough to receive immunotherapy at the auxiliary starting point. The results showed the same trend but they did not all reach statistical significance. In addition, given the imbalance in MYCN status and its prognostic value, adjusted analyses on this factor were requested and also supported a positive effect of immunotherapy on survival (HRs = 0.52 and 0.60).

First historical comparison (Garaventa historical controls)

Table 32: Kaplan Meier results of overall survival

		APN311-202 + APN311-303 (N=48)	Historic Controls (N=29)	Total (N=77)
Deaths	n (%)	26 (54.2)	25 (86.2)	51 (66.2)
Censored[b]	n (%)	22 (45.8)	4 (13.8)	26 (33.8)
Overall survival[a] (days)	Mean[c]	921	541.7	777.5
	Standard error	68.5	93.5	58.7
	Median	1254	318	715
	95% CI	686[d]	191-667	441-1254
Overall survival rate[a] at: 1 year	KM estimate	0.83	0.45	0.69
2 years	KM estimate	0.6	0.31	0.49
3 years	KM estimate	0.5	0.24	0.4
Log-rank test	p-value (two-tailed)	0.0031		

CI = confidence interval; KM = Kaplan Meier
[a] Overall survival was defined as time from the starting point (see Statistical Analysis Plan in Appendix 8.2 and Listing 1.1) to the date of death from any cause
[b] For patients having no event (=death), censoring was done at the last date at which the patient was known to be alive
[c] The mean survival time and its standard error were underestimated for both groups and total because the largest observation was censored and the estimation was restricted to the largest event time
[d] Estimation of the upper limit was not possible

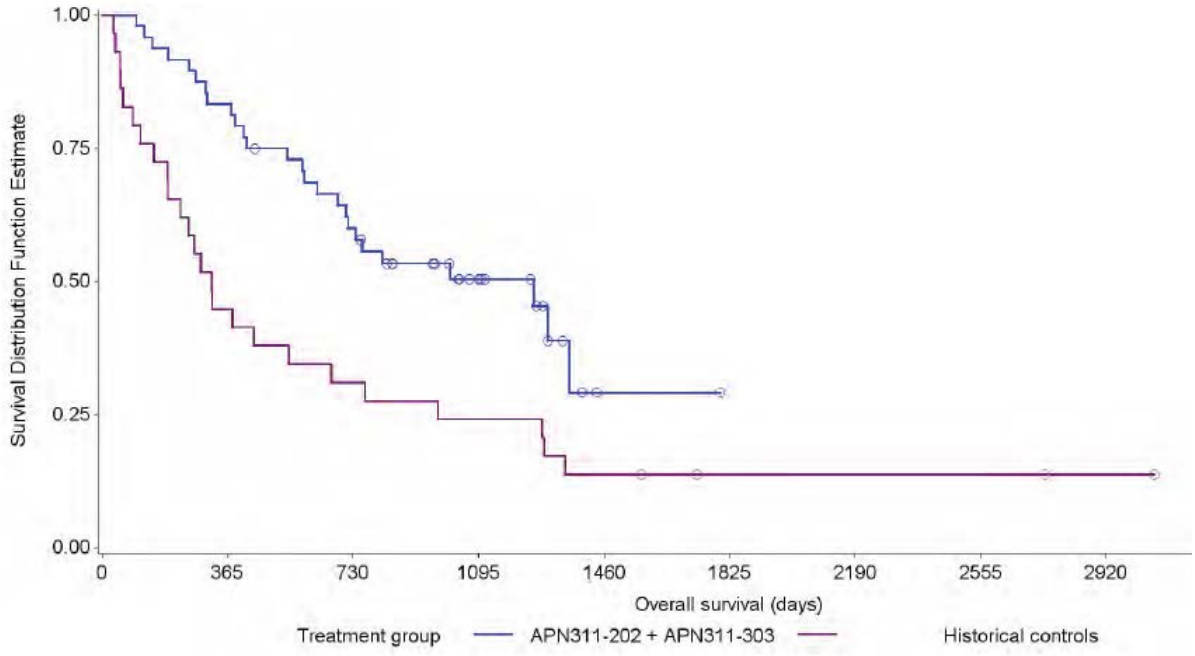


Figure 5 Kaplan-Meier Overall survival curves

Second historical comparison (R1 controls)

Table 33: Kaplan Meier results of overall survival

		Treatment group 2		
		APN311-202 + APN311-303 (N=48)	Historic Control R1 (N=52)	Total (N=100)
Deaths	n (%)	26 (54.2)	39 (75)	65 (65)
Censored[b]	n (%)	22 (45.8)	13 (25)	35 (35)
Overall survival[a] (days)	Mean[c]	921	911.4	1102.6
	Standard error	68.5	136.4	105.9
	Median	1254	630	757
	95% CI	686[d]	281-838	588-1004
Overall survival rate[a] at: 1 year	KM estimate	0.83	0.56	0.69
2 years	KM estimate	0.6	0.46	0.53
3 years	KM estimate	0.5	0.28	0.39
Log-rank test	p-value (two-tailed)	0.0302		

CI = confidence interval; KM = Kaplan Meier
[a] Overall survival was defined as time from the starting point (see Statistical Analysis Plan in Appendix 8.2 and Listing 1.1.2) to the date of death from any cause
[b] For patients having no event (=death), censoring was done at the last date at which the patient was known to be alive
[c] The mean survival time and its standard error were underestimated for both groups and total because the largest observation was censored and the estimation was restricted to the largest event time
[d] Estimation of the upper limit was not possible

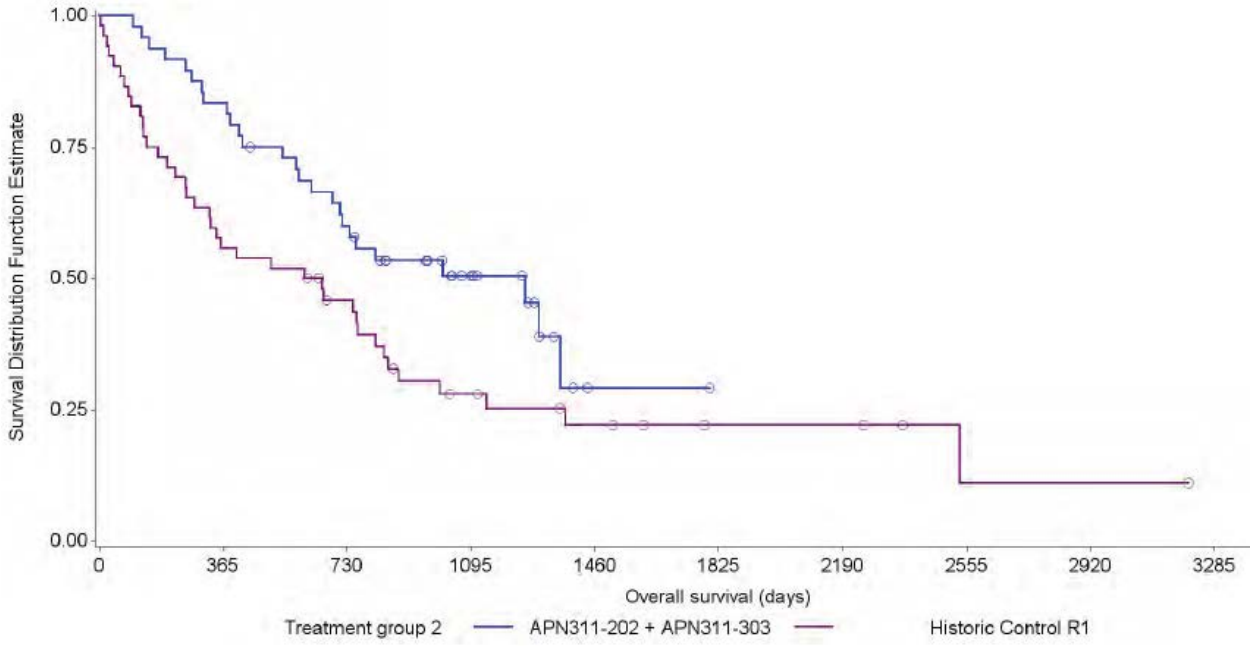


Figure 6: Kaplan-Meier Overall survival curves

Supportive studies

Study APN311-201

This is an ongoing feasibility study using of ch14.18/CHO and IL-2 after haploidentical stem cell transplantation. In this setting, anti-tumour activity is exerted by the donor-derived NK cells and IL-2 is used at a low dose from Cycle 4 to prevent graft vs. host disease. The short-infusion regimen was administered up to 9 cycles in this trial.

Overall, 35 patients with histologically confirmed neuroblastoma had experienced at least one relapse between the time of their initial diagnosis and the allogeneic haploidentical stem cell transplantation (ASCT), when they were enrolled into this study.

Their age ranged from 3 to 20 years (median = 6 years) and included more females than males (69% vs. 31%). Most patients (94%) had evidence of distant relapse, particularly metastases to the skeleton and bone marrow. Most tumours (71%) were MYCN non-amplified. Nearly all patients (97%) had received chemotherapy, approximately half of the patients (43%) had undergone surgery, half of the patients (46%) radiation therapy and approximately three-quarters of the patients (77%) had ^{131}I-radiolabelled mIBG therapy for treatment of recurrent neuroblastoma.

After haplo-HSCT, the patient status was the following: 11 patients had CR, 2 had VGPR, 17 had PR, 1 had MR (mixed response), 3 had NR (no response) and 1 had PD.

In 22 evaluable patients with evidence of disease prior to immunotherapy, 11 (50%) had a complete response at the end of treatment. However, the results of patients initially negative should also be taken into account in the analysis. Out of 28 evaluable patients, 12/28 (43%) showed an improvement, 8/28 (29%) worsened and 8/28 (29%) had no change. A more conservative estimate of the proportion of patients improved would be based on the whole patient population, i.e. 12/35 (34%).

Comparison to historical controls

Data from 20 historic control patients were selected from the CD3/CD19 depleted Haplotransplantation Study, which was performed at the University Hospital Tübingen. Patients were selected if they had relapsed neuroblastoma and had undergone Haplo-SCT from 2004.

The patients in the historic control group were diagnosed with neuroblastoma between 1999 and 2009. Patients in study APN311-201 were initially diagnosed with histologically confirmed neuroblastoma between 2005 and 2011 as reported by Tübingen.

The starting point of the analysis for the historic control patients was equal to the date of Haplo-SCT plus the median time between Haplo-SCT and start of antibody therapy for the APN311-201 patients (83 days).

Patients with relapse/progression or who died between haplo-SCT and the auxiliary starting point (i.e., date of haplo-SCT plus the median time between transplantation and start of antibody therapy for the APN311-201 patients) were excluded from the historical control group.

The immunotherapy cohort was slightly older with more females but importantly with more MYCN amplified tumours (29% vs 12%) than the controls, i.e. with less favourable prognosis.

Table 34: APN311-201 vs. historical controls: Patient characteristics

		APN311-201 N = 35	Historic Control N = 17
Period of diagnosis		2005-2011	1999-2009
Period of Haplo-SCT	Range	2010-2013	2004-2012
Gender, n (%)	Male	24 (68.6)	13 (76.5)
	Female	11 (31.4)	4 (23.5)
Age (years)[1]	N	35	17
	Mean (SD)	8.17 (3.79)	7.54 (3.86)
	Median	7.10	6.30
	Min, Max	4.0, 20.9	2.7, 19.3
Age category (years)[1], n (%)	> 1.5 - 5	4 (11.4)	3 (17.6)
	> 5	31 (88.6)	14 (82.4)
MYCN amplification, n (%)	Yes	10 (28.6)	2 (11.8)
	No	25 (71.4)	15 (88.2)
mIBG therapy	Yes	27 (77.1)	11 (64.7)
	No	8 (22.9)	6 (35.3)

MYCN = N-myc proto-oncogene protein also known as N-Myc or basic helix-loop-helix protein 37 (bHLHe37), is a protein that in humans is encoded by the MYCN gene; N = total number of patients in each group; n =number of patients in a category.
[1] Age (yrs) = (date of starting point – birth date) / 365.25. Age (yrs) was rounded to one decimal place.
Starting point for APN311-201 patients: day antibody therapy started.
Starting point for historic controls: date of Haplo-SCT plus 83 days (median time between Haplo-SCT and start of antibody therapy for APN311-201 patients).

Table 35: APN311-201 vs. historical controls: Event-free survival

		APN311-201 (N=35)	Historic Control (N=17)
Total number of patients	N	35	17
Patients with events[a]	n (%)	20 (57.1%)	13 (76.5%)
Censored[b]	n (%)	15 (42.9%)	4 (23.5%)
EFS[c] (days)	Mean[d]	716.3	174.3
	SE[d]	97.5	28.6
	Median	584	135
	95% CI	250[e]	86-337
EFS at: 1 year	KM estimate	0.62	0.24
2 years	KM estimate	0.46	0.24
3 years	KM estimate	0.40	0.24
Log-rank test	p-value	0.0570	

CI = confidence interval; EFS = event free survival; KM = Kaplan Meier; N = total number of patients in each group; n =number of patients in a category; SE = standard error

[a] Patients who had relapse/progression/death.

[b] For survivors without relapse/progression/death the patient was censored at the date of latest follow-up, at which the patient was known to be event free.

[c] Event free survival was defined as time from the starting point (see SAP, provided in Appendix 8.2, and listing 2.1) to the date of first relapse/progression or death from any cause. Date of event is taken from first relapse/ progression or death, whichever comes first.

[d] The mean survival time and its standard error were underestimated for both treatment groups and in total because the largest observation was censored and the estimation was restricted to the largest event time.

[e] Estimation of upper limit was not possible.

Increased survival (EFS and OS) was shown in the immunotherapy cohort, which was statistically significant for OS (p = 0.026). As some patients had already received ch14.18 before haplo-SCT (7 in the APN311-201 trial and 5 in the historical cohort, respectively), a sub-group analysis was also conducted excluding these patients and showed similar results.

Table 36: APN311-201 vs. historical controls: Overall survival

		APN311-201 (N=35)	Historic Control (N=17)
Total number of patients	N	35	17
Deaths	n (%)	12 (34.3)	13 (76.5)
Censored[a]	n (%)	23 (65.7)	4 (23.5)
Overall survival[b] (days)	Mean[c]	749.6	383.3
	SE[c]	54.3	46.2
	Median	-[d]	368
	95% CI	733[e]	237-498
Overall survival[b] at: 1 year	KM estimate	0.8	0.53
2 years	KM estimate	0.76	0.21
3 years	KM estimate	0.55	0.21
Log-rank test	p-value	0.0026	

CI = confidence interval; KM = Kaplan Meier; N = total number of patients in each group; n =number of patients in a category; SE = standard error.

[a] For patients having no event (=death), censoring was done at the date of the latest follow up.

[b] Overall survival was defined as time from the starting point (see SAP, provided in Appendix 8.2, and listing 2.1) to the date of death from any cause.

[c] The mean survival time and its standard error were underestimated for both treatment groups and in total because the largest observation was censored and the estimation was restricted to the largest event time.

[d] Estimation was not possible.

[e] Estimation of maximum was not possible.

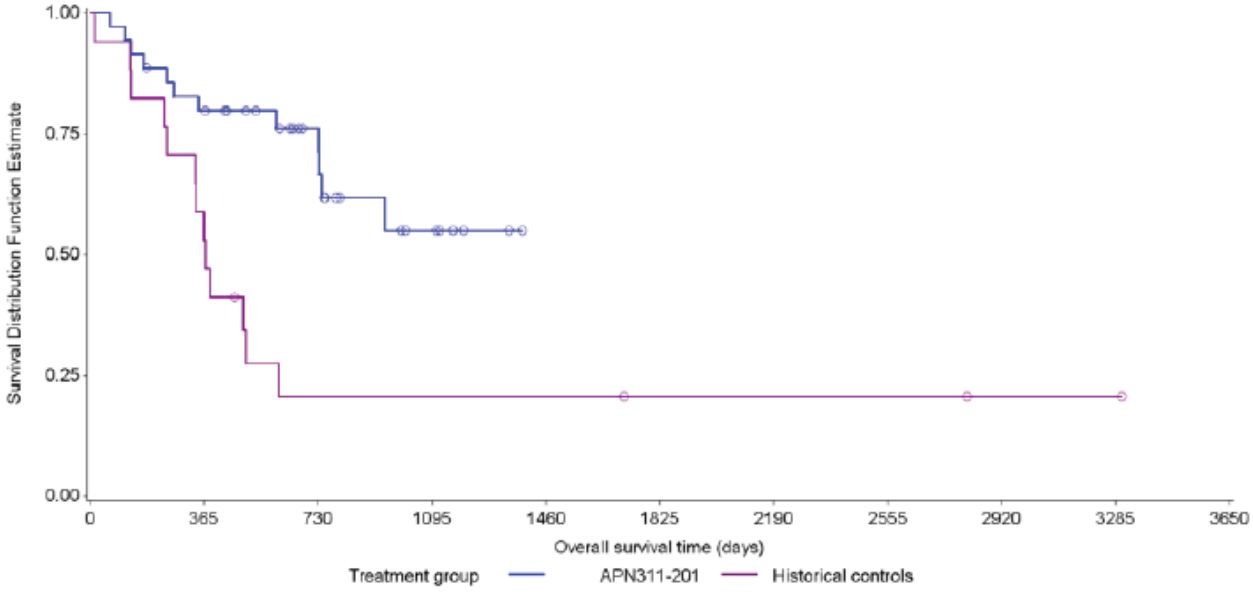


Figure 7: APN311-201 vs. historical controls: OS Kaplan Meier curve (all subjects)

Study APN311-301

This was the first phase of the SIOPEN study which compared ch14.18/CHO+13-cis RA with 13-cis RA alone. The number of patients enrolled was very small: 34 patients in the ITT set (16 patients in the 13-cis RA arm and 18 patients in the 13-cis-RA+ch14.18/CHO arm) and 25 patients in the FAS (full analysis set), which only included patients having received treatment; 11 patients in the 13-cis-RA arm and 14 patients in the 13-cis-RA+cis14.18/CHO arm.

At 2-year, overall survival was higher in the 13-cis RA alone arm (82% vs 71%) whereas afterwards, the opposite trend was reported; at 3- and 4-year, OS appeared higher in the ch14.18/CHO + 13-cis RA arm: 71% vs 64% and 48% vs 35%, respectively.

2.5.2. Discussion on clinical efficacy

Design and conduct of clinical studies

The clinical development of ch14.18/CHO has been conducted by the International Society of Paediatric Oncology Europe Neuroblastoma (SIOPEN). The clinical dossier consists of six studies of which five (APN311-101, APN311-201, APN311-202, APN311-301, APN311-303) were small (54 or less patients treated); one was actually a retrospective data collection of a compassionate use programme. It is noteworthy that none of the three single-arm investigator-sponsored trials had efficacy as a primary endpoint since these were Phase I/II trials to investigate the safety, pharmacokinetics, pharmacodynamics, and anti-tumour response. Their objectives were first to bridge to previous ch14.18 produced in other cell lines and subsequently to evaluate a new way of delivering the cycle dose, as a continuous infusion rather than daily 8-hour infusions, in an attempt to reduce the well-known pain toxicity of anti-GD2 mAbs. The last trial is a feasibility trial investigating a new experimental approach including haploidentical SCT followed by ch14.18/CHO monotherapy, with a low dose IL-2 regimen subsequently added to prevent GvHD.

The sixth study (APN311-302) enrolled in total 406 patients with high-risk neuroblastoma who were randomised to study the effect of IL-2 added to the combination of ch14.18 and 13-cis RA after first-line therapy. The first part of this study (APN311-301) enrolled 34 patients who were randomised to receive

ch14.18/CHO and RA or RA monotherapy. This trial was stopped and the data have been submitted following CHMP request; however, these are too limited to enable any conclusion.

Historical comparisons

Overall, none of the submitted studies included a comparative arm with patients who did not receive ch14.18/CHO except for the very small APN311-301 trial (25 evaluable patients). In the absence of internal controls, the assessment of the efficacy of immunotherapy was performed by comparison to historical control data.

Following CHMP request, the two studies in the relapsed setting were compared to historical controls; additional data on the relapses were collected to strengthen the evidence that these are reasonably matched with the patients treated in the APN311 studies for the most relevant baseline characteristics that are known from the literature. Two historical cohorts were identified: one from the Italian Neuroblastoma Registry (Garaventa et al, 2009) with enrolment between 1999 and 2004, the other included relapsed patients extracted from the SIOPEN high risk neuroblastoma study (HRNBL1) in an early phase (R1; 2002-2010) before immunotherapy was introduced. This whole second cohort was also used for the historical comparison in the first-line setting.

Comparative analyses were conducted between controls and individual APN311 studies as well as pooled studies for the relapsed setting. A sensitivity analysis was conducted for all these comparisons excluding from the control arm those patients who died (and progressed for the first-line setting) within 60 days of the follow-up starting point; this criterion is used as a surrogate of the performance status/clinical condition of the controls as patients eligible to receive Dinutuximab beta Apeiron/IL-2 are anticipated to be in good condition to be able to tolerate it.

Based on the literature, there are four key individual prognostic factors of survival in the relapsing patients: age at diagnosis, INSS stage of the tumour, time to first relapse and MYCN amplification status. When pooled together, the relapsed patients in the APN311 studies appear reasonably well matched to both cohorts (Italian Registry and HRNBL1-R1) except for the MYCN amplification status (fewer amplified in the APN311 studies, which would favour them against the controls). Therefore, a sensitivity analysis adjusting for this factor was requested by the CHMP.

Quality of the data and analyses

As expected for compassionate use (study APN311-303), the patient population is extremely heterogeneous, including both first-line and refractory/relapsed patients. Furthermore, due to the retrospective nature of the data collection, there was a substantial amount of missing data, especially for prognostic factors; these data could not be retrieved in spite of the Applicant's review of the data. As a consequence of this design, population (selection bias), application of treatment (no prospective treatment protocol), data recording (possible lack of standardization) and measurement of outcomes could potentially be affected.

Furthermore, a GCP inspection was carried out, which identified critical and major findings. In particular, the retrospective informed consent procedure was not appropriate. The Applicant had to conduct a re-consent procedure and to present its outcomes to the Ethics Committee, which finally agreed that all data from the compassionate use programme could be used if anonymised. Regarding the accuracy of the data, the inspectors concluded that the data could be considered reliable, which was endorsed by the CHMP.

Efficacy endpoints

In study APN311-202, the primary endpoint was the number of CD16/CD56 positive activated NK cells but this endpoint is not acceptable as efficacy endpoint because the correlation of this PD parameter with

clinical outcomes is unclear. In fact, no correlation with tumour response could be found by the Applicant, rather a trend towards longer OS in patients with high expression of CD16/CD56+ activated NK cells.

Tumour response, especially the occurrence of complete response (CR) may indicate anti-tumour activity of the treatment regimen but does not always relate to clinical benefit e.g. prolonged survival. Moreover, ch14.18/CHO was combined with 13-cis-RA and IL-2 in most studies. Thus, it is not clear whether the reported responses are exclusively the result of ch14.18/CHO treatment or of the other components of the applied regimen. Responses, including complete response, have also been reported for 13-cis-RA therapy. Given the fixed number of treatment cycles 5 (or 6), which was applied to the vast majority of patients except in case of early disease progression, the best response is not necessarily the most clinically relevant outcome as it takes into account responses of very short duration. Evaluation at the end of treatment, which was usually performed 6-8 months after treatment initiation (except in case of early disease progression), provides a valuable indication of the disease outcome after such a period of time.

Event free survival is generally considered as an important efficacy endpoint, and this might even be used as primary endpoint when there is a good correlation between EFS and OS and a long median OS is anticipated. However, this endpoint is complicated by several methodological issues, including the exact definition of events and methods of disease status determination. The time points at which disease status was assessed during treatment and follow-up were not strictly pre-specified; consequently, it is not clear whether the exact time of disease progression was determined.

As efficacy data in terms of EFS and OS were not planned in the protocols, these have been collected retrospectively and presented by the Applicant. The value of EFS results is considered limited due to the methodological issues previously mentioned and OS is considered the most important efficacy endpoint. As the proportion of censored subjects is high after 2 years, only outcomes during the first 2 years after treatment are currently considered reliable in the R/R setting.

Efficacy data and additional analyses

No proper dose finding trial was conducted. The first trial investigated only three treatment cycles in a very small number of heterogeneous patients and without defined timing of efficacy assessments. The second trial tested only one dose.

Refractory/Relapsed setting

For the total of both studies APN311-303 and 202, the overall response (CR+PR) at the end of treatment was 26/72 (36%; 95%CI [25%, 48%]) in patients with detectable disease, out of whom 9 (13%) had a CR, while disease had progressed in 29/72 patients (40%; 95%CI [19%, 52%]).

Survival data (EFS/OS) showed comparable results in both studies at one year. At 2-year, inconsistent results were observed across the studies: EFS was lower but OS was higher in study APN311-303 compared with the outcomes in study APN311-202; the 2-year OS rate was 75% in study -303 and 63% in study -202 (for the total population). Three-year results are not reliable due to high level of censoring.

EFS/OS rates tended to be higher in refractory disease compared to relapses. This finding is in line with a retrospective study in patients treated with 131I-MIBG but in contrast with older data from the Italian registry.

EFS/OS rates were higher in patients with no evidence of disease compared to those with evidence of disease, as would be expected, in one of the studies (APN311-303), while no difference was observed in the other study (APN311-202). There is no explanation for the latter finding although the sample size is small.

Historical control comparisons were conducted for relapsed patients.

A sensitivity analysis adjusting for MYCN amplification status supported a favourable effect of ch14.18/CHO on survival. Another sensitivity analysis was conducted excluding from the control cohorts, the patients who died within 60 days of the follow-up starting point; this criterion is used as a surrogate of the performance status/clinical condition of the controls as patients eligible to receive Dinutuximab beta Apeiron/IL-2 are anticipated to be in good condition to be able to tolerate it.

Due to the small samples of treated patients and controls, these historical comparisons lack power but the point estimate and confidence interval do indicate survival benefit. In conclusion, the magnitude of the effect may differ but the trends are consistent and the replication of the results provides reassurance about the benefit even if its extent cannot be accurately quantified.

The results of the feasibility study on haploidentical SCT followed by ch14.18/CHO monotherapy (and IL-2 to prevent GvHD) are promising. In this study, the 1-year rates were higher for EFS (62%) but lower for OS (80%) compared to the two other trials, which is likely due to the different population and complications of the treatment modalities.

The additive effect of IL-2 is not known for patients with relapsed/refractory disease as the effect of omitting IL-2 in the treatment has not been studied in this population. This will be addressed in the context of a specific obligation (see Annex II).

First-line setting

Study APN311-302 compared ch14.18/CHO with or without IL-2 in the first-line setting (patients with high-risk neuroblastoma having previously received induction chemotherapy and achieved at least a partial response, followed by myeloablative therapy and stem cell transplantation. It was used to justify ch14.18/CHO monotherapy as the survival results suggest limited added value of IL-2.

However in this study, treatment compliance to IL-2 was poor; moreover, compliance with ch14.18/CHO was worse in the combination arm (ch14.18/CHO + IL-2) than in the ch14.18/CHO monotherapy arm. It is noteworthy that compliance in study APN311-302 is relativity poor when compared to compliance in the R/R setting, which makes the interpretation of these results and their extrapolation to other treatment settings difficult.

The 3-year EFS (primary endpoint) showed rates of 55% without IL-2 and 61% with IL-2 while the 3-year OS rates were 64% and 69%, respectively. It is agreed that the differences in EFS and OS were small between the treatment arms although the rates in the +IL-2 arm tended to be (numerically) higher than the rates in the –IL-2 arm, in particular at the later time points. Nevertheless, it is agreed that the data indicate that there is no or only limited added benefit of the addition of IL-2 to the treatment with ch14.18/CHO in the first-line setting without residual disease (at 3 years, EFS: 62% vs 66% and OS: 71% vs 72%, respectively). Whether this is also true for patients with residual disease is doubtful based on the APN311-302 study results. In a post-hoc analysis, a numerical difference (8-9%) in 3-year survival rates was reported for patients with evidence of disease before immunotherapy (EFS: 46% vs 54% and OS: 54% vs 63%, respectively) in contrast to those without evidence of disease, i.e. with complete response to previous therapies). These outcomes were in line with other data: a higher relapse rate in the arm receiving only ch14.18: 41% (74/180) vs 35% (67/190), with more relapses in multiple sites: 35% (38/109) vs 25% (26/104); in patients with disease before immunotherapy, a lower tumour response rate in the arm receiving only ch14.18: 33% (22/66) vs 46% (32/70) (missing data excluded).

A comparison to an historical control group obtained from study HRNBL1-R1 was performed using 450 patients diagnosed with neuroblastoma between 2002 and 2010. Given the relatively high number of patients it is expected that these patients are representative for the patients with high risk neuroblastoma seen in clinical practice during this period. Furthermore, control and study groups seemed to be

reasonably balanced for the main prognostic factors like MYCN status, INSS stage at initial diagnosis and age of diagnosis.

This comparison showed that the percentage of patients that were still alive after 3 year of follow up was 12 % (59% vs 71%, for MAT and MAT+ immunotherapy respectively) higher after APN311 treatment than for patients who did not receive immunotherapy. Such difference is considered clinically relevant. With the sensitivity analysis excluding patients who had relapse/progression or death within the first two months (60 days) after the auxiliary starting point, still 9% more patients treated with immunotherapy than with 13-cis-RA alone, were alive after 3 years of follow up and after 5 years of follow-up the survival rate was 12% higher for the immunotherapy group.

IL-2 combination

Overall, the data indicated that the added value of IL-2 to the treatment effect of ch14.18/CHO is doubtful in the overall population of study APN311-302, while a significant contribution to the safety profile of treatment is noted. Thus, omitting IL-2 from the treatment schedule for some of these patients could be considered.

Whether this is also true for relapsed/refractory patients is uncertain as the effect of omitting IL-2 in the treatment has not been studied in this population. One should be extremely cautious with extrapolation of the findings of the APN311-302 study to the relapsed/refractory setting, i.e. a patient population with higher risk, especially given the results observed in patients with residual disease.

Based on these data, it can be accepted that in the first-line setting and if there is no residual disease, IL-2 has no benefit. In all other circumstances, first-line with residual disease (PR) and R/R setting, it is not currently possible to dismiss an add-on benefit of IL-2, and therefore, the combination of Dinutuximab beta Apeiron to IL-2 is recommended until new data become available.

Additional efficacy data needed in the context of a MA under exceptional circumstances

Taking into account the totality of the available data, the CHMP was of the view that the data set on the clinical efficacy of Dinutuximab beta Apeiron under normal conditions of use could not be considered comprehensive due to the absence of any randomised head-to-head comparison with a placebo or active comparator in any clinical setting. The use of historical controls has major weaknesses especially in this case given the scarcity of the information available to support that patients treated with Dinutuximab beta Apeiron and controls were matched.

However, it is not considered feasible to generate a comprehensive data set due to ethical considerations preventing the conduct of a randomised placebo-controlled trial. At the time of this report, dinutuximab is standard of care in the treatment of high risk neuroblastoma, whereby neither physicians nor patients would be prepared to participate in a placebo-controlled trial.

In addition, a comparative trial against the previously authorised dinutuximab product Unituxin (the only one potential active comparator) is not expected to be feasible given the withdrawal of the marketing authorisation of Unituxin.

Therefore, the current situation prevents the generation of new controlled data to confirm the outcomes of studies APN311-202, -303, and -302.

The CHMP was therefore of the view that a marketing authorisation under exceptional circumstances should be granted subject to a number of obligations in line with a programme of ongoing studies and analyses to be further proposed by the Applicant. Within this programme, additional PK/PD and immunogenicity data should be generated, especially about the impact of HACAs on efficacy, since the product being highly toxic, the benefit of completing all treatment cycles after developing HACAs should

be evaluated. Furthermore, updated survival data should be generated as they are currently immature. Finally, one Phase II study is further investigating whether the addition of IL-2 to Dinutuximab beta Apeiron improves anti-tumour response and survival in the R/R setting.

In addition, a drug exposure registry should be implemented. While this study is mainly intended for the generation of further safety data, data on short-term anti-tumour response and long-term survival should also be generated. These specific obligations will be reviewed annually and continuation of the authorisation shall be linked to these annual re-assessments.

2.5.3. Conclusions on the clinical efficacy

R/R setting

In a pooled analysis of the two APN311 studies, a response rate of 36% was achieved at the end of treatment, with 13% of complete response. This result looks promising and strongly suggests anti-tumour activity, although a sustained effect of previous therapies of the relapse cannot be completely ruled out.

All historical control comparisons showed that immunotherapy improves survival in relapsed disease. The magnitude of the effect may differ but the trends are consistent and the replication of the results provides reassurance about the benefit even if its extent cannot be accurately quantified.

While none of these outcomes on its own would be sufficient to establish efficacy, it is considered that, by the combination of the provided evidence (early tumour response to treatment and long-term survival data), the efficacy of ch14.18/CHO is demonstrated, even though the exact effect size is not fully known.

First-line setting

The use of ch14.18/CHO in patients with high-risk neuroblastoma who have previously received induction chemotherapy and achieved at least a partial response, followed by myeloblative therapy and stem cell transplantation, is supported by the results of study APN311-302. A comparison with a historical control group showed that the percentage of patients that were still alive after 3 years of follow-up was 12% higher after ch14.18/CHO treatment (with or without IL-2) than for patients who did not receive immunotherapy, a difference that is considered clinically relevant.

Therefore, it is considered that in this population also, immunotherapy increases long term survival.

However, the CHMP considered that the available data set on the clinical efficacy was not comprehensive and that the following measures are necessary to generate additional efficacy data in the context of a marketing authorisation under exceptional circumstances:

- The MAH should provide PK/PD and immunogenicity data, including the effect of HACAs on efficacy, using data collected from several ongoing trials (e.g. APN311-202v1-2 and -304)
- The MAH should provide survival update from studies APN311 -202 and -302.
- The MAH should provide the results of the Phase II study comparing Dinutuximab beta Apeiron monotherapy vs combination with IL-2 in the R/R setting.
- The MAH should follow up patients in the SAFARY drug registry and provide annual results.

2.6. Clinical safety

Patient exposure

The overall safety database currently includes **514 patients** that received ch14.18/CHO: 98 patients as a continuous infusion over 10 days and 416 patients as short 8h-infusions. Overall, 281 patients received it in combination with IL-2 and 207 patients received antibody treatment without IL-2; 26 patients received both single-agent and combination cycles, but with a low IL-2 dose. In addition, 13-cis retinoic was also administered to patients in studies APN311-202, -302 and -303.

The dosing regimens of the three products are shown in the next table.

It should be emphasized that the method of AE collection varied across studies. In particular, in the largest study (APN311-302) only SAEs were fully reported while for other AEs, a pre-defined list of 31 specific toxicities was used. A similar system was used in study APN311-201 where safety was recorded by toxicity forms and AEs that were not already included in the form were to be reported as TEAEs. All adverse events that were listed as toxicity were classified as at least possibly related to treatment. As the main study is a retrospective analysis of patients enrolled into the compassionate use programme, safety was also retrospectively assessed from adverse events recorded from time of enrolment until 30 days after last study treatment.

Therefore, except for SAEs and some ADRs of specific interest, the evaluation will mainly focus on the 98 patients of the continuous infusion studies due to the specific toxicities of the transplantation setting, the restricted number of cycles administered in the PK bridging study.

Table 37: Summary of ch14.18/CHO, IL-2 and 13-cis-RA administration in APN311 studies

Study		Ch14.18 - i.v.	IL-2 – s.c.	13-cis-RA – p.o.	Cycles
APN311-303 (Compassionate Use)	Patient 1-4	Days 1-11 (10 days) Continuous (24h) 5-10 mg/m^2/day [a]	Days 1-5 (5 days) 6 x 10^6 IU/m^2/day	Days 15-28 (14 days) 80 mg/m^2/day b.i.d.	3-6 cycles, 1 cycle = 28-35 days
	Patient 5-54	Days 8-18 (10 days) Continuous (24h) 10 mg/m^2/day [a]	Days 1-5 & 8-12 (2 x 5 days) 6 x 10^6 IU/m^2/day	Days 19-32 (14 days) 80 mg/m^2/day b.i.d	5/6 cycles, 1 cycle = 35 days
APN311-101		Days 1-5 (5 days) Short-term (8h) Dose escal.: 10, 20, 30 mg/m^2/day	NA	NA	3 cycles 1 cycle = 4 weeks (28 days)
APN311-201	Cycle 1-3	Days 1-5 (5 days) Short-term (8h) 20 mg/m^2/day	NA	NA	9 cycles 1 cycle = 4 weeks (28 days)
	Cycle 4-9		Day 6, 8, 10 (3 days) 1 x 10^6 IU/m^2/day		
APN311-202		Days 8-18 (10 days) Continuous (24h) 10 mg/m^2/day [a]	Days 1-5 & 8-12 (2 x 5 days) 6 x 10^6 IU/m^2/day	Days 19-32 (14 days) 80 mg/m^2/day b.i.d.	5 cycles, 1 cycle = 35 days
APN311-302	- IL2	Days 8-12 (5 days) Short-term (8h) 20 mg/m^2/day [b]	NA	14 days 80 mg/m^2/day b.i.d. Weeks: 1, 2, 5, 6, 9, 10, 13, 14, 17, 18, 21, 22	5 cycles ch14.18 & IL-2, 6 cycles RA, start with RA 1 cycle = 4 weeks (28 days)
	+ IL2		Days 1-5 & 8-12 (2 x 5 days) 6 x 10^6 IU/m^2/day 2 h after stop of ch14.18 infusion		

In the main (continuous infusion) studies 68/98 (69%) patients completed the planned 5-6 cycles. The main reason for treatment discontinuation was progressive disease and the number of patients having stopped treatment due to an ADR, as only reason or associated with PD, was 6 (6%).

Table 38: Summary of completed cycles – Studies APN311-101, -201, -202, -303

Completed cycles	Number (%) of Patients			
	APN311-303 (N=54)	APN311-202 (N=44)	APN311-101 (N=15)	APN311-201 (N=35)
0	-	-	-	1 (2.9%)
1	1 (1.9%)	3 (6.8%)	1 (6.7%)	6 (17.1%)
2	2 (3.7%)	7 (15.9%)	6 (40.0%)	1 (2.9%)
3	9 (16.7%)	3 (6.8%)	8 (53.3%)	2 (5.7%)
4	3 (5.6%)	2 (4.5%)	NA	4 (11.4%)
5	29 (53.7%)	29 (65.9%)	NA	2 (5.7%)
6	10 (18.5%)	NA	NA	4 (11.4%)
7	NA	NA	NA	2 (5.7%)
8	NA	NA	NA	4 (11.4%)
9	NA	NA	NA	9 (25.7%)

NA: not applicable

Table 39: Summary of completed cycles (cont'd) - Study APN311-302

Cycle	Number (%) of Patients		
	ch14.18/CHO + 13-cis-RA (N = 183)	ch14.18/CHO + 13-cis-RA + IL-2 (N = 183)	All (N = 366)
1	183 (100.0)	183 (100.0)	366 (100.0)
2	180 (98.4)	179 (97.8)	359 (98.1)
3	175 (95.6)	176 (96.2)	351 (95.9)
4	170 (92.9)	167 (91.3)	337 (92.1)
5	169 (92.3)	161 (88.0)	330 (90.2)
6	163 (89.1)	153 (83.6)	316 (86.3)
Treatment completed [1]	112 (78.3)	61 (39.4)	173 (58.1)
Treatment not completed	31 (21.7)	94 (60.6)	125 (41.9)
Unknown [2]	40 -	28 -	68 -

13-cis-RA = 13-cis retinoic acid, IL-2 = aldesleukin, N = number of patients, SAF = safety set.

[1] Treatment was considered completed if at least 50% of the planned doses of ch14.18/CHO and IL-2 were administered in 1 to 5 cycles.

[2] For patients with missing information concerning weight and/or height the BSA and therefore the planned dosages could not be calculated, treatment completion was judged as unknown.

Adverse events

The overall summary of TEAEs is presented hereafter.

Table 40: Overall summary of treatment-emergent adverse events (TEAEs)

Patients with	APN311-303	APN311-202	APN311-101	APN311-201
	N (%) patients (N=54)	N (%) patients (N=44)	N (%) patients (N=15)	N (%) patients (N=35)
Any AE	**54 (100.0%)**	**44 (100.0%)**	**15 (100.0%)**	**34 (97.1%)**
Any AE possibly related to study drug [a]	54 (100.0%)	44 (100.0%)	15 (100.0%)	29 (82.9%)
Any AE possibly related to IL-2	54 (100.0%)	44 (100.0%)	NA	ND
Any AE possibly related to ch14.18/CHO	54 (100.0%)	44 (100.0%)	15 (100.0%)	ND
Any AE possibly related to 13-cis-RA	27 (50.0%)	ND	NA	NA
Any serious AE	**12 (22.2%)**	**26 (59.1%)**	**2 (13.3%)**	**25 (71.4%)**
Any SAE possibly related to study drug [a]	6 (11.1%)	22 (50.0%)	1 (6.7%)	13 (37.1%)
Any SAE possibly related to IL-2	4 (7.4%)	18 (40.9%)	NA	ND
Any SAE possibly related to ch14.18	6 (11.1%)	20 (45.5%)	1 (6.7%)	ND
Any SAE possibly related to 13-cis-RA	-	ND	NA	NA
Any AE leading to discontinuation of study drugs[b]	**5 (9.3%)**	**10 (22.7%)**	**-**	**4 (11.4%)**
Maximal NCI CTCAE Grade				
Grade 1 (mild)	-	-	-	9 (25.7%)
Grade 2 (moderate)	3 (5.6%)	2 (4.5%)	-	7 (20.0%)
Grade 3 (severe)	32 (59.3%)	20 (45.5%)	13 (86.7%)	15 (42.9%)
Grade 4 (life threatening/disabling)	19 (35.2%)	22 (50.0%)	2 (13.3%)	2 (5.7%)
Grade 5 (death)	-	-	-	1 (2.9%)
Any AE leading to death	-	**1 (2.3%)**	-	**1 (2.9%)**
Deaths *	22 (40.7%)	20 (45.5%)	10 (66.7%)	12 (34.3%)

* All documented deaths, including deaths during follow-up period
a Depending on the study design refers to ch14.18/CHO only or to the combination of ch14.18/CHO and IL-2 and 13-cis-RA. For APN311-202 refers to ch14.18/CHO and IL-2 treatment.
b Permanent or temporary discontinuation in studies APN311-303 and -202, permanent discontinuation in study APN311 201.
AE=adverse event, N=number of subjects, NA = not applicable, NCI CTC=National Cancer Institute Common Toxicity Criteria, ND = not determined.
Possibly related AEs: AEs with relationship coded as 'Possible', Probable', 'Definite' or with missing relationship

While the number of TEAEs decreased significantly over treatment cycles, the proportion of patients with any TEAE remained high throughout the study (data not shown).

The most frequent TEAEs are presented for cycle 1 in studies APN311-202 and -303 below. General disorders, namely pyrexia, were the most frequently documented TEAEs, followed by investigation-related TEAEs in study APN311-202 and by gastrointestinal disorders in study APN311-303.

In study APN311-202, the most frequent PTs were pyrexia, pain, vomiting, cough, increased weight, and laboratory abnormalities (increased alanine aminotransferase and gammaglutamyltransferase, anaemia, leukopenia, thrombopenia).

In study APN311-303, the most frequent PTs included also skin reactions, constipation, tachycardia, hypotension, and capillary leak syndrome (only during the first cycle).

Table 41: Most frequent TEAEs reported in cycle 1 (studies APN311-303 and APN311-202)

Cycle	SYSTEM ORGAN CLASS Preferred term	N (%) patients	
		APN311-202 (N=44)	APN311-303 (N=54)
Cycle 1	Patients with events	44 (100.0%)	54 (100.0%)
	GENERAL DISORDERS	**44 (100.0%)**	**54 (100.0%)**
	Pyrexia	44 (100.0%)	36 (66.7%)
	Pain	23 (52.3%)	20 (37.0%)
	Fatigue	12 (27.3%)	18 (33.3%)
	GASTROINTESTINAL DISORDERS	**33 (75.0%)**	**50 (92.6%)**
	INVESTIGATIONS	**36 (81.8%)**	**32 (59.3%)**
	RESPIRATORY DISORDERS	**32 (72.7%)**	**37 (68.5%)**
	Cough	20 (45.5%)	17 (31.5%)
	Hypoxia	14 (31.8%)	15 (27.8%)
	BLOOD AND LYMPHATIC SYSTEM DISORDERS	**28 (63.6%)**	**32 (59.3%)**
	METABOLISM AND NUTRITION DISORDERS	**19 (43.2%)**	**12 (22.2%)**
	SKIN DISORDERS	**26 (59.1%)**	**40 (74.1%)**
	Pruritus	14 (31.8%)	31 (57.4%)
	VASCULAR DISORDERS	**22 (50.0%)**	**45 (83.3%)**
	Hypotension	14 (31.8%)	18 (33.3%)
	Capillary leak syndrome	12 (27.3%)	39 (72.2%)
	MUSCULOSKELETAL DISORDERS	**4 (9.1%)**	**35 (64.8%)**
	INFECTIONS AND INFESTATIONS	**14 (31.8%)**	**15 (27.8%)**
	NERVOUS SYSTEM DISORDERS	**9 (20.5%)**	**19 (35.2%)**
	CARDIAC DISORDERS	**7 (15.9)**	**23 (42.6%)**
	RENAL AND URINARY DISORDERS	**9 (20.5%)**	**14 (25.9%)**

Severe (grade 3 & 4) events

The most frequent severe (grade 3/4) AEs were pain, abnormal haematological and liver function tests, pyrexia, infections, allergic reactions and capillary leak syndrome.

Table 42: Summary of grade 3 and 4 TEAEs occurring in > 1 subject in any study APN311-202 & -303

SYSTEM ORGAN CLASS / PT	APN311-303		APN311-202	
Maximum toxicity grade	3	4	3	4
GASTROINTESTINAL DISORDERS				
Abdominal pain upper	6 (11.1%)	3 (5.6%)	-	-
Vomiting	5 (9.3%)	-	3 (6.8%)	-
Diarrhea	3 (5.6%)	1 (1.9%)	1 (2.3%)	-
Nausea	2 (3.7%)	-	3 (6.8%)	-
Abdominal pain	3 (5.6%)	-	2 (4.5%)	-
Abdominal distension	2 (3.7%)	-	-	-
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS				
Pyrexia	5 (9.3%)	-	15 (34.1%)	-
Pain	14 (25.9%)	1 (1.9%)	5 (11.4%)	2 (4.5%)
Inflammation	2 (3.7%)	-	-	-
SKIN AND SUBCUTANEOUS TISSUE DISORDERS				
Pruritus	8 (14.8%)	-	-	-
Urticaria	5 (9.3%)	-	-	-
Rash	3 (5.6%)	-	1 (2.3%)	-
VASCULAR DISORDERS				
Hypotension	2 (3.7%)	-	9 (20.5%)	-
Capillary leak syndrome	7 (13.0%)	-	2 (4.5%)	1 (2.3%)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS				
Pain in extremity	11 (20.4%)	4 (7.4%)	-	-
Back pain	6 (11.1%)	-	-	-
Arthralgia	4 (7.4%)	-	-	-
Bone pain	2 (3.7%)	-	-	-
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS				
Cough	9 (16.7%)	-	1 (2.3%)	-
Hypoxia	3 (5.6%)	-	8 (18.2%)	-
Bronchospasm	3 (5.6%)	-	1 (2.3%)	-
Acute respiratory distress syndrome	-	-	1 (2.3%)	1 (2.3%)
Pleural effusion	2 (3.7%)	-	-	-
BLOOD AND LYMPHATIC SYSTEM DISORDERS				
Anemia	18 (33.3%)	2 (3.7%)	18 (40.9%)	-
Neutropaenia	20 (37.0%)	6 (11.1%)	6 (13.6%)	4 (9.1%)
Febrile neutropaenia	-	-	6 (13.6%)	-
Thrombocytopaenia	9 (16.7%)	7 (13%)	2 (4.5%)	2 (4.5%)
Leukopaenia	7 (13.0%)	-	-	-
CARDIAC DISORDERS				
Tachycardia	2 (3.7%)	-	-	-
Pericardial effusion	2 (3.7%)	-	-	-
INFECTIONS AND INFESTATIONS				
Device related infection	1 (1.9%)	-	11 (25.0%)	-
Sepsis	-	-	-	5 (11.4%)
Urinary tract infection	1 (1.9%)	-	1 (2.3%)	-
Skin infection	-	-	2 (4.5%)	-
INVESTIGATIONS				
Increased ALT	8 (14.8%)	2 (3.7%)	13 (29.5%)	2 (4.5%)
Increased GGT	7 (13.0%)	-	14 (31.8%)	1 (2.3%)
Decreased neutrophil count	-	-	17 (38.6%)	3 (6.8%)
Decreased platelet count	-	-	10 (22.7%)	9 (20.5%)
Increased AST	1 (1.9%)	-	4 (9.1%)	-
Increased weight	2 (3.7%)	-	1 (2.3%)	2 (4.5%)
Increased CRP	3 (5.6%)	1 (1.9%)	-	1 (2.3%)
Decreased white blood cell count	-	-	3 (6.8%)	2 (4.5%)
Increased blood bilirubin	1 (1.9%)	-	2 (4.5%)	1 (2.3%)
Decreased urine output	-	-	2 (4.5%)	-
Decreased haemoglobin	-	-	1 (2.3%)	1 (2.3%)
NERVOUS SYSTEM DISORDERS				
Headache	4 (7.4%)	-	1 (2.3%)	-
METABOLISM AND NUTRITION DISORDERS				
Decreased appetite	1 (1.9%)	-	3 (6.8%)	-
Hyperkalaemia	1 (1.9%)	2 (3.7%)	-	
Hyponatraemia	-	-	1 (2.3%)	1 (2.3%)
Hypokalaemia	1 (1.9%)	1 (1.9%)	-	-
HAEMATOLOGY INVESTIGATIONS				
Prolonged ATPP	2 (3.7%)	-	1 (2.3%)	-
IMMUNE SYSEM DISORDERS				
Cytokine release syndrome	-	-	2 (4.5%)	-
Anaphylactic reaction	-	-	2 (4.5%)	-

Serious adverse event/deaths/other significant events

Deaths

Seven patients died for a reason other than disease progression.

For three patients, death occurred several months after the end of treatment: one as a result of an accident and two as the result of an infection.

Four deaths could be considered as possibly treatment-related as they occurred as a result of an AE that started under therapy.

- Two deaths occurred in study APN311-302 due to capillary leak syndrome and acute respiratory distress syndrome, which may have been the result of an anaphylactic reaction.
- One death in study APN311-201 is more likely related to the previous allogeneic stem cell transplantation (herpes encephalitis); the patient developed pneumonia leading to pulmonary failure with fatal outcome about 2 months later.
- One death in study APN311-202 was due to septic shock and was attributed to delayed antibiotic treatment in an outpatient who presented with repeated fever episodes and was subsequently hospitalised. It does not seem that, in this case, home treatment could be directly incriminated in the delayed antibiotic therapy, but according to the Applicant, rather the fact that the patient was not followed in a specialised environment.

SAEs

The SAE profile was broadly similar in studies APN311-201 and 202; the most frequent SAEs were infections, pyrexia, hypotension, thrombocytopaenia. However, a high occurrence of serious hypoxia/respiratory distress was reported specifically in study APN311-202.

The patient incidence of SAEs decreased significantly over the treatment cycles: from 39% (cycle 1) to 7% (cycle 5) in study APN311-202 and from 15% (cycle 1) to 0% (cycle 5) in study APN311-303.

Study APN311-302 allowed to compare the safety profile of ch14.18/CHO (+13-cis RA) alone and combined with IL-2. SAEs were reported more frequently in patients receiving IL-2 compared to patients not receiving IL-2: 46% vs 27%. More patients who received IL-2 experienced at least 1 SAE leading to the discontinuation of ch14.18/CHO, 13-cis-RA, and/or IL-2, if applicable: 17% vs 6% of patients (47 vs 16 SAEs).

Table 43: Summary of SAEs occurring in > 1 subject in any study

SYSTEM ORGAN CLASS Preferred term	Number (%) Patients			
	APN311-303 (N=54)	APN311-202 (N=44)	APN311-101 (N=15)	APN311-201 (N=35)
OVERALL	**12 (22.2%)**	**25 (56.8%)**	**2 (13.3%)**	**25 (71.4%)**
BLOOD AND LYPMPHATIC SYSTEM DIS	**-**	**3 (6.8%)**	**-**	**3 (8.6%)**
Thrombocytopenia	-	-	-	2 (5.7%)
Anaemia	-	2 (4.5%)	-	-
GASTROINTESTINAL DISORDERS	**5 (9.3%)**	**5 (11.4%)**	**2 (13.3%)**	**2 (5.7%)**
Vomiting	2 (3.7%)	3 (6.8%)	-	-
Diarrhea	1 (1.9%)	3 (6.8%)	-	2 (5.7%)
GENERAL DISORDERS	**3 (5.6%)**	**7 (15.9%)**	**-**	**4 (11.4%)**
Pain	1 (1.9%)	2 (4.5%)	-	1 (2.9%)
Pyrexia	1 (1.9%)	6 (13.6%)	-	2 (5.7%)
IMMUNE SYSTEM DISORDERS	**-**	**2 (4.5%)**		**1 (2.9%)**
Anaphylactic reaction	-	2 (4.5%)	-	1 (2.9%)
INFECTIONS AND INFESTATIONS	**3 (5.6%)**	**9 (20.5%)**	**-**	**16 (45.7%)**
Bronchitis	1 (1.9%)	-	-	2 (5.7%)
Gastroenteritis	-	-	-	3 (8.6%)
Pneumocystis jirovecii pneumonia	-	-	-	3 (8.6%)
Device related infection	-	3 (6.8%)	-	1 (2.9%)
Sepsis	-	4 (9.1%)	-	1 (2.9%)
INVESTIGATIONS	**-**	**6 (13.6%)**	**-**	**2 (5.7%)**
Platelet count decreased	-	2 (4.5%)	-	-
METABOLISM AND NUTRITION DIS	**1 (1.9%)**	**3 (6.8%)**	**-**	**1 (2.9%)**
Hyponatremia	-	2 (4.5%)	-	-
NERVOUS SYSTEM DISORDERS	**2 (3.7%)**	**1 (2.3%)**	**-**	**4 (11.4%)**
Convulsion	1 (1.9%)	-	-	3 (8.6%)
RESPIRATORY DISORDERS	**1 (1.9%)**	**8 (18.2%)**	**-**	**1 (2.9%)**
Hypoxia	-	5 (11.4%)	-	-
Acute respiratory distress syndrome	1 (1.9%)	2 (4.5%)	-	-
SKIN DISORDERS	**-**	**-**	**1 (6.7%)**	**1 (2.9%)**
VASCULAR DISORDERS	**-**	**5 (11.4%)**	-	**4 (11.4%)**
Hypotension	-	3 (6.8%)	-	2 (5.7%)

Treatment-related events

Since most TEAEs documented during the studies were judged to have at least a possible relationship to the study treatment (any of ch14.18/CHO, IL-2, 13-cis-RA), treatment-related TEAEs were generally comparable to overall TEAE incidences.

Study APN311-302 allowed to compare the safety profile of ch14.18/CHO (+13-cis RA) alone and combined with IL-2. Toxicities were generally more frequent in patients who received IL-2 compared to patients who did not receive IL-2 in particular capillary leak syndrome, platelet abnormalities, hypotension, infections, nausea or vomiting, fever, and pain related to ch14.18/CHO. Constipation however was observed less frequently with concomitant IL-2 treatment than without IL-2 treatment.

Table 44: Toxicities in study APN311-302 (SAF; N=366)

SYSTEM ORGAN CLASS Toxicities	Number (%) of Patients ch14.18/CHO+ 13-cis-RA (N = 183)		ch14.18/CHO+13-cis-RA + IL-2 (N = 183)		All (N = 366)	
ANY	181	(98.9)	181	(98.9)	362	(98.9)
GENERAL CONDITION	140	(76.5)	164	(89.6)	304	(83.1)
GUT TOXICITY	135	(73.8)	145	(79.2)	280	(76.5)
Stomatitis	29	(15.8)	40	(21.9)	69	(18.9)
Nausea or vomiting	99	(54.1)	121	(66.1)	220	(60.1)
Diarrhea	92	(50.3)	114	(62.3)	206	(56.3)
Constipation	76	(41.5)	47	(25.7)	123	(33.6)
SKIN TOXICITY	147	(80.3)	159	(86.9)	306	(83.6)
Skin	124	(67.8)	138	(75.4)	262	(71.6)
Allergy	101	(55.2)	119	(65.0)	220	(60.1)
LIVER TOXICITY	118	(64.5)	126	(68.9)	244	(66.7)
Bilirubin	15	(8.2)	35	(19.1)	50	(13.7)
SGOT and SGPT	118	(64.5)	121	(66.1)	239	(65.3)
CARDIAC TOXICITY	61	(33.3)	88	(48.1)	149	(40.7)
Cardiac function	6	(3.3)	10	(5.5)	16	(4.4)
ECHO: LV-SF	1	(0.5)	8	(4.4)	9	(2.5)
Hypotension	48	(26.2)	78	(42.6)	126	(34.4)
Hypertension	24	(13.1)	11	(6.0)	35	(9.6)
INFECTIONS	147	(80.3)	170	(92.9)	317	(86.6)
Infections	106	(57.9)	132	(72.1)	238	(65.0)
Fever	145	(79.2)	168	(91.8)	313	(85.5)
HEMATOLOGICAL TOXICITY	164	(89.6)	174	(95.1)	338	(92.3)
Hemoglobin	162	(88.5)	174	(95.1)	336	(91.8)
WBC	148	(80.9)	153	(83.6)	301	(82.2)
Granulocytes	140	(76.5)	154	(84.2)	294	(80.3)
Platelets	124	(67.8)	156	(85.2)	280	(76.5)
RENAL TOXICITY	46	(25.1)	56	(30.6)	102	(27.9)
Creatinine	25	(13.7)	35	(19.1)	60	(16.4)
Proteinuria	16	(8.7)	11	(6.0)	27	(7.4)
Hematuria	18	(9.8)	24	(13.1)	42	(11.5)
GFR	14	(7.7)	10	(5.5)	24	(6.6)
Tubular phosphate reabsorption	1	(0.5)	3	(1.6)	4	(1.1)
NEUROLOGICAL TOXICITY	28	(15.3)	44	(24.0)	72	(19.7)
Central neurotoxicity	19	(10.4)	28	(15.3)	47	(12.8)
Peripheral neurotoxicity	13	(7.1)	25	(13.7)	38	(10.4)
VASCULAR TOXICITY	70	(38.3)	116	(63.4)	186	(50.8)
Capillary leak syndrome	45	(24.6)	91	(49.7)	136	(37.2)
Cytokine release syndrome	49	(26.8)	64	(35.0)	113	(30.9)
PAIN	115	(62.8)	138	(75.4)	253	(69.1)
Pain related to ch14.18/CHO	115	(62.8)	138	(75.4)	253	(69.1)
OCULAR TOXICITY	33	(18.0)	45	(24.6)	78	(21.3)
Dilated pupils	23	(12.6)	40	(21.9)	63	(17.2)
Accommodation defects	15	(8.2)	23	(12.6)	38	(10.4)
Papilloedema	5	(2.7)	3	(1.6)	8	(2.2)

13-cis-RA = 13-cis retinoic acid, ECHO: LV-SF = echocardiogram: left ventricle - systolic function, GFR = glomerular filtration rate, IL-2 = aldesleukin, N = number of patients, SGOT = serum glutamic oxaloacetic transaminase (= AST), SGPT = serum glutamic pyruvic transaminase (= ALT), WBC = white blood cells.

Adverse drug reactions

The list of ADRs has been established by the applicant and reviewed by the CHMP. Due to different methods of AE collection across studies, ADR frequencies were calculated either on the totality of the safety database (N=514) when possible/relevant or on the subpopulation of studies APN311-101, -201, -202, -303 (N=148).

Table 45: Adverse drug reactions reported in the clinical trials submitted

System organ class	ADR Preferred Term	Frequency
Infections and infestations	infection (including pneumonia, skin infection, herpes virus infection, myelitis, encephalomyelitis)	53.3% (N=274/514)
	device related infection	10.1% (N=15/148)
	Sepsis	1.4% (N=7/514)
Blood and lymphatic system disorders	anaemia	77.4% (N=398/514)
	leucopenia	66.5% (N=342/514)
	neutropenia	10.1% (N=15/148)
	thrombocytopenia	62.3% (N=320/514)
	lymphopenia	2.0% (N=3/148)
	disseminated intravascular coagulation	0.4% (N=2/514)
	eosinophilia	0.7% (N=1/148)
Immune system disorders	hypersensitivity	62.8% (N=323/514)
	cytokine release syndrome	32.1% (N=165/514)
	anaphylactic reaction	5.4% (N=828/514)
	serum sickness	0.7% (N=1/148)
Metabolism and nutrition disorders	fluid retention	20.9% (N=31/148)
	decreased appetite	4.1% (N=21/514)
	hypoalbuminaemia	5.4% (N=8/148)
	hyponatraemia	5.4% (N=8/148)
	hypokalaemia	4.7% (N=7/148)
	hypophosphataemia	4.7% (N=7/148)
	hypomagnesaemia	4.1% (N=6/148)
	hypocalcaemia	3.4% (N=5/148)
	dehydration	1.4% (N=2/148)
Psychiatric disorders	agitation	2.0% (N=3/148)
	anxiety	1.4% (N=2/148)
Nervous system disorders	headache	11.5% (N=17/148)
	peripheral neuropathy	9.5% (N=49/514)
	seizure	2.9% (N=15/514)
	paraesthesia	4.7% (N=7/148)
	dizziness	4.1% (N=6/148)
	tremor	1.4% (N=2/148)
	increased intracranial pressure	0.4% (N=2/514)
	posterior reversible encephalopathy syndrome	0.4% (N=2/514)
Eye disorders	mydriasis	12.5% (N=64/514)
	pupillotonia	10.1% (N=15/148)
	eye oedema (eyelid, periorbital)	11.5% (N=17/148)
	ophthalmoplegia	2.3% (N=12/514)
	papilloedema	1.6% (N=8/514)
	accommodation disorder	7.0% (N=36/514)
	blurred vision	3.4% (N=5/148)
	photophobia	2.7% (N=4/148)
Cardiac disorders	tachycardia	15.8% (N=81/514)
	cardiac failure	1.8% (N=9/514)
	left ventricular dysfunction	1.9% (N=10/514)
	pericardial effusion	1.4% (N=2/148)
Vascular disorders	hypotension	39.1% (N=201/514)
	capillary leak syndrome	40.5% (N=208/514)
	hypertension	8.2% (N=42/514)
	hypovolaemic shock	0.2% (N=1/514)
	venoocclusive disease	0.7% (N=1/148)
Respiratory, thoracic and mediastinal disorders	hypoxia	25.7% (N=38/148
	cough	16.3% (N=84/514)
	bronchospasm	4.1% (N=21/514)

	dyspnoea	7.4% (N=11/148)
	respiratory failure	1.4% (N=7/514)
	lung infiltration	2.0% (N=3/148)
	pulmonary oedema	2.0% (N=3/148)
	pleural effusion	7.4% (N=11/148
	tachypnoea	1.4% (N=2/148)
	laryngospasm	2.0% (N=3/148)
Gastrointestinal disorders	vomiting	57.2% (N=294/514)
	diarrhoea	51.2% (N=263/514)
	constipation	32.5% (N=167/514)
	stomatitis	16.7% (N=86/514)
	nausea	7.2% (N=37/514)
	lip oedema	3.4% (N=5/148)
	ascites	5.4% (N=8/148)
	abdominal distension	2.7% (N=4/148)
	ileus	1.4% (N=7/514)
	dry lips	2.7% (N=4/148)
	enterocolitis	0.8% (N=4/514)
Hepatobiliary disorders	hepatocellular injury	0.4% (N=2/514)
Skin and subcutaneous tissue disorders	urticaria	17.6% (N=26/148)
	pruritus	49.3% (N=73/148)
	rash	22.3% (N=33/148)
	dermatitis (including exfoliative)	2.7% (N=4/148)
	erythema	7.4% (N=11/148)
	dry skin	6.1% (N=9/148)
	hyperhidrosis	2.7% (N=4/148)
	petechiae	2.0% (N=3/148)
	photosensitivity reaction	1.4% (N=2/148)
Musculoskeletal and connective tissue disorders	muscle spasms	1.4% (N=2/148)
Renal and urinary disorders	oliguria	4.1% (N=6/148)
	urinary retention	1.9% (N=10/514)
	hyperphosphaturia	1.0% (N=5/514)
	haematuria	8.6% (N=44/514)
	proteinuria	7.6% (N=39/514)
	renal failure	0.6% (N=3/514)
General disorders and administration site conditions	pyrexia	87.9% (N=452/514)
	chills	19.6% (N=29/148)
	pain*	77.4% (N=398/514)
	peripheral oedema	27.0% (N=40/148)
	face oedema	19.6% (N=29/148)
	injection site reaction	4.7% (N=7/148)
Investigations	increased weight	37.8% (N=56/148)
	increased transaminases	52.9% (N=272/514)
	increased gamma glutamyltransferase	16.2% (N=24/148)
	increased blood bilirubin	13.2% (N=68/514)
	increased blood creatinine	14.2% (N=73/514)
	decreased weight	3.4% (N=5/148)
	decreased glomerular filtration rate	4.5% (N=23/514)
	hypertriglyceridaemia	3.4% (N=5/148)
	prolonged activated partial thromboplastin time	1.4% (N=2/148)
	prolonged prothrombin time	1.4% (N=2/148)
	prolonged thrombin time	1.4% (N=2/148)

*includes abdominal pain, pain in extremity, musculoskeletal pain, chest pain, arthralgia

Adverse events of interest

Pain-related AEs and intravenous morphine use

The main expected toxicity of ch14.18 antibody treatment is related to neuropathic pain symptoms. In studies APN311-303 and -202, pain self-assessment was performed using specific paediatric scales (e.g., Wong-Baker Faces Pain Rating Scale or Faces Pain Scale – Revised). The investigator documented the occurrence of pain on a daily basis in study APN311-101 and in study APN311-201 the incidence of pain was documented per treatment cycle only.

Pain assessment

The total number of patients with at least one documentation of pain in each treatment cycle is provided for the continuous infusion studies APN311-303 and -202, using a dosage of 10 mg/m^2/day, and for the short infusion study APN311-101, using a dosage 10 to 30 mg/m^2/day.

In the continuous infusion studies, around 90% of the patients experienced pain in cycle 1. The percentage of patients with pain decreased in subsequent treatment cycles, to about 60% in cycle 5. In study APN311-101, 67% of the patients experienced pain in cycle 1 and 22% in cycle 3.

Table 46: Total number of patients with pain at any day of the cycle

Cycle	APN311-101		APN311-303		APN311-202	
	N	Patients with pain n (%)	N	Patients with pain n (%)	N [1]	Patients with pain n (%)
Cycle 1	15	10 (66.7%)	54	49 (90.7%)	24	21 (87.5%)
Cycle 2	14	3 (24.4%)	53	36 (67.9%)	25	17 (68.0%)
Cycle 3	9	2 (22.2%)	49	36 (73.5%)	20	9 (45.0%)
Cycle 4	-	NA	41	29 (70.7%)	18	12 (66.7%)
Cycle 5	-	NA	38	22 (57.9%)	17	10 (58.8%)

N = number of patients with pain assessment, NA = not applicable
[1] Patients with pain based on parent assessment score

Table 47: Occurrence of pain-related events reported by investigators and parents by cycle

	Pooled pain events		Parent assessment	
	APN311-202 (N=44) n/N* (%)	APN311-303 (N=54) n/N* (%)	APN311-202 (N=44) n/N# (%)	APN311-303 (N=54) n/N# (%)
Cycle 1	29/44 (65.9%)	51/54 (94.4%)	21/24 (87.5%)	49/54 (91%)
Cycle 2	20/40 (50.0%)	27/53 (50.9%)	17/25 (68.0%)	36/53 (68%)
Cycle 3	10/32 (31.3%)	24/51 (47.1%)	9/20 (45.0%)	36/49 (73%)
Cycle 4	14/31 (45.2%)	14/42 (33.3%)	12/18 (66.7%)	29/41 (71%)
Cycle 5	9/29 (31.0%)	13/39 (33.3%)	10/17 (58.8%)	22/38 (58%)

n= number of patients in category, N* = number of patients exposed to ch14.18/CHO, N# = number of patients with assessment.

In study APN311-201 (short infusions), there was a clear decrease in the incidence of pain from Cycle 1 (100%) to Cycle 6 (65%); as there was a drop in the number of patients after Cycle 6, the apparent increase in pain incidence during the last cycles should be interpreted with caution.

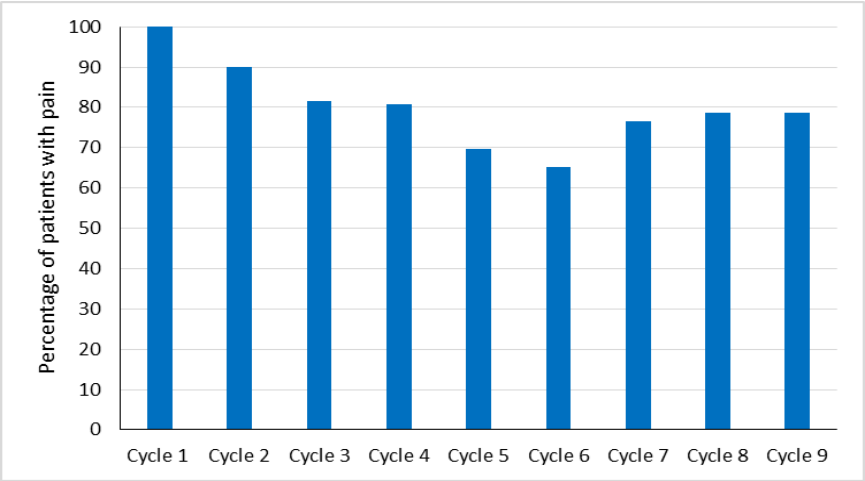


Figure 8: Occurrence of pain in study APN311-201

Intravenous morphine use

Intravenous morphine is generally used in ch14.18 treatment to prevent expected severe pain events. The proportion of patients requiring iv morphine in the continuous infusion studies decreased over the cycles but much more in study 303 (from 96 to 11%) than in study 202 (from 100 to 72%).

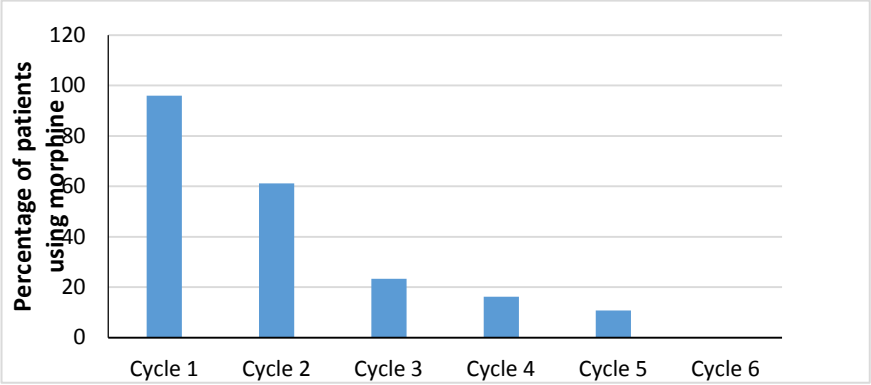


Figure 9: Use of morphine in study APN 311-303

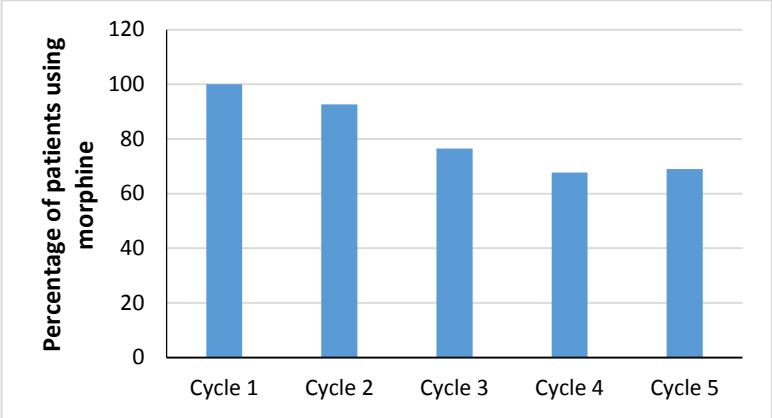


Figure 10: Use of morphine in study APN 311-202

The mean morphine dose also declined over cycles, especially in the continuous infusion studies.

Table 48: Intravenous morphine use (mean) per cycle in patients receiving morphine

Cycle	APN311-101		APN311-303		APN311-202	
	N[1]	Cumulative morphine dose (mg)	N	Cumulative morphine dose (mg)	N	Cumulative morphine dose (mg)
Cycle 1	15	91.0	48	54.0	44	41.5
Cycle 2	14	76.7	30	33.2	38	35.5
Cycle 3	9	71.2	11	35.7	26	33.5
Cycle 4	-	NA	7	17.8	22	30.0
Cycle 5	-	NA	4	26.9	21	26.7

[1]All patients receiving ch14.18/CHO treatment received morphine.
N = number of patients with morphine use, NA = not applicable.

In study APN311-201, the proportion of patients receiving morphine declined from 100% to 87% by cycle 5, although it is unclear if it was intravenously in all cases.

Pain medications other than morphine were allowed in most studies. These included gabapentin, acetaminophen, NSAID, ketamine and fentanyl patches (a morphine analogue). In study APN311-303, a number of patients (44/54; 81%) received prophylactic naloxone, which is often used to counteract the effects of morphine.

Hypersensitivity reactions

In the continuous infusion studies APN311-202 and -303, infusion-associated and allergic reactions were reported in 73% and 89% of the patients, respectively. Most were of mild or moderate severity. Grade 3 reactions were reported in 18% and 17% of the patients, respectively. No grade 4 reaction was reported. Their incidence decreased from cycle 1 (52% and 74%, respectively) to cycle 5 (29% and 41%, respectively).

Manifestations included hypotension (45% and 63%, respectively), facial, periorbital or lip oedema (2% to 44%), bronchospasm (14% and 11%, respectively), urticaria (23% and 24%, respectively) and rashes (20% and 31%, respectively). The rate of hypotension, however, needs to be interpreted with caution as hypotension is a common complication of iv morphine, non-steroidal anti-inflammatory drugs and antihistamines, which – at least in early treatment cycles- virtually every patient received as concomitant medication.

In study APN311-302, skin allergies were reported in 55% of the patients in the arm without IL-2 and 65% of patients in the arm with IL-2.

Across all studies, 46 serious infusion-associated and allergic reactions were reported in 34 patients (7%). Reactions reported by more than 2 patients included hypersensitivity (18 patients), hypotension (7 patients), anaphylactic reaction (5 patients), and bronchospasm (4 patients). All resolved with the exception of bronchospasm/laryngospasm, and acute respiratory distress syndrome, further complicated by pneumonia and sepsis in one patient (ch14.18/CHO alone), who eventually died of multi-organ failure during cycle 3.

In the total safety database, only 6 patients (1.2%) had a coded anaphylactic reaction/shock, definitely diagnosed by the investigator. Following CHMP request, a comprehensive search using PTs terms suggestive of an anaphylactic reaction according to literature consensus, chronology and treatment with adrenaline resulted in 16/148 patients (from studies APN311-101, -201, -202, -303), i.e. 11% overall and 6/148 (4%) with serious reactions. The last figure is consistent with an incidence of 12/366 (3%) in study APN311-303.

Hypoxia/respiratory distress

In the continuous infusion studies APN311-202 and -303, hypoxia/respiratory failure were reported in 43% and 44% of the patients, respectively. They were mostly grade 1 or 2. The majority occurred in the first two cycles.

Overall, there were 12 serious hypoxia/respiratory distress events in 11 patients; only 4 cases were reported in study APN311-302 (3/4 without IL-2), one of which was eventually fatal (see death section). Most SAEs led to interruption or discontinuation of treatment.

Cytokine release syndrome

Ten episodes of CRS in 8 patients had been coded by the diagnosis CRS or SIRS and 70 sequences of events were considered as possible episodes of CRS. Therefore, in summary 80 episodes of CRS were identified. The majority of events (95%) were graded as mild to moderate

CRS was reported in 36% and 56% of the patients in studies APN311-202 and -303, respectively. Most episodes occurred during the first treatment cycle of ch14.18/CHO. In the following cycles the incidence of CRS gradually decreased. The majority of events (95%) were graded as mild or moderate and no life-threatening event was observed during the episodes of CRS.

In study APN311-302, CRS occurrence was only slightly increased by the addition of IL-2: 27% of the patients in the arm without IL-2 and 35% of patients in the arm with IL-2. Their frequency decreased over cycles. Half of CRS events were grade 1 while 10% were severe (grade 3-4).

Capillary leak syndrome

A specific analysis was conducted to identify patients who experienced the full clinical picture of CLS including the main symptoms of fluid extravasation (oedema), hypoalbuminemia, haemoconcentration, and/or hypotension. Variable rates of CLS were reported across studies reflecting the lack of standardisation in data reporting and emphasis on this particular ADR, e.g. 36% in study APN311-202 and 83% in study -303. More than half of the events occurred in the first cycle and their incidence gradually decreased over cycles; 10% of the events were reported as serious and 18% as severe (grade 3-4) with 2 events being life-threatening.

IL-2 is well known to induce CLS and, in study APN311-302, CLS occurrence was doubled by the addition of IL-2: 25% of the patients in the arm without IL-2 and 50% of patients in the arm with IL-2. Their frequency decreased over cycles and 10% of the events were severe (grade 3-4) overall, but more with IL-2 (12%) than without IL-2 (7%).

Neurological disorders

Potentially severe reactions to ch14.18 treatment include neurological disorders of the eye, based on binding of the antibody to optic nerve cells.

In the continuous infusion studies APN311-202 and -303, neurological eye disorders were reported in 23% and 28% of the patients, respectively. These figures are consistent with the eye toxicities reported in study APN311-302: 18% without IL-2 vs 25% with IL-2. Mydriasis and accommodation defects with blurred vision were the most common manifestations. Two grade 4 cases were reported, ophtalmoplegia (which resolved after 10 months) and optic atrophy.

Across all studies, SAEs related to neurological eye disorders were reported in 3% of the patients. They were resolving or resolved, some with sequelae.

In the continuous infusion studies APN311-202 and -303, both motor and sensory neuropathies were reported with an incidence of 9% and 5%, respectively. These figures are consistent with the peripheral

neurotoxicities reported in study APN311-201 (9%) and in study APN311-302: 7% without IL-2 vs 14% with Il-2. Most events were of grade 1-2 and resolved; two patients discontinued treatment.

Infections

In the continuous infusion studies APN311-202 and -303, infections were reported in 61% and 76% of the patients, respectively. These figures are consistent with the infections reported in study APN311-302: 58% without IL-2 vs 72% with Il-2.

However, severe infections (grade 3-4) were more frequent in study APN311-202 (50% of patients) compared to 15% in study APN311-303. The reason for this finding is unknown. However, the Applicant commented that "study ANP311-202 allowed outpatient treatment with ch14.18/CHO if treatment was well tolerated, which may lead to delayed diagnosis and eventually to delayed treatment". The great majority of grade 3 events were device-related infections and more than half of these patients were treated at the same centre. According to the Applicant, device-related infections are well-known and frequent complications of central venous catheterization and are thus presumably rather related to the catheter than to ch14.18/CHO administration. Nevertheless, one patient died of septic shock, which was attributed to delayed antibiotic treatment; this outpatient presented with repeated fever episodes and was subsequently hospitalised. It does not seem that, in this case, home treatment could be directly incriminated in the delayed antibiotic therapy, but according to the Applicant, rather the fact that the patient was not followed in a specialised environment.

Overall, there were 84 serious infection-related events in 63 patients (12%). The occurrence was especially high in study APN311-201 after haploidentical SCT (46%), compared to 6% - 23% in the other studies. IL-2 did not increase the serious infection rate in study APN311-302: 8% without IL-2 vs 10% with IL-2. Overall, three patients died of infectious complications (see death section).

Others

Posterior reversible encephalopathy syndrome (PRES) was diagnosed in 3 patients; however in one case, the causality between ch14.18/CHO and PRES was assessed as unlikely due to the chronology of the event and alternative possible causes.

Laboratory findings

The most common haematological changes assessed as clinically relevant by the investigator were decreases in neutrophils, in platelet count and in haemoglobin. The most common clinical chemistry abnormalities were abnormal liver function tests (elevated transaminases and gamma-GT) as well as elevated CRP.

Safety in special populations

N/A

Safety related to drug-drug interactions and other interactions

No studies have been performed.

Discontinuation due to adverse events

In the continuous infusion study, the number of patients having stopped treatment due to an ADR, as only reason or associated with progressive disease, was 6 (6%). These were hypoxia, bronchospasm, anaphylactic reaction (3), peripheral neuropathy (1), visual disorder (1), and infection (1).

In study APN311-201, this occurred in 4 patients due to CLS, peripheral neuropathy, psychotic disorder, and herpes zoster (unrelated).

2.6.1. Discussion on clinical safety

Overall, 514 patients received ch14.18/CHO: 98 patients as a continuous infusion over 10 days and 416 patients as five daily short 8h-infusions. Overall, 281 patients received ch14.18/CHO in combination with IL-2 and 207 patients received antibody treatment as monotherapy; 26 patients received both single-agent and combination cycles, but with a very low IL-2 dose used to prevent GvHD. The combination also included 13-cis retinoic acid in most studies. In principle, the size of the safety data base is acceptable for a rare disorder, especially since the safety profile of anti-GD2 is already known to a large extent from published literature.

It should be emphasized that the method of AE collection varied across studies. In particular, in the largest study (APN311-302) only SAEs were fully reported while for other AEs, a pre-defined list of 31 specific toxicities was used. A similar system was used in study APN311-201 where safety was recorded by toxicity forms and AEs that were not already included in the form were to be reported as TEAEs. The SAE incidence in study APN311-303 was much lower than in the other R/R studies. A comprehensive Applicant's assessment of grade 4 events did not provide an explanation for the difference in SAE reporting compared to study APN311-202. It is likely that, the patients being already hospitalised, the difference is mainly due to a different appreciation of "medically significant" events.

Dosing regimen and pain

The vast majority of patients across the studies received a dose of 100 mg/m^2/cycle, which is recommended in the SmPC (i.e. 10 mg/m^2/day for 10 days). The target number of cycles and their duration differed between studies, but in the continuous infusion studies, the majority of patients received 5 (or 6) 35 day-treatment cycles.

Pain is an anticipated side effect of anti-GD2, which has been managed by standard pain prophylaxis, including i.v. morphine. While the morphine dosage somewhat varied across studies, the recommended dose is in general at the high end of the paediatric guidelines of pain management.

There is variation in the infusion scheme (short term (8hr/5 days) vs. continuous infusion (24 hr/10 days). According to the Applicant, the continuous infusion concept (24h) is associated with an extended exposure and lytic pressure leading to a reduction of pain/morphine use, and is therefore proposed as the post-marketing administration scheme. However, there is no study directly comparing the continuous with the short infusions, and the differences in study design, assessment methodology and data presentation severely hamper comparison across studies.

The two studies using continuous infusion showed similar percentages of patients suffering from pain according to parent assessment, which decreased from approximately 90% in cycle 1 to 60% in cycle 5. These figures are consistent with the incidence of pain-related events over treatment cycles, although parents tended to report pain more often than investigators.

Simultaneously, the proportion of patients given iv morphine decreased but, much more in study APN311-303 (from 96 to 11%) than in study -202 (from 100 to 72%). In those patients receiving morphine, the mean cumulative morphine dose was approximately halved by cycle 5 in both studies.

In the small study using short infusions (APN311-101), the proportion of patients with pain was much lower but the Applicant argued that the morphine dose was higher. In the second study using short infusions (APN311-201) a decrease in pain incidence from 100% (cycle 1) to 70% (cycle 5) was observed, hence slightly worse than in study APN311-202 and -303 (~60%). The proportion of patients receiving

morphine declined from 100% to 87% by cycle 5, although it is unclear if it was intravenously in all cases; this proportion was indeed slightly higher than in study APN311-202 (72%). As there was no obvious effect on morphine tolerance, the Applicant did not propose to recommend prophylactic use of naloxone.

The data available indicate that the correlation between pain and morphine use/dose is not straightforward and difficult to interpret. Nevertheless, these data suggest that the pain and morphine use were more frequent with short infusions than continuous long-lasting infusion although the difference did not appear substantial, and the evidence lacks robustness given the limited sample size. Furthermore, the good results regarding morphine use in the single-centre study were not replicated in the multicentre study therefore undermining the strength of the conclusion.

Based on these data, the continuous infusion is not recommended as the only mode of administration as this also implies doubling the duration of ch14.18 treatment. No data on the duration of hospitalisation with the short infusions have been submitted to compare with that of the continuous infusion (median 8-10 days).

As a consequence of the occurrence of pain, premedication with analgesics, including intravenous opioids, prior to each infusion of dinutuximab beta is required. A triple therapy, including nonopioid analgesics (according to WHO guidelines), gabapentin and opioids, is recommended for pain treatment. The individual dose may vary widely. Nonopioid analgesics should be used permanently during the treatment, e.g. paracetamol or ibuprofen (see section 4.4 of the SmPC).

The patient should be primed with 10 mg/kg/day, starting 3 days prior to dinutuximab beta infusion. The daily dose of gabapentin is increased to 2×10 mg/kg/day orally, the next day and to 3×10 mg/kg/day orally, the day before the onset of dinutuximab beta infusion and thereafter. The maximum single dose of gabapentin is 300 mg. This dosing schedule should be maintained for as long as required by the patient.

Oral gabapentin should be tapered off after weaning off intravenous morphine infusion, at the latest after dinutuximab beta infusion therapy has stopped.

Treatment with opioids is standard with dinutuximab beta. The first infusion day and course usually requires a higher dose than subsequent days and courses.

- Before initiation of a continuous intravenous morphine infusion, a bolus infusion of 0.02 to 0.05 mg/kg/hour morphine should be started 2 hours before dinutuximab beta infusion.
- Subsequently, a dosing rate of 0.03 mg/kg/hour is recommended concomitantly with dinutuximab beta infusion.
- With daily infusions of dinutuximab beta, morphine infusion should be continued at a decreased rate (e.g. 0.01 mg/kg/h) for 4 hours after the end of dinutuximab beta infusion.
- With continuous infusion, in response to the patient's pain perception, it may be possible to wean off morphine over 5 days by progressively decreasing its dosing rate (e.g. to 0.02 mg/kg/hour, 0.01 mg/kg/hour, 0.005 mg/kg/hour).
- If continuous morphine infusion is required for more than 5 days, treatment should be gradually reduced by 20% per day after the last day of dinutuximab beta infusion.

After weaning off intravenous morphine, in case of severe neuropathic pain, oral morphine sulphate (0.2 to 0.4 mg/kg every 4 to 6 hours) can be administered on demand. For moderate neuropathic pain, oral tramodol may be administered (see section 4.4 of the SmPC).

Home treatment

There are currently no sufficient data to allow for the administration of ch14.18 in an outpatient setting (home treatment). This seems to have been successful in a very specialised centre but has not been readily generalised in the multicentre study. In particular, this is likely to have increased the risk of severe infections, mostly related to the device used for the infusion, and resulted in a child death due to delayed antibiotic therapy in a small hospital not specialised in oncology.

Safety profile

The most frequent treatment-emergent AEs were pyrexia, pain, vomiting, cough, increased weight, skin reactions, constipation, tachycardia, hypotension, and capillary leak syndrome. Laboratory abnormalities were frequent, including increased alanine aminotransferase and gammaglutamyltransferase, anaemia, leukopenia, thrombopenia.

SAEs were reported in 22% of the patients in the compassionate use programme compared with 59% in study APN311-202 and 71% in study APN311-201. In study APN311-302, SAEs were reported more frequently in patients receiving IL-2 (46%) compared to patients not receiving IL-2 (27%) and led to discontinuation of ch14.18/CHO, 13-cis-RA, and/or IL-2 in 17% vs 6% of patients, respectively. However, the discontinuation rate in the continuous infusion studies using IL-2 was the same as the latter (6%) and about 10% in study APN311-201.

In the continuous infusion studies APN311-202 and -303, infusion-associated and allergic reactions were reported in 73% and 89% of the patients, respectively. Most were of mild or moderate severity. The most frequent manifestations included hypotension, urticaria and rashes, bronchospasm and oedema of the face.

In study APN311-302, where only skin allergies were identified, the addition of IL-2 increased their frequency only marginally, from 55% to 65%. Across all studies, 46 serious infusion-associated and allergic reactions were reported in 34 patients (7%). The occurrence of serious anaphylactic reactions is estimated at 3.5%.

In the continuous infusion studies APN311-202 and -303, hypoxia/respiratory failure were reported in 43% and 44% of the patients, respectively. Most were mild or moderate and occurred during the first two cycles.

Severe infusion-related reactions, including CRS, anaphylactic and hypersensitivity reactions, may occur despite the use of premedication. Occurrence of a severe infusion related reaction (including CRS) requires immediate discontinuation of dinutuximab beta therapy and may necessitate emergency treatment.

Cytokine release syndrome frequently manifests itself within minutes to hours of initiating the first infusion and is characterised by systemic symptoms such as fever, hypotension and urticaria.

Anaphylactic reactions may occur as early as within a few minutes of the first infusion with dinutuximab beta and are commonly associated with bronchospasm and urticaria (see section 4.4 of the SmPC).

Antihistamine premedication (e.g. diphenhydramine) should be administered IV approximately 20 minutes before starting each dinutuximab beta infusion. It is recommended that antihistamine administration be repeated every 4 to 6 hours as required during dinutuximab beta infusion. Patients should be closely monitored for anaphylaxis and allergic reactions, particularly during the first and second treatment course.

Intravenous antihistamine, epinephrine (adrenaline) and prednisolone for intravenous administration should be immediately available at the bedside during administration of dinutuximab beta to manage life-threatening allergic reactions. It is recommended that treatment for such reactions include

prednisolone administered by intravenous bolus, and epinephrine administered by intravenous bolus every 3 to 5 minutes as necessary, according to clinical response. In case of bronchial and/or pulmonary hypersensitivity reaction, inhalation with epinephrine (adrenaline) is recommended and should be repeated every 2 hours, according to clinical response (see section 4.4 of the SmPC).

CLS was reported more frequently in study APN311-303 (83%) than in study APN311-202 (36%), with no clear explanation. The vast majority (82%) was grade 1 or 2 and their incidence decreased over treatment cycles. In study APN311-302, CLS occurrence was doubled by the addition of IL-2, from 25% to 50%. In addition, their severity also increased with IL-2. IL-2 is well known for inducing CLS. CLS usually develops within hours after initiation of treatment, while clinical symptoms (i.e. hypotension, tachycardia) are reported to occur after 2 to 12 hours. Careful monitoring of circulatory and respiratory function is required (see section 4.4 of the SmPC).

Infections were very frequent in this immunocompromised population: 61%-76% in the continuous infusion studies. Severe infections (grade 3-4) were more frequent in study APN311-202 (50% of patients) compared to 15% in study APN311-303. The occurrence of severe catheter-related infections in these immunocompromised patients is a concern that currently precludes the recommendation of home treatment. Patients should have no evidence of systemic infection and any identified infection should be under control before starting therapy.

Neurological disorders of the eye (23%-28%) and peripheral neuropathies (5%-9%) have been reported in line with the known toxicities of ch14.18 to the nerve. No dose modification is necessary in the case of an impaired visual accommodation that is correctable with eye glasses, as long as this is judged to be tolerable. Treatment must be interrupted in patients who experience Grade 3 vision toxicity (i.e. subtotal vision loss per toxicity scale). In case of any eye problems, patients should be referred promptly to an ophthalmology specialist (see section 4.4 of the SmPC).

Cases of motor or sensory neuropathy lasting more than 4 days must be evaluated and non-inflammatory causes, such as disease progression, infections, metabolic syndromes and concomitant medication, should be excluded. Treatment should be permanently discontinued in patients experiencing any objective prolonged weakness attributable to dinutuximab beta administration. For patients with moderate (Grade 2) neuropathy (motor with or without sensory), treatment should be interrupted and may be resumed after neurologic symptoms resolve (see section 4.4 of the SmPC).

Occurrence of haematologic toxicities has been reported with Dinutuximab beta Apeiron, such as erythropenia, thrombocytopenia or neutropenia. Grade 4 haematologic toxicities, improving to at least Grade 2 or baseline values by start of next treatment course, do not require dose modification.

Regulatory monitoring of liver function and electrolytes is recommended (see section 4.4 of the SmPC).

A theoretical risk for indirect reduction of CYP activity due to higher TNF α and IL-6 levels during immunotherapy cannot be ruled out.
Use of systemic corticosteroids is not recommended due to possible interference with immune activation, which is necessary for the therapeutic action of the antibody. Vaccinations and the use of intravenous immunoglobulins, which may interfere with ch14.18-dependent cellular toxicity, are not recommended during immunotherapy (see section 4.5 of the SmPC).
From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

Additional safety data needed in the context of a MA under exceptional circumstances

Taking into account the totality of the available data, the CHMP was of the view that the data set on the clinical safety of Dinutuximab beta Apeiron under normal conditions of use could not be considered comprehensive due to the absence of any randomised head-to-head comparison with a placebo. Furthermore, the method of data collection in the largest data set (study APN311-302) was incomplete as only SAEs were fully reported while for other AEs, a pre-defined list of 31 specific toxicities was used.

However, it is not considered feasible to generate a comprehensive data set due to ethical considerations preventing the conduct of a randomised placebo-controlled trial. At the time of this report, dinutuximab is standard of care in the treatment of high risk neuroblastoma, whereby neither physicians nor patients would be prepared to participate in a placebo-controlled trial.

In addition, a comparative trial against the previously authorised dinutuximab product Unituxin (the only potentially suitable active control) would not be feasible given the withdrawal of the marketing authorisation and since it is unlikely that patients would be willing to participate in a study comparing dinutuximab to dinutuximab beta.

Therefore, the current situation prevents the generation of new controlled data to confirm the outcomes of studies APN311-202, -303, and -302.

The CHMP was therefore of the view that a marketing authorisation under exceptional circumstances should be granted subject to specific obligations, including the implementation of the drug registry SAFARY proposed by the Applicant. In addition to general longer-term safety information, data will be specifically generated on pain intensity and need for pain medications, effect on peripheral and central nervous system, including visual impairment, anti-tumour response and longer-term effectiveness.

The specific obligations will be reviewed annually and continuation of the authorisation shall be linked to these annual re-assessments.

2.6.2. Conclusions on the clinical safety

The evaluation of safety is hampered by the absence of controlled trials without ch14.18 and by the heterogeneity of data collection across the academic trials. However, the safety profile of anti-GD2 antibodies is already known from the literature. Following CHMP request, the Applicant has performed numerous analyses of the safety data and most characteristics of the safety profile of the product are now adequately described.

The CHMP considered that the safety data were not comprehensive and that the following measure is necessary to generate additional safety data in the context of a marketing authorisation under exceptional circumstances:

- A drug registry (SAFARY) will collect specific data on pain intensity and need for pain medications, effect on peripheral and central nervous system, including visual impairment, and longer-term safety as well as on anti-tumour response and longer-term effectiveness.

2.7. Risk Management Plan

Safety concerns

Table 49: Summary of the safety concerns

Summary of safety concerns Risk	Safety concern
Important identified risks	• Pain • Serious infusion reactions including hypersensitivity, hypotension, and cytokine release syndrome • Neurological eye disorders • Peripheral neuropathy • Capillary leak syndrome • Hypoxia, respiratory distress and respiratory failure • Hematological toxicities
Important potential risks	• Cardiotoxicity • Immunogenicity • Medication errors
Missing information	• Drug-drug interactions • Use in adolescents, adults, and elderly • Use in patients with an ethnic origin other than Caucasian • Use in patients with hepatic and renal impairment • Potential harm from overdose • Long-term effects of treatment in early childhood

Pharmacovigilance plan

Table 50: Ongoing and planned studies in the PhV development plan

Activity / Study title (type of activity, study title [if known] category 1-3)*	**Objectives**	**Safety concerns addressed**	**Status Planned, started**	**Date for submission of interim or final reports (planned or actual)**
A Patient Registry in patients with neuroblastoma being treated with APN311 (Category 2)	Collect long term safety data	Long term safety, Pain intensity and need of appropriate medication for pain relief. Potential effect on peripheral and central nervous system and	Planned	Data will be regularly reviewed

Activity / Study title (type of activity, study title [if known] category 1-3)*	Objectives	Safety concerns addressed	Status Planned, started	Date for submission of interim or final reports (planned or actual)
		visual impairment		
Evaluation of PK + immunogenicity profile from ongoing studies (Category 2)	Collect PK and immunogenicity data, provide evidence of robustness of the drug assay drug tolerance of the binding and neutralizing HACA assays.	Missing PK data to support the posology in children over the entire age range. Missing exposure – efficacy relationship. Insufficient data on the impact of HACAs on PD, efficacy and safety To evaluate benefit of completing all treatment cycles after developing HACAs	Planned	2019
Study APN311-202 after Amendment 3 multi-center, open-label, randomized, controlled study in R/R and high-risk patients (Category 2)	Assess efficacy and safety of APN311, 10 mg/m²/day administered as continuous infusion over 10 days s.c. IL-2 + 13-cis-RA (five 35-day cycles).	Differentiate between APN311 and IL 2 related AEs in the R/R setting	Ongoing	First interim report including 3-year follow-up: 2021[a]
Survival results of Studies APN311-202 and -302 (at least 5 years) (Category 2)	Currently survival data are immature and long-term survival cannot be estimated.	To provide further survival data	Ongoing	Reports 2021

Risk minimisation measures

Safety concern	Routine risk minimization measures	Additional risk minimization measures
Pain	Sections 4.4 and 4.8 of the SmPC	None.
Serious infusion reactions including hypersensitivity, hypotension, and cytokine release syndrome	Sections 4.2; 4.4. and 4.8 of the SmPC	None.

Safety concern	Routine risk minimization measures	Additional risk minimization measures
Neurological eye disorders	Sections 4.4 and 4.8 of the SmPC	None.
Capillary leak syndrome (CLS)	Sections 4.2; 4.4. and 4.8 of the SmPC	None.
Peripheral neuropathy	Sections 4.4 and 4.8 of the SmPC	None.
Hypoxia, respiratory distress and respiratory failure	Section 4.8 of the SmPC	None
Hematological toxicities	Sections 4.4 and 4.8 of the SmPC	None
Cardiotoxicity	Section 4.8 of the SmPC	None
Immunogenicity	Section 5.1 of the SmPC	3.7.3.
Medication errors	Section 4.2 of the SmPC	None
Drug-drug interactions	Section 4.5 of the SmPC	None.
Use in adolescents, adults, and elderly	There is currently no scientific evidence for additional risks to adolescents, adults and elderly patients when exposed to APN311.	None.
Use in patients with an ethnic origin other than Caucasian	There is currently no scientific evidence for additional risks to non-Caucasian patients when exposed to APN311.	None.
Use in patients with hepatic and renal impairment	Section 5.2 of the SmPC	None.
Potential harm from overdose	Section 4.9 of the SmPC	None
Long-term effects of treatment in early childhood	Section 4.2 of the SmPC	None

Conclusion

The CHMP and PRAC considered that the risk management plan version 7.0 (signed 22 March 2017) is acceptable.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.9. New Active Substance

2.9.1. Problem statement

This application was submitted in accordance with Article 8(3) of Directive 2001/83/EC and it contained a claim and discussion as to why the active substance dinutuximab beta should be regarded as new.

The applicant requested the active substance dinutuximab beta contained in the above medicinal product to be considered a new active substance in comparison to the known dinutuximab previously authorised in the European Union as Unituxin, and claimed that dinutuximab beta differs significantly in properties with regard to safety and efficacy from the already authorised substance.

2.9.2. Scientific evaluation

2.9.2.1. Quality aspects

Position of the Applicant

Dinutuximab beta (ch14.18/CHO, APN311) is a mouse-human chimeric monoclonal anti GD2 IgG1 antibody expressed in Chinese Hamster Ovary (CHO) cells, which is the active substance used to manufacture the APN311 drug product (Dinutuximab beta Apeiron). Dinutuximab beta specifically binds to the disialoganglioside GD2. This glycolipid is expressed on neuroblastoma cells. Dinutuximab beta binds to cell surface GD2 and induces cell lysis of GD2-expressing cells via complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC).

Dinutuximab (Unituxin), a mouse-human chimeric monoclonal anti GD2 IgG1 antibody expressed in an SP2-0 murine cell line sharing the same amino acid sequence and targeting the same antigen. Unituxin received marketing authorization in the European Union on August 14, 2015.

Due to the alternative expression systems, there are significant differences in glycosylation between the two products. Dinutuximab (Unituxin) contains galactose-α-1,3-galactose (Gal1-alpha3Gal) epitopes.

Terminal Gal1-alpha3Gal-glycostructures on proteins have been well demonstrated for potential to cause allergic/hypersensitivity reactions (up to anaphylaxis) in humans ((Commins et al., 2016), (Steinke et al., 2015); (Commins et al., 2014); (Berg et al., 2014); (Chung et al., 2008)). These allergic reactions can be induced by repeated exposure (i.e. classical immunization) to the drug but more importantly they can also be triggered immediately during/after the first contact to the respective protein as most humans already exhibit a pre-formed anti-Gal1-alpha3Gal antibody immune response. One very well documented

pharmaceutical case is the SP2/0 cell-derived monoclonal anti-EGFR antibody cetuximab which has been approved for the treatment of colon carcinoma and SCCHN cancers ((Maier et al., 2015); (Chung et al., 2008))

Proof of presence of Gal1-alpha3Gal epitopes on dinutuximab: During the assessment of the marketing authorization application for dinutuximab (Procedure No. EMEA/H/C/002800/0000) by the European Medicines Agency (EMA), the EMA clearly confirmed the presence of galactose alpha-1,3-galactose glycosylation patterns on dinutuximab (Unituxin) and their potential role in triggering of allergic (including anaphylaxis) reaction by requesting the following information from United Therapeutics: “An update on the measurement of antibodies against non-human glycans as ch14.18, which is produced in rodent cells, contains galactose alpha-1,3-galactose (Gal1-alpha3Gal) and N-glycolylneuraminic acid and natural occurring antibodies to non-human glycans may be involved in some of the allergic reactions reported with ch14.18” (cited from (EMA_D120_List_of_Clinical_Questions_to_dinutuximab, 2015)).

The presence of galactose alpha-1,3-galactose residues on dinutuximab was further confirmed in the EMA assessment report for dinutuximab ((EMA_EPAR_dinutuximab, 2015), p. 17) as well as a presentation given by the lead author of the dinutuximab clinical trial publications, Prof. Yu, at the 2014 ANR (Advances in Neuroblastoma Association) meeting held in Cologne, Germany (Yu, 2014). On this occasion it was presented that based on the results from COG Study ANBL 0032 performed with Unituxin, the association of total anti- α-Gal antibody levels with ≥ grade 1 allergic reaction is statistically significant.

In contrast, Apeiron has demonstrated with highly sensitive bioanalytical methods, that no Gal1-alpha 3Gal-epitopes are present on several batches of ch14.18/CHO. This result was (partly) expected as CHO cells (like human cells) produce no or only very low levels of terminal Gal1-alpha3Gal-glycoforms in contrast to murine SP2/0 cells (Zhong, 2012).

The results of a head-to-head comparative glycosylation analysis performed on Dinutuximab beta Apeiron (Ref. SR-951.PI) vs Unituxin (batch 2600694) and “NCI-like” material (derived from a fermentation using the initial SP2-0 research cell line as applied for manufacture of clinical ch14.18/SP2-0 material by the NCI) are provided in Table 1, clearly illustrating the differences in the glycosylation pattern of both monoclonal antibodies, Dinutuximab beta Apeiron and Unituxin. Dinutuximab beta Apeiron completely lacks alpha-gal N-glycosylation, whereas Unituxin carries 1.12% alpha-gal determinants.

Table 51:

Table 1: Head-to-head comparison of glycosylation patterns of APN311 vs. Unituxin and ch14.18 derived from a fermentation using the initial SP2-0 research cell line as applied for manufacture of clinical ch14.18/SP2-0 material by the NCI (NCI-like)

Component	APN311 (ch14.18/CHO) SR-951PI (batch GMP-1)	Unituxin (ch14.18/SP2-0) (batch 2600694)	"NCI-like" (ch14.18/SP2/0) (batch 240914B9)
A1	0.36	0.32	-
F(6)A1	0.60	0.71	-
A2	2.90	1.92	1.25
F(6)A2	35.55	58.81	27.50
M5	4.68	3.89	-
A2[6]G(4)1	-	-	0.97
A2[3]G(4)1	-	-	0.97
A2G(4)1	0.38	-	-
F(6)A2[6]G(4)1	29.00	19.44	30.61
F(6)A2[3]G(4)1	9.61	7.47	12.60
F(6)A1G(4)1Ga1	-	0.35	-
M6	1.49	0.90	-
A2G(4)2	-	-	0.69
F(6)A2G(4)1Ga(3)1 isomer	-	0.26	1.17
F(6)A2G(4)2	10.61	4.64	16.71
F(6)A2G(4)1Ga(3)1 isomer	-	-	0.61
M5A1G1	-	0.41	-
M7 D3	1.34	-	-
F(6)A2G(4)2S(3)1	1.91	-	-
M7	-	0.38	-
F(6)A2G(4)2Ga(3)1 isomer	-	0.51	2.42
F(6)A2G(4)2Ga(3)1 isomer	-	-	0.97

Head-to-head batch comparison data on glycosylation patterns performed for different batches of Dinutuximab beta Apeiron T65, T90, T110, GMP2, GMP3, GMP4, GMP5 and reference standard SR-951PI) confirm that the differences in glycosylation are not effects of batch-to-batch variability, and therefore represent unique molecular features of the different antibodies due to the different expression hosts.

In support of the Applicant's claim, an expert opinion provided by Prof. Dr. Uri Galili, who spent major parts of his scientific career in investigating α-gal epitopes and its immunological features, which was acknowledged by the naming "Galili epitopes", is included.

In conclusion, and in line with the expert opinion of Prof. Dr. Galili, the absence of α-gal epitopes is the only guarantee to avoid possible α-gal epitope associated anaphylactic reactions, which may have a fatal outcome for patients. It also may help to reduce the severity and frequency of treatment associated allergic reactions, which could be clearly attributed to be associated with the Unituxin combination therapy and the presence of anti-Gal antibodies by the principal investigator of COG study ANBL0032 (Yu, A.L., ANR, 2014).

Based on the facts listed above, it has been sufficiently demonstrated that the absence of α-gal epitopes in ch14.18/CHO is a specific feature of the utilized CHO production cell line, consistently resulting in differences in critical quality attributes (CQAs).

Discussion on quality aspects

Dinutuximab beta Apeiron (ch14.18/CHO) dinutuximab beta has the **same amino acid sequence as Unituxin** (ch14.18) dinutuximab. The plasmid used for the transfection of CHO cells to generate the RCB and subsequent MCB for dinutuximab beta (Dinutuximab beta Apeiron) was the same as that used previously for the generation of ch14.18-producing murine myeloma cells SP2/0 (ch14.18/SP2/0) and NS0 (ch14.18/NS0) in early development. Therefore, these have the same primary molecular structure.

These products are manufactured according to a different manufacturing process and they have a slightly different glycosylation profile. The difference in expression system and the subsequent difference in the presence/absence of galactose-1,3α-galactose structures (Unituxin 1.12%; Dinutuximab beta Apeiron Not Detectable) are noted, but the absolute amount of Gal-1,3α-Gal structures is considered small. Additional data from the 'NCI-like' material from the ch14.18/SP2-0 cell line is not relevant in this context, since the comparison is only with the product (Unituxin) to be used clinically.

It has been the policy of the CHMP that changes in the manufacturing process of a given product, or differences between the manufacturing processes of two different products (e.g. a biosimilar product and its Reference Medicinal Product) do not require a new INN, New Active Substance (NAS) status, and/or another qualification that the two active substances are somehow different. Such a status is only warranted if the differences in manufacturing processes result in relevant differences between the two products. Therefore, the mere fact that the manufacturing processes of dinutuximab (Unituxin) and dinutuximab beta (Dinutuximab beta Apeiron) are different is not sufficient to grant NAS status.

Conclusions on quality aspects

From a quality perspective, it is accepted that differences in the glycosylation profile exist.

However, the mere fact that the manufacturing processes of dinutuximab-beta (Dinutuximab beta Apeiron) and dinutuximab (Unituxin) are different (leading to differences in the glycosylation profile) is not sufficient to grant NAS status. According to Notice to Applicants Volume 2A, Chapter 1, Annex I, indent 3, a new active substance includes "*a biological substance previously authorised in a medicinal product in the European Union, but differing significantly in properties with regard to safety and/or efficacy which is due to differences in one or a combination of the following: in molecular structure, nature of the source material or manufacturing process".*

2.9.2.2. Non-clinical aspects

Position of the Applicant

Following i.v. application both monoclonal antibodies dinutuximab and dinutuximab beta trigger complement-dependant (CDC) resulting in activation of the complement cascade and lysis of target cells, as well as antibody-dependant cellular cytotoxicity (ADCC), mediated by recruitment of natural killer (NK) cells via the Fc-receptor (CD16, FcγR) interaction of the constant region of the heavy chains and finally also leading to target cell lysis.

However, in contrast to CDC activity, a 10-fold difference was observed with regard to induction of ADCC measured with dinutuximab beta triggering a significantly stronger ADCC activity than ch14.18/SP2/0 and ch14.18/NS0 at the lower concentration range (Zeng 2005). In addition a head-to-head comparison of Unituxin (batch 2600694) and Dinutuximab beta Apeiron (batch 102092; GMP-4) revealed differences

in CD16 (FcγR) and FcRn binding activity. The lower CD16 binding activity may be the reason for lower ADCC activity of Unituxin, although such difference could not finally be proven for the Unituxin batch investigated, which may be a result of the variability of the ADCC assay itself.

Furthermore, Unituxin showed a different FcRn binding kinetic (lower K_a values), compared to Dinutuximab beta Apeiron. Whether this would translate into a clinically meaningful difference in PK parameters is not clear, however these results provide additional evidence of differences in important parameters associated with the mechanism of action of both entities.

The cause for these observations in favour of dinutuximab beta probably is to be found in the different glycosylation patterns of the antibodies as their amino acid sequences/CDRs are identical. A reduction of fucose content within the glycosylation site of antibody Fc domains is reported to result in an increase of ADCC activity (see also Module 2.4, section 2.1.2). This effect is caused by the higher affinity binding of the fucose-reduced antibody to the FcgammaRIIIa receptor on natural killer cells.

In conclusion, glycosylation and differences in the glycosylation profile represent an important part of the chemical structure of biologic molecules. In addition to the differences in the chemical composition/structure, differences in the glycosylation patterns also determine differences in the biologic activity and the safety profile of biologic molecules, which for Dinutuximab beta Apeiron vs Unituxin is demonstrated by respective differences in the safety profile, Dinutuximab beta Apeiron (dinutuximab beta, ch14.18/CHO) is considered to qualify for the New Active Substance status.

Discussion and conclusion on non-clinical aspects

A head-to-head comparison of Dinutuximab beta Apeiron (batch 102092; GMP-4) and Unituxin (batch 2600694) showed differences in CD16 (FcγRIIIa) and FcRn binding activity. The lower CD16 binding activity may be the reason for a lower ADCC activity of Unituxin (reported in the literature, Zeng 2005), although such difference was not proven for the Unituxin batch investigated. A reduction of fucose content within the glycosylation site of antibody Fc domains is reported to result in higher affinity binding of CD16 (FcγRIIIa) and a subsequent increase of ADCC activity. However, no difference in impact on biological activity has been demonstrated.

Dinutuximab beta Apeiron showed different FcRn binding kinetics to Unituxin (lower K_a values for Unituxin). Whether this would translate into a clinically meaningful difference in PK parameters is not established.

There are no meaningful differences between ch14.18/CHO and ch14.18/SP2-0 from a non-clinical point of view.

2.9.2.3. Clinical aspects

Position of the Applicant

The presence of a1.3-Gal structures is commonly known to being associated with the occurrence of treatment related allergic/anaphylactic reactions.

Due to the severity of the potential outcome, which may be fatal, and the rapid onset of anaphylactic reactions, any possibility to reduce their frequency/probability has to be considered to be of significant benefit for patient safety. The frequency of anaphylactic reactions observed in clinical studies performed with APN311 (ch14.18/CHO), are discussed extensively in the D180 response to Q37. In conclusion, the results of a detailed search for anaphylactic reactions, based on a consensus paper driven search algorithm, revealed a potential worst case frequency of anaphylactic reactions of 4.1% (n=148) in the studies APN311-101, APN311-201, APN311-202, and APN311-303. This is in line with a frequency of

3.3% reported from the supportive study APN311-302. Overall, among all studies only 6 patients (i.e. 1%), had a coded anaphylactic reaction/shock, definitely diagnosed by the investigator. For Unituxin the frequency of reported anaphylaxis was reported to be 10% (see EMA EPAR Dinutuximab, 2015, p79). In any case the difference in the reported anaphylactic reactions is magnitudes more beneficial for APN311 vs. Unituxin (10-fold). Even in case of a worst case scenario comparison of potential anaphylactic reactions identified by the implementation of the above mentioned search algorithm, in comparison to the actually reported anaphylactic reactions for Unituxin, the result for APN31 remains significantly better (about 2.5-fold).

However, it cannot be proven that this significant difference can solely be attributed to the presence α- gal epitopes in Unituxin (ch14.18/SP2-0) or if potential synergistic effects of the concomitant GM-CSF administration plays a role.

Discussion on clinical aspects

Cross-study comparison between Unituxin and Dinutuximab beta Apeiron studies is hampered by major uncertainties around AE definition, collection, reporting and analyses. For both products, studies were led and sponsored by clinicians, and generally no standardised methods were applied across studies and centres. Importantly, in the largest trial with Dinutuximab beta Apeiron (study APN311-302), only serious anaphylactic reactions were isolated, as for non-serious reactions, they were all combined under generic toxicity terms (e.g. “allergy” as part as “skin toxicity”).

Nevertheless, cross-study comparisons have been attempted. The overall rate of anaphylactic reactions, using an algorithm in the case of Dinutuximab beta Apeiron (based on preferred terms and concomitant use of adrenaline) is 16/148 (10.8%) in the pooled small trials. In the Unituxin trials, the rate of anaphylactic reaction reports is 176/984 (17.9%) in the pooled population and 28/114 (24.6%) in the pivotal trial ANBL0032 (EMA EPAR Dinutuximab, 2015, p75). Therefore, a very conservative comparison (given the different evaluation method and the small sample for Dinutuximab beta Apeiron) would provide an approximate 2-fold ratio in favour of Dinutuximab beta Apeiron.

When focusing on serious anaphylactic reactions, a comparison of the rates of anaphylactic reactions reported as such by the investigators would provide a 4- to 8-fold ratio in favour of Dinutuximab beta Apeiron. However, when using a conservative estimate in the case of Dinutuximab beta Apeiron (using the same algorithm), the ratio becomes 1.6 in the comparison of the first-line pivotal trial (5.3% vs 3.3%) and 2.7 when comparing the pooled populations (9.5% vs 4.1%), hence again an approximate 2-fold ratio.

Overall, a 2-fold ratio is not is not sufficiently high to conclude on a safety difference given the uncertainties about cross-study comparisons previously highlighted. Moreover, there are other differences between the studies that need to be taken into account as they could have affected the occurrence/frequency of anaphylactic reactions; these include differences in treatment modalities (presence/absence of GM-CSF co-administration, differences in premedication or dosing schedule) and the experience gained with anti-GD2 treatment and management of ADRs. Therefore, the relationship between the reported difference in anaphylactic reactions and the presence/absence of alpha-gal epitopes is not considered established.

Based on the available quality, non-clinical and clinical data, the CHMP considers that dinutuximab beta, which have some differences in molecular structure, nature of the source material or manufacturing process, does not differ significantly in properties with regard to safety and/or efficacy from dinutuximab contained in medicinal product Unituxin previously authorised within the European Union on 14 August 2015 and therefore is not considered to be a new active substance.

2.10. Product information

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.10.2. Additional monitoring

- Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Dinutuximab beta Apeiron (dinutuximab beta) is included in the additional monitoring list as:
- It is a biological product authorised after 1 January 2011;
- It is approved under exceptional circumstances [REG Art 14(8), DIR Art (22)]
- It has a PASS imposed at the time of authorisation; [REG Art 9(4)(cb), DIR Art 21a(b)].

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

The indication as recommended by CHMP is as follows:

Treatment of high-risk neuroblastoma in patients aged 12 months and above, who have previously received induction chemotherapy and achieved at least a partial response, followed by myeloablative therapy and stem cell transplantation, as well as patients with history of relapsed or refractory neuroblastoma, with or without residual disease. Prior to the treatment of relapsed neuroblastoma, any actively progressing disease should be stabilised by other suitable measures.

In patients with a history of relapsed/refractory disease and in patients who have not achieved a complete response after first line therapy, Dinutuximab beta Apeiron should be combined with interleukin 2 (IL 2).

Neuroblastoma is an embryonal tumour of the autonomic nervous system, meaning that the cell of origin is thought to be a developing and incompletely committed precursor cell derived from neural-crest tissues. Neuroblastomas generally occur in very young children; the median age at diagnosis is 17 months. The tumours arise in tissues of the sympathetic nervous system, typically in the adrenal medulla or paraspinal ganglia, and thus can present as mass lesions in the neck, chest, abdomen, or pelvis.

3.1.2. Available therapies and unmet medical need

For high-risk neuroblastomas, the first-line treatment can be divided into three distinct phases:

- induction of remission with intensive chemotherapy. The backbone of the most commonly used induction therapy includes dose-intensive cycles of cisplatin and etoposide alternating with vincristine, cyclophosphamide, and doxorubicin. Topotecan was added to this regimen based on the

anti-neuroblastoma activity seen in relapsed patients. After a response to chemotherapy, resection of the primary tumour is usually attempted.

- consolidation of the remission with myeloablative chemotherapy which attempts to eradicate minimal residual disease using lethal doses of chemotherapy followed rapidly by rescue with autologous hematopoietic progenitor cells to repopulate the bone marrow.
- and finally a maintenance phase used to treat potential minimal residual disease (MRD) following HSCT to reduce the risk of relapse, e.g. with ch14.18 (dinutuximab) and isotretinoin, a molecule that induces terminal differentiation of neuroblastoma cell lines.

In the recurrent setting, treatment has evolved in the recent era and comprises salvage chemotherapy, radiotherapy and surgery, ^{131}I-MIBG therapy, and ch14.18 monoclonal antibody therapy with aldesleukin-2 (IL-2) and oral isotretinoin (13-cis RA).

Therefore, in both the first-line and relapsed/refractory settings, ch14.18 has become standard of care to treat residual disease.

3.1.3. Main clinical studies

The main studies are:

- Study APN311-303, a retrospective data collection from the compassionate use of the product in a single centre where a heterogeneous population of patients with neuroblastoma in the relapsed/refractory setting was treated.

- Study APN311-302, a randomised controlled Phase III trial comparing the effect of IL-2 added to the combination of ch14.18 and 13-cis RA after first-line therapy.

A supportive phase I/II study evaluating ch14.18 /CHO administered with IL-2 in patients with relapsed/refractory neuroblastoma has also been submitted (Study APN311-202).

3.2. Favourable effects

Relapsed/refractory setting

For the total population of studies APN311-303 and 202, the overall response (CR+PR) at the end of treatment (i.e. approximately 6 to 8 months after treatment initiation or earlier in case of progressive disease) was 26/72 (36%; 95%CI [25, 48]) in patients with detectable disease, out of whom 9 (13%) had a CR, while disease had progressed in 29/72 patients (40%; 95%CI [29, 52]). In patients with R/R disease, the ORR was 42% (14/33) in study APN311-202 and 28% (10/36) in study APN311-303 but given the small numbers, confidence intervals are largely overlapping.

At 1-year, event-free survival (EFS) was 52% in both studies and overall survival (OS) was 89% and 92%, respectively. At 2-year, EFS was lower in study APN311-303 (35%) than in study APN311-202 (47%) while the opposite trend was observed for OS: 75% and 63%, respectively.

First-line setting

Study APN311-302 provided survival data with and without the addition of IL-2. The 3-year EFS (primary endpoint) showed rates of 55% without IL-2 and 61% with IL-2 while the 3-year OS rates were 64% and 69%, respectively.

3.3. Uncertainties and limitations about favourable effects

The evaluation of drug exposure and its potential impact on efficacy is not considered robust. Measurement of ch14.18/CHO concentrations has been conducted at different analytical centres using different dilutions, capturing antibodies; interference/matrix effects have been observed with all applied methods and as such comparison of serum APN311 levels between (and even within) studies is uncertain and this hampers the PopPK analysis and investigation of exposure-effect relationships.

Due to poor drug tolerance of the new validated HACA assay, the immunogenicity data are not considered reliable. The Applicant's analyses of the impact of HACAS on PD, efficacy and safety can only be considered exploratory. Extensive work is requested post-authorisation (see Annex II) to further characterise the PK and immunogenicity profile of the product; in particular, since the product is highly toxic, the benefit of completing all treatment cycles after developing HACAs should be investigated.

The OS data currently available are immature and are only reliable for up to 2 years in the R/R studies and 3 years for the first-line study. Updated survival data to confirm the effectiveness of immunotherapy are requested post-authorisation (see Annex II).

There is still some level of uncertainty about the add-on effect of IL-2. It is agreed that the differences in EFS and OS reported in study APN311-302 were small although the rates in the +IL-2 arm tended to be (numerically) higher than the rates in the –IL-2 arm, in particular at the later time points. This would indicate that there is no or only limited added benefit of the addition of IL-2 to the treatment with APN311 in the first-line setting without residual disease. Whether this is also true for patients with residual disease is doubtful based on the APN311-302 study results. In a post-hoc analysis, a numerical difference (8-9%) in 3-year survival rates was reported for patients with evidence of disease before immunotherapy in contrast to those without, i.e. with complete response to previous therapies. Importantly also, the additive effect of Il-2 is not known for patients with relapsed/refractory disease as the effect of omitting IL-2 in the treatment has not been studied in this population. Therefore, the MAH should provide the results of the Phase II study comparing Dinutuximab beta Apeiron monotherapy vs combination with IL-2 in the R/R setting (see Annex II).

3.4. Unfavourable effects

In the two studies using the dosing regimen with continuous infusion in combination with IL-2 and 13-cis RA, all 98 patients experienced treatment-emergent AEs; the most frequent were pyrexia, pain at various sites (especially abdominal and extremities), gastrointestinal (constipation, vomiting, diarrhoea), cutaneous (pruritus, dry skin, rash, urticaria), haematological (anaemia, neutropaenia, thrombocytopaenia), abnormal liver function tests (ALT, GGT), capillary leak syndrome (with oedema, increased weight), hypotension, tachycardia, and cough (possibly allergic).

Most patients experienced at least one severe (grade 3/4) AE (94%) in the compassionate use programme although out of 3700 TEAEs, only 12% were grade 3 and 1% grade 4, which indicates that this treatment is associated with significant toxicity. The most frequent severe AEs were pain, abnormal haematological and liver function tests, pyrexia, infections, allergic reactions and capillary leak syndrome. Most severe and serious AEs were considered related to the treatment.

Despite the regimen of a continuous infusion over 10 days, severe pain (score ≥ 7) was reported by one third to half of the patients, although its incidence decreased over the cycles: from approximately 90% in cycle 1 to 60% in cycle 5. Simultaneously, the proportion of patients given iv morphine decreased but, much more in study APN311-303 (from 96 to 11%) than in study -202 (from 100 to 72%). In those patients receiving morphine, the mean cumulative morphine dose was approximately halved by cycle 5 in both studies.

Currently, in the whole database (514 patients), four deaths could be considered as treatment-related and resulted from a capillary leak syndrome, an acute respiratory distress syndrome (possibly related to an anaphylactic reaction), an herpes encephalitis (probably related to the prior allogeneic stem cell transplantation), and septic shock likely to be device-related in an outpatient.

The safety data generated in study APN311-302 enable to evaluate the additional toxicity of IL-2 combined with ch14.18/CHO. The most important difference relates to SAEs, which were reported in 46% of the patients receiving IL-2 compared to 27% of the patients not receiving IL-2. The addition of IL-2 doubled the occurrence of CLS (from 25% to 50%) although only 10% were severe. This translated into a higher discontinuation rate due to toxicity: 17% vs 6%. Therefore, a safety benefit is notable for monotherapy over the combination with IL-2.

3.5. Uncertainties and limitations about unfavourable effects

The evaluation of safety is hampered by the absence of control arms without ch14.18 in the trials and by the heterogeneity of data collection across the academic trials. However, the safety profile of anti-GD2 monoclonal antibodies is already known to a large extent and the available data are largely in line with current knowledge.

Reliable immunogenicity data have not been provided due to bioanalytical issues. There were no consistent relationships between ADA positivity and efficacy measure response, EFS and OS between studies. Neither was there a correlation between ADA positivity and TEAE. As indicated by the Applicant, the current analyses are considered exploratory only and further research will be conducted to enable conclusive interpretations (see Annex II).

No long term safety data are currently available but this will be addressed with a post-authorisation drug registry (see Annex II).

3.6. Effects Table

Table 52: Effects Table for Dinutuximab beta Apeiron in the treatment of refractory/relapsed neuroblastoma (data cut-off: 2014 or 2015)

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References Studies
Favourable Effects						
Anti-tumour response	CR + PR	N (%) % (CI)	12/39 (31) 31/33 (42) 36 (25; 48)	None	No control Limited information on salvage therapies	APN311-303 APN311-202 Pooled 303+202
Event-free survival	At 2 years	%	35 47	None	No control Limited information on prognostic factors	APN311-303 APN311-202
Overall survival In relapsed patients	At 2 years	%	75 63 60	 Historical control 31 46	 Some imbalance in MYCN status favouring APN311	APN311-303 APN311-202 Pooled 303+202 Ital controls R1 controls
Overall survival In first-line	At 3 years	%	 71	Historical control 59	 Main prognostic factors matched	APN311-302 R1 controls

Effect	Short Description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References Studies
Unfavourable Effects						
SAEs		N (%)	12/54 (22) 26/44 (59)		Unexplained discrepancy between studies 6% of the patients discontinued treatment due to ADRs	APN311-303 APN311-202
Pain	Patients with pain (parent assessment)	N (%)	Cycle 1 49/54 (91) 21/24 (88) Cycle 5 22/38 (58) 10/17 (59)		Sub-study	APN311-303 APN311-202 APN311-303 APN311-202
	Pain-related events	N (%)	Cycle 1 51/54 (94) 29/44 (66) Cycle 5 13/39 (33) 9/29 (31)			APN311-303 APN311-202 APN311-303 APN311-202
	Patients receiving iv morphine	%	Cycle 1: 96 100 Cycle 5: 11 72		Difficulties in comparing with short infusions due to different methodology of evaluation Notable differences between the two studies in the last cycles	APN311-303 APN311-202 APN311-303 APN311-202
Infusion/allergic reactions	Hypotension Hypoxia/Resp distress Urticaria Bronchospasm	%	63 45 44 43 24 23 11 14			APN311-303 APN311-202 APN311-303 APN311-202 APN311-303 APN311-202 APN311-303 APN311-202
Cytokine release syndrome	Incidence	%	56 36			APN311-303 APN311-202
Capillary leak syndrome	Incidence	%	83 36		Unexplained discrepancy between studies	APN311-303 APN311-202
Human anti-chimeric antibodies	Incidence	%	56 64		Unreliable results due to bioanalytical issues	APN311-303 APN311-202

Abbreviations: CR: complete response; PR: partial response; HACA: human anti-chimeric antibody

Historical controls: Italian Neuroblastoma Registry (Garaventa et al, 2009) and SIOPEN high risk neuroblastoma study (HRNBL1) in an early phase (R1)

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Advances in neuroblastoma treatment over the last three decades have resulted in ability to cure over 70% of children using risk-directed therapeutic approaches. However, until very recently, the outcome of high-risk neuroblastoma, especially in children older than 18 months with metastatic disease, remained poor. Despite intensive multimodal therapy, over 50% of patients with high-risk neuroblastoma relapsed with a dismal long-term outcome. In recent years, they have been offered a wide variety of salvage

treatments following disease recurrence. Up to half of these patients achieved some response or stable disease, and survival after relapse was longer in patients who had received salvage therapy. In an INRG analysis of 2,266 patients who experienced first progression/relapse, the median time to relapse was 13 months, and 5-year OS from the time of first relapse was 20% (London & Castel et al, 2011). The longer survival after relapse is also likely due to early detection of disease recurrence as a result of employing more sophisticated surveillance studies in recent years. Many of the factors at diagnosis that are prognostic of survival also influence survival after disease progression or relapse. In addition, time to first relapse is a significant adverse factor for survival.

Therefore, for patients categorized as high risk disease and for those with low or intermediate risk disease that do not respond or have relapsed on appropriate front-line treatment, there is still an unmet medical need.

As anti-GD2 became standard practice after the publication of the ECOG trial in 2009, it became very difficult for the Applicant to conduct randomised studies.

The efficacy of Dinutuximab beta Apeiron is supported by anti-tumour response at the end of the treatment cycles and overall survival compared to historical controls. While none of these outcomes on its own would be sufficient to establish efficacy, it is considered that, by the combination of the provided evidence (early tumour response to treatment and long-term survival data), the efficacy of APN311 is made plausible, even though the exact effect size is not fully known.

Ch14.18 and IL-2 is a regimen with well-known severe toxicities, the management of which requires a considerable level of medication. Pain, hypersensitivity reactions and capillary leak syndrome are major adverse events associated with anti-GD2 therapies. However, the safety profile is comparable to other anti-GD2 treatment.

3.7.2. Balance of benefits and risks

The overall B/R of Dinutuximab beta Apeiron for the

"Treatment of high-risk neuroblastoma in patients aged 12 months and above, who have previously received induction chemotherapy and achieved at least a partial response, followed by myeloablative therapy and stem cell transplantation, as well as patients with history of relapsed or refractory neuroblastoma, with or without residual disease. Prior to the treatment of relapsed neuroblastoma, any actively progressing disease should be stabilised by other suitable measures.

In patients with a history of relapsed/refractory disease and in patients who have not achieved a complete response after first line therapy, Dinutuximab beta Apeiron should be combined with interleukin 2 (IL 2)."

is positive.

First-line indication

All patients included in study APN311-302 were treated with immunotherapy. In total 370 patients were enrolled. A comparison to an historical control group obtained from study HRNBL1-R1 showed that the percentage of patients that were still alive after 3 year of follow up was 12 % (59% vs 71%, for MAT and MAT+ immunotherapy respectively) higher after APN311 treatment than for patients who did not receive immunotherapy. Altogether, it is considered that in this population, immunotherapy increases survival of these patients, and therefore, the benefit-risk balance of APN311 for this patient population is positive. However, for patients with residual disease after previous therapies, available data suggest that the combination to IL-2 may be of benefit, and therefore, should be recommended in these patients.

Relapse / Refractory setting

For patients with a history of relapsed disease, two main studies were submitted. Both studies were single arm studies and included patients who had a history of relapsed disease but had CR or VGPR/PR/SD on latest treatment; patients with PD were excluded from the study.

Two cohorts of controls were identified by the Applicant in an Italian Registry and in the earlier phase (R1 – 2002-2010) of the SIOPEN High Risk Neuroblastoma Study (HRNBL1) before immunotherapy was introduced.

Comparative analyses have been conducted between these controls and individual APN311 studies as well as pooled studies (for the relapse setting). All these analyses point out in the same direction, i.e. showing that immunotherapy improves survival in relapsed patients. The replication of the results provides reassurance about the treatment benefit even if its extent cannot be accurately quantified.

In the R/R setting, anti-tumour activity has been evaluated in patients with residual disease after salvage therapy. In a pooled analysis of the two APN311 studies, a response rate of 36% is achieved at the end of treatment, with 13% of complete response.

In the absence of a randomised controlled trial, none of these outcomes on its own would be sufficient to establish efficacy. However, it is considered that taken together, early anti-tumour response and long-term survival provide plausible evidence of efficacy.

As the combination to IL-2 has been used in both trials, it is recommended in this setting before new data become available.

As safety and efficacy has not been established in patients less than 12 months of age (although patients in this age category are likely to be rare), the indication is limited to patients above this age.

The CHMP furthermore considered that Dinutuximab beta Apeiron should be restricted to hospital-use only and must be administered under the supervision of a physician experienced in the use of oncological therapies. It must also be administered by a healthcare professional in an environment where full resuscitation services are immediately available.

3.7.3. Additional considerations on the benefit-risk balance

Marketing authorisation under exceptional circumstances

Taking into account all available data, the CHMP considers that the benefit-risk balance of Dinutuximab beta Apeiron in the treatment of high-risk neuroblastoma is positive.

However, the CHMP is of the view that the data set on the clinical efficacy and safety of Dinutuximab beta Apeiron cannot be considered comprehensive.

The CHMP agreed that it was not feasible to produce comprehensive data under normal conditions of use of Dinutuximab beta Apeiron due to ethical considerations preventing the conduct of a randomised placebo-controlled trial. In addition, a comparative trial against Unituxin (the only acceptable potential active control) is not expected to be feasible given the withdrawal of the marketing authorisation. In addition, it is unlikely that patients would be willing to participate to a study comparing Unituxin to dinutuximab beta.

The CHMP is therefore of the view that a marketing authorisation under exceptional circumstances should be granted subject to a number of specific obligations:

- In order to further investigate the benefits of Dinutuximab beta Apeiron in the treatment of high-risk neuroblastoma a drug registry (SAFARY) has been proposed by the Applicant. The objective is to collect more data on pain and its management, effect on peripheral and central

nervous system, including visual impairment, long-term safety as well as short- and long-term effectiveness. Annual reports should be submitted as part of the annual re-assessment.

- A PK/PD and immunogenicity evaluation should be conducted in order to better define the posology in children over the entire age range and the impact of HACAs on PD, efficacy and safety. This will be based on an analysis of plasma samples collected from patients in studies APN311-202v1-2-3 and APN311-304 according to an agreed protocol. Fully validated analytical drug and HACA assays that cover the time course of exposure should be used for this evaluation. When submitting these data, the Applicant should provide further evidence of the robustness of the drug assay and of the drug tolerance of the binding and neutralising HACA assays.
- In order to evaluate the add-on effect of IL-2 in patients with high risk relapsed/refractory neuroblastoma, the MAH will submit the results of study APN311-202v3.
- In order to further estimate the long-term survival effect of dinutuximab beta, the MAH will submit at least 5-year survival data for patients included in studies APN311-202 and APN311-302.

Data from these studies should be generated on a regular basis for review in the context of the annual re-assessments.

Therefore, recommending a marketing authorisation under exceptional circumstances is considered appropriate.

3.8. Conclusions

The overall B/R of Dinutuximab beta Apeiron is positive.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Dinutuximab beta Apeiron is favourable in the following indication:

"Treatment of high-risk neuroblastoma in patients aged 12 months and above, who have previously received induction chemotherapy and achieved at least a partial response, followed by myeloablative therapy and stem cell transplantation, as well as patients with history of relapsed or refractory neuroblastoma, with or without residual disease. Prior to the treatment of relapsed neuroblastoma, any actively progressing disease should be stabilised by other suitable measures.

In patients with a history of relapsed/refractory disease and in patients who have not achieved a complete response after first line therapy, Dinutuximab beta Apeiron should be combined with interleukin-2 (IL22)."

The CHMP therefore recommends the granting of the marketing authorisation under exceptional circumstances subject to the following conditions:

Other conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Specific Obligation to complete post-authorisation measures for the marketing authorisation under exceptional circumstances

This being an approval under exceptional circumstances and pursuant to Article 14(8) of Regulation (EC) No 726/2004, the MAH shall conduct, within the stated timeframe, the following measures:

Description	**Due date**
Non-interventional post-authorisation safety study (PASS): In order to collect data on pain and its management, effect on peripheral and central nervous system, including visual impairment, long-term safety and long-term effectiveness, the MAH should submit the results of a study based on data deriving from a registry of patients with high risk neuroblastoma.	Annual reports to be submitted
In order to better define the posology in children over the entire age range and the impact of HACAs on PD, efficacy and safety, the MAH will submit the results of an evaluation of plasma samples collected from patients in studies APN311-202v1-2-3 and APN311-304 according to an agreed protocol.	31 December 2019
In order to evaluate the add-on effect of IL-2 in patients with relapsed refractory neuroblastoma, the MAH will submit the results of study APN311-202v3.	31 December 2021
In order to evaluate the long-term survival effect of dinutuximab beta, the MAH will submit at least 5-year survival data for patients included in studies APN311-202 and APN311-302.	31 December 2021

New Active Substance Status

Based on the review of the available data, the CHMP considers that dinutuximab beta, which have some differences in molecular structure, nature of the source material or manufacturing process, does not differ

significantly in properties with regard to safety and/or efficacy from dinutuximab contained in medicinal product previously authorised within the European Union and therefore is not considered to be a new active substance.

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0094/2014 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.