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EMA/CHMP/408316/2015
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Unituxin

International non-proprietary name: dinutuximab

Procedure No. EMEA/H/C/002800/0000

Note

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

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List of abbreviations

aa	Amino acid
AAA	Amino acid analysis
ADCC	Antibody dependent cellular cytotoxicity
AdEERS	Adverse Event Expedited Reporting System
ADME	Absorption, Distribution, Metabolism and Excretion
AE	Adverse event
alpha-gal	galactose alpha-1,3-galactose
ALT	Alanine aminotransferase
APC	Absolute phagocyte count
ASAEL	Agent specific adverse event list
ASCT	Autologous stem cell transplantation
AST	Aspartate aminotransferase
ATRA	All-trans-retinoic acid
AUC	Area under the curve
BI	Biological indicator
BUN	Blood urea nitrogen
CAEPR	Comprehensive Adverse Event and Potential Risks
CBC	Complete blood count
CCOP	Clinical Trials for Cooperative Groups
CD	Circular dichroism
CDC	Complement-dependent cytotoxicity
CDR	Complementarity determining regions
CE	Capillary electrophoresis
CFU	Colony forming unit
ch14.18	Chimeric 14.18
CHGA	Chromogranin
CHMP	Committee for Medicinal Products for Human Use
CHO	Chinese Hamster Ovary
CI	Confidence interval
cIEF	Capillary isoelectric focussing
Cmax	Maximum peak concentration
CNS	Central nervous system
COG	Children's Oncology Group
CPP	Critical Process Parameter
CQA	Critical Quality Attribute
CR	Complete response
CRADA	Cooperative Research and Development Agreement
CT	Computed tomography
CTCAE	Common Toxicity Criteria for Adverse Events
CTM	Clinical Trial Material
CTSU	Clinical Trials Support Unit
Da	Dalton
DARF	Drug accountability record form
DBP	Diastolic blood pressure
DCTD	Division of Cancer Treatment and Diagnosis
DCX	Doublecortin
DDC	Dopadecarboxylase
DIC	Disseminated intravascular coagulation
DNA	Deoxyribonucleic Acid
DO	Dissolved oxygen
DoE	Design of Experiment
DP	Drug product
DS	Drug substance
DSMC	Data Safety Monitoring Committee
ECG	Electrocardiogram
ECHO	Echocardiogram
eCRF	Electronic case report form
EFS	Event-free survival

ELISA	Enzyme-linked immunosorbent assay
EM(E)A	European Medicines Agency
EP	European Pharmacopoeia
EPCB	End of Production Cell Bank
FBS	Fetal Bovine serum
FDA	Food and Drug Administration
FDG-PET	fluoro-2-deoxy-D-glucose positron emission tomography
FEV1	Forced expiratory volume in one second
FHO	Fleming-Harrington-O'Brien
FMEA	Failure modes and effect analysis
FTIR	Fourier transformed infrared spectroscopy
FVC	Forced vital capacity
G0F	Glycan with core fucose and no galactose
G1F.3	Glycan with core fucose and one galactose (α1,3 linkage)
G1F.6	Glycan with core fucose and one galactose (α1,6 linkage)
G2F	Glycan with core fucose and two terminal galactose
GC	Gas chromatography
GCP	Good clinical practice
GD2	Disialoganglioside
GFR	Glomerular filtration rate
GGT	Gamma-glutamyl transferase
GM-CSF	Granulocyte macrophage colony-stimulating factor
GMP	Good Manufacturing Practice
HACA	Human anti-chimeric antibody
HAMA	Human anti-mouse antibody
HC	Heavy chain
HCP	Host Cell Protein
HILIC	Hydrophilic interaction liquid chromatography
HMW	High molecular weight
HPLC	High performance liquid chromatography
hr	Hour
HR	Heart rate
HRQOL	Health Related Quality of Life
HVA	Homovanillic acid
IC	Immunocytology
ICF	Informed consent form
ICH	International Conference on Harmonisation
IEF	Isoelectric focussing
IgG1	Immunoglobulin G, subtype 1
IL-2	Interleukin-2
IND	Investigational New Drug Application
INRC	International Neuroblastoma Response Criteria
INSS	International Neuroblastoma Staging System
IRB	Institutional Review Board
ITT	Intent to Treat
IV	Intravenous
IVIG	Intravenous immunoglobulin
IVP	Intravenous push
kg	Kilogram
L	Litre
LAK	Lymphokine-activated killer
LC	Light chain
LD	Lan-DeMets
LDH	Lactate dehydrogenase
LMW	Low molecular weight
LOEL	Lowest Observed Effect Level
LRV	Log_{10} reduction value
mAb	Monoclonal antibody
MAGE-1	Melanoma antigen-1
Man 5	Mannose 5
max	maximum
MCB	Master Cell Bank

mcg	Microgram
MedDRA	Medical Dictionary for Regulatory Activities
MFI	Microflow imaging
mg	Milligram
MIBG	Meta-iodobenzylguanidine
min	minimum
mL	Millilitre
mM	Millimolar
MR	Mixed response
MRD	Minimal residual disease
MRI	Magnetic resonance imaging
MS	Mass spectrometry
MTD	Maximum tolerated dose
MUGA	Multiple gated acquisition scan
MVM	Minute virus of mouse
n.r.	Not reported
N/A	Not applicable
NANA	N-acetyl neuraminic acid
NCI	National Cancer Institute
NGNA	N-glycolyl neuraminic acid
NK	Natural killer cells
NLT	Not less than
NMT	Not more than
NOEL	No Observed Effect Level
NOS	Not otherwise specified
NS	Normal saline
NSC	National Service Center
OD	Optical density
OS	Overall survival
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PD	Progressive disease
PE	Physical examination
PFT	Pulmonary function test
PGP 9.5	Protein gene product 9.5
Ph. Eur.	European Pharmacopoeia
PHOX2B	Paired-like homeobox 2B
pI	Isoelectric point
PIP	Paediatric Investigational Plan
PK	Pharmacokinetic
PR	Partial response
QARC	Quality Assurance Review Center
QC	Quality Control
QP	Qualified Person
RA	Isotretinoin, cis-retinoic acid
Rt	Retention time
RT-PCR	Reverse transcriptase polymerase chain reaction
S/D	Solvent/detergent
SAE	Serious adverse event
SAIC	Science Applications International Corporation
SBP	Systolic blood pressure
SC	Subcutaneous
SCr	Serum creatinine
SD	Standard deviation
SD	Stable disease
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SE-HPLC	Size-exclusion HPLC
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SmPC	Summary of product characteristics
SpO2	Oxygen saturation
SPR	Surface plasmon resonance

TEM	Transmission electron microscopy
TH	Tyrosine hydroxylase
TLDA	Taqman® low density array
TNBP	Tributyl phosphate
TSH	Thyroid stimulating hormone
t1/2α	Distribution half-life
t1/2β	Terminal elimination half-life
TTC	Threshold of Toxicological Concern
ULN	Upper limit of normal
USP	United States Pharmacopoeia
UTC	United Therapeutics Corporation
UV	Ultraviolet
VGPR	Very good partial response
VMA	Vanillyl-mandelic acid
WBC	White blood cell count
WCX	Weak cation exchange
WFI	Water for injection
wv	Working volume
µL	Microlitre

1. Background information on the procedure

1.1. Submission of the dossier

The applicant United Therapeutics Europe Ltd submitted on 5 December 2013 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Unituxin, 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 20 September 2012.

Unituxin, was designated as an orphan medicinal product EU/3/11/879 on 21 June 2011. Unituxin was designated as an orphan medicinal product in the following indication: Treatment of neuroblastoma

The applicant applied for the following indication: Unituxin is indicated in infants, children, and adolescents aged 11 months to 17 years for the treatment of high-risk neuroblastoma, following myeloablative therapy and autologous stem cell transplant (ASCT), in combination with granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-2 (IL-2), and isotretinoin.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Unituxin 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/Rare disease designations.

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 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/0208/2013, on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0208/2013 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 not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Applicant's request(s) for consideration

New active Substance status

The applicant requested the active substance dinutuximab, United Therapeutics contained in the above medicinal product to be considered as a new active substance in itself, as the applicant claims that it is not a constituent of a product previously authorised within the Union.

Protocol Assistance

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

Licensing status

A new application was filed in the following countries: USA

The product was not licensed in any country at the time of submission of the application.

1.2. Manufacturers

Manufacturer of the active substance

United Therapeutics Corporation
1040 Spring Street
Silver Spring, Maryland 20910
USA

Manufacturer responsible for batch release

Penn Pharmaceutical Services Limited
23-24 Tafarbaubach Industrial Estate, Tredegar, Gwent
NP22 3AA
United Kingdom

1.3. Steps taken for the assessment of the product

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

Rapporteur: Robert James Hemmings Co-Rapporteur: Joseph Emmerich

- The application was received by the EMA on 5 December 2013.
- The procedure started on 26 December 2013.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 14 April 2014. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 13 April 2014.
- During the meeting on 10 April 2014 the Pharmacovigilance Risk Assessment Committee (PRAC) adopted the PRAC Advice on the submitted Risk Management Plan.
- During the meeting on 25 April 2014, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 25 April 2014.
- On 8 May 2014, the Applicant requested an additional 3-month clock-stop to provide responses to the consolidated List of Questions.
- The summary report of the inspection carried out at the following sites Seattle Children's (USA), National Cancer Institute (USA) and The Children's Mercy Hospital (USA) between 18 July and 07 August 2014 was issued on 18 August 2014.

- The applicant submitted the responses to the CHMP consolidated List of Questions on 19 November 2014.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 31 December 2014.
- During the meeting on 9 January 2015 the Pharmacovigilance Risk Assessment Committee (PRAC) adopted the PRAC Advice on the submitted Risk Management Plan.
- During the CHMP meeting on 19-22 January 2015, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant.
- On 16 February 2015, the Applicant requested an additional one month clock-stop to provide responses to the list of outstanding issues.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 17 April 2015.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CHMP members on 6 May2015.
- During the meeting on 7 May 2015 the Pharmacovigilance Risk Assessment Committee (PRAC) adopted the PRAC Advice on the submitted Risk Management Plan.
- During the meeting on 18-21 May 2015, 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 Unituxin.

2. Scientific discussion

2.1. Introduction

Problem statement

Short description of the disease

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. As may be expected with a disease of developing 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. 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.

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).

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).

Current therapy

Current treatment for high-risk neuroblastomas 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[1]. Topotecan was added to this regimen based on the anti-neuroblastoma activity seen in relapsed patients[2]. Response to therapy at the end of induction chemotherapy correlates with EFS at the completion of high-risk therapy[3]. 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. Randomized controlled studies have shown an improvement in 3-year EFS for HSCT (31% to 47%) versus conventional chemotherapy (22% to 31%)[4][5][6].
- and finally a maintenance phase used to treat potential minimal residual disease following HSCT to reduce the risk of relapse[7], e.g. with isotretinoin, a molecule that induces terminal differentiation of neuroblastoma cell lines; this therapy had been shown to increase the event-free survival from 29% to 46% at 3 years. Overall survival was not significantly different between the two groups, with 3-year estimates for patients assigned to isotretinoin and no further therapy of 56±6 % and 50±6 %, respectively[4].

There is still a high unmet medical need in this category of young patients for more strategies able to eradicate residual neuroblastoma cells at the completion of cytotoxic therapy.

About the product

Dinutuximab (also referred to as ch14.18) is a chimeric human/mouse monoclonal antibody (mAb), produced in a murine myeloma cell (SP2/0 hybridoma cell), that 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).

Mode of action

Disialoganglioside is a surface glycolipid antigen that is normally found on neurons, peripheral pain fibres, and skin melanocytes; it is ubiquitously expressed on most neuroblastoma tumours regardless of disease stage and is abundant on the cell surface, providing a tractable target for passive immunotherapy approaches. It is also expressed by some melanomas, brain tumours, small cell carcinomas of the lung, and some sarcomas.

[1] Kushner BH, LaQuaglia MP, Bonilla MA, et al.: Highly effective induction therapy for stage 4 neuroblastoma in children over 1 year of age. J Clin Oncol 12 (12): 2607-13, 1994.
[2] Park JR, Scott JR, Stewart CF, et al.: Pilot induction regimen incorporating pharmacokinetically guided topotecan for treatment of newly diagnosed high-risk neuroblastoma: a Children's Oncology Group study. J Clin Oncol 29 (33): 4351-7, 2011.
[3] Cheung NK, Heller G, Kushner BH, et al.: Stage IV neuroblastoma more than 1 year of age at diagnosis: major response to chemotherapy and survival durations correlated strongly with dose intensity. Prog Clin Biol Res 366: 567-73, 1991.
[4] Matthay KK, Villablanca JG, Seeger RC, et al.: Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. Children's Cancer Group. N Engl J Med 341 (16): 1165-73, 1999.
[5] Berthold F, Boos J, Burdach S, et al.: Myeloablative megatherapy with autologous stem-cell rescue versus oral maintenance chemotherapy as consolidation treatment in patients with high-risk neuroblastoma: a randomised controlled trial. Lancet Oncol 6 (9): 649-58, 2005.
[6] Pritchard J, Cotterill SJ, Germond SM, et al.: High dose melphalan in the treatment of advanced neuroblastoma: results of a randomised trial (ENSG-1) by the European Neuroblastoma Study Group. Pediatr Blood Cancer 44 (4): 348-57, 2005.
[7] Matthay KK, Reynolds CP, Seeger RC, et al.: Long-term results for children with high-risk neuroblastoma treated on a randomized trial of myeloablative therapy followed by 13-cis-retinoic acid: a children's oncology group study. J Clin Oncol 27 (7): 1007-13, 2009.

Dinutuximab reacts specifically with GD2 and has been shown to induce both antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) *in vitro* and has been found to mediate the lysis of several neuroblastoma cell lines in a dose-dependent manner. It may also prevent attachment of circulating malignant cells to the extracellular matrix.

Claimed indication and recommendation for use and posology

The applicant applied for the following indication: treatment of high-risk neuroblastoma, following myeloablative therapy and autologous stem cell transplant (ASCT), in combination with granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-2 (IL-2) and isotretinoin.

The final indication following CHMP review of this application is:

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). Unituxin is administered in combination with granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-2 (IL-2), and isotretinoin.

Unituxin is restricted to hospital-use only and must be administered under the supervision of a physician experienced in the use of oncological therapies. It must be administered by a healthcare professional prepared to manage severe allergic reactions including anaphylaxis in an environment where full resuscitation services are immediately available.

Unituxin is intended to be administered by intravenous (IV) infusion for four consecutive days during five monthly courses at a daily dosage of 17.5 mg/m^2. Unituxin is presented as a concentrate for solution for infusion, with the active substance dinutuximab at 3.5 mg/mL in a clear, Type I glass vial (5mL) with a latex-free bromobutyl rubber stopper and 20mm aluminium flip-off overseal. The proposed shelf life is 18 months at 2-8°C, to be used immediately after dilution.

The treatment regimen consists of dinutuximab, GM-CSF, IL-2, and isotretinoin, administered over six consecutive courses. The complete dosing regimen is outlined in Table 2

Table 1: Courses 1, 3, and 5 dosing schedule for Unituxin, GM-CSF and isotretinoin

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15-24
GM-CSF[1]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Dinutuximab[2]				X	X	X	X								
Isotretinoin[3]											X	X	X	X	X

[1.] Granulocyte macrophage colony-stimulating factor (GM-CSF): 250 µg/m^2/day, administered by either subcutaneous injection (strongly recommended) or intravenous infusion over 2 hours.
[2.] Dinutuximab: 17.5 mg/m^2/day, administered by intravenous infusion over 10–20 hours.
[3.] Isotretinoin: for body weight greater than 12 kg: 80 mg/m^2 administered orally twice daily for a total dose of 160 mg/m^2/day; for body weight up to 12 kg: 2.67 mg/kg administered orally twice daily for a total daily dose of 5.33 mg/kg/day (round dose up to nearest 10 mg).

Table 2: Courses 2 and 4 dosing schedule for Unituxin and IL-2; Courses 2, 4, and 6 dosing schedule for isotretinoin

Day	1	2	3	4	5	6	7	8	9	10	11	12-14	15-28
IL-2[1]	X	X	X	X				X	X	X	X		
Dinutuximab[2]								X	X	X	X		
Isotretinoin[3]													X

[1.] Interleukin-2 (IL-2): 3 MIU/m^2/day administered by continuous intravenous infusion over 96 hours on Days 1-4 and4.5 MIU/m^2/day on Days 8-11.
[2.] Dinutuximab: 17.5 mg/m^2/day, administered by intravenous infusion over 10-20 hours.
[3.] Isotretinoin: for body weight greater than 12 kg: 80 mg/m^2 administered orally twice daily for a total dose of 160 mg/m^2/day; for body weight up to 12 kg: 2.67 mg/kg administered orally twice daily for a total daily dose of 5.33 mg/kg/day (round dose up to nearest 10 mg).

Unituxin should not be administered as an intravenous push or bolus. It should be administered by intravenous infusion over 10 hours. The infusion is started at a dose rate of 0.875 mg/m2/h and continued at this rate for 30 minutes; the rate is then increased to 1.75 mg/m2/h and continued at this rate for the remainder of the infusion, if tolerated. The infusion duration may be extended up to 20 hours to help minimise reactions during infusion (see sections 4.4 and 4.8) that do not respond adequately to other supportive measures. The infusion must be terminated after 20 hours, even if the full dose cannot be delivered within this timeframe.

Prior to starting each treatment course, refer to Table 3 for a list of criteria that must be evaluated.

Table 3: Clinical criteria that must be evaluated prior to the start of each treatment course of Unituxin

Central nervous system (CNS) toxicity
• Delay course initiation until CNS toxicity is Grade 1 or resolved and/or seizure disorder is well controlled
Hepatic dysfunction
• Delay initiation of first course until alanine aminotransferase (ALT) is less than 5 times upper limit of normal (ULN). Delay initiation of courses 2-6 until ALT is less than 10 times ULN.
Thrombocytopenia
• Delay course initiation until platelet count is at least 20,000/µL. • If patient has CNS metastases, delay course initiation and give platelet transfusion to maintain platelet count at least 50,000/µL.
Respiratory dysfunction
• Delay course initiation until dyspnoea at rest has been resolved and/or peripheral oxygen saturation is at least 94 % on room air.
Renal dysfunction
• Delay course initiation until creatinine clearance or glomerular filtration rate (GFR) is at least 70 mL/min/1.73 m^2
Systemic infection or sepsis
• Delay course initiation until systemic infection or sepsis has resolved.
Leukopaenia
• Delay initiation of first course until absolute phagocyte count (APC) is at least 1,000/µL.

In addition to the above criteria, clinician judgement must be exercised in the evaluation of the patient's cardiovascular functions (see section 4.2 of the SmPC).

The development programme/compliance with CHMP guidance/scientific advice

Clinical development programme

The U. S. National Cancer Institute (NCI) has led the development of ch14.18 for more than 20 years with the pivotal Phase III trial being conducted by the Children's Oncology Group (COG). Further to the significant results reported in that study, the NCI sought a commercial partner to complete the required development activities to bring ch14.18 to the market.

In July 2010, United Therapeutics Corporation (UTC) entered into a Cooperative Research and Development Agreement with the NCI to collaborate on the late-stage development and commercialization of ch14.18. As such, UTC has exclusive rights to the clinical study data from all NCI-sponsored ch14.18 studies as well as the technical information needed to manufacture comparable ch14.18.

As the whole clinical development programme was conducted using product manufactured for NCI, a bioanalytical comparison of ch14.18 produced by the two processes has been conducted to ensure that UTC-manufactured ch14.18 is comparable to the ch14.18 that NCI-produced. In addition, UTC is also performing a PK comparability study in neuroblastoma patients to demonstrate that systemic exposure to ch14.18 is similar with both products.

Since the premature discontinuation of the pivotal trial (DIV-NB-301) conducted by the COG and the release of its results in the New England Medical Journal (Yu, 2010), ch14.18 has become part of the standard therapy for the treatment of high risk neuroblastoma. In Europe, the International Society of Paediatric Oncology (SIOPEN) has introduced ch14.18 in their ongoing protocol on high risk neuroblastoma (HR-NBL-1/SIOPEN). Because GM-CSF is not available in Europe and IL-2 may be more effective than GM-CSF, only the combination with IL-2 is applied (besides isotretinoin). Moreover, due to the lack of randomised trial showing that the antibody alone was not effective and due to the enhanced toxicity of the combination, which may need dose reduction of ch14.18, a randomised trial comparing ch14.18 alone vs. ch14.18 + IL-2 is currently ongoing. It is important to note that the antibody used in this multinational trial (20 countries) has been manufactured in a CHO cell line, and as such, is not exactly the same as dinutuximab produced in a murine myeloma cell line (SP2/0 hybridoma cells).

Scientific Advice / Protocol Assistance

Dinutuximab having been granted Orphan designation, protocol assistance was received from the CHMP in 2011 (EMEA/H/SA/2232/I/2011/PA/III) about the comparability approach between the NCI and UTC products, including a limited *in vivo* study in a relevant species, and the presentation of the clinical dossier with emphasis on several points to be addressed more specifically (the primary endpoint of EFS in an open-label trial without an independent blinded review committee; the real contribution of ch14.18 to the favourable results; the lack of long-term safety data).

2.2. Quality aspects

2.2.1. Introduction

Dinutuximab (also referred to as ch14.18) is a glycosylated chimeric IgG1 human/mouse monoclonal antibody (mAb), produced in a murine myeloma cell (SP2/0 hybridoma cell), that 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 heavy chain has 443 amino acids and one glycosylation site (Asn 293), molecular weight 49750 Da to 50075.4 Da. The light chain has 220 amino acids and a molecular weight of 24070.9 Da. The molecular weight of dinutuximab is in the range of 147,625 – 150,744 Da.

Disialoganglioside is ubiquitously expressed on most neuroblastoma tumours regardless of disease stage and is abundant on the cell surface. Dinutuximab reacts specifically with GD2 and has been shown to induce both antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) *in vitro* and has been found to mediate the lysis of several neuroblastoma cell lines in a dose-dependent manner.

Unituxin is intended to be administered by intravenous (IV) infusion. Unituxin is presented as a concentrate for solution for infusion, with the active substance dinutuximab at 3.5 mg/mL (*i.e.* 17.5 mg/5mL), in a clear, Type I glass vial (5mL) with a Fluorotec stopper, and an aluminium flip-off overseal. The product should to be used immediately after dilution.

2.2.2. Active Substance

General Information

Dinutuximab (also referred to as ch14.18) is a glycosylated chimeric IgG1 human/mouse monoclonal antibody (mAb), produced in a murine myeloma cell (SP2/0 hybridoma cell), that 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 heavy chain has 443 amino acids and one glycosylation site (Asn 293), molecular weight 49750 Da to 50075.4 Da. The light chain has 220 amino acids and a molecular weight of 24070.9 Da. The molecular weight of dinutuximab is in the range of 147,625 – 150,744 Da.

Manufacture, characterisation and process controls

Manufacture

The active substance is manufactured at United Therapeutics Corporation, 1040 Spring Street, Silver Spring, MD 20910, USA.

The use of a single-tiered cell banking system, consisting only of the MCB, was initially proposed for the manufacture of dinutuximab. A working cell bank (WCB) has since been manufactured to allow use of a two-tiered system; release testing is completed, but characterisation is still ongoing. The WCB for ch14.18 production will be implemented once the WCB is released for GMP use and should be introduced into the dossier via a variation procedure.

The upstream process consists of the thawing of a cell bank vial, cell expansion in a series of flasks, production in a bioreactor and recovery of the active substance. The active substance is purified with a series of chromatography, viral inactivation and filtration and ultra-/diafiltration steps.

Process validation was based on a full scale approach and no claims with regards to a design space or real-time product release is made. The process description and process controls include the revised flow chart as well as the controls of critical steps and intermediates; CPPs and IPCs are defined and correspond to what is expected to describe and control the process. The establishment of the process parameters ranges based on Design of Experiment (DoE) studies is deemed acceptable.

ICH Q8/Q9/Q10 principles were employed during the development and control strategy for ch14.18. During manufacturing process development, both traditional and enhanced (DoE) strategies were employed. Process validation was based on a full-scale approach and no claims with regards to a design space or real-time product release is made.

Some definitions were only considered for internal use (e.g. well-controlled critical process parameter or non-critical process parameter) as they are not defined in ICH regulatory guidance (Q8) and thus not fully supported. The establishment of the control parameters of the harvest bioreactor and the chromatography conditions based on DoE studies submitted with the dossier are considered acceptable and the corresponding steps at commercial scale are sufficiently detailed.

Hold time studies for the cell culture and downstream purification process were performed, including cumulative hold times. Validation of the hold times for the intermediates was submitted in order to support the requested hold times.

Process validation was successfully performed for the active substance manufacturing process.

Characterisation

Characterisation of dinutuximab has been performed through analysis of primary, secondary and tertiary structure, which demonstrates that this is consistent with the expected structure for a monoclonal antibody. The expected primary structures of the heavy and light chain sequences were confirmed. C-terminal lysine heterogeneity is observed, in common with other monoclonal antibodies.

The active substance is glycosylated and exhibits various glycoforms. The single N-linked carbohydrate structure is the expected diantennary structure with microheterogeneity, including variability in the terminal galactose residues. The main glycan structures are G0F, G1F and G2F, with lower levels of Mannose 5. The carbohydrate moieties are mostly fucosylated.

Biological activity for active substance was assessed. Product- and process-related impurities were adequately investigated.

Specification

Dinutuximab is tested for batch release by methods selected to address product characteristics (identity, purity, potency, general quality), as well as product and process related impurities and safety.

Description and validation of the analytical methods for release and stability testing of the active substance are in general considered adequate.

A number of new analytical methods were introduced since the time of the initial submission or improved regarding to the former method used), or still undergoing development. Validation data has been provided for release and stability assays, with the establishment of acceptance criteria justified, in line with ICH Q6B requirements. The active substance acceptance criteria are mainly built upon compendial requirements or established based on statistical analysis of UTC lots history and process validation studies

A number of the proposed acceptance criteria have been revised based on UTC batch data. Limits for excipients have been added to the specifications. Acceptance criteria have been set for the five major glycans as part of the control process for this monoclonal antibody. For alpha-(1,3)-galactose linked sugars, the acceptance criterion was tightened based on batch data. Acceptance criteria for levels of afucosylated glycans and ADCC have also been included.

Container closure system

The primary container for dinutuximab active substance is a 10 litre bag, comprising a multilayer film, with low density polyethylene for the product contact surface, ethyl vinyl alcohol as a gas barrier and nylon to provide structural integrity. The bag material is certified to meet USP Class VI requirements for plastics and to be free of animal-derived components. The primary closure materials also comply with the requirements of CHMP/QWP/4359/03. Bulk drug containers are translucent and stored at 2-8°C in the dark, which is considered acceptable to prevent photodegradation from occurring during bulk storage.

The Applicant has provided details of extractable studies, showing that levels of extractable from the bags, filter and tubing are within acceptable limits.

Stability

Stability data under long-term (5° ± 3°C/ambient humidity) were provided for three active substance batches manufactured at United Therapeutics facility in Silver Spring, MD, USA. All the stability data provided were within active substance acceptance criteria. Additional tests to further assess specific degradation products are under development. Further stability data has been provided and the limit for storage of active substance is proposed as 6 months at 2 - 8°C.

Under accelerated conditions changes were observed in spectrophotometric based assays which could be associated with changes in protein structure or aggregation. This will be further investigated.

Data to support 6 months storage of active substance has been provided and further studies are scheduled up to 18 months at 2 - 8°C. However, this is the same storage condition as the finished product and the cumulative storage time for dinutuximab needs to be considered. Based on the stability results, the cumulative maximum storage limit is proposed as 24 months, comprising 6 months for active substance and 18 months for finished product.

2.2.3. Finished Medicinal Product

Description of the product and Pharmaceutical Development

Dinutuximab finished product is provided as a sterile, buffered, aqueous solution of the antibody at 3.5 mg/mL, with Histidine, Sodium Chloride, and Polysorbate 20 in Water for Injections (WFI). An appropriate titrant is used, as required, to adjust the solution to a target pH of 6.8. There are no overages in the dinutuximab formulation and the finished product is supplied in a single use 5 mL vial (17.5mg dinutuximab). The primary container for dinutuximab is a 5mL/20mm Type 1 glass vial, with a 20mm stopper and 20mm aluminium flip-off over seal. For administration, the appropriate dosage is removed from the vial and diluted into a 100 mL infusion bag containing 0.9% sodium chloride. The diluted solution is held at ambient temperature prior to and during the infusion.

Manufacture of the product and process controls

Manufacture of the product

The finished product manufacturing process mainly consists in a simple 0.22 µm filtration followed by an aseptic filling into vials. The final batch size of the bulk finished product will vary with yield from the active substance manufacturing process. Active substance batches are not pooled for manufacture of finished product.

Controls are applied for the finished product manufacturing process, including equipment and container closure sterilisation and depyrogenation, dispensing and pre-filtration limits (including maximum hold times prior to filtration and filling) and final filling (including maximum fill duration, 100% fill weight check and 100% automated stopper presence and placement prior to capping). Defect inspection controls are also applied for the filled vials.

Validation of the finished product manufacturing process was successfully demonstrated.

Control of excipients

All the excipients (histidine, sodium chloride, Polysorbate 20 and hydrochloric acid) used in the formulation of the finished product comply with compendial requirements, including Ph. Eur. This is acceptable.

Specification

Dinutuximab is tested for batch release by methods selected to address product characteristics (identity, purity, potency, general quality), as well as product- and process-related impurities and safety.

Analytical methods are described in the active substance section, except for extractable volume and dosage uniformity, endotoxin and sterility. The sterility assay was validated using Bacteriostasis/ Fungistasis (B/F) testing, performed according to USP <71> using the Membrane Filtration Sterility Test, but all these assays conform to the Ph. Eur. requirements.

A number of the proposed acceptance criteria have been revised based on UTC batch data. Excipient content is determined in the active substance specification and are not included in the finished product specification; this is acceptable.

Reference standards or materials

Reference Standard Lot S110601 appears to be representative of the batches of finished product (from the lot release data) and analytical testing shows this to be suitable as a reference standard.

Container closure system

The container closure system (5 mL/20 mm Type 1 glass vial with a pre-washed Flurotec B2-40 20 mm stopper, and an aluminium flip-off overseal) has been described in sufficient detail. Extractables testing was provided for the stopper, which is acceptable. The vials are stored in a box due to light sensitivity of the product. Considering the potential photodegradation of the finished product, instructions to keep the vial in the carton to protect from light are included in the SmPC to avoid this degradation pathway.

Container closure integrity was demonstrated using liquid immersion of the samples into a solution containing the microorganism *Brevundimonas diminuta*. Details have been provided regarding the test, including the amount of time, pressure and vacuum used in the test.

Stability of the product

Five lots of finished product (in vials) were placed on stability under long-term (5 ± 3ºC/ambient humidity) and accelerated storage conditions (25 ± 2ºC/ 60 ± 5% Relative Humidity). All samples were stored in the inverted position. The lots were manufactured at UTC between October 2011 and March 2013. Each lot satisfied all stability acceptance criteria to the last time point tested (four lots were tested to 24 months, one lot to 18 months)

Considering the proposed long term storage conditions of the active substance are the same as those of the finished product, the combined storage times should be clearly stated. The cumulative maximum storage time has been set at 24 months, comprising 6 months for active substance and 18 months for finished product at 2-8°C. This shelf life is supported by batch data. The in-use stability after dilution of the finished product in 0.9% sodium chloride is 24 hours at ambient temperature (less than 25°C).

Adventitious agents

The MCB and EOP cells were assayed for adventitious and endogenous agents; it was confirmed that the *in vivo* assays were performed on newborn and adult mice for the MCB according to the "Note for guidance on quality of biotechnological products: viral safety evaluation of biotechnology products derived from cell lines of human or animal origin" (CPMP/ICH/295/95 or ICH Topic Q5A (R1). Regarding the retroviral contamination detected, it is typical and expected with this type of cells.

A number of orthogonal steps in the manufacturing process have been evaluated for removal or inactivation of virus. Appropriate model viruses were selected for the study. Cytotoxicity/ interference assays demonstrated that the process matrix used did not interfere with the tests. Spike and recovery studies were performed on qualified scale-down models using process intermediates taken from a representative full- scale manufacturing lots.

Chromatography columns are treated with a standard process for cleaning/regeneration with solutions containing sodium hydroxide. Summary reports of the viral validation study sanitisation process for the chromatography columns have been provided.

During the manufacturing process, no product of biological origin is used. Foetal Bovine Serum (FBS) was used in generation of the initial Master Cell Bank from which the current Master Cell Bank was derived.

The Applicant has provided details for the FBS used in the initial MCB and the new MCB and a revised TSE assessment. The source of the FBS in both cases is considered satisfactory.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Comparability has been demonstrated between the NCI material used in clinical studies and the proposed commercial process (UTC material), although the comparability studies were complicated by the age of the initial NCI material at the time of testing in comparability studies and changes in the analytical methods. It can be concluded that sufficient comparability has been demonstrated between more recent batches (post-2010) of ch14.18 manufactured by NCI using the original process and batches of ch14.18 manufactured by UTC using the proposed commercial process. This comparability issue, initially raised as a Major Objection was satisfactorily addressed by the Applicant and is considered resolved.

The Applicant was not able to provide the required information concerning the source, history and generation of the cells substrate, which is not in line with the ICH Q5D guideline, but considering the length of time since the generation of the cell line and submission of the dossier, this can be accepted. Data based on peer-reviewed literature were provided for the vector development and a plasmid map were provided. Characterisation tests performed for the MCB are the identity assay, the coding sequence and the determination of the copy number, which is acceptable.

Confirmation has been given that the genetic stability performed using cells from the manufacturing process and expanded at small scale is representative of the genetic stability at commercial scale, although the scale-down fermenter details have not been provided to support this. When generating the new WCB, the Applicant is recommended to make an end of production cell bank and characterise the genetic stability of both the WCB and EPCB. The use of the new WCB will have to be authorised with a variation.

The manufacturing control strategy for the active substance is considered satisfactory; process validation studies demonstrated that upstream and downstream manufacturing processes were well controlled. Critical process parameters and in-process controls are applied to the cell culture process, bioreactor harvest and purification steps. Validation of the analytical methods used for the control of the active substance and finished product has been completed; acceptance criteria for active substance and finished product have been tightened in line with batch data and justified. Both the active substance and finished product are stored at 2-8°C and a maximum cumulative shelf life of 2 years has been proposed (6 months for active substance and 18 months for finished product).

A number of issues remained regarding inadequate validation and approach for the replacement, improvement and development of the analytical methods. Following the additional information provided by the Applicant, these issues are considered resolved.

Concerning stability, a 6-month and 18-month shelf life at 2-8°C have been justified for active substance and finished product, respectively. Considering that the activity assays are now considered validated, this is acceptable. The required in-use stability of 24 hours at ambient temperature (less than 25°C) after dilution of the finished product in 0.9% sodium chloride is also considered acceptable. The following statement, in line with the Note for guidance on maximum shelf life for sterile products for human use after first opening or following reconstitution, has also been included in the SmPC: "*From a microbiological point of view, (…) the product should be used immediately. If not used immediately, in-use storage times and conditions are the responsibility of the user*".

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

Overall, the quality of Unituxin 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 Unituxin is considered acceptable.

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 several points for further investigation.

2.3. Non-clinical aspects

2.3.1. Introduction

The data summarized includes studies conducted with ch14.18 and/or its murine anti-human GD2 predecessors (murine Mab 14.18 or 14.G2a). These studies evaluated the in vitro and in vivo binding profiles, mechanism of action and in vivo anti-tumor activity of anti-GD2 therapy. In addition, secondary pharmacology data were presented to describe the proposed mechanism responsible for the pain associated with administration of ch14.18 (as observed during clinical studies). Based on the data submitted, which includes data to describe similarities in binding affinities and similar in vitro and in vivo biological activities; pharmacodynamic data generated with murine mab 14.18 or 14.G2a is relevant to ch14.18. Hence, published data which describe the effects of these antibodies have been submitted in support of this application.

2.3.2. Pharmacology

Primary pharmacodynamic studies

The primary pharmacology data submitted in support of this application are summarised in Table 4.

Table 4: Summary of primary pharmacology data

Test Article: ch14.18 (murine Mab 14.18 and/or 14.G2a)[a]				
Type of Study	**Test System(s)**	**Method of Administration/Test Articles**	**Testing Facility**	**Reference/ Study Number**
Primary Pharmacodynamics				
Binding to tumors	tumor tissues and cell lines	In vitro murine Mab 14.18	NA[b]	Mujoo 1987
Binding to GD2 antigen	M-21 melanoma cells Neuroblastoma cells: IMR-6, SMS-KCNR, CHP-134, NMB-7, SK-N-AS, SMS-KAN	In vitro ch14.18, 14.G2a, murine Mab 14.18	NA[b]	Mueller 1990 Mujoo 1987
Antibody dependent cytotoxicity (ADCC)	GD2$^+$ neuroblastoma cells: NMB-7, IMR-32, IMR-6, SMS-KCNR, SK-N-AS GD2$^+$ melanoma cells: M-21, A375	In vitro ch14.18, murine Mab 14.8, 14.G2a,	NA[b]	Barker 1991 Mueller 1990 Kendra 1999 Mujoo 1987 Mujoo 1989
Antibody dependent cytotoxicity (ADCC)	GD2$^+$ neuroblastoma cells: SMS-KCN, SMS-LHN and LA-N-1 GD2$^-$ neuroblastoma cells: SK-N-SH	In vitro ch14.18*	NA[b]	Chen 2000
Binding to tumors	tumor tissues and cell lines	In vitro murine Mab 14.18	NA[b]	Mujoo 1987
Binding to GD2 antigen	M-21 melanoma cells Neuroblastoma cells: IMR-6, SMS-KCNR, CHP-134, NMB-7, SK-N-AS, SMS-KAN	In vitro ch14.18, 14.G2a, murine Mab 14.18	NA[b]	Mueller 1990 Mujoo 1987
Antibody dependent cytotoxicity (ADCC)	GD2$^+$ neuroblastoma cells: NMB-7, IMR-32, IMR-6, SMS-KCNR, SK-N-AS GD2$^+$ melanoma cells: M-21, A375	In vitro ch14.18, murine Mab 14.8, 14.G2a,	NA[b]	Barker 1991 Mueller 1990 Kendra 1999 Mujoo 1987 Mujoo 1989
Antibody dependent cytotoxicity (ADCC)	GD2$^+$ neuroblastoma cells: SMS-KCN, SMS-LHN and LA-N-1 GD2$^-$ neuroblastoma cells: SK-N-SH	In vitro ch14.18*	NA[b]	Chen 2000

In vitro studies

Binding of murine mAb 14.18 to various tumours

To characterise the presence of GD2 on different tumour types and identify potential tumour types that may derive some benefit from ch14.18 therapy, reactivity of murine mab 14.18, the predecessor of ch14.18, to a series of cultured tumour cells and frozen tumour tissues was evaluated by ELISA or immunohistochemical analysis, respectively. Murine mab 14.18 reacted strongly with neuroblastoma, melanoma, glioblastoma and small cell lung cancer tissues, while other tumour types showed no detectable immunohistochemistry staining. No detectable staining to weak staining was observed for Ewing's sarcoma. The staining pattern of murine mab 14.18 measured by ELISA with various human tumour cell lines confirmed that the antibody recognizes tumours of neuroectodermal origin: neuroblastoma, melanoma, glioma and small cell lung carcinoma (Table 5)

Table 5: Murine mAb 14.18 reactivity towards cultured cells

Tumor	Absorbance
Neuroblastoma	
LAN-1	++++
LAN-5 Duke	++++
SMS-KCNR	++++
CHP-134	++++
SMS-KAN	++++
NMB-7	++++
IMR-6	++++
SK-N-AS	+++
Melanoma	
Melur	+
A375 P	+++
A375 Met	+++
FM8	+++
FM9 Met	+++
M-14	++++
M-21	++++
Glioma	
U138 M6	++++
Small Cell Lung Carcinoma	
T293	++++
NIH-N417	++++
NIH-H-82	++
NIH-H-69	++
Other tumor cell lines	
Pancreatic carcinoma (fast growing)	-
Pancreatic carcinoma (slow growing)	-
Pancreatic carcinoma 1320 (metastatic)	-
Adenocarcinoma (UCLA-P3)	-
Lymphoblastoid cell lines	
L-14 (B cell)	-
LG-2 (B cell)	-
MOLT-4 (T cell)	-
HPB-ALL (T cells)	-

Absorbance at 492nm: 0.15-0.3 (+); 0.3-0.6 (++); 0.6-0.9 (+++; 0.9->2.0 (++++); 0-0.050 (-)

[Modified from Table 1 in Mujoo 1987)]

Anti-GD2 binding sites in tumour cell lines

Binding of ch14.18, murine Mab 14.18 and/or 14.G2a to a series of neuroblastoma cell lines and the melanoma M-21 cell line was assessed by Scatchard analysis (Mujoo 1987; Mueller 1990). Tumour cells were incubated with increasing amounts of ^{125}I-labeled ch14.18 or 125I-labeled 14.G2a.

With respect to the M-21 cells, similar results were obtained with the murine and chimeric GD2 antibodies. The number of binding sites per M-21 cell was 1.5 x 10^7 for ch14.18 and 1.4 x 10^7 for 14.G2a. The K_D was 11.2 nM for ch14.18 and 11.9 nM 14.G2a. Non-specific binding was 5-10% total bound radiolabel. Murine mab 14.18 bound to all of the neuroblastoma cells tested; however, the number of GD2 binding sites was variable for the different neuroblastoma cell types, ranging from 110,000 to 267,000 sites/cell.

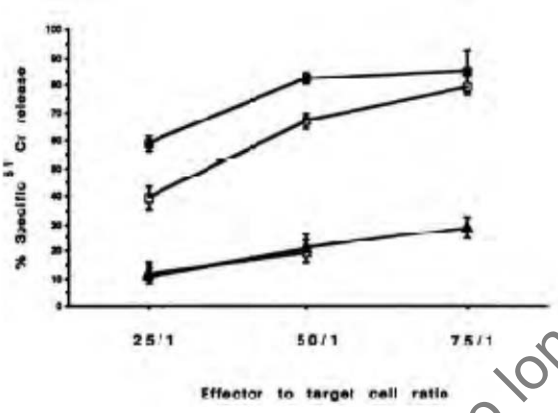
Effects on antibody-dependent cell cytotoxicity (ADCC)

The ability of ch14.18, murine mab 14.18 or 14.G2a to induce ADCC was evaluated with GD2+ neuroblastoma cell lines NMB-7, IMR-32, SMS-KCNR, IMR-6, SKN-AS and Kelly (Mujoo 1987; Mujoo 1989; Barker 1991; Zeng 2005) and two GD2+ melanoma cell lines, M21 and A375 (Mueller 1990; Kendra 1999). In the Barker study, ch14.18 or 14.G2a, at concentrations ranging from 0.001 to 2.5 µg/mL, was added to the tumour cells, followed by addition of human peripheral blood mononuclear cells

(PBMCs) or granulocytes harvested from the venous blood of healthy adult donors or of stage IV neuroblastoma patients at least 4 weeks after the last therapy.

ch14.18 was capable of mediating the destruction of tumour target cells in the presence of non-adherent PBMCs (i.e., PBMC fraction enriched for lymphocytes) or granulocytes; while adherent PBMCs did not stimulate cell lysis. With the use of PBMCs from healthy donors, a ch14.18 dose-dependent increase of NMB-7 cytotoxicity was observed with the percentage of tumour cells killed ranging from approximately 20% with to approximately 70%. With patient PBMCs, at a E:T ratio of 10:1; the percentage of IMR-32 tumour cells killed was similar across the ch14.18 concentration range of 0.005 to 2.5 µg/mL, ranging from 33-58%. Background lysis of the tumour cells without ch14.18 was 0% with PBMCs from healthy donors and ranged from 0-22% with PBMCs isolated from neuroblastoma patients.

As Figure 1 demonstrates, ch14.18 was able to induce more tumour cell lysis in the presence of neuroblastoma patient granulocytes than in the presence of patient PBMCs(see section 5.3 of the SmPC).



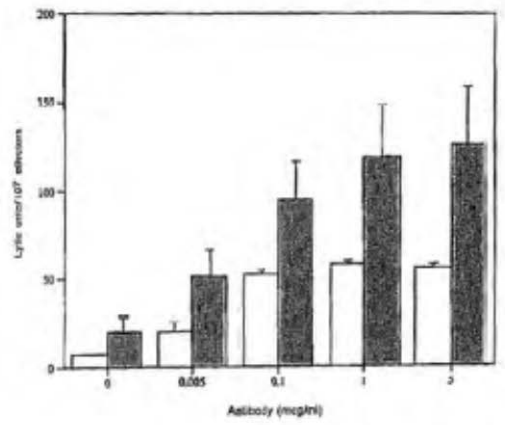
Ch14.18-mediated Lysis of NMB-7 Neuroblastoma Tumor Cells by Same Patient Granulocytes (boxes) or PBMCs (triangles) in the Absence (open symbols) or Presence (closed symbols) of Recombinant Human GM-CSF. The concentration of ch14.18 and rhGM-CSF was 1 µg/mL and 100 ng/mL, respectively. The E:T ratio was 25:1, 50:1 or 75:1. Percent of cells killed (mean ± SD) over a 4 hour incubation period are shown. [Figure 7 in Barker 1991]

Figure 1: ch14.18-mediated lysis of NMB-7 neuroblastoma cells

Zeng, *et al* (Zeng 2005) and Mujoo, *et al* (Mujoo 1987) conducted similar experiments except that human PBMCs were either pre-incubated with IL-2 (200 IU/mL) or added directly to ^{51}Cr labelled neuroblastoma cells at various E:T ratios for 4 or 20 hours. Zeng, *et al* demonstrated that the effectiveness of the ADCC to lyse tumor cells was greater with a longer incubation time, higher E:T ratios and higher ch14.18 concentrations. The maximum cytotoxicity was 30% for SK-N-AS neuroblastoma cells and 80% for M-21 melanoma cells. Mujoo and coworkers reported maximum cytotoxicity of 49%, 35% and 20% for SMS-KCNR, IMR-6 and SL-N-AS neuroblastoma cells with murine Mab 14.18.

In other experiments by Mueller, et al (Mueller 1990) and Kendra, et al (Kendra 1999), ^{51}Cr-labeled M-21 or A375 melanoma tumour cells were incubated with human PBMCs (effector cells) in the presence or absence of IL-2 and ch14.18 or 14.G2a for 4 hours at 37 °C. The E:T ratios ranged from 6:1 to 200:1, and the anti-GD2 antibody concentrations ranged from 0.001 to 50 µg/mL. In both sets of experiments a dose-dependent increase in ADCC was apparent for the two melanoma cell lines across the anti-GD2 antibody concentration range tested within each E:T ratio (see section 5.3 of the SmPC). Kendra, et al demonstrated that ch14.18, at concentrations as low as 0.005 µg/mL (without IL-2 supplementation), enhanced the lysis of M-21 cells by fresh human PBMCs (Figure 2). A dose-dependent increase in cell lysis occurred over the ch14.18 concentration range of 0.005 – 0.1 µg/mL; lysis then remained similar

between 0.1 and 5 µg/mL ch14.18. IL-2 supplementation of the effector cells further increased the lytic potential of ch14.18 at all mAb concentrations.

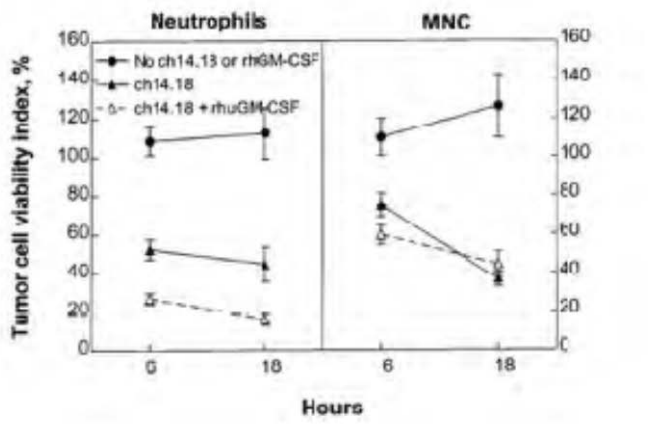


Cytotoxicity of M-21 cells by ch14.18 without IL-2 (open bars) and by ch14.18 with IL-2 (closed bars). Each bar represents the mean ± SE of 5 normal lymphocyte donors. [Figure 1 in Kendra 1999]

Figure 2: ch14.18-mediated cytotoxicity of M-21 cells in the absence and presence of IL-2

Similarly, Mueller and co-workers showed that maximum specific lysis of 25-55% of the M-21 cells was achieved with ch14.18 at concentrations ranging between 0.5 and 10 µg/mL without IL-2 supplementation and that at least 0.1 µg/mL of ch14.18 (without IL-2) was necessary to obtain approximately 25% or greater specific lysis of A375 cells. It is noted that ADCC was also observed with 14.G2a under the same conditions, although with 50- to 100-fold less efficiency (whereby 50- to 100-fold higher concentrations of antibody were required).

Chen et al evaluated the ability of ch14.18 and/or effector cells (i.e., neutrophils or PBMCs isolated from healthy human volunteers) to induce ADCC in a series of GD2-positive neuroblastoma cell lines, SMS-KCN, SMS-LHN and LA-N-1, and of the GD2-negative cell line SK-N-SH. The effector cells (neutrophils or PBMCs; 0.1 – 100:1 E:T ratio), ch14.18 at 0.1- 10 µg/mL and/or rhGM-CSF at 0-250 ng/mL were added to the wells and incubated. The results are summarised in Figure 3. ch14.18 in the presence of either neutrophils or PBMCs induced ADCC (approximately 40% and 20% tumour cell cytotoxicity after 6 hours, respectively, and approximately 60% cytotoxicity after 18 hours for both). The addition of rhGM-CSF augmented the ch14.18-mediated tumour cell killing only when neutrophils were the effector cells (approximately 80 and 90% of the cells were killed after 6 and 18 hours, respectively).



Calcein-AM labeled SMS-LHN neuroblastoma cells were incubated for 6 or 18 hours with no additions (closed circles), 10 µg/mL ch14.18 (closed triangles) or 10µg/mL ch14.18 + 250 ng/mL rhGM-CSF (open triangles). Cell survival is plotted as mean ± SE. [Figure 3 in Chen 2000]

Figure 3: Time course of ch14.18-mediated tumour cell cytotoxicity

Table 6 depicts the ADCC effect after 7 days of co-culturing neuroblastoma cells with neutrophils (50:1 E:T ratio), ch14.18 at 10 µg/mL, in the presence or absence of rhGM-CSF.

Table 6: Tumour cell viability after 7 days of treatment with neutrophils, ch14.18 and rhGM-CSF

Cell line	Exp	Neutrophils alone (% tumor cell viability)	Neutrophils + ch14.18 (% tumor cell viability)	Neutrophils+ ch14.18 + rhGM-CSF (% tumor cell viability)
SMS-KCN (GD2+)	1	156*	23*,#	17*,#
	2	104	32*,#	17*,#
	3	130	47#	72#
	4	140	130	61#
SMS-LHN (GD2+)	1	238*	33#	40*,#
	2	170	31#	41#
	3	177*	92#	45*,#
	4	137	76#	80#
LA-N-1 (GD2+)	1	66*	19*,#	9*,#
	2	19*	9*	1*
SK-N-SH (GD2-)	1	475*	448*	563*
	2	370*	ND	203*
	3	170*	141*	168*
	4	316*	264*	295*

EXP = experiment; ND = not determined; neutrophils = 50:1 E:T ratio; ch14.18 = 10 µg/mL; rhGM-CSF= 250 ng/mL
*P < 0.05 (Student's t-test) for tumor growth compared to that of control (tumor cells alone)
#P < 0.05 (Student's t-test) for growth inhibition compared to the effect of neutrophils alone
[adapted from Table 2 in Chen 2000]

Effects on complement-dependent cytotoxicity

The ability of ch14.18 (0.05 to 50 µg/mL) to induce complement-dependent cytotoxicity (CDC) was evaluated using melanoma and neuroblastoma cells (Mujoo 1987; Mueller 1990; Zeng 2005). M-21 GD2+ melanoma cells were more sensitive to ch14.18-mediated CDC than A375 GD2+ melanoma cells.

The Kelly cell line was the most sensitive with cytotoxicity of 60 to 80% at 0.1 to 10 µg/mL, whereas the maximum cytotoxicity of M-21 cells was 80% at 1 µg/mL ch14.18 (with 25% complement). CDC ranging

from 50 to 95% was also reported for IMR-6, SMS-KCNR, CHP-134, NMB-7, SKN-AS and SMS-KAN neuroblastoma cells with murine mab 14.18 at concentrations of 5-10 µg/mL (Mujoo 1987).

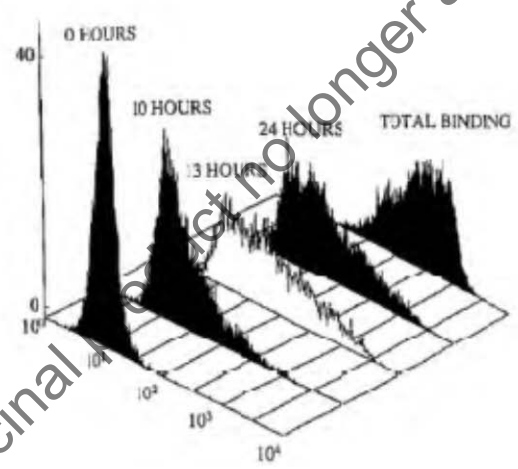
In vivo studies

Binding of ch14.18 in a melanoma xenograft mouse model

To measure ch14.18 binding to tumour cells *in vivo*, three separate experiments were performed (Kendra 1999). SCID mice were inoculated IP with M-21 melanoma cells/mouse. For flow cytometry assessments, animals were sacrificed at 0, 10, 13 or 24 hours after a single IP injection of 0.01, 0.1 or 1.0 mg (~0.5, 5 or 50 mg/kg based on 0.02 kg mouse) ch14. For immunohistochemistry assessments, the animals were sacrificed 24 hours after antibody administration, and the presence of GD2 antigen or ch14.18 was detected by immunohistochemical staining.

Figure 7 illustrates the flow cytometry analysis of M-21 tumour cells treated with ch14.18.

In addition, immunohistochemical staining performed on tumours excised 24 hours after antibody administration demonstrated that the GD2 antigen was homogenously present throughout the entire tumour. The highest dose of ch14.18 tested (1.0 mg) bound to the periphery of the tumour and penetrated the tumour to a depth of approximately 20 cells. At the mid-dose of 0.1 mg, ch14.18 binding was only observed at the periphery of the tumour, and at the low dose of 0.01 mg, no staining was observed.



One mouse was evaluated per time point. Total binding was determined by harvesting nodules from mice not treated with ch14.18 and staining with ch14.18 ex vivo. [Figure 2 in Kendra 1999]

Figure 4: Flow cytometry analysis of M-21 tumor cells extracted from SCID bearing xenografts and treated with ch14.18

Ch14.18 anti-tumour activity in a melanoma xenograft model

Two experiments (Kendra 1999) were conducted in which C.B.-17 scid/scid female mice (2-8 mice/group) were injected SC into the right flank with (1) human melanoma cell line M-21(10^6 cells), (2) M-21 cells + IL-2 (1,500 IU), (3) M-21 cells + human PBMCs + ch14.18 (0.5 mg), (4) M-21 cells + IgG (isotype control, 0.5 mg) or (5) M-21 cells + IL-2 + ch14.18 + PBMCs. The PBMCs were used at E:T ratios of 3:1, 10:1 or 30:1.

Results are summarised in Table 7.

Table 7: Effects of ch14.18, IL-2 and/or PBMCs on tumour growth in a mouse model of melanoma

Experiment	# animals	ch14.18[a]	IL-2[b]	PBMC (E:T)[c]	Tumor size (cm^2)[d]
1	7	-	-	-	0.96, 1.05. 0.8. 1.65. 1.4, 1.9, 1.4 (Mean = 1.3 ± 0.4)
1	8	+	-	-	2.04, 0.42, 1.05, 0.63, 0.65, 1.68, 2.70, 1.28 (Mean = 1.3 ± 0.8)
1	7	Isotype	-	-	1.08, 2.4,9.0 1.36, 2.8, 3.99, 3.3 (Mean = 3.4 ± 2.7)
2	2	-	-	-	0.54, 0.34
2	2	-	+	30:1	0.2, 0.49
2	2	-	+	10:1	0.32, 0.0
2	2	-	+	3:1	0.72, 0.72
2	2	+	+	30:1	0.0, 0.0
2	2	+	+	10:1	0.0, 0.0
2	2	+	+	3:1	0.0, 0.0

[a]Animals received either 0.5 mg ch14.18 in PBS (+) or PBS alone (-)
[b]Animals received either 1,500 Units IL-2 in PBS (+) or PBS alone (-)
[c]Animals were injected with either PBS (-) alone or PBMCs in PBS at the stated E:T ratio
[d]Tumor nodules were measured 29 days after inoculation in experiment 1 and 24 days after inoculation in experiment 2
[adapted from Table 1 in Kendra 1999]

Prophylactic anti-tumour activity in a neuroblastoma xenograft mouse model

To assess the ability of ch14.18 to inhibit neuroblastoma establishment and growth, BALB/c athymic (nu/nu) mice (n=6 animals/group) were given subcutaneous injections of SK-N-AS neuroblastoma cells. One day later, irrelevant mAb C281 (anti-GD3 mAb) or murine mab or PBS control was administered IP and repeating on days 2, 4, 6, 8, 13 and 18 after initial tumour inoculum (Mujoo, 1987).

Starting on Day 10, all animals in the PBS group began to rapidly develop large tumours that ranged in volume from 1600 to 6800 mm^3 on the last day of the experiment (Day 23). Similarly, after 9 days, animals receiving mAb C281 (control) rapidly developed large tumours with volumes on Day 23 between 600 and 4500 mm^3. In contrast, murine mab 14.18 delayed the development of tumours. Tumours slowly developed starting on Day 20 with volumes of 200 to 900 mm^3 on Day 23. There was no statistically significant difference between the PBS control and mAb C281 groups, whereas statistical significance was achieved on Days 8-23 when tumour volumes of murine mab 14.18 groups were compared to the two control groups.

The experiment was repeated whereby the neuroblastomas were allowed to develop and grow for 9 days. Animals were given 200 µg/dose (~ 10 mg/kg) of irrelevant Mab C281 (anti-GD3 mAb) or murine mab 14.18 on Days 10, 13, 15 and 18. Tumours developed in both groups however animals treated with murine mab 14.18 developed tumours that were statistically significantly smaller when compared with the irrelevant mAb control on Day 10 and 23.

Secondary pharmacodynamic studies

Severe pain is associated with the administration of ch14.18 in the clinic (Yu, 2010). The mechanism behind this neurotoxicity was explored during in vivo and ex vivo studies utilising mice, rats, rabbits and dogs (Vriesendorp, 1997).

Dogs that had been administered 14.G2a displayed significantly reduced distal motor amplitudes when compared to animals administered low doses of 14.G2a (~0.2 mg/kg) and to control animals, findings that are indicative of distal axonal dysfunction. In immunohistochemistry studies, 14.G2a was found to bind to the granular layer of the cerebellum, to the vagus nerves and many of the sciatic nerve fibres in all of the animals (Vriesendorp, 1997). Despite the reduced distal motor amplitudes and the observation that 14.G2a binds to many normal nervous system tissues, there was no evidence of histopathologic

abnormalities, such as demyelination, axonal degeneration, or inflammation in sciatic nerves, vagus nerves, brachial plexus, lumbosacral roots, sural nerve, or cervical or myenteric ganglia of the dogs administered 14.G2a.

In addition, three independent studies demonstrated that ch14.18 or 14.G2a administered IV at 0.1 to 3 mg/kg or intrathecally (IT) at 0.01 – 0.1 ng induced rapid and prolonged mechanical allodynia (Slart, 1997; Xiao, 1997; Sorkin, 2002). Bolus administration of ch14.18 at dose levels ≥0.1 mg/kg resulted in a clear, reproducible mechanical allodynia in the rat. The effect was fully developed within an hour after administration and then plateaued for at least 3 hours. Allodynia was still present 24 to 48 hours after ch14.18 administration, albeit to a lesser extent. This change in somatosensory processing was accompanied by increases in heart rate and blood pressure.

Safety pharmacology programme

Safety pharmacology studies were performed to investigate the effects of ch14.18 on cardiovascular and respiratory systems.

Table 8: Summary of Respiratory and Cardiovascular Systems studies

Study type / Study Number / GLP status	Species / Strain	Route of administration	Doses	Noteworthy findings
Effects on the Respiratory and Cardiovascular systems SBL354-005 Yes	Cynomolgus Monkey	10 hour IV Infusion	ch14.18: 14 mg/kg 3 Male	No changes in respiratory parameters. Increases in heart rate (2 of 3) and blood pressure (1 of 3). Shortening of PR and QT intervals related to increased heart rate. No change in corrected QT (QTc).

Pharmacodynamic drug interactions

No Pharmacodynamic drug interaction studies were conducted.

2.3.3. Pharmacokinetics

Formal pharmacokinetic studies with ch14.18 have not been conducted, and instead data has been obtained and summarized from the public literature. Data summarized include studies conducted with ch14.18 and/or its murine anti-human GD2 predecessors (murine mAb 14.18 or 14.G2a).

Two methods for analysis of ch14.18 were developed: a validated method for analysis of ch14.18 in Sprague Dawley rat plasma and a qualified method for analysis of ch14.18 in Cynomolgus monkey plasma.

A 28-day repeat-dose toxicity and toxicokinetic study (SBL354-003) was conducted. In this study, vehicle or ch14.18 was administered to Sprague Dawley rats via IV infusion at increasing doses.

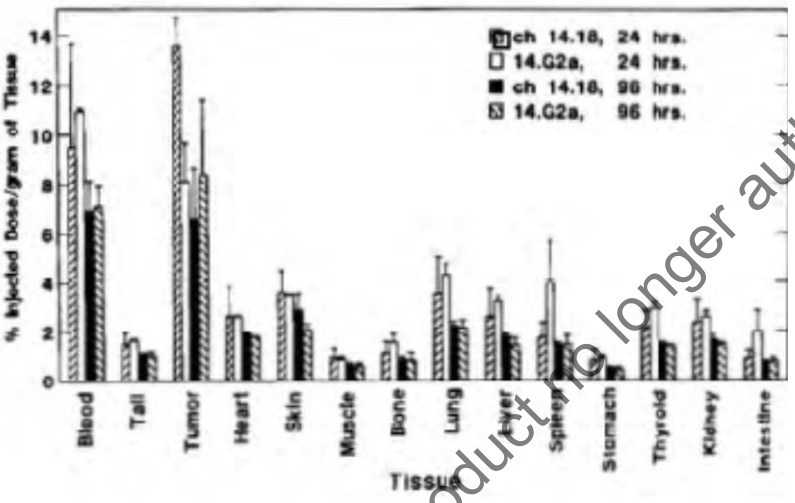
Table 9: TK data of the Toxicological Study SBL354-003

Daily dose (mg/kg)	0 (Control)		5		15		45	
Nb of animals	M: 8	F: 8	M: 8	F: 8	M: 8	F: 8	M: 8	F: 8
C_0 (ng/mL) Day 1 Day 22	 NC NC	 NC NC	 98000 1690	 179000 36300	 295000 324000	 318000 171000	 774000 1550000	 1020000 1120000
$AUC_{0-4\ h}$								

(ng·hr/mL)								
Day 1	NC	NC	358000	307000	930000	939000	2800000	2950000
Day 22	NC	NC	12500	33200	889000	418000	5440000	3640000
$T_{1/2}$ (hr)	NC	NC	NC	NC	70.1	70.2	98.2	132
$AUC_{24h-inf}$ (ng·hr/mL)	NC	NC	NC	NC	15000000	1390000	159000000	153000000

After the final dose (Day 25), plasma concentrations of ch14.18 decreased rapidly at 5 mg/kg (undetectable within 48 hours), gradually at 15 mg/kg (generally undetectable by the third week of recovery) and were still detectable at 45 mg/kg at the end of the recovery period.

Following IV injection in mice, 125I-ch14.18 targeted and accumulated in the GD2-expressing tumour. With the exception of the blood, which had similar levels as the tumour, non-targeted, normal tissues had substantially lower levels of radioactivity at all time-points evaluated. By 4 days post-administration, the level of 125I-ch14.18 within all tissues was slightly reduced, with the tumour maintaining the highest levels. Similar results were observed with 125I-14.G2a.



[Figure 2 in Mueller 1990]

Figure 5: Distribution of 125I-labelled ch14.18 in melanoma tumour bearing mice.

In the dog, 2 animals at the maximum dose of 202 mg (~20 mg/kg 14.G2a) had a terminal half-life that was 14 hours faster than that observed at the low dose of 2 mg (i.e. Tβ½ = 22 vs. 36 hours, respectively; Vriesendorp 1997).

Table 10: Distribution of 14.G2a in the dog

Group	No. of dogs	^{111}In-14.G2a dose	Unlabeled-14.G2a dose	Blood $t\alpha_{1/2}$	Blood $t\beta_{1/2}$	Liver %ID	Liver $t_{1/2}$	Urine %ID
H	2	2 mg	0	3 hours	36 hours	46%	70 hours	4%
I	2	2 mg	200 mg	3 hours	22 hours	53%	58 hours	7%

$t\alpha_{1/2}$: initial rapid clearance from blood; $t\beta_{1/2}$: slower elimination; % ID: percentage of injected dose [adapted from Table 2 in (Vriesendorp 1997)]

Bone marrow, spleen and thymus do not show specific targeting with high or low doses of 14.G2a. Following the high-dose administration of unlabelled 14.G2a uptake occurred in lymphoid tissues including the tonsils and popliteal lymph nodes similar to uptake at the low-dose while distribution into

the mesenteric lymph nodes was much more prominent at the high-dose compared to the low-dose. For the kidney, adrenal glands and liver, ratios for low-dose 14.G2a were similar to the control antibodies (data not shown). As discussed previously, the significantly elevated liver ratio for the high-dose group (I group) was likely due to the use of anesthesia. Uptake ratios for the heart, lung, endocrine and gastrointestinal tissues were between 3 and 7 (data not shown). In contrast, the ratios in CNS tissues were 10-fold lower, attributable to the blood-brain barrier. The ratio in the pituitary gland (which is not affected by the blood-brain barrier) was 5, and ratios within the periphery nervous system were 2-7 times higher than in the CNS tissues.

Administration of a high-dose of 14.G2a was associated with an enhanced uptake of the mAb by the liver and lymphoid tissue, which was associated with lymph node hyperplasia. While GD2 antigen is present on tissues of the peripheral and central nervous system 14.G2a did not accumulate to any great extent in these tissues.

No formal metabolism, excretion or pharmacokinetic drug interaction studies have been conducted.

2.3.4. Toxicology

Single dose toxicity

While no formal single-dose toxicology studies were conducted, studies that have been published demonstrating that major adverse events or deaths were not reported following single dose administration of ch14.18 and 14.G2a in mice, rats, rabbits, and dogs.

Table 11: Summary of single-dose toxicity studies

Species / Study ID / GLP	Method of Administration / Doses (mg/kg) / Duration	Major findings
Mice		
SJL Mouse Vriesendorp 1997 No	IP 14.G2a: 8-32 mg (400-1600 mg/kg based on 0.02 kg mouse) 4x female 1-day	Observed Maximum Nonlethal Dose = 1600 mg/kg Maximum nonlethal daily dose = 4800 mg/m2 ; multiple of Human = 274 14.G2a bound to the granular layer in the cerebellum, myenteric ganglia and dorsal root ganglia, sciatic nerves. 14.G2a did not result in clinical, electrophysiological, or histological findings of neuropathy.
Rat		
Lewis rat Vriesendorp 1997 No	IV 14.G2a: 2-20 mg (8-80 mg/kg based on 0.25 kg rat) 11x Female 1-day	Observed Maximum Nonlethal Dose = 80 mg/kg Maximum nonlethal daily dose = 480 mg/m2 ; multiple of Human = 27 14.G2a bound to the granular layer in the cerebellum, myenteric ganglia and dorsal root ganglia, sciatic nerves. 14.G2a did not result in clinical, electrophysiological, or histological findings of neuropathy.
Sprague-Dawley rat Slart 1997 No	IV ch14.18: 0, 0.1, 1, 3, 10 mg/kg 4 to 6x Male 1-day	Observed Maximum Nonlethal Dose = 10 mg/kg Maximum nonlethal daily dose = 60 mg/m2 ; multiple of Human = 3 *0.1, 1, 3, 10 mg/kg:* Animals treated with all 4 dose levels of ch14.18 had statistically significantly lower pain thresholds and thus higher percent maximum allodynia than the saline control animals. Touch Evoked Agitation (TEA) was significantly increased in the 0.1, 1 and 3.0 mg/kg groups on Day 1 and increased but not statistically significantly in the 10 mg/kg group. By Day 2-3 after antibody injection, 1, 3 and 10 mg/kg groups showed statistically significant increases in TEA compared to saline control. *1, 3 and 10 mg/kg:* An immediate and precipitous drop in mechanical threshold which plateaued about an hour after antibody injection and was maintained for

Species / Study ID / GLP	Method of Administration / Doses (mg/kg) / Duration	Major findings
		the remainder of the 2.5 hour of testing. 24 and 48 hours after ch14.18 administration thresholds increased compared to the acute response; however, there was still statistically significant allodynia for animals that had received 1.0 and 3.0 mg/kg of ch14.18, but not 10.0 mg/kg when compared to the saline group.
Sprague-Dawley rat Xiao 1997 No	IV ch14.18: 0, 1 mg/kg 14.G2a: 0, 1 mg/kg 4 to 8x Male 1-day	Observed Maximum Nonlethal Dose = 1 mg/kg Maximum nonlethal daily dose = 6 mg/m2 ; multiple of Human = 0.3 After ch14.18 administration, animals presented with allodynia starting within the first 15 minutes, which then plateaued between 45-60 minutes. 14.G2a showed the same trend and timing, but at a smaller magnitude. Animals treated with ch14.18 or 14.G2a had limited locomotor activity in general and did not stand upright against the side of the cage. Following ch14.18 or 14.G2a administration, a high incidence of background activity (i.e., electrical activity prior to mechanical stimulation) was observed in primary afferent fibers in the sural nerve. There was minimal activity in Aβ fibers (slowly adapting), whereas background discharge was apparent in a significant percentage of Aδ fibers rapidly and slowly adapting) and was the prevalent condition in C-fibers. A bolus injection of 15 mg/kg of lidocaine or a continuous infusion of lidocaine suppressed the ch14.18-mediated background activity >50% when lidocaine plasma concentration was greater than 0.3 µg/mL.
Sprague-Dawley rat Sorkin 2002 No	IV or IT 14.G2a IV: 0, 1.0, 3.0 mg/kg 14.G2a IT: 0, 0.01, 0.05, 0.1, 0.5 ng 4 to 8x Male 1-day	Observed Maximum Nonlethal Dose = 3 mg/kg Maximum nonlethal daily dose = 18 mg/m2 ; multiple of Human = 1 IV bolus of 14.G2a displayed a statistically significant decrease in 50% probability mechanical withdraw threshold over the 3 hours following administration. Thermal withdrawal thresholds were unaffected by 14.G2a at either 1.0 or 3.0 mg/kg dose levels. Intrathecal administration of 14.G2a resulted in a dose-dependent decrease in mechanical threshold for the three lower doses (0.01, 0.05 and 0.1 ng). However, at the highest dose level (0.5ng) the mechanical threshold did not differ from baseline, although the animals reacted with whole body piloerections and/or startling response but did not lift the stimulated paw, a reaction not observed at any other dose level. All animals pretreated with capsaicin then with intrathecal 14.G2a reacted to mechanical stimulation in a similar manner to that at baseline throughout the study. Intrathecal administration of 14.G2a at any dose level did not produce a change in thermal threshold.
Rabbit		
New Zealand white Rabbit Vriesendorp 1997 No	IV 14.G2a: 1.5-40 mg (0.8-22 mg/kg based on 1.8 kg rabbit) 5 x Male 1-day	Observed Maximum Nonlethal Dose = 22 mg/kg Maximum nonlethal daily dose = 264 mg/m2 ; multiple of Human = 15 14.G2a bound to the granular layer in the cerebellum, myenteric ganglia and dorsal root ganglia, sciatic nerves. 14.G2a did not result in clinical, electrophysiological, or histological findings of neutopathy.
Dog		
Beagle dog Vriesendorp 1997 No	IV 14.G2a: 2 mg (0.2 mg/kg based on 10 kg dog) 2 sex not reported 1-day	Observed Maximum Nonlethal Dose = 0.2 mg/kg Maximum nonlethal daily dose = 4 mg/m^2 ; multiple of Human = 0.2 14.G2a bound to the granular layer in the cerebellum, myenteric ganglia and dorsal root ganglia, sciatic nerves. High-dose of 14.G2a in dogs produced signs of neurotoxicity, characterized as reduced distal motor amplitudes, indicative of distal axonal dysfunction.
Monkey		
Cynomolgus Monkey SBL354-004 No	IV ch14.18: 10.5 or 21 mg/kg 2M/group 1-day	Observed Maximum Nonlethal Dose = 21 mg/kg Maximum nonlethal daily dose = 252 mg/m^2 ; multiple of Human = 14 Foreskin swelling noted in both ch14.18 dose groups. Vomiting noted in high dose group within 30 minutes of dosing

Repeat dose toxicity

Table 12: Summary of repeat-dose toxicity studies

Species / Study ID / GLP	Method of Administration /Duration / Doses (mg/kg)	Non-Lethal dose (mg/kg/d)	Major findings
Dog			
Beagle dog Vriesendorp 1997 No	IV 14.G2a: ~ 40 mg/day (4 mg/kg based on 10 kg dog) 2, sex not reported 5-day	202 mg/kg over 5 days	14.G2a bound to the granular layer in the cerebellum, myenteric ganglia and dorsal root ganglia, sciatic nerves. High-dose of 14.G2a in dogs produced signs of neurotoxicity, characterized as reduced distal motor amplitudes, indicative of distal axonal dysfunction.
Rat			
Sprague-Dawley rat SBL354-003 Yes PIVOTAL STUDY	IV ch14.18: 5, 15, or 45 mg/kg 10/sex/group duration of dosing: 4 weeks duration of post-dose: 6 weeks	NOAEL < 5 mg/kg	Erythrocyte count, hemoglobin concentration, and/or hematocrit values were decreased slightly in males at 15 mg/kg and in females at 5 and 15 mg/kg. In addition, changes indicating hematopoiesis (high reticulocyte ratio and/or platelet count, increased cellularity of the hematopoietic cells in the femoral and sternal bone marrow, and/or extramedullary hematopoiesis in the liver and spleen) were observed in both sexes at doses ≥5 mg/kg. High spleen weight was noted in both sexes at doses ≥ 15 mg/kg. Leukocyte counts (mainly lymphocyte and neutrophil counts) were increased in males at 15 mg/kg and above, and in females at 45 mg/kg. In immunophenotyping of lymphocytes, CD3-NKR-P1A+ cells (NK cells) were increased in both sexes at doses ≥15 mg/kg. In histopathology, granulomatous inflammation in the lung, ileum, and Peyer's patch was observed in both sexes at 45 mg/kg, and hemorrhage in the lamina propria/muscular layer of the ileum was observed in males at 45 mg/kg. Increased lung weight was also noted in females at 45 mg/kg. Other changes in hematology and blood chemistry included low eosinophil count and A/G ratio, and high total protein, globulin, and Ca+2 in both sexes at doses ≥5 mg/kg. Prolongation of prothrombin time (PT) and activated partial thromboplastin time (APTT) in males at doses ≥5 mg/kg, and high monocyte and basophil counts and low potassium in females at 45 mg/kg were noted. Other changes in organ weight and histopathology included increased adrenal weight noted in females at doses ≥5 mg/kg, increased number of germinal centers in the white pulp in the spleen in males at 45 mg/kg and in females at doses ≥15 mg/kg. LIVER CHANGES: In histopathology, abnormal cell division (anisonucleosis of the hepatocytes, increased number of mitosis, and diffuse basophilic change of the hepatocytes) was observed in males at doses of ≥15 mg/kg and in females at doses ≥5 mg/kg. Centrilobular congestion and increased number/hypertrophy of the Kupffer cells (potentially the result of circulatory disturbances) were observed in both sexes at doses ≥5 mg/kg. Hepatocellular necrosis and pericentral vein/interlobular fibrosis were observed in 3 and 1 male respectively at 45 mg/kg, and pericentral vein/interlobular fibrosis was observed in 2 females at 5 mg/kg. In organ weight, increased liver weight was observed in both sexes at doses ≥ 15 mg/kg. In blood chemistry, high aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), and total cholesterol in both sexes and high ALP in females were noted at 45 mg/kg, high ALT and total cholesterol were noted in both sexes at 15 mg/kg, and high total cholesterol was noted in females at 5 mg/kg. (NOAEL) for males and females was lower than 5 mg/kg/day based on the liver-related changes, the slight decrease in erythrocyte parameters, and changes related to hematopoiesis

Species / Study ID / GLP	Method of Administration /Duration / Doses (mg/kg)	Non-Lethal dose (mg/kg/d)	Major findings
			observed in both sexes at 5 mg/kg and above.

Genotoxicity

No *in vitro/in vivo* genotoxicity studies have been conducted or are planned with ch14.18.

Carcinogenicity

No carcinogenicity studies have been conducted.

Reproduction Toxicity

No reproductive and developmental toxicity studies have been conducted.

Toxicokinetic data

The following table summarises the toxicokinetic data with ch14.18

Table 13: Overview of TK data

Dose Level	C_{max} µg/mL	Multiple of Human (C_{max})	AUC[c] µg*hr/mL	Multiple of Human (AUC)
Human [a] 25 mg/m^2	14	-	2600[d]	-
Rat (SBL354-003) 30 mg/m^2	19[b]	1.4	ND	-
90 mg/m^2	248[b]	17.7	8195	3.2
270 mg/m^2	1335[b]	95	156000	60

ND - Not Determined
a - Desai *et al.* J Clin Oncol 30, 2012 (S; abstr 9576)
b - Determined as C0 on Day 22 (day 1 of cycle 4), mean of male and female rats
c - $AUC_{24\text{-}inf}$ after 4th weekly cycle of treatment, mean of male and female rats (therefore 24 hrs of exposure post the final dose is missing)
d - Mean $AUC_{0\text{-}inf}$

Local Tolerance

No local tolerance studies have been conducted.

Other toxicity studies

Tissue cross-reactivity studies were conducted on normal or malignant frozen tissue.

Table 14: Summary of repeat-dose toxicity studies

Species / Study ID / GLP	Method of Administration /Duration / Doses (mg/kg)	Non-Lethal dose (mg/kg/d)	Major findings
Tissue Cross-Reactivity Mujoo 1987 No	Fresh frozen normal human tissues *in vitro* Murine Mab 14.18 (concentration used for staining was not reported)		Murine MAb 14.18 bound to adult cerebellum and fetal brain. All other tissues tested showed negative staining.
Tissue Cross-Reactivity Cheresh 1986 No	Fresh frozen normal human tissues *in vitro*		Little to no reactivity could be detected with murine Mab 14.18.

Species / Study ID / GLP	Method of Administration /Duration / Doses (mg/kg)	Non-Lethal dose (mg/kg/d)	Major findings
	Murine Mab 14.18 (concentration used for staining was not reported)		
Tissue Cross-Reactivity IM2038 Yes	Fresh frozen normal human tissues (juvenile and adult) *in vitro* ch14.18: 2 and 10 µg/ml		*Staining with ch14.18 was present in the juvenile human tissue panel as summarized below:* • Membrane/membrane granules in the following tissue elements: o epithelium in the kidney (tubules), skin (epidermis), and thymus (reticular) o endothelium in the majority of tissues o mononuclear cells in the gastrointestinal tract (colon, esophagus, small intestine, and stomach), heart, liver (may include Kupffer cells), lung, pancreas, skin, spleen, thymus, tonsil, and urinary bladder o reticuloendothelium in the spleen o reticular cells in GALT in the small intestine o chromaffin cells in the adrenal medulla o glomerular tuft cells in the kidney o hilus cells in the ovary o granulosa cells in the ovary • Cytoplasm/cytoplasmic granules in the following tissue elements: o epithelium in the esophagus (submucosal glands), kidney (tubules), lung (pneumocytes), prostate, skin (sweat glands), thymus (reticular), and tonsil (mucosa and crypts) o myoepithelium in the skin (sweat glands) o endothelium in the majority of tissues o stroma/stromal cells in the majority of tissues o smooth myocytes in the gastrointestinal tract (esophagus and stomach), prostate, urinary bladder, and uterus (endometrium) o mononuclear cells in the gastrointestinal tract (colon, esophagus, small intestine, and stomach), heart, liver (may include Kupffer cells), lung, pancreas, skin, spleen, thymus, tonsil, and urinary bladder o reticular cells in GALT in the small intestine o adipocytes in the adrenal, colon, pancreas, skin, and spinal cord o Schwann cells in the adrenal, gastrointestinal tract (esophagus, small intestine, and stomach), heart, kidney, liver, ovary, pancreas, prostate, skin, spinal cord, spleen, striated (skeletal) muscle, testis, thyroid, urinary bladder, and uterus (endometrium) o endoneurium in the heart, ovary, striated (skeletal) muscle, and urinary bladder o myenteric plexus (including neurons [ganglion cells] and reserve cells) in the gastrointestinal tract (colon, esophagus, small intestine, and stomach) o ganglion cells (including neurons and reserve cells) in the prostate o neurons in the brain (cerebrum) o glial cells in the brain (cerebrum) and spinal cord o neuropil in the brain (cerebrum) and spinal cord o chromaffin cells in the adrenal medulla o glomerular tuft cells in the kidney o granulosa cells in the ovary o theca cells in the ovary *Staining with ch14.18 was present in the adult human ovary and testis as summarized below:* • Membrane/membrane granules in the following tissue elements: o hilus cells in the ovary o seminiferous epithelium in the testis o interstitial (Leydig) cells in the testis o endothelium in the ovary and testis • Cytoplasm/cytoplasmic granules in the following tissue

Species / Study ID / GLP	Method of Administration /Duration / Doses (mg/kg)	Non-Lethal dose (mg/kg/d)	Major findings
			elements: o granulosa cells in the ovary o luteal cells in the ovary o theca cells in the ovary o seminiferous epithelium in the testis o interstitial (Leydig) cells in the testis o endothelium in the ovary and testis o stroma/stromal cells in the ovary and testis *Conclusion:* Staining with ch14.18 was present in numerous tissue elements throughout the juvenile tissue panel, as well as in adult human ovary and testis. In general this staining was consistent with reported sites of G_{D2} expression. Then in general, the staining was consistent with reported sites of G_{D2} expression. However no previous reports describing the expression of G_{D2} by reproductive elements of the ovary and testis, endothelium, adipocytes or glomerular tuft cells were identified, therefore staining in these tissue elements may represent previously unreported sites of G_{D2} expression or cross-reactivity with another epitope(s) closely related to G_{D2}.
Tissue Cross-Reactivity IM2189 Yes	Fresh frozen normal rat tissues *in vitro* ch14.18: 2 and 10 µg/ml		Staining with ch14.18 generally consistent with literature reports. However no previous reports describing the expression of G_{D2} by endothelium or perithelium, cardiac muscle, chondrocytes or reproductive elements of the ovary, placenta or testis were identified, therefore staining in these tissue elements may represent previously unreported sites of G_{D2} expression or cross-reactivity with another epitope(s) closely related to G_{D2}.
Tissue Cross-Reactivity IM2190 Yes	Fresh frozen normal rabbit tissues *in vitro* ch14.18: 2 and 10 µg/ml		Staining with ch14.18 generally consistent with literature reports. However no previous reports describing the expression of G_{D2} by mesothelium, endothelium or perithelium, skeletal muscle, chondrocytes, or reproductive tissue elements of the ovary, placenta or testis were identified, therefore staining in these tissue elements may represent previously unreported sites of G_{D2} expression or cross-reactivity with another epitope(s) closely related to G_{D2}.

2.3.5. Ecotoxicity / environmental risk assessment

No environmental risk assessment was submitted for dinutuximab.

2.3.6. Discussion on non-clinical aspects

Dinutuximab (or the murine monoclonal antibody 14.18) has been administered to mice, rabbits, rats, and dogs in single- or repeat-dose regimens that exceed the dose that is used clinically (see section 5.3 of the SmPC).

The binding of murine mab 14.18 (the predecessor of ch 14.18) with various human tumour cell lines was evaluated and the antibody was shown to recognise tumours of neuroectodermal origin: such as neuroblastoma, melanoma, glioma and small cell lung carcinoma. The murine mab 14.18 bound to all of the neuroblastoma cells tested; however, the number of GD2 binding sites was variable for the different neuroblastoma cell types.

Data from the literature demonstrate that ch14.18, and to a lesser extent 14.G2a, have the ability to kill both neuroblastoma and melanoma cells via an ADCC mechanism. There is also some evidence to suggest a CDC mechanism. Lymphocytes and granulocytes, particularly neutrophils, may be the primary cell populations responsible for ch14.18 mediated tumour cell cytotoxicity, and the activity of these

effector cells was maintained in neuroblastoma patients that have been on induction therapy. Finally, soluble cytokines such as rhGM-CSF and rhIL-2 enhance the ch14.18-mediated lysis of tumour cells, which may be useful in optimizing the anti-tumour effect of ch14.18.

In vitro, both neutrophils and PBMCs have been shown to mediate ADCC with ch14.18 in the short-term (up to 18 hours). Increased ADCC was dependent upon ch14.18 concentration, effector cell number, and the addition of rhGM-CSF increased cytotoxicity of neutrophils but not PBMCs. Over the long-term (7 days), at a high effector-to-target cell ratio and high ch14.18 concentration, neutrophils, particularly in the presence of rhGM-CSF, inhibited growth of GD2-positive cell lines, whereas under the same conditions growth of the GD2 negative cell line, in which ADCC could not be triggered, was stimulated. However, any tumour stimulatory effect induced by the hematopoietic cells alone on GD2 positive cell lines was overcome by the addition of ch14.18 in the presence or absence of rhGM-CSF.

The augmentation of cell lysis and ADCC in the presence of IL-2 and GM-CSF, respectively, therefore aims to optimise cytotoxicity in vivo and is therefore supportive of the proposed treatment regimen.

In vivo, in SCID mice bearing M21 xenografts, following intraperitoneal administration of ch14.18, there was a dose-dependent distribution within the tumour. In a separate study, administration of ch14.18 with PBMCs and IL-2 prevented the establishment and growth of GD2+ melanoma tumours, whereas treatment with ch14.18 alone or PBMCs and IL-2 alone had no effect on tumour growth.

Administration of anti-GD2 antibodies IV and intrathecally led to a rapid onset of mechanical, but not thermal, allodynia that was prolonged, for at least 48 hours. This was accompanied with an increase in the peripheral nerve background activity of Aβ, Aδ and C-type fibres, which was reduced with lidocaine and fully blocked with capsaicin, demonstrating an involvement of peptidergic fine afferent fibres. Most likely the antibody induces pain (mechanical allodynia) by binding to GD2 antigen on peripheral nerves and/or myelin. Although, the administration of dinutuximab did not seem to be associated with peripheral neuropathy, it is noted that these studies involved the administration of a single dose only. A cardiovascular and respiratory safety pharmacology study conducted in the cynomolgus monkey demonstrates that continuous intravenous administration of ch14.18 resulting in plasma concentrations greater than 10- fold (C_{max}) those observed clinically (Desai, 2012) showed minimal effects on the cardiovascular system, consisting of moderate increases in blood pressure and heart rate, and there were no observed effects on ECG parameters or on the respiratory system. It is possible that increased heart rate and blood pressure are the result of indirect mechanisms such as a response to pain.

In the rat, rapid elimination of ch14.18 after the final dose indicates a potential anti-drug antibody response which was observed only at 5 mg/kg/day. After the final dose, plasma concentrations of ch14.18 decreased rapidly at 5 mg/kg (undetectable within 48 hours), gradually at 15 mg/kg (generally undetectable by the third week of recovery) and were still detectable at the maximum dose of 45 mg/kg at the end of the 6-week recovery period. In these studies, the Applicant has indicated that ch14.18 plasma concentrations were up to ~100-fold greater than the observed clinical exposures.

Following IV injection, ^{125}I-ch14.18 targeted and accumulated in the GD2-expressing tumour. With the exception of the blood, which had similar levels as the tumour, non-targeted, normal tissues had substantially lower levels of radioactivity at all time-points evaluated. The chimeric ch14.18 and murine 14.G2a antibodies displayed identical blood clearance kinetics, and there appeared to be no difference in their ability to target GD2-expressing tumour xenografts in nude mice.

A large difference in half-life (14 hours) was observed in a study investigating distribution of 14.G2a in dogs. Initially, a potential mechanism for this large difference in half-life was requested. However the PK profile associated with administration of a murine antibody (14.G2a) in the dog was considered to have limited clinical relevance considering the pharmacokinetics of the actual drug product, ch14.18, has been

more recently well characterized in man. Hence, no further clarification on the difference in PK profile was sought.

In separate studies conducted in the dog, six days post-injection of the maximum dose (202 mg) of 14.G2a was associated with an enhanced uptake by the liver and mesenteric lymph nodes. The anaesthesia used caused reversible blood pooling in splanchnic organs and may have contributed to the liver accumulation. The high 14.G2a levels in the lymph node correlated with microscopic changes of mild distension of the subcapsular sinusoids and large follicles with pale germinal centres in the cortex. Very little 14.G2a penetrated the blood-brain barrier, and 2 - 7 times more radioactivity was detected in the peripheral nervous system than in the central nervous system. Although these data suggest that ch14.18 does not cross the blood-brain barrier in appreciable amounts, during immunohistochemistry studies, 14.G2a was found to bind to the granular layer of the cerebellum, to the vagus nerves and many of the sciatic nerve fibers. Moreover, as outlined previously, single doses of the proposed product, caused a significant reduction in distal motor amplitude. Low motor amplitudes are not fully explained; however it has been suggested that this could be due to binding of 14G2a to GD2 in the axon disrupting normal axonal function. It has also been postulated that the observed effect could be a result of cytokine production and that the corresponding hyperplastic lymph nodes are indicative of a robust immune response to a mouse antibody. The release of cytokines has been described as a potential mechanism for the pain experienced in man.

No formal metabolism studies have been conducted however IgG antibodies are predominantly cleared via catabolism to small peptides and individual amino acids.

The repeated-dose study in the rat appears to be adequate in terms of the maximum dose evaluated. No clinical signs of CNS toxicity were observed. Findings of note included treatment related adverse reactions of the liver (characterized by centrilobular congestion, abnormal cell division, hepatocellular necrosis and pericentral vein/interlobular fibrosis) which may be related to circulatory disturbances and changes indicative of increased hematopoiesis (high reticulocyte ratio and/or platelet count, increased cellularity of the hematopoietic cells in the femoral and sternal bone marrow, and/or extramedullary hematopoiesis in the liver and spleen). These changes were noted to be very slight to slight in severity and recovered or tended to recover following the cessation of dosing. The CHMP notes that a NOAEL has not been identified for the 28-day study in the rat. The principal findings appear to affect the liver, and it is noted that the increased ALT for e.g. is listed as one on the adverse reactions within the SmPC.

However, to date, only a study of 28 days duration has been conducted. Given that this is the first molecule of its class, the CHMP was of the view that the incidence and extent of this peripheral neuropathy following repeated administration, the underlying pathophysiology, and the potential for reversibility would benefit from further investigation. The Applicant will conduct further non-clinical investigations post-authorisation, in order to fully characterise the effects of dinutuximab on peripheral nerves (see RMP). The Applicant has suggested that a juvenile toxicity study in monkeys of 5 months duration will be performed in order to evaluate the effects of dinutuximab on the central and peripheral nervous system. However, the CHMP are of the view that studies in non-human primates are not necessary to fulfil this request. Non-clinical data generated in a single study using one species, along with additional monitoring in patients should provide additional information that would lead to improved information and advice for the prescriber.

GLP tissue cross-reactivity studies were conducted with rat, rabbit and human tissues: staining with ch14.18 was generally consistent with reported sites of GD2 expression. This information confirms the relevance of rat and rabbit as relevant species in accordance with ICH S6. However, no previous reports describing the expression of GD2 by endothelium or perithelium, cardiac muscle, chondrocytes or reproductive elements of the ovary, placenta or testis were identified, therefore staining in these tissue

elements may represent previously unreported sites of GD2 expression or cross-reactivity with another epitope(s) closely related to GD2.

The Applicant's rationale for not performing genotoxicity studies is deemed to be acceptable.

There is no direct evidence to suggest that anti-GD2 therapies do not have the potential to stimulate cell proliferation of healthy/normal cells. However, there is a wealth of evidence to support the fact that anti-GD2 therapies such as dinuxituximab suppress the growth of not only neuroblastomas, but small cell lung cancer cells, and melanoma cells as well. The Applicant has also confirmed that a positive correlation exists between GD2 expression and cell proliferation in a cell type that is not related to the proposed indication. Hence, on the basis of the data provided, it is agreed that the potential for the dinuxtuximab to cause carcinogenicity is low and that the absence of carcinogenicity studies is justified.

The lack of studies to determine the effects on fertility and embryofoetal development is accepted, given the proposed indication. In the repeat-dose toxicity studies, administration of dinutuximab in male and female rats resulted in no adverse effects on reproductive organs at exposures that were at least 60 fold higher than those observed clinically. However, in light of the potential expression of GD2 in the reproductive tract and the fact that the proposed product could be administered to patients up to the age of 17, at the request of the CHMP, the Applicant has discussed the effects (or lack thereof) on male and female fertility as observed during the repeated-dose studies and the level of information available has been included within the SmPC (see sections 4.6 and 5.3 of the SmPC).

In addition, because of the limited reproductive toxicity data, dinutuximab is not recommended during pregnancy and in women of childbearing potential not using contraception. It is also recommended that women of childbearing potential use contraception for 6 months after discontinuation of treatment with dinutuximab.

Injection site reaction is listed as a common adverse event within the SmPC, so from a non-clinical perspective, no further action is warranted.

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 for an environmental risk assessment. Therefore, no environmental risk assessment was provided and none is required.

2.3.7. Conclusion on the non-clinical aspects

In conclusion, the non-clinical studies (pharmacology, pharmacokinetics and toxicology), submitted for the marketing authorisation application for dinutuximab were considered acceptable for the assessment of non-clinical aspects. Non-clinical data reveal no special hazard for humans based on conventional studies conducted to date. These studies support the current dinutuximab dosing regimen of 17.5 mg/m^2/day administered for four consecutive days during five monthly courses.

The CHMP considers the following measures necessary to address the non-clinical issues:

- To conduct additional nonclinical research to evaluate the incidence and extent of peripheral and central nervous system toxicity following repeated administration of dinutuximab, the underlying pathophysiology, and the potential for reversibility.

2.4. Clinical aspects

2.4.1. Introduction

The NCI has sponsored 11 clinical trials with ch14.18 including four in adult subjects with melanoma and seven in paediatric subjects with neuroblastoma. All these studies have used the product manufactured for the NCI. Four additional studies have been conducted in subjects with neuroblastoma outside the NCI. Finally, UTC has performed a pharmacokinetic study comparing the product to be commercialised with the NCI product.

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.

- Tabular overview of clinical studies

Study Identifier	Study Description	Sample Size	Test Product(s); Dosage Regimen	Duration of Treatment
NCI Sponsored Studies in Subjects with Neuroblastoma				
POG-9347 (Yu 1997)	Open-label efficacy and tolerability study of ch14.18 + GM-CSF in subjects with recurrent neuroblastoma	Recurrent Neuroblastoma (n = 32)	ch14.18: 50 mg/m^2/day IV over 5 hours x 4 days GM-CSF: 10 mcg/kg/day x 14 days IV or SC	14 days
CCG-0935 (Ozkaynak 2000)	Open-label, dose escalation and tolerability study of ch14.18 + GM-CSF in subjects with neuroblastoma	Neuroblastoma (n = 23)	ch14.18: 20-50 mg/m^2/day IV over 5 to 10 hours x 4 days GM-CSF: 250 mcg/m^2/day IV or SC	Up to six 4-day courses every 28 days
CCG-0935A (Gilman 2009)	Open-label, dose escalation and tolerability study of ch14.18 + GM-CSF + IL-2 in subjects with neuroblastoma	Neuroblastoma (n = 25)	ch14.18: 20-40 mg/m^2/day IV over 5 to 10 hours x 4 days GM-CSF: 250 mcg/m^2/day IV or SC Courses 1, 3, and 5 IL-2: up to 4.5 MIU/m^2/day IV x four days Courses 2 and 4 RA: 80 mg/m^2/dose BID x 14 days, PO Courses 1-6	Up to five 4-day courses (a 6th with RA alone) every 28 days
DIV-NB-301 (ANBL0032) (Yu 2010)	Open-label, randomized, efficacy and safety study of ch14.18 + GM-CSF + IL-2 in subjects with neuroblastoma	High-risk Neuroblastoma (n = 251)	ch14.18: 25 mg/m^2/day IV over 5 to 20 hours x 4 days Courses 1-5 GM-CSF: 250 mcg/m^2/day IV or SC Courses 1, 3, and 5 IL-2: up to 4.5 MIU/m^2/day IV x four days Courses 2 and 4 RA: 80 mg/m^2/dose BID x 14 days, PO Courses 1-6	Approximately 6 months
DIV-NB-302 (ANBL0032) (Ongoing)	Open-label, non-randomized, efficacy and safety study of ch14.18 + GM-CSF + IL-2 in subjects with neuroblastoma	High-risk Neuroblastoma 737 subjects enrolled since the close of randomization (13 Jan 2009) through 30 June 2013	ch14.18: 25 mg/m^2/day IV over 5 to 20 hours x 4 days Courses 1-5 GM-CSF: 250 mcg/m^2/day IV or SC Courses 1, 3, and 5 IL-2: up to 4.5 MIU/m^2/day IV x four days Courses 2 and 4 RA: 80 mg/m^2/dose BID x 14 days, PO Courses 1-6	Approximately 6 months
DIV-NB-30	Open-label, safety study of ch14.18 +	High-risk	ch14.18: 25 mg/m^2/day IV over 10 to	Approxi

Study Identifier	Study Description	Sample Size	Test Product(s); Dosage Regimen	Duration of Treatment
3 (ANBL0931)	GM-CSF + IL-2 in subjects with neuroblastoma	Neuroblastoma (n = 105)	20 hours x 4 days Courses 1-5 GM-CSF: 250 mcg/m^2/day IV or SC Courses 1, 3, and 5 IL-2: up to 4.5 MIU/m^2/day IV x four days Courses 2 and 4 RA: 80 mg/m^2/dose BID x 14 days, PO Courses 1-6	mately 6 months
CHP1002 (Desai 2014)	Open-label PK correlative laboratory sub-study of the ANBL0032 study.	High-risk Neuroblastoma (n = 14)	ch14.18: 25 mg/m^2/day IV over 10 to 20 hours x 4 days Courses 1-5 GM-CSF: 250 mcg/m^2/day IV or SC Courses 1, 3, and 5 IL-2: up to 4.5 MIU/m^2/day IV x four days Courses 2 and 4 RA: 80 mg/m^2/dose BID x 14 days, PO Courses 1-6	Approximately 6 months
ANBL1221 (Ongoing)	Randomized, open-label efficacy and tolerability study.	Refractory, relapsed or progressive neuroblastoma (n = 74)	Regimen A: Temozolomide (100 mg/m^2/dose PO daily, days 1-5), Irinotecan (50 mg/m^2/dose IV daily, days 1-5) and Temsirolimus (35 mg/m^2/dose IV on days 1 and 8) Regimen B: Temozolomide (100 mg/m^2/dose PO daily, days 1-5), Irinotecan (50 mg/m^2/dose IV daily, days 1-5) and ch14.18 (25 mg/m^2/dose IV days 2-5) and GM-CSF (250 mcg/m^2/dose SC days, 6-12)	Up to 1 year (17 courses)
NANT 2011 04 (Ongoing)	Open-label, uncontrolled, dose escalation study of lenalidomide in combination with ch14.18	Refractory, relapsed or progressive neuroblastoma (n = 62)	Lenalidomide (doses from 18 mg/m^2/dose, to 100 mg/m^2/dose PO, for days 1-21), ch14.18 (25 mg/m^2/dose IV days 8-11), isotretinoin (80 mg/m^2/dose, BID, PO days 15-28)	Up to 6 months
UTC Sponsored Study in Subjects with Neuroblastoma				
DIV-NB-201	Open-label, randomized, pharmacokinetic study of ch14.18 + GM-CSF + IL-2 in subjects with neuroblastoma	High-risk Neuroblastoma (n = 28)	ch14.18: 17.5 (UTC) or 25 (NCI) mg/m^2 IV over 10-20 hours x 4 days x 5 courses; GM-CSF: 250 mcg/m^2/day IV or SC Courses 1, 3, and 5 IL-2: up to 4.5 MIU/m^2/day IV x four days Courses 2 and 4 RA: 80 mg/m^2/dose BID x 14 days, PO Courses 1-6	Approximately 6 months
Non-NCI or UTC Sponsored Studies in Subjects with Neuroblastoma				
Handgretinger 1995	Open-label, dose escalation and tolerability study in subjects with neuroblastoma	Neuroblastoma (n = 9)	ch14.18: 30-50 mg/m^2/day IV over 8 hours x 5 days	One to four courses separated by 8 to 12 week intervals
Uttenreuther-Fischer 1995 and Yu 1998	Open-label, dose escalation and tolerability study in subjects with neuroblastoma or osteosarcoma	Neuroblastoma (n = 10) Osteosarcoma (n = 1)	ch14.18: 10-200 mg IV over 1 to 4 hours x 1 to 4 days	One to five courses separated by

Study Identifier	Study Description	Sample Size	Test Product(s); Dosage Regimen	Duration of Treatment
				two to three week intervals
NB90 and NB97 (Simon 2004; Simon 2005; Simon 2011)	Retrospective analysis of open-label ch14.18 administered in the NB90 and NB97 studies	Neuroblastoma (n = 334)	ch14.18: 20 mg/m^2/day over 8 to 12 hours x 5 days	Six cycles separated by a two month interval
Klingebiel 1998	Open-label safety and tolerability study of iodine-131-meta-iodobenzylguanidine (^{131}I-MIBG), high dose chemotherapy, and ch14.18	Neuroblastoma (n = 11)	iodine-131-meta-iodobenzylguanidine followed by ch14.18: 20-40 mg/m^2/day over 8 hours x 5 days	Two to five courses
National Cancer Institute (NCI) Sponsored Studies in Subjects with Melanoma				
B89-0005 (Saleh 1992)	Part A: Open-label, dose escalation and tolerability study of ch14.18 in subjects with melanoma	GD2-positive metastatic melanoma (n = 13)	ch14.18: 5-100 mg IV over 1 to 4 hours x one day (100 mg dose split over 2 days)	Single dose
	Part B: Open-label, single dose study with ch14.18 in subjects with melanoma	GD2-positive metastatic melanoma (n = 6)	ch14.18: 30 mg/m^2 IV over 1 to 4 hours x 2 days x up to 3 consecutive weeks (up to a maximum dose of 180 mg/m^2)	Repeat doses up to 3 weeks
B90-0014 (Albertini 1997)	Open-label, dose escalation and tolerability study of ch14.18 in combination with IL-2 in subjects with melanoma	Melanoma (n = 24)	ch14.18: 2-10 mg/m^2/day IV over 4 hours x 5 days x 2-3 cycles IL-2: 1.5 MIU/m^2/day IV x 4 days; continuous 96-hr infusion x 2-3 cycles	Up to 10 weeks
B93-0009 (Murray 1996)	Open-label, dose escalation and tolerability study of ch14.18 in combination with GM-CSF in subjects with melanoma	Malignant Melanoma (n = 16)	ch14.18: 15-60 mg/m^2 IV over 4 hours GM-CSF: 250 mcg/m^2/day SC x 14 days	2 weeks
B94-0002 (Choi 2006)	Open-label, dose escalation and tolerability study of ch14.18 and R24 (an anti-GD3 mAb) in combination with IL-2 in subjects with melanoma and sarcoma	Melanoma (n = 23) Sarcoma (n = 4)	ch14.18: 2-7.5 mg/m^2/day IV over 4 hours x 5 days R24: 1-10 mg/m^2/day IV x 5 days IL-2: 1.5 MIU/m^2/day IV (n = 26); 4.5 MIU/m^2/day IV (n = 1)	3 weeks

2.4.2. Pharmacokinetics

The pharmacokinetics of ch14.18 have only been evaluated in patients, either adults with melanoma or mostly children with neuroblastoma. The PK data collected in trials sponsored by NCI or UTC are summarised in Table 15; most children enrolled in these trials were 1 to 15 years old.

Table 15: Summary of NCI/UTC sponsored studies providing PK data for ch14.18

Study Identifier	Study Description	Sample Size with PK data-age	Test Products; Dosage Regimen	Source of the data
Children with neuroblastoma				

Study Identifier	Study Description	Sample Size with PK data-age	Test Products; Dosage Regimen	Source of the data
CCG-0935 Ozkaynak 2000 NCI	Open-label, dose escalation and tolerability study of ch14.18 + GM-CSF	Neuroblastoma n = 18 2 – 15 years	ch14.18: 20-50 mg/m^2/day IV over 5 to 10 hours x 4 days GM-CSF: 250 mcg/m^2/day IV or SC x 7 days	***Publication*** Data from Course 1
CCG-0935A Gilman 2009 NCI	Open-label, dose escalation and tolerability study of ch14.18 + GM-CSF + IL-2 *+ RA (80 mg/m^2/dose BID x 14 days, PO Courses 1-6)	Neuroblastoma n = 12 2 – 14 years	Regimen 3 ch14.18: 25 mg/m^2/day IV over 5 to 10 hours x 4 days GM-CSF: 250 mcg/m^2/day IV or SC Courses 1, 3, and 5 IL-2: 4.5 MIU/m^2/day IV x 4 days Courses 2 and 4	***Publication*** Data from Course 1 to 5
DIV-NB-301 (ANBL0032) NCI	Open-label, randomized, efficacy and safety study of ch14.18 + GM-CSF + IL-2 *+ RA (80 mg/m^2/dose BID x 14 days, PO Courses 1-6)	High-risk Neuroblastoma N = 138 1 – 15 years	ch14.18: 25 mg/m^2/day IV over 5 to 20 hours x 4 days Courses 1-5 GM-CSF: 250 mcg/m^2/day IV or SC Courses 1, 3, and 5 IL-2: up to 4.5 MIU/m^2/day IV x four days Courses 2 and 4	***CSR*** Data from Course 1, 4, 5
CHP1002 Desai 2014 NCI	Open-label PK correlative laboratory sub-study of the ANBL0032 study. *+ RA (80 mg/m^2/dose BID x 14 days, PO Courses 1-6)	High-risk Neuroblastoma n = 14 1 – 7 years	ch14.18: 25 mg/m^2/day IV over 10 to 20 hours x 4 days Courses 1-5 GM-CSF: 250 mcg/m^2/day IV or SC Courses 1, 3, and 5 IL-2: up to 4.5 MIU/m^2/day IV x four days Courses 2 and 4	***Publication*** Data from Course 1, 3, 5
DIV-NB-302 (ANBL0032) NCI	Open-label, efficacy and safety study of ch14.18 + GM-CSF + IL-2 *+ RA (80 mg/m^2/dose BID x 14 days, PO Courses 1-6)	High-risk Neuroblastoma n = 277 1 – 29 years	ch14.18: 25 mg/m^2/day IV over 5 to 20 hours x 4 days Courses 1-5 GM-CSF: 250 mcg/m^2/day IV or SC Courses 1, 3, and 5 IL-2: up to 4.5 MIU/m^2/day IV x 4 days Courses 2 and 4	***Safety Summary*** Data from Course 1, 4, 5
DIV-NB-303 (ANBL0931) NCI	Open-label, safety study of ch14.18 + GM-CSF + IL-2 *+ RA (80 mg/m^2/dose BID x 14 days, PO Courses 1-6)	High-risk Neuroblastoma n = 103 1 – 27 years	ch14.18: 25 mg/m^2/day IV over 5 to 20 hours x 4 days Courses 1-5 GM-CSF: 250 mcg/m^2/day IV or SC Courses 1, 3, and 5 IL-2: up to 4.5 MIU/m^2/day IV x 4 days Courses 2 and 4	***Safety Summary*** Data from Course 1, 4, 5
DIV-NB-201 UTC	Open-label, crossover, randomized, pharmacokinetic study of ch14.18 + GM-CSF + IL-2 *+ RA (80 mg/m^2/dose BID x 14 days, PO Courses 1-6)	High-risk Neuroblastoma n = 28 1 – 9 years	ch14.18: 17.5 (UTC) or 25 (NCI) mg/m^2 IV over 10-20 hours x 4 days x 5 courses; GM-CSF: 250 mcg/m^2/day IV or SC Courses 1, 3, and 5 IL-2: up to 4.5 MIU/m^2/day IV x four days Courses 2 and 4	***CSR*** UTC vs. NCI PK profile during Course 1 and 3
Adults with melanoma				
B89-0005 Saleh 1992 NCI	Part A: Open-label, dose escalation and tolerability study of ch14.18	GD2-positive metastatic melanoma n = 13 29 – 80 years	ch14.18: 5, 15, 45, and 100 mg IV over 1 to 4 hours x one day (100 mg dose split over 2 days)	***Publication*** Single dose

Study Identifier	Study Description	Sample Size with PK data-age	Test Products; Dosage Regimen	Source of the data
B93-00091 Murray 1996 NCI	Open-label, dose escalation and tolerability study of ch14.18 in combination with GM-CSF	Malignant Melanoma n = 5 30 – 77 years	ch14.18: 15, 30, 45, 60 mg/m^2 IV over 4 hours GM-CSF: 250 mcg/m^2/day SC x 14 days	***Publication*** Single dose

The Applicant performed a population PK analysis on the data from Study DIV-NB-201 where a validated bioanalytical assay was used to measure dinutuximab concentrations. A two compartmental model was fitted and the following parameters were estimated for the product manufactured by UTC.

Table 16: Summary of the PK parameters – popPK analysis

	Weight kg	BSA m2	CL L/day	CL L/day/m2	V1 L	V2 L	Vss L	t1/2 h
N	26	26	26	26	26	26	26	26
Mean	14.8	0.635	0.683	1.09	1.43	3.94	5.38	240
SD	4.13	0.138	0.307	0.457	0.403	1.1	1.47	135
CV%	27.9	21.8	44.9	41.9	28.2	27.9	27.4	
Geometric Mean	14.2	0.62	0.602	0.971	1.38	3.79	5.18	
Geometric CV%	29.3	22.3	60	59	29	29.3	28.6	

Absorption

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

Distribution

The pharmacokinetics of dinutuximab were evaluated in clinical study DIV-NB-201. In this study, 27 children with high-risk neuroblastoma (age: 3.9 ± 1.9 years) received up to 5 cycles of Unituxin at 17.5 mg/m^2/day as an intravenous infusion over 10 to 20 hours for 4 consecutive days every 28 days. The mean (± standard deviation) maximum plasma concentration observed after the 4th infusion was 11.5 (± 2.3) mcg/mL. In a population pharmacokinetic analysis, the geometric mean volume of distribution at steady state was estimated at 5.2 L.

Elimination

Classical biotransformation studies have not been performed with dinutuximab. In children, mean terminal half-life estimated in the population PK analysis was 240 (± 135) hours, consistent with that found in adults. The mean geometric clearance was determined to be 0.025 L/h.

Dose proportionality and time dependencies

Dose proportionality

In adult subjects, the AUC appeared to be dose-proportional when the dose (5 mg, 15 mg, 45 mg, or 100 mg) was normalized to the body weight based on data from Saleh (1992). In children, Uttenreuther-Fischer et al (1995) found that peak concentrations and AUCs strongly correlated with the administered dose. In Study DIV-NB-201, at the recommended dose, the mean observed C_{max} after the last infusion of the treatment course was 11.5 (± 2.3) mcg/mL.

Time dependency

Ch14.18 measurements in consecutive treatment courses were carried out in several paediatric studies.

Uttenreuther-Fischer et al (1995) noted that the mean $t_{1/2}\beta$ values significantly decreased in five subjects receiving a second course of ch14.18 as compared to the first course: 72.9 ± 19.8 hours (median 70 hours) versus 31.7 ± 18.4 hours (median 24 hours). The investigators suggested that development of a HACA response against the antigen-binding site of ch14.18 in three of five subjects could, in part, account for these changes, another possible cause being progressive growth of bulky tumours in another patient (increased antibody uptake and/or metabolism by this tumour).

In Study CHP002, where 7 children were studied on courses 1 and 3, there was no change in clearance except in one subject that developed HACA between courses 1 and 3; this subject had a 41% decrease in exposure (AUC) from course 1 to course 3.

In the pivotal Phase III trial (DIV-NB-301) and its further open-label extensions (DIV-NB-302& -303), subjects assigned to ch14.18 immunotherapy had samples taken for the determination of ch14.18 and HACA at the following time points: pre-treatment and in Course 1 (before the 4th infusion), 4 (one week prior the 1st infusion and before the 4th infusion), 5 (one week prior the 1st infusion and before the 4th infusion), and after Course 6 (see Table 5). Median ch14.18 concentrations after the first treatment course were lower in subjects that developed HACAs, especially trough levels. Furthermore, one patient in the bioequivalence study (DIV-NB-201) who developed neutralising antibodies had no detectable ch14.18 levels during the 3rd treatment course.

Finally, in a population PK analysis performed on all available data (DIV-NB-301, -302, -303, -201, and CHP1002) the presence of HACA increased clearance by 60%. The analysis of antibody titre with post-hoc PK parameters showed some correlation with clearance however there is high variability.

In the absence of antibodies, the clearance and exposure to ch14.18 did not change over treatment courses. In particular, the population PK analysis did not show an impact of concomitant medication (GM-CSF and IL-2).

Table 17: Summary of ch14.18 plasma concentrations (ng/mL) in Studies DIV-NB-302 and-303 by antibody status

Study Day		HACA Positive Subjects (N=64)	HACA Negative Subjects (N=318)
Day -1	n	46	231
	Mean	141.92	105.34
	SD	2599.82	424.2

	Median	12.78	18.37
	Min, Max	0.0, 17660.0	0.0, 3739.4
Day 6	n	55	265
	Mean	5481.29	5949.33
	SD	2237.56	2331.43
	Median	5081.22	5739.00
	Min, Max	271.2, 13454.0	20.6, 15011.3
Day 80	n	53	230
	Mean	403.19	706.01
	SD	903.36	1144.50
	Median	121.09	440.59
	Min, Max	0.9, 5520.0	26.9, 10910.0
Day 90	n	56	219
	Mean	3765.09	5259.20
	SD	2772.56	2086.70
	Median	3746.34	5093.04
	Min, Max	23.3, 10370.8	124.5, 13148.4
Day 111	n	43	211
	Mean	227.63	616.65
	SD	448.43	946.90
	Median	90.41	432.51
	Min, Max	1.9, 2712.0	1.5, 11630.0
Day 118	n	49	204
	Mean	5228.35	6738.40
	SD	3376.22	2353.95
	Median	5027.79	6583.96
	Min, Max	5.9, 13354.6	277.6, 17415.6
Final	n	40	208
	Mean	80.85	268.74
	SD	109.49	507.36
	Median	30.71	157.35
	Min, Max	0.0, 416.0	0.6, 5347.3

Comparability of the NCI and UTC products (Study DIV-NB-201)

Overall, 28 subjects were enrolled in this randomised cross-over trial: 2 courses of one product followed by 3 courses of the other product. ch14.18 manufactured by UTC was administered at a dose of 17.5 mg/m^2/day intravenously (IV) x four days in each course (either Courses 1 and 2 or Courses 3, 4, and 5) whereas ch14.18 manufactured by NCI was administered at a dose of 25 mg/m^2/day IV x four days in each course (either Courses 1 and 2 or Courses 3, 4, and 5). Doses of ch14.18 are equivalent between the two manufacturers in terms of total ch14.18 protein amount.

The mean age of subjects at randomisation was 3.9 years (range 0.76 to 8.8 years). The majority of subjects were male (16/28; 57%) and white (23/28; 82%). There were no notable differences in the demographics of subjects by treatment sequence.

Blood sampling was performed during Courses 1 and 3 (ch14.18 co-administered with GM-CSF). All 28 subjects received at least one dose of study therapy and were included in the safety population. Twenty-seven subjects were included in the PK population as one subject was excluded from the PK analysis due to a HACA response that was inhibiting in the PK assay during Course 3.

A model-based approach was employed by the Applicant to account for the complexities of the study in a paediatric oncology population. PK parameters estimates were based on a nominal ch14.18 dose of 17.5 mg/m^2 and an infusion time of 10 hours. Product was determined not to be a significant covariate on clearance or volume of distribution. Simulations were performed with population values of body weight (14.2 kg) and body surface area (BSA; 0.62 m^2). The primary comparability analysis is summarised in Table 6. A conventional non-compartmental analysis was also performed and the geometric mean ratio for AUC was 108 (90% CI: 97-120) and for C_{max} 133 (90% CI: 104- 169). When excluding the patient with neutralising antibodies, the C_{max} ratio was substantially reduced and the variability of C_{max} is expected given the variability of the duration of infusion. In totality, it is considered that these data demonstrate comparable exposure between the NCI and UTC products.

Table 18: Primary comparability analysis of NCI and UTC products

PK Parameter	Geometric Mean UTC (Comparator)	Geometric Mean NIC (Reference)	Ratio	90% CI of Ratio	
				Lower	Upper
AUC_{inf} (mcg*h/mL)	431.2	413.5	1.04	0.98	1.11
C_{max} (ng/mL)	6568.2	6876.9	0.96	0.88	1.04

Of the 27 subjects evaluated for HACA, six (22%) had confirmed HACA titres during the study with only one testing positive for neutralising antibody. There were more patients developing antibodies on the UTC product (5) than on the NCI product (1); however, the study population was relatively small and 3/5 patients had low titre antibodies to the UTC product.

There was no substantial difference in the AE profile of the two products; in particular, the rate of allergic reactions appeared similar.

Special populations

Important covariates on PK were explored in a population PK analysis based on data from the following clinical studies: ANBL0032 (DIV-NB-301 and DIV-NB-302), ANBL0931 (DIV-NB-303), DIV-NB-201, and CHP1002. Some data imputation was required for this model. This analysis confirmed that dosing based on BSA is appropriate. The important covariates identified in the model are the presence of antibodies and manufacturer, although the latter is attributed to the known difference in nominal dosing units, as previously described. Of the other covariates tested there was no effect of age, race, gender, concomitant medications (IL-2, GM-CSF) or presence of capillary leak syndrome. Finally, there was no apparent significant correlation between CL (or V1) with any of the renal and hepatic impairment measures.

Pharmacokinetic interaction studies

No Pharmacokinetic interaction studies were conducted with dinutuximab

Pharmacokinetics using human biomaterials

2.4.3. Pharmacodynamics

No human pharmacodynamic data have been presented in this application. The immunogenicity response to ch14.18 is presented in the safety section of the report.

In addition, the Applicant refers to ch14.18's ability to mediate an antibody-dependent cellular cytotoxicity (ADCC) response, which has been evaluated in several non-clinical studies described from the published literature in the Non-clinical part of the application.

Mechanism of action

GD2 is a surface glycolipid antigen that is normally found on neurons, peripheral pain fibres, and skin melanocytes. In neuroblastoma, GD2 is expressed homogeneously and abundantly on 100% of neuroblastoma cells and facilitates the attachment of tumour cells to the extracellular matrix. GD2 is also expressed by some melanomas, brain tumours, small cell carcinomas of the lung, and some sarcomas.

The mechanism of action for ch14.18 is through antibody dependent cell-mediated cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). *In vitro* studies have demonstrated that anti-GD2 antibodies effectively lyse tumour cells through ADCC (Mujoo 1987, Mueller 1990) and CDC (Mujoo 1987). Anti-GD2 therapies may also prevent attachment of circulating malignant cells to the extracellular matrix.

Multiple anti-GD2–targeted antibodies have been developed: murine antibodies (3F8 and 14G2a), a chimeric antibody (ch14.18), humanised antibodies (Hu3F8 and hu14.18 K3222A). The murine IgG3 monoclonal antibody 3F8 was discovered in 1985 and represents the first anti-GD2 antibody. During the 1990s, 14.G2a, an IgG2a murine anti-GD2 antibody, was evaluated in Phase I and II clinical trials, but research ceased after the less-immunogenic chimeric preparation (ch14.18) became available for clinical evaluation; ch14.18 demonstrated 50-100 times more potency for the GD2 antigen and less immunogenicity than 3F8.

Rationale for the combination to GM-CSF and IL-2

In clinical studies investigating the safety and efficacy of ch14.18 monotherapy, results were mixed (see supportive studies in the Efficacy section). Given the less than optimal responses for subjects treated with ch14.18 monotherapy, investigators posited whether the addition of GM-CSF to ch14.18 could improve outcomes in patients with high-risk neuroblastoma.

The rationale for including GM-CSF is multi-fold. It is known to promote antitumor immunity through the activation of monocytes, macrophages, and dendritic cells, thereby enhancing ADCC which is a known mechanism for ch14.18. In addition, peripheral blood mononuclear cells (PBMC), T cells, and antigen presenting cells cultured with GM-CSF exhibit increased production of Type-1 cytokines (i.e., IL-12, interferon-γ, and tumour necrosis factor-α), which stimulate inflammatory and cellular responses, and decreased production of Type-2 cytokines (IL-10 and IL-4), which are mediators of immune suppression. GM-CSF has been consistently demonstrated *in vitro* that granulocytes are more efficient at inducing ADCC to neuroblastoma cells when combined with GM-CSF and anti-GD2 antibodies. Importantly, GM-CSF has not been shown to induce the proliferation of neuroblastoma cells nor does it appear on its own to kill neuroblastoma cells. The clinical effect of the combination of ch14.18 and GM-CSF has been investigated in Phase I-II studies (see supportive studies in the Efficacy section).

The reason for including IL-2 is multi-fold. It causes activation of natural killer (NK) cells, generation of lymphokine-activated killer (LAK) cells, and augments ADCC, a mechanism of action for ch14.18.

Increased natural killer and ADCC induced tumour lysis has been shown *in vitro* when murine 14.18 was applied with PBMC cells incubated with recombinant IL-2. In a melanoma xenograft mouse model, IL-2 alone had no effect on tumour growth while the combination of both ch14.18 and IL-2 was effective at eradicating tumours. In a Children's Cancer Group (CCG) Phase II clinical trial, IL-2 was administered to children with refractory solid tumours; no antitumour effects were observed in children with sarcomas or neuroblastomas, whereas one of five children with renal cell carcinoma had a complete response. Early clinical studies with the murine antibody 14.G2a and IL-2 demonstrated the combination was well tolerated and induced ADCC-mediated tumour lysis. A Phase I study was subsequently conducted with ch14.18 and IL-2 in patients with melanoma; immune activation was confirmed following ch14.18 and IL-2 administration through the induction of LAK cells and ADCC (Albertini 1997). Based on these results, IL-2 was added to the ch14.18 regimen included in the CCG-0935A study (see supportive studies in the Efficacy section).

2.4.4. Discussion on clinical pharmacology

All PK data have been collected in patients, mostly in the target population of children and adolescents. This is acceptable given the toxicity of the product.

PK information is extracted from published literature but mostly from the recently completed studies CHP1002 and DIV-NB-201 using validated bioanalytical assays and non-compartmental methods as well as PK modelling. In addition, plasma concentrations are available from sparse sampling in the pivotal Phase III trial (DIV-NB-301) and its extensions. Finally, a population PK analysis taking into account all available paediatric data was conducted.

The PK profile of ch14.18 appears bi-exponential with a mean terminal half-life of 10 days. Geometric mean estimate for the volume of distribution at steady state is 5.2 L, consistent with other monoclonal antibodies, and for the clearance 0.025 L/h.

The kinetics of ch14.18 appear roughly dose-proportional over the range of 10 – 100 mg/m^2.

In the absence of antibodies, the clearance and exposure to ch14.18 do not change over treatment courses. The development of HACAs decreases the exposure to ch14.18, which may become undetectable in the presence of neutralising antibodies.

Dinutuximab is a protein for which the expected metabolic pathway is degradation to small peptides andindividual amino acids by ubiquitous proteolytic enzymes.

A population PK analysis demonstrated that dosing based on BSA is appropriate. The important covariate identified in the model is the presence of antibodies; the presence of HACA increases clearance by 60%. The role of neutralising antibodies is currently unclear as the number of subjects affected was too small to draw any conclusion. Therefore, the applicant will develop and validate an assay for the detection of neutralising antibodies in the presence of dinutuximab and conduct a study to assess the neutralising ADA response to dinutuximab (see RMP).

A major concern was raised during the procedure regarding the comparability between the UTC commercial product (administered at a dose of 17.5 mg/m^2/day) and the NCI product (administered at a dose of 25 mg/m^2/day) that was used in the clinical trials. However the Population PK and conventional non compartmental analyses of data from Study DIV-NB-201 demonstrate that there is not a significant difference in exposure between the two products. Limited data from this study suggest that the safety profile of the two products is comparable although the UTC product appeared slightly more immunogenic.

There was no effect of age, race, gender, concomitant medications (IL-2, GM-CSF) and presence of capillary leak syndrome, renal or hepatic impairment on the PK of dinutuximab. No interaction studies have been performed. A risk for interactions with concomitantly used medicinal products (other than those cited above) cannot be excluded.

Pharmacodynamics

The mechanism of action of ch14.18 is through antibody dependent cell-mediated cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). Pharmacodynamic data (ADCC and CDC) from serum of patients treated with ch14.18 +/- cytokine are available in the literature. In the NCI trials (including the pivotal trial), ADCC was planned to be measured in patient blood samples; the applicant is recommended to submit these data post-authorisation, including the impact of HACAs on these results.

Following intensive chemotherapy, the immune system of patients with high-risk neuroblastoma is weakened in terms of functioning neutrophils, macrophages, and lymphocytes. Therefore, the rationale for combining ch14.18 with a cytokine stimulating neutrophils and other white blood cells (GM-CSF) and a cytokine stimulating NK and LAK cells (IL-2) is acknowledged from a PD perspective. Furthermore, *in vitro* and experimental animal model data have shown the effects of these combinations on tumour cell lysis and tumour growth.

Available clinical data indicate that the cytokines on their own have no relevant anti-tumour effect in patients with neuroblastoma. In the early monotherapy trials with anti-GD2 monoclonal antibodies clinicians did not consider the treatment benefit sufficiently important, and therefore, based on *in vitro* and experimental animal models, investigated the combination to GM-CSF, and subsequently to IL-2.

2.4.5. Conclusions on clinical pharmacology

The PK profile of ch14.18 has been well characterised in the target population

Exposure is considered comparable following the administration of the NCI and UTC products, therefore the clinical data generated with the NCI product can be extrapolated to the UTC product.

Further information on the UTC product is required post-authorisation to determine the type and kinetics of the antibody response and its impact on dinutuximab exposure (see Annex II obligation).

2.5. Clinical efficacy

This application is supported by a single pivotal open-label randomised trial, which evaluated the addition to isotretinoin of ch14.18 in combination with GM-CSF and IL-2 in comparison to isotretinoin alone (control).

2.5.1. Dose response study(ies)

- A Phase I pilot trial was conducted in 10 children with neuroblastoma and one adult with osteosarcoma where escalating doses of 10 to 200/mg/m2 ch14.18 were administered and PK data were obtained (Uttenreuther-Fischer, 1995). Although the maximum tolerated dose (MTD) was not reached, clinical responses were observed and the biologic activity of ch14.18 was demonstrated in vivo using post-treatment sera against ch14.18 coated tumour target cells to mediate lysis in a complement dependent cytotoxicity assay (Yu, 1998).

• The first efficacy trial was a Phase II study (open-label, uncontrolled) sponsored by the NCI and conducted by the Pediatric Oncology Group from 1994 to 1997, which evaluated the addition of GM-CSF to ch14.18 (POG-9347): 4 daily IV doses of ch14.18 50 mg/m2 over 5 hours (total 200 mg/m2) and 14 SC daily doses of 10 mcg/kg of GM-CSF. 32 subjects were enrolled and treated for 1 to 12 courses. As 6/28 evaluable patients exhibited some kind of response, the product was considered worthy of further evaluation.

• Over the same period from 1995 to 1997, a Phase I study also sponsored by the NCI but conducted by the Children's Cancer Group (CCG-0935) evaluated the tolerability of ch14.18 at daily doses of 20 to 50 mg/m2 administered for 4 days in combination with GM-CSF 250 mcg/m2 (IV or SC) for 7 days. 22 patients received at least one dose of ch14.18 and the MTD was determined to be 40 mg/m2/day. Subsequently, from 1997 to 2001, the same group conducted another study (CCG-0935A) to evaluate the tolerability of the addition of IL-2 to the previous combination, and from 2000, the addition of 13-cis-retinoic acid (RA). Three regimens were tested consecutively in 23 patients, and regimen 3 with an MTD of 25 mg/m2/day for ch14.18 was the one to be further used in the Phase III pivotal trial, which was performed by the cooperative group (COG) after POG and CGC had merged.

2.5.2. Main study

DIV-NB-301 (ANBL0032): Phase III Randomized Study of Chimeric Antibody 14.18 (ch14.18) in High-Risk Neuroblastoma Following Myeloablative Therapy and Autologous Stem Cell Rescue

Methods

Study Participants

The main selection criteria were as follows:

- patients diagnosed with neuroblastoma, and categorized as high-risk at the time of diagnosis and < 40 years of age at diagnosis
- having completed therapy (in various other protocols) including intensive induction followed by ASCT and radiotherapy, AND achieved CR, VGPR, or PR (according to International Neuroblastoma Response Criteria (INRC) at pre-ASCT evaluation
- with no more than 9 months from the date of starting the first induction chemotherapy after diagnosis to the date of ASCT
- after assessment of residual disease by tumour imaging (CT, MRI, MIBG scan), bone marrow aspiration & biopsy
- enrolment and randomisation between Day 50 and Day 85 (maximum Day 100 if significant post-ASCT complications) post final-ASCT procedure, when the APC (APC = neutrophils + monocytes) was at least 1000/µL (cytokine support allowed), at least 7 days after completing radiotherapy
- patients could not have PD
- with a Lansky or Karnofsky Performance Scale score of ≥ 50% and a life expectancy of > two months
- with adequate renal, hepatic, cardiac, pulmonary, CNS function (defined according to set criteria)

- Females of childbearing potential were required to have a negative pregnancy test. Subjects of childbearing potential were required to use an effective birth control method. Female subjects who were lactating were required to stop breast-feeding.

Treatments

Standard Therapy (regimen A)

Patients in the standard-therapy group received isotretinoin (RA) given orally at a dose of 160 mg/m^2 of body-surface area per day, divided into two daily doses, for 14 consecutive days within each of 6 consecutive 28-day courses.

Immunotherapy (regimen B - see scheme below)

- Patients received ch14.18 at a dose of 25 mg/m^2 per day IV (initially over 5 hours; from 2009 extended to 10-20 hours) for 4 consecutive days during each of five consecutive 4-week courses.
- During the last 2 weeks in each of the five courses, they also received isotretinoin orally at a dose of 160 mg/m^2); this dose of isotretinoin was also given by itself during a final sixth course.
- During courses 1, 3, and 5, GM-CSF (sargramostim, Leukine, Berlex or other manufacturer) was given daily at a dose of 250 µg/m^2 per day SC or IV for 14 days, starting 3 days before ch14.18 was started.
- During courses 2 and 4, IL-2 (aldesleukin, Proleukin, Chiron) was given, by means of continuous IV infusion, for 4 days during week 1 at a dose of 3.0×10^6 IU/m^2 per day, as well as for 4 days during week 2 at a dose of 4.5×10^6 IU/m^2 per day, concurrent with ch14.18.

Course 1	Course 2	Course 3	Course 4	Course 5	Course 6
Ch14.18	Ch14.18	Ch14.18	Ch14.18	Ch14.18	
GM-CSF	IL-2	GM-CSF	IL-2	GM-CSF	
13cisRA	13cisRA	13cisRA	13cisRA	13cisRA	13cisRA

Treatment schema for courses 1, 3, & 5 with GM-CSF (28 days per course)

Day	0	1	2	3	4	5	6	7	8	9	10-13	24
GM-CSF	X	X	X	X	X	X	X	X	X	X	X	
ch14.18				X	X	X	X					
13cisRA											X	
Begin Course 2&4												X

Treatment Schema for Courses 2 & 4 with IL2

Day	0	1	2	3	4-6	7	8	9	10	11-13	14-27	28
IL2	X	X	X	X		X	X	X	X			
Ch14.18						X	X	X	X			
13cisRA											X	
Begin course 3&5												X

Figure 6: Treatment scheme of immunotherapy with ch14.18 (regimen B)

Ch14.18 was as a sterile solution in single-dose vials containing 25 mg/5 mL (5 mg/mL) in phosphate buffered saline.

Dose modifications for GM-CSF, IL-2, ch14.18, and RA were planned for toxicities during the study. In addition to dose modifications, the protocol included standard of care recommendations/nursing guidelines for the treatment of toxicities expected with study therapy including fever, neuropathic pain, capillary leak syndrome, electrolyte imbalances, allergic type reactions, visual changes, and weight loss/anorexia and joint pains.

Concomitant therapy

Appropriate antibiotics, blood products, anti-emetics, fluids, electrolytes and general supportive care measures were allowed. In addition, the following pre-medications were required during all ch14.18 courses for the prevention of anticipated toxicities associated with ch14.18 including neuropathic pain and allergic reactions: hydroxyzine OR diphenhydramine IV, oral acetaminophen, morphine sulfate IV (hydromorphone or fentanyl also allowed), lidocaine or gabapentin if needed. Epinephrine and hydrocortisone had to be available for the treatment of anaphylactic reactions. Pre-medications were also required with IL-2 infusions; recommendations were given for the treatment of chills (meperidine), anti-emetics and signs of capillary leak syndrome (furosemide).

Prohibited treatment included other anti-cancer therapies, immunosuppressive drugs, corticosteroids except for treatment of acute life-threatening allergic reactions and anaphylaxis, cytokines or growth factors and intravenous immunoglobulin post-ASCT were discouraged (due to possible interference with ADCC).

However, concomitant medications were not recorded during this study, except for steroid use, which was infrequently reported (2-5% depending on the courses with highest use during Courses 2 and 4 with IL-2).

Treatment compliance

ch14.18 was administered in an inpatient setting. However, other components of therapy including GM-CSF, IL-2, and RA were administered in both the inpatient and outpatient settings and not recorded in the electronic case report form.

Objectives

The primary objective was to determine if monoclonal ch14.18 in combination with GM-CSF, IL-2 and RA improved event-free survival (EFS) after myeloablative therapy and stem cell rescue as compared to RA alone, in high-risk neuroblastoma subjects who achieved a pre-ASCT response of complete response (CR), very good partial response (VGPR), or PR.

The main secondary objectives included:

- the comparison of overall survival (OS) between treatment arms,
- the comparison of EFS and OS in the subgroup of high-risk International Neuroblastoma Staging System (INSS) Stage 4 neuroblastoma subjects,
- the correlation of tumour biology characteristics with EFS and OS,
- the determination of the toxicities of the combination of ch14.18 with cytokines,
- the determination of a descriptive profile of HACA during treatment,
- the comparison of the outcome data of the subjects with persistent disease documented by biopsy (Stratum 07) to the historical data for the analogous subjects from past protocol CCG-3891.

Outcomes/endpoints

The primary endpoint was event-free survival (EFS) defined as the time from study enrolment until the first occurrence of relapse, progressive disease (PD), secondary malignancy, death, or date of last contact (if no event occurred).

Progressive disease was defined according to INRC and included the development of any new lesion, the increase of a measurable lesion by > 25%, previously negative bone marrow testing positive for tumour, or an increase from ≤ 10% tumour in marrow to > 10% tumour. Elevated urine catecholamine metabolite levels and tumour cell invasion of bone marrow were also considered in the assessment of tumour response.

Disease status was evaluated prior to the start of study therapy and within two weeks after the last dose of RA in Course 6. This included tumour imaging (CT, MRI, MIBG, and FDG-PET scan or bone scan if tumour was not MIBG avid) and bone marrow aspirates/biopsies. Tumour imaging was repeated every 3 months until 1 year post-treatment, then every 6 months for the next 2 years (approximately 3.5 years after enrolment) and then as clinically indicated. Another bone marrow biopsy/aspiration was collected 3 months after the completion of study therapy and again at relapse. Copies of MIBG were required to be submitted to the Quality Assurance Review Center (QARC) for central review at enrolment and post-treatment.

Secondary endpoints included Overall survival (OS) defined as the time from enrolment until death (or time of last contact in the absence of death).

Sample size

Following FDA advice, the sample size was increased to support a one-sided alpha level of 0.025 (previously 0.05) to address the primary objective, involving the intent-to-treat (ITT) cohort of all eligible randomised subjects. For at least 80% power, this required 386 subjects.

Randomisation

Patients were stratified according to pre-ASCT response ("CR" vs. "VGPR" vs. "PR"), stem cells received ("purged" vs. "unpurged"), and various frontline chemotherapies (25 strata in total). Patients were randomised to immunotherapy or standard therapy treatment arms except those from stratum 07, which consisted of patients with biopsy-confirmed post-ASCT persistent disease; these were not randomised but assigned to the immunotherapy treatment. Stratified permuted blocks were used for randomisation, which was centrally performed using the COG Remote Data Entry system.

Blinding (masking)

N/A

Statistical methods

All efficacy analyses were performed on the Intent-to-Treat (or ITT) population, which was defined as all eligible subjects randomised into the study; which did not include Stratum 07 subjects. All ITT subjects were analysed in the arm to which they were randomised, regardless of the treatment they were actually given.

A two-sided log-rank test with a significance level of 0.05 was used to test for a difference between the EFS/OS distributions of the ch14.18 immunotherapy and RA arm versus the RA alone arm.

Results

Participant flow

Overall, 257 subjects were enrolled in the trial prior to the close of randomisation (13 January 2009) including:

- 230 subjects that were randomised: 114 to immunotherapy + RA and 116 to RA alone
    - Two subjects randomised to the RA alone arm had no data recorded in the eCRF at the time of the data cut to confirm the start of study therapy and one subject was removed from the database due to ineligibility; 113 subjects were included in the ITT analysis (2009).
    - One subject in the immunotherapy + RA arm had no data in the database; 113 subjects were included in the ITT analysis (2009).
- 27 subjects (Stratum 07) non-randomly assigned to receive ch14.18 immunotherapy and RA as these subjects had post-ASCT biopsy-proven residual disease; only 25 subjects had data at the time of the primary analysis (2009).

The patient disposition (2009) is shown in Figure 10 and Table 22.

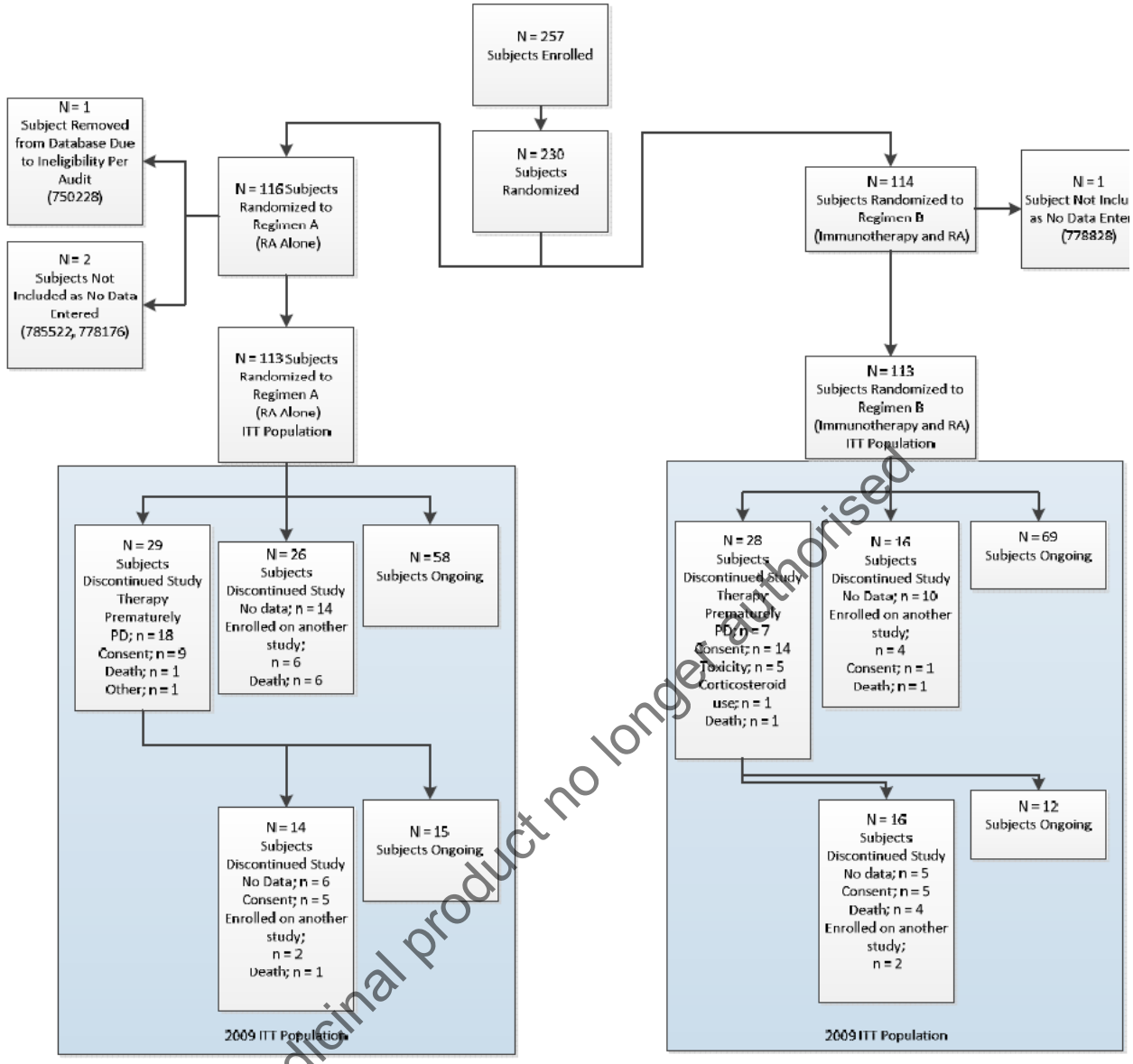


Figure 7: Patient disposition in the randomised trial (2009 analysis)

Subjects receiving ch14.18 immunotherapy and RA were followed for a median (range) of 830 (4-2522) days after study enrolment as compared to RA alone subjects who were followed for 731 (7-2495) days after study enrolment.

Of the 249 subjects who were reported to have received at least one dose of study therapy at the time of the data cut:

- 179 subjects were reported to have completed study therapy
- 70 subjects were reported to have discontinued study therapy prematurely: 41 subjects in the immunotherapy + RA arm and 29 subjects in the RA alone arm.

Table 19: Summary of subject disposition (30 June 2009)

	30 June 2009 Data Cut		
	RA Alone	Immunotherapy + RA	Overall
Number of subjects enrolled	113	138	251
Number of subjects randomized	113	113	226
Number of Stratum 07 Subjects	0	25	25
Number of subjects completed	82	97	179
Safety Population	111	138	249
Intent-to-Treat Population	113	113	226
No. of Subjects that discontinued Protocol Therapy (Safety Population)			
Missing[1]	0	6 (4.3%)	6 (2.4%)
Course 1	12 (10.8%)	11 (8.0%)	23 (9.2%)
Courses 2	1 (0.9%)	6 (4.3%)	7 (2.8%)
Courses 3	7 (6.3%)	7 (5.1%)	14 (5.6%)
Courses 4	5 (4.5%)	7 (5.1%)	12 (4.8%)
Courses 5	1 (0.9%)	2 (1.4%)	3 (1.2%)
Courses 6	3 (2.7%)	2 (1.4%)	5 (2.0%)
Reason For Discontinuation of Protocol Therapy (Safety Population)			
PD	18 (16.2%)	12 (8.7%)	30 (12.0%)
Withdrawal By Subject	9 (8.1%)	14 (10.1%)	23 (9.2%)
Missing[1]	0	6 (4.3%)	6 (2.4%)
Toxicity	0	6 (4.3%)	6 (2.4%)
Corticosteroid Use	0	2 (1.4%)	2 (0.8%)
Death	1 (0.9%)	1 (0.7%)	2 (0.8%)
Other	1 (0.9%)	0	1 (0.4%)
No. of Subjects that discontinued Study (Safety Population)			
Missing[1]	71 (64.0%)	96 (69.6%)	167 (67.1%)
Discontinued	40 (36.0%)	42 (30.4%)	82 (32.9%)
Reason For Discontinuation of Study (Safety Population)			
Missing[1]	71 (64.0%)	96 (69.6%)	167 (67.1%)
Death	7 (6.3%)	7 (5.1%)	14 (5.6%)
Enrollment onto another COG therapeutic study with tumor therapeutic intent (e.g., at recurrence)	8 (7.2%)	7 (5.1%)	15 (6.0%)
No Data Entered[2]	20 (18.0%)	22 (15.9%)	42 (16.9%)
Subject Lost to Follow-up	0	0	0
Withdrawal of Consent for further data submission	5 (4.5%)	6 (4.3%)	11 (4.4%)

[1] Subjects with "missing" values were ongoing at the time of the data cut.
[2] Subjects with "no data entered" values were reported to have discontinued the study; however, a reason for study discontinuation was not reported.

The Applicant became aware later that 5 subjects in the RA alone arm and 4 subjects in the immunotherapy + RA arm had not received any treatment. Therefore, these subjects were later excluded from the safety analyses.

After the data cut-off date for the 2009 analysis, four subjects originally randomised to RA alone and after having completed their full course regimen were crossed-over during the study protocol follow-up and received ch14.18 immunotherapy and RA (start dates in July and August 2009).

The long-term efficacy analysis (June 2012) was performed on 114 subjects in each randomised treatment arm. In the RA alone arm, a second subject was removed from the database due to ineligibility but the two subjects initially without data in 2009 had their data completed. In the immunotherapy + RA arm, the subject initially without data in 2009 had the data completed.

Recruitment

Conduct of the study

A total of 19 versions of the protocol have been submitted including 18 amendments (1, 2, 3, 4, 4a, 4b, 5, 6, 7, 8, 9b, 10, 11a, 12, 13, 13a, 14, 15) up to 07 December 2012.

Study data were monitored by the COG according to the NCI's Guidelines. A minimum of 10% of accrued study subjects (across all study protocols) participating at each institution were required to be reviewed every 36 months.

A Study Data Review Committee was established to ensure data quality during the conduct of the study. This committee was comprised of the Study Chair, COG Study Statistician, and COG Research Coordinator. Study data were reviewed by this committee on a regular basis according to the Study Data Review Plan.

A Data Safety Monitoring Committee (DSMC) consisting of NCI, COG, and independent members was established to review safety and efficacy data during the study. This DSMC consisted of a minimum of nine voting members, the majority of which were not affiliated with the COG; however, the Chair and Vice-chair were COG members. This committee was responsible for evaluating subject safety during the conduct of the study and for reviewing study data against the specific predefined monitoring boundaries.

After the data transfer to UTC, the Applicant re-monitored 28 of the top enrolling institutions (during the randomised portion of the study) in US and Canada for targeted study endpoints reviewed against source documents and audited approximately 10% of study sites.

Interim analyses

A brief outline of relevant landmarks from the study derived from information scattered throughout the dossier is given below.

October 2001	First patient enrolled. Interim analyses planned every 6 months after the first 23 events with the intention to stop for futility only.
12 March 2004	Revised study endpoints and sample size increased from 322 (one-sided 0.05 test) to 386 subjects (one-sided 0.025 test) (amendment 4).
28 November 2005	Stopping rules changed to allow early stopping for efficacy; the lower bound is calculated based on repeated testing of the alternative hypothesis that the relative risk is equal to 1.6 at a p-value of 0.005 and the upper bound uses an alpha*t^2 spending function for a cumulative alpha level of 0.05 (amendment 6).
June 2007	Upper boundaries re-calculated using alpha = 0.025 (one-sided) changing from 0.05 (one-sided).
November 2008	Study to be temporarily closed due to concern for increased incidence of allergic reactions in the experimental arm; decision to confirm the interim analysis after review of the EFS events.
13 January 2009:	Interim analysis conducted on the basis of which it was decided to close the study to randomisation.
30 June 2009	Cut-off for the confirmatory efficacy analysis
30 June 2012	Cut-off for the long-term efficacy analysis

The table below gives a summary of the stopping boundaries used at each interim analysis.

Monitoring Time point	Observed cumulative number of events	Observed proportion of total expected information	Observed upper boundary z-value	Observed p-value	Upper boundary z-value, cumulative alpha = 0.05	Upper boundary z-value, cumulative alpha = 0.025	Nominal alpha
Nov-05	29	0.212	1.963	0.0495	2.853	--	0.0043
Jun-06	39	0.285	1.905	0.0567	2.758	--	0.0058
Nov-06	49	0.360	2.257	0.0240	2.633	--	0.0085
Jun-07	57	0.416	2.450	0.0143	--	2.764	0.0057
Dec-07	62	0.453	2.120	0.0340	--	2.717	0.0066
Jun-08	70	0.511	2.550	0.0108	--	2.700	0.0069
Nov 2008 (Jan 2009)	**83**	**0.606**	**2.528**	**0.0115**	**--**	**2.550**	**0.0108**
Jun-09	94	0.686		0.0330			

Baseline data

The demographics of the ITT population (i.e. all patients randomised) are summarised in Table 23 and the baseline prognostic factors in Table 24.

For several prognostic factors, the baseline differences between treatment arms pointed towards worse prognosis in the control arm compared to the immunotherapy + RA arm as reflected by more tumours showing MYCN amplification (40% vs. 32%), diploidy (41% vs. 31%), and unfavourable histology (72% vs. 60%), respectively. The proportion of CR was also marginally higher in the immunotherapy + RA arm (35.4% vs. 33.6%).

Table 20: Summary of patient demographics

Characteristics	ITT Population		
	Treatment		
	RA Alone n = 113	Immunotherapy + RA n = 113	Overall n = 226
Age at Enrollment (years): mean (range)	4.0 (0.94 – 13.29)	4.3 (0.95 – 15.29)	4.1 (0.94 – 15.29)
Gender: Male/Female (%)	57/43	63/37	60/40
Ethnicity: n (%)			
Hispanic or Latino	11 (10%)	11 (10%)	22 (10%)
Not Hispanic or Latino	96 (85%)	100 (89%)	196 (87%)
Unknown	6 (5%)	2 (2%)	8 (4%)
Race: n (%)			
White	90 (80%)	95 (84%)	185 (82%)
Black or African American	8 (7%)	8 (7%)	16 (7%)
Asian	4 (4%)	2 (2%)	6 (3%)
Native Hawaiian or Other Pacific Islander	2 (2%)	0	2 (1%)
Multiple	2 (2%)	1 (1%)	3 (1%)
Other / Unknown	7 (6%)	7 (6%)	14 (6%)

Number of Days Post-Final ASCT: mean ± SD	77 ± 12.0	75 ± 8.7	76 ± 10.5

Table 21: Summary of baseline prognostic factors

Baseline Prognostic Factor	RA Alone (n=113)	Immunotherapy + RA (n=113)	Chi-square test p-value
Age at Enrollment	--	--	1.0000
<18 months	4 (3.5%)	4 (3.5%)	--
>=18 months	109 (96.5%)	109 (96.5%)	--
Age at Diagnosis Category			0.2308
Adolescent (12-18 y)	1 (0.9%)	4 (3.5%)	--
Child (2-12 y)	71 (62.8%)	79 (69.9%)	--
Infant/Toddler (28 d-2 y)	35 (31.0%)	28 (24.8%)	--
Unknown	6 (5.3%)	2 (1.8%)	--
Age at Enrollment Category	--	--	0.2179
Adolescent (12-18 y)	1 (0.9%)	4 (3.5%)	
Child (2-12 y)	94 (83.2%)	97 (85.8%)	--
Infant/Toddler (28 d-2 y)	18 (15.9%)	12 (10.6%)	--
INSS Stage	--	--	0.0604
Stage 2a	0	4 (3.5%)	--
Stage 3	16 (14.2%)	10 (8.8%)	--
Stage 4s	0	2 (1.8%)	--
Stage 4	92 (81.4%)	89 (78.8%)	--
Unknown	5 (4.4%)	8 (7.1%)	--
MYCN amplification	--	--	0.4155
Amplified	45 (39.8%)	36 (31.9%)	--
Non-amplified	51 (45.1%)	52 (46.0%)	--
Unknown	17 (15.0%)	25 (22.1%)	--
DNA ploidy	--	--	0.3309
Diploid	46 (40.7%)	35 (31.0%)	--
Hyperdiploid	48 (42.5%)	49 (43.4%)	--
Unknown	19 (16.8%)	29 (25.7%)	--
Histology	--	--	0.9444
Favorable	5 (4.4%)	4 (3.5%)	--
Unfavorable	81 (71.7%)	68 (60.2%)	--
Unknown	27 (23.9%)	41 (36.3%)	--
Pre ASCT Response	--	--	0.9546
CR	38 (33.6%)	40 (35.4%)	--
VGPR	49 (43.4%)	47 (41.6%)	--
PR	26 (23.0%)	26 (23.0%)	--
Stem Cell Type	--	--	0.7907
Purged	29 (25.7%)	28 (24.8%)	--
Unpurged	58 (51.3%)	61 (54.0%)	--
Unknown	26 (23.0%)	24 (21.2%)	--

Numbers analysed

Outcomes and estimation

Event-free survival

The results of the primary analysis (13 January 2009), confirmatory analysis (30 June 2009), and follow-up analysis (30 June 2012) are shown in Table 20 and the Kaplan-Meier plots (June 2009 and 2012) are shown in Figures 8 and 9.

Table 22: Summary of EFS results

	Immunotherapy+RA	**RA alone**
13 January 2009	n=113	n=113
2-year EFS (95% CI)	66.3% (56.2%, 76.3%)	46.4% (35.8%, 57.1%)
Log-rank test p-value	0.0115	
Hazard ratio (95% CI)	0.57 (0.37; 0.89)	
30 June 2009	n=113	n=113
2-year EFS (95% CI)	65.6% (56.1%, 75.2%)	48.1% (38.0%, 58.2%)
Log-rank test p-value	0.0330	
Hazard ratio (95% CI)	0.64 (0.43; 0.97)	
30 June 2012	n=114	n=114
3-year EFS (95% CI)	62.8% (53.9%, 71.7%)	50.9% (41.6%, 60.2%)
Log-rank test p-value	0.0990	
Hazard ratio (95% CI)	0.69 (0.47; 1.01)	

Of note, the 2-year EFS estimate increased between January and June 2009 in the RA alone arm due to correction of data errors.

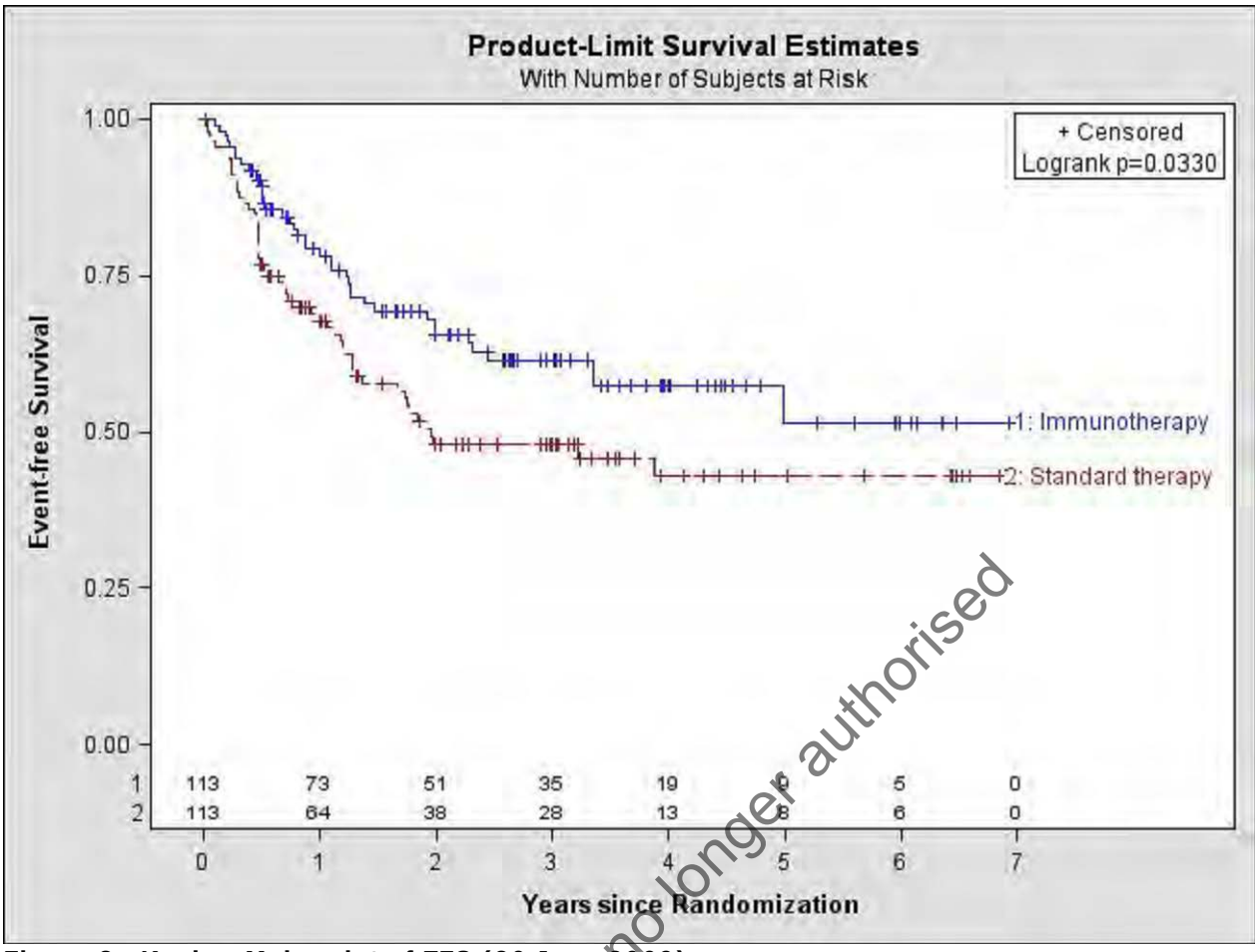


Figure 8: Kaplan-Meier plot of EFS (30 June 2009)

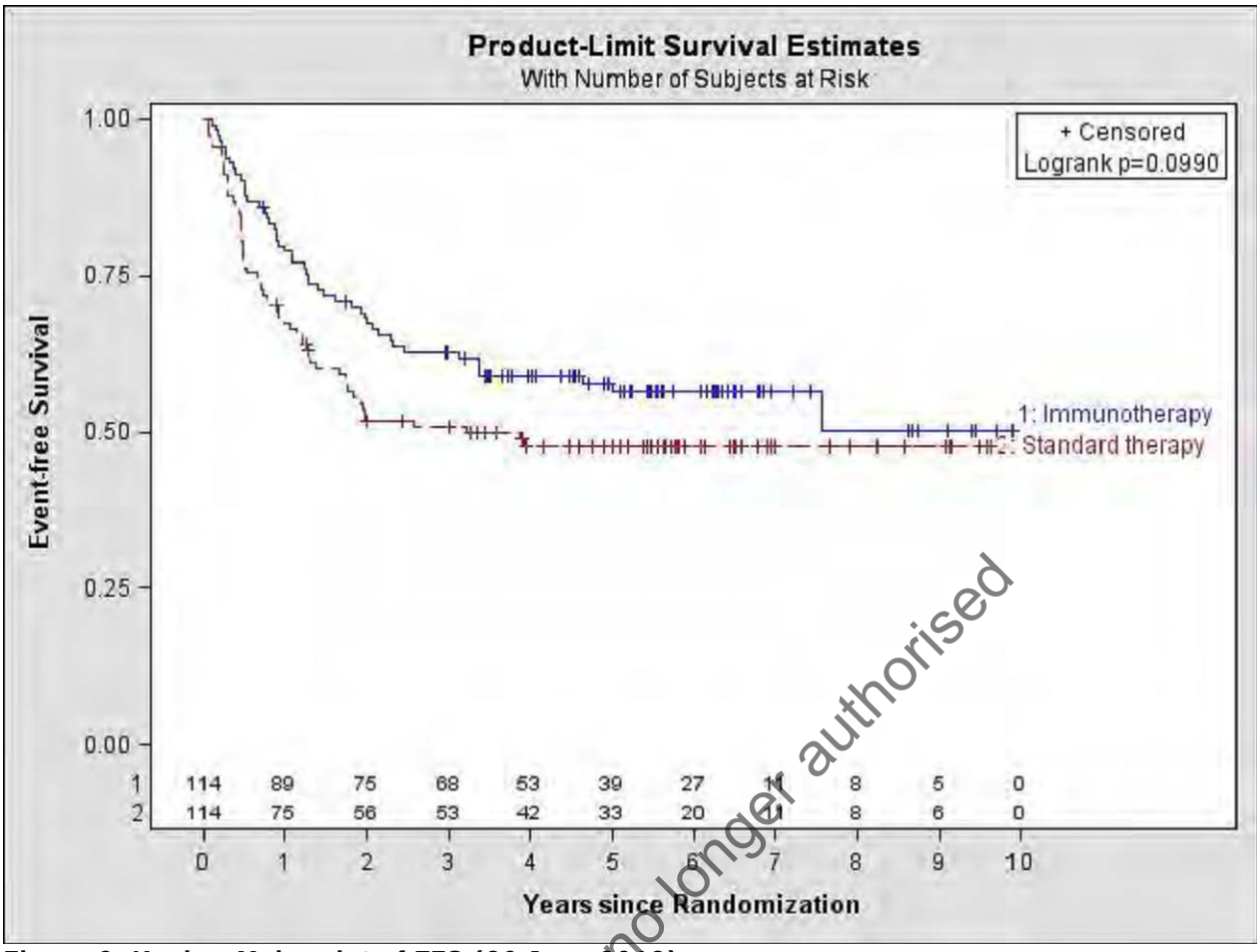


Figure 9: Kaplan-Meier plot of EFS (30 June 2012)

Overall survival

The results of the primary analysis (13 January 2009), confirmatory analysis (30 June 2009), and follow-up analyses (30 June 2012 and March 2014) are shown in Table 23 and the Kaplan-Meier plot of the most recent analysis (March 2014) is shown in Figure 10.

Table 23: Summary of OS results

	Immunotherapy+RA	**RA alone**
13 January 2009	n=113	n=113
2-year OS (95% CI)	86.2% (78.8%, 93.6%)	74.5% (65.2%, 83.9%)
Log-rank test p-value	0.0223	
Hazard ratio (95% CI)	0.52 (0.30; 0.92)	
30 June 2009	n=113	n=113
2-year OS (95% CI)	85.4% (78.2%, 92.6%)	75.3% (66.4%, 84.2%)
Log-rank test p-value	0.0213	
Hazard ratio (95% CI)	0.54 (0.32; 0.92)	
30 June 2012	n=114	n=114
3-year OS (95% CI)	79.5% (72.1%, 87.0%)	67.3% (58.4%, 76.1%)
Log-rank test p-value	0.0165	
Hazard ratio (95% CI)	0.57 (0.36, 0.89)	
March 2014	n=114	n=114
5-year OS (95% CI)	74.2% (66.1%, 82.3%)	57.0% (47.5%, 66.4%)
Log-rank test p-value	0.0301	
Hazard ratio (95% CI)	0.62 (0.40, 0.96)	

Of note, the 2-year OS estimate increased between January and June 2009 in the RA alone arm due to correction of data errors.

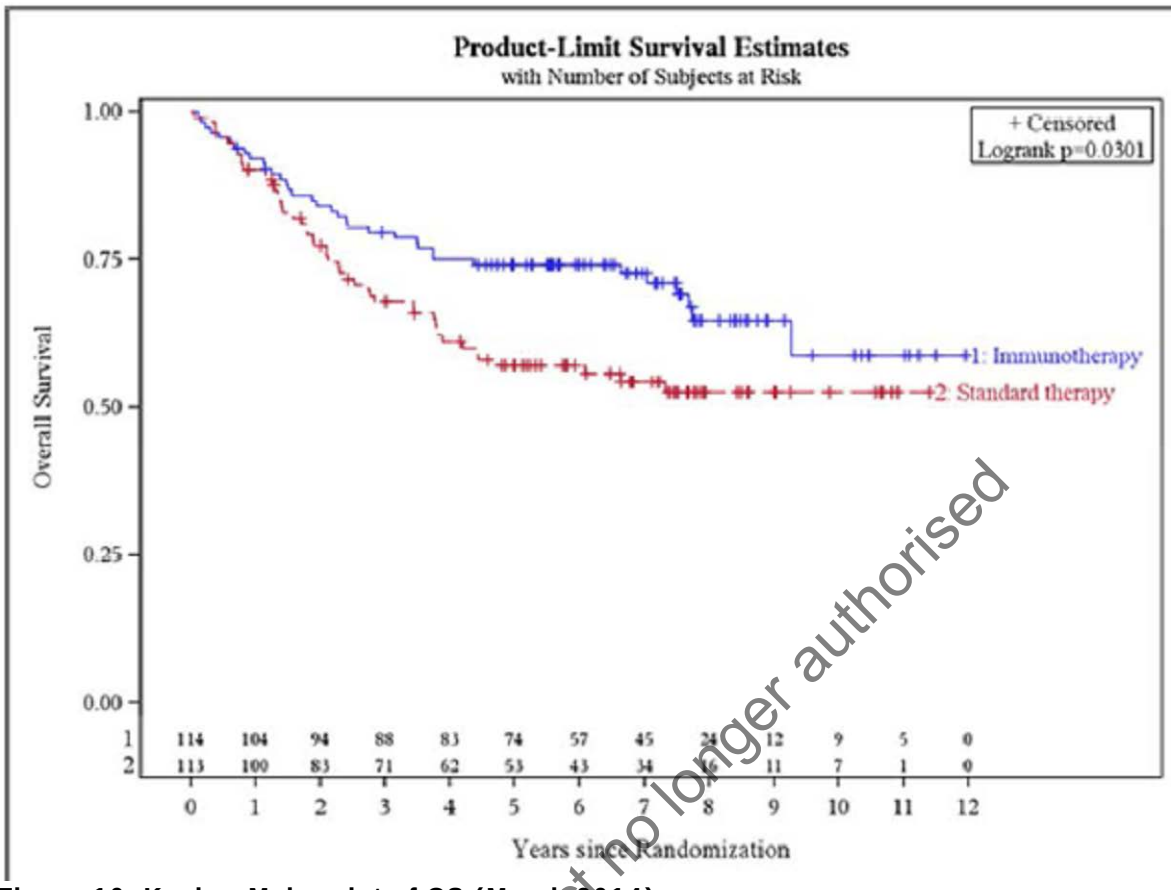


Figure 10: Kaplan-Meier plot of OS (March 2014)

Results in INSS stage 4 patients

The EFS and OS results of the confirmatory analysis (30 June 2009) and follow-up analysis (30 June 2012) are shown in Table 24 and 25, respectively.

Table 24: Summary of EFS results in INSS stage 4 patients

	Immunotherapy+RA	**RA alone**
30 June 2009	n=89	n=92
2-year EFS (95% CI)	62.6% (51.8%, 73.4%)	43.7% (32.8%, 54.7%)
Log-rank test p-value	0.0422	
Hazard ratio (95% CI)	0.64 (0.42, 0.99)	
30 June 2012	n=90	N=93
3-year EFS (95% CI)	59.5% (49.3%, 69.7%)	46.5% (36.3%, 56.8%)
Log-rank test p-value	0.0971	
Hazard ratio (95% CI)	0.71 (0.47, 1.07)	

Table 25: Summary of OS results in INSS stage 4 patients

	Immunotherapy+RA	RA alone
30 June 2009	n=89	n=92
2-year OS (95% CI)	83.3% (74.9%, 91.7%)	75.8% (66.2%, 85.4%)
Log-rank test p-value	0.0813	
Hazard ratio (95% CI)	0.62 (0.36, 1.07)	
30 June 2012	n=90	n=93
3-year OS (95% CI)	79.6% (71.2%, 88.0%)	64.3% (54.3%, 74.2%)
Log-rank test p-value	0.0149	
Hazard ratio (95% CI)	0.55 (0.34, 0.90)	

Results according to pre-ASCT status

The EFS and OS results of the confirmatory analysis (30 June 2009) and follow-up analysis (30 June 2012) are shown in Table 26.

Table 26: Summary of EFS results in patients with pre-ASCT CR/VGPR vs. PR

	Immunotherapy+RA	RA alone
30 June 2009		
CR/VGPR	n=87	n=87
2-year EFS (95% CI)	68.5% (57.9%, 79.1%)	51.8% (40.0%, 63.5%)
Log-rank test p-value	0.1236	
Hazard ratio (95% CI)	0.68 (0.42; 1.11)	
PR	n=26	n=26
2-year EFS (95% CI)	56.7% (36.0%, 77.4%)	35.7% (16.5%, 54.9%)
Log-rank test p-value	0.0987	
Hazard ratio (95% CI)	0.54 (0.26; 1.13)	
CR/VGPR	n=87	n=87
2-year OFS (95% CI)	91.2% (84.9%, 97.5%)	80.9% (71.8%, 90.0%)
Log-rank test p-value	0.0340	
Hazard ratio (95% CI)	0.49 (0.25; 0.96)	
PR	n=26	n=26
2-year OFS (95% CI)	67.4% (47.3%, 87.5%)	58.3% (37.3%, 79.3%)
Log-rank test p-value	0.2773	
Hazard ratio (95% CI)	0.62 (0.26; 1.49)	
30 June 2012		
CR/VGPR	n=88	n=88
3-year EFS (95% CI)	68.1% (58.4%, 77.9%)	54.5% (43.9%, 65.1%)
Log-rank test p-value	0.2265	
Hazard ratio (95% CI)	0.71 (0.45; 1.12)	
PR	n=26	n=26
3-year EFS (95% CI)	44.3% (24.8%, 63.8%)	38.5% (19.8%, 57.2%)
Log-rank test p-value	0.2158	
Hazard ratio (95% CI)	0.65 (0.33; 1.29)	
CR/VGPR	n=88	n=88
3-year OS (95% CI)	82.9% (75.0%, 90.8%)	71.4% (61.7%, 81.1%)
Log-rank test p-value	0.0265	
Hazard ratio (95% CI)	0.55 (0.32; 0.94)	
PR	n=26	n=26
3-year OS (95% CI)	67.3% (48.6%, 85.9%)	53.8% (34.7%, 73.0%)
Log-rank test p-value	0.3715	
Hazard ratio (95% CI)	0.69 (0.31; 1.56)	

Results in patients with persistent disease documented by biopsy (stratum 07)

The 3-year point estimate of EFS (95% CI) for Stratum 07 subjects treated with ch14.18 immunotherapy + RA was 33% (16%, 51%) as compared to an historical control with a 3-year EFS estimate of 12% ± 6%.

Subgroup analyses by prognostic factors

Certain factors including age at diagnosis, international neuroblastoma staging system (INSS) stage, MYCN oncogene amplification, DNA ploidy, pre-ASCT response, tumor histology, and stem cell type have been associated with overall outcomes among patients diagnosed with neuroblastoma. Specifically, patients diagnosed at greater than 18 months of age, with stage 4 disease, or with MYCN amplification have a poorer prognosis as compared to patients diagnosed at less than 12 months of age, with a lower tumor stage (i.e., stage 1-3) or without MYCN oncogene amplification. In addition, subjects with diploid DNA ploidy, unfavorable tumor histology, and a pre-ASCT response of PR are also expected to have a poorer prognosis.

The forest plots of EFS and OS for the main prognostic factors are presented in Figures 11 and 12.

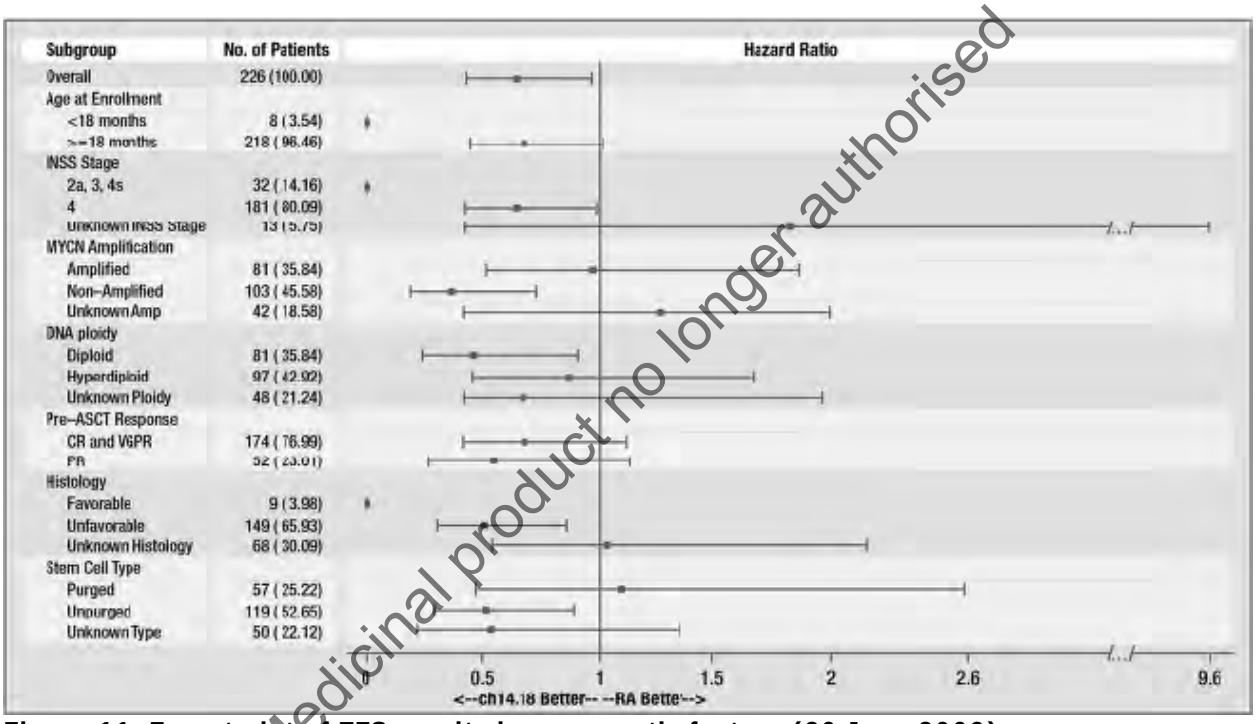


Figure 11: Forest plot of EFS results by prognostic factors (30 June 2009)

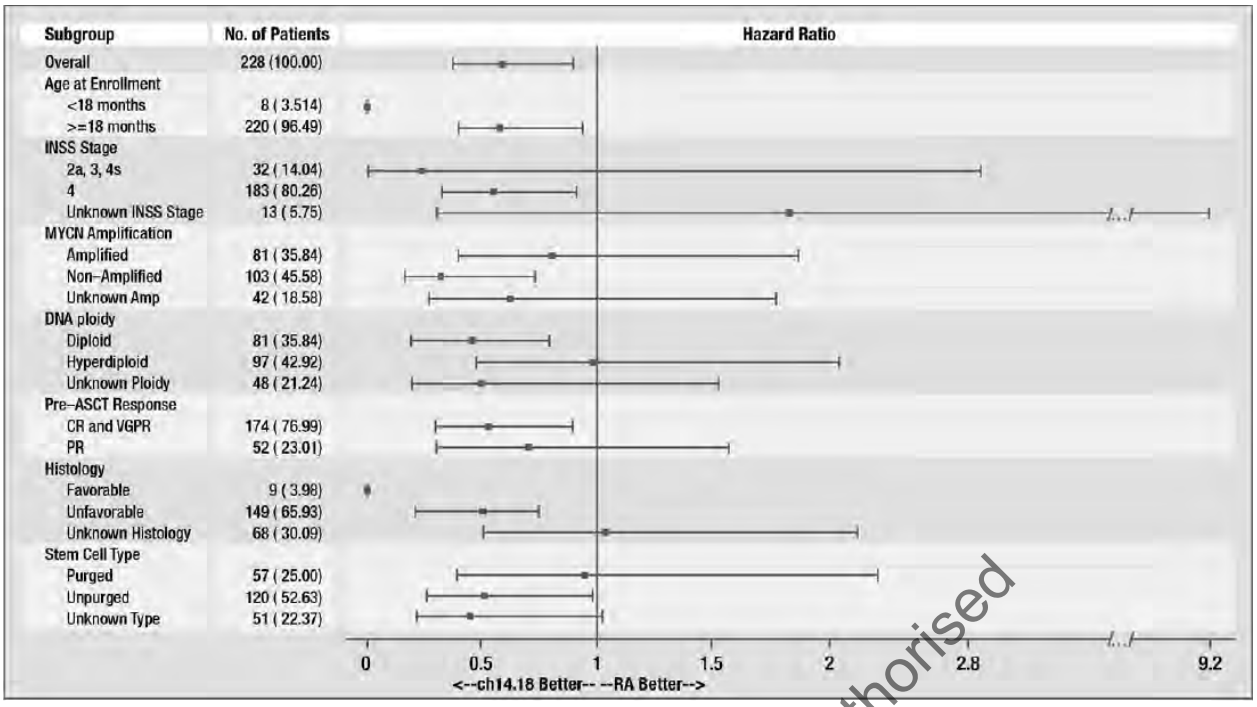


Figure 12: Forest plot of OS results by prognostic factors (30 June 2012)

In addition, an evaluation of survival according to the existence of minimum residual disease at baseline was performed. Patients enrolled in the trial had to have a baseline MIBG scan (up to 30 days before starting therapy) and data were available from 197 patients (97 in the RA alone arm and 100 in the experimental arm). Patients were evaluated at 10 different sites: Head, T-Spine, Chest, L-Spine, Pelvis, Upper Arms, Lower Arms, Femurs, Lower Legs, and Extra-osseous. A patient's Curie score was calculated as the sum of their scores over all individual sites. Patients were categorized by Curie score (0 vs. >0). The results of this analysis are shown in Table 27.

Table 27: EFS and OS by treatment and Curie score

Patient cohort	N (%)	3-year EFS ± std error (%)	EFS p-value	3-year OS ± std error (%)	OS p-value
Treatment					
RA Only	97 (49%)	46.4 ± 5.1	0.0493	64.7 ± 4.9	0.0033
GD2+RA	100 (51%)	63.9 ± 4.9		81.9 ± 3.9	
Curie Score =0 – Treatment					
RA Only	82 (49%)	47.5 ± 5.6	0.0202	66.8 ± 5.3	0.0019
GD2+RA	85 (51%)	70.5 ± 5.0		83.5 ± 4.1	
Curie Score >0 – Treatment					
RA Only	15 (50%)	40.0 ± 12.6	0.9305*	53.3 ± 12.9	0.5875
GD2+RA	15 (50%)	26.7 ± 11.4		73.3 ± 11.4	

*Apparent violation of proportional hazards assumption; log-rank test invalid

Summary of main study

The following table summarises the efficacy results from the main studies supporting the present application. This summary should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 28: Summary of efficacy for trial DIV-NB-301

Title: Phase III Randomized Study of Chimeric Antibody 14.18 (ch14.18) in High-Risk Neuroblastoma Following Myeloablative Therapy and Autologous Stem Cell Rescue			
Study identifier	DIV-NB-301 (COG ANBL0032)		
Design	Multicentre, open-label randomised as add-on to isotretinoin		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	7 years (Oct 2001 – Jan 2009) not applicable Ongoing	
Hypothesis	Superiority		
Treatments groups	RA alone	Isotretinoin, 6courses, 113	
	Immunotherapy + RA	Ch14.18 + GM-CSF + IL-2 + isotretinoin, 6 courses, 113	
Endpoints and definitions	Primary endpoint	EFS	Event-free survival
	Secondary endpoint	OS	Overall survival
Database lock	13 January 2009		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent to treat 6-monthly interim analyses with stopping rules for efficacy using Fleming Harrington-O'Brien boundaries with cumulative alpha of 0.025 one-sided, later changed to Lan-DeMets boundaries		
Descriptive statistics and estimate variability	Treatment group	Imunotherapy+RA	RA alone
	Number of subject	113	113
	2-year EFS	66.3%	46.4%
	95%CI	56.2%, 76.3%	35.8%, 57.1%
	2-year OS	86.2%	74.5%
	95%CI	78.8%, 93.6%	65.2%, 83.9%
Effect estimate per comparison	Primary endpoint (EFS)	Log-rank	
		P-value (one-sided)	0.0115
	Secondary endpoint (OS)	Log-rank	
		P-value (one-sided)	0.0223

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

Clinical studies in special populations

Supportive studies

Study DIV-NB-302 (ANBL0032)

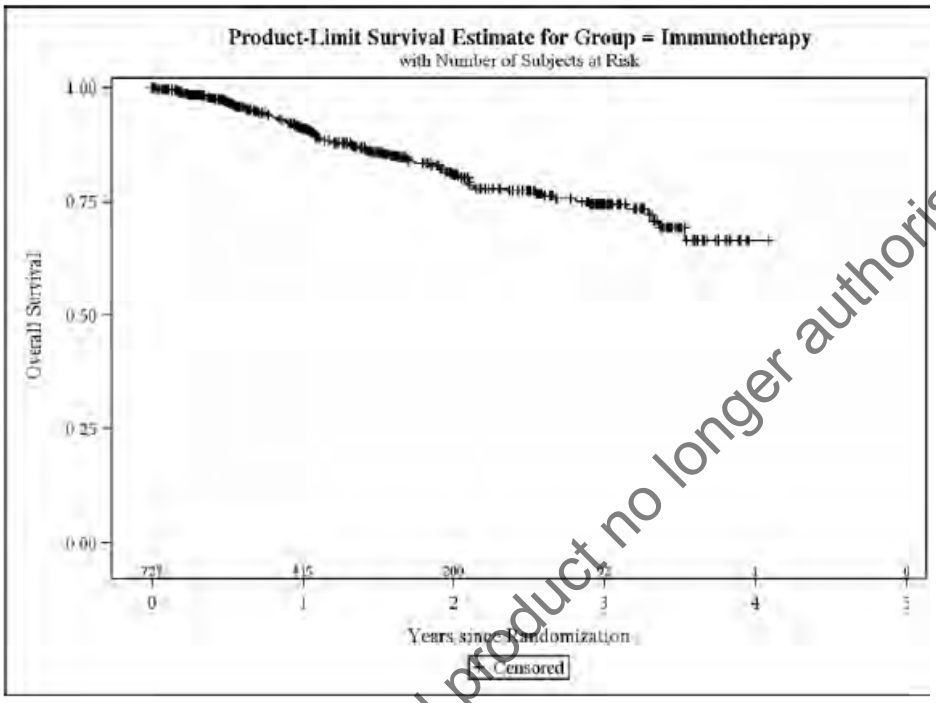
Efficacy was also evaluated in 737 subjects in the large extension study (DIV-NB-302) enrolled after the close of randomization in January 2009.

Results were consistent with those of the pivotal trial with a similar estimate for 2-year EFS of 65.2% (95% CI 60.7%, 69.8%) and a 2-year survival rate of 81% (95%CI 77%; 85%).

Table 29: Summary of Time to EFS

	Immunotherapy + RA n = 726
Two-year EFS % (95% CI)	65.2% (60.7%, 69.8%)
Censored Observations n (%)	547 (75%)

Data Source: Tables 14.2.1.1 and 14.2.1.2 and Listing 16.2.6.1.1



Data Source: Figure 14.2.4

Figure 13: Kaplan-Meier Curve of OS

Some information is also provided by the results of the early development of ch14.18 (see tabulated overview of clinical studies).

German development

Evidence of the efficacy of ch14.18 administered alone has been provided by the retrospective analysis of a combination of two German trials sponsored by Deutsche Krebshilfe. In two trials (NB90 and NB97), all subjects having successfully completed induction chemotherapy could proceed to receive either low-dose maintenance chemotherapy or myeloablative high-dose chemotherapy followed by ASCT but only if they did not relapse, progress, or develop a second malignancy. All NB97 subjects and some NB90 ASCT pilot subjects were subsequently treated with either a 12-month maintenance treatment of low-dose chemotherapy (12 alternating cycles of oral melphalan/etoposide and vincristine/cyclophosphamide) or ch14.18 at a median dose of 20 mg/m^2 (12 – 40) on Days 1-5 of a two-month cycle for a total of six cycles.

A retrospective analysis compared EFS and OS rates between three groups of subjects (>1 year old) with neuroblastoma stage 4:

- subjects who received maintenance treatment with ch14.18 (n = 166);
- subjects who received 12-month low-dose maintenance chemotherapy (n = 99);
- subjects who received no further treatment after the induction chemotherapy (n = 69, control group).

Univariate analysis showed similar EFS in all three subject groups at three years (46.5 ± 4.1%, 44.4 ± 4.9%, 37.1 ± 5.9%, respectively; p = 0.314). Superior OS was noted at three years in the ch14.18 treatment group (68.5 ± 3.9%) compared to either the maintenance therapy group (56.6 ± 5.0%; p = 0.029) or the control group (46.8 ± 6.2%; p = 0.009). However, multivariate analysis revealed no clear benefit for patients treated with ch14.18 and consequently this treatment was discontinued in the neuroblastoma trials. In addition, the investigators concluded that ch14.18 maintenance treatment was associated with considerable but manageable adverse events (pain, elevated CRP and fever, rash and pruritus, signs of capillary leak syndrome) (Simon, 2004). Similar results were observed in smaller cohorts of infants < 1 year old (Simon, 2005).

A follow-up of the same subjects (> 1 year old) was published by Simon et al in 2011 (median observation time: 11 years). The 9-year EFS rates were 41 ± 4%, 31 ± 5%, and 32 ± 6% for subjects who received maintenance therapy with ch14.18, maintenance therapy with oral chemotherapy, or no maintenance chemotherapy, respectively (p = 0.098). At 9 years, OS was significantly better in subjects treated with ch14.18 (46 ± 4%) as compared to subjects treated with low-dose maintenance chemotherapy (34 ± 5%) or subjects who did not receive maintenance therapy (35 ± 6%) (p = 0.019). A multivariate analysis found that maintenance treatment with ch14.18 significantly improved EFS and OS compared to no maintenance therapy (EFS: p = 0.021; OS: p = 0.011); however, no difference was observed in EFS and OS between maintenance treatment with ch14.18 as compared to low-dose chemotherapy (EFS: p = 0.688; OS: p = 0.182). The authors concluded that ch14.18 may prevent late relapses in subjects with minimal residual disease, which may explain why the survival benefit was observed at nine years and not at the initial three-year follow-up.

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

This application is supported by a single open-label pivotal trial sponsored by the NCI and conducted by the COG in the US, Canada, and Australia. An open-label design was the only feasible given the complexity of the immunotherapy regimen. The study population of high risk neuroblastoma is the target population (the majority being INSS stage 4) although the criteria used to define “high risk” were not exactly those currently used (as defined by the International Neuroblastoma Risk Group (INRG) task force) however the two classifications are broadly overlapping, the cut-off age, which is one of the main criteria to define high risk, was 12 months in the COG classification rather than 18 months in the INRG classification. Only 8 children were less than 18 months old. It is therefore accepted that most study patients would be considered with high risk neuroblastoma. In principle, the design of the study is considered acceptable with EFS as the primary endpoint. However, the study was open-label, no central review of tumour imaging was conducted, the clinicians and radiologists were aware of the treatment, and the type of contact accepted for the determination of the absence of relapse is considered questionable.

A DSMC was established to review safety and efficacy data during the study but it is not considered fully independent as the Chairs were COG members. Interim analyses were planned every 6 months. The trial was stopped prematurely because, according to the DSMC, superiority of immunotherapy with ch14.18 over the control had been established on EFS. However, this was not justified as it is obvious that the

stopping boundary was not crossed; hence superiority of immunotherapy was not demonstrated in January 2009, when the decision to stop the randomisation was taken. The GCP inspection revealed that the reason was that there was only very small chance that the final result would not meet the 0.025 significance level for licensure and that the data had been trending in the same direction throughout the study. However, the DSMC documentation also revealed that the trial accrual rate had been much slower than expected and the perspective of its temporary closure due to an increase in allergic reactions is likely to have also impacted the decision. The GCP inspection also concluded that the efficacy data were reliable. For all the reasons previously listed, the EFS results have to be interpreted with caution and the overall survival results are considered paramount to evaluate the treatment benefit.

Efficacy data and additional analyses

The baseline prognostic factors with respect to tumour biology were not well balanced across the treatment arms and pointed towards worse prognosis in the control arm. However, this was taken into account in additional statistical analyses with adjustment for prognostic factors.

When the trial was stopped by the DMSC in January 2009, the stopping boundary had not been crossed; the observed p-value was 0.0115, which was superior to the nominal alpha calculated (0.0108). Therefore, the trial should not have been stopped. Estimates for 2-year EFS were 66.3% vs. 46.4% for the immunotherapy and control arms, respectively. In the analysis conducted 5 months later, these estimates were 65.6% vs. 48.1%, respectively (p = 0.0330) with an HR of 0.64 (0.43; 0.97); the apparent increase in EFS rate in the RA alone treatment arm was due to the correction of errors in the dataset. In the large extension study (737 patients), a similar estimate of 65.2% (95% CI 60.7%, 69.8%) was reported.

In contrast with these EFS results, overall survival results in four analyses (from 2009 to 2014) unequivocally demonstrate the benefit of immunotherapy, which is both statistically and clinically relevant with a survival benefit of 14% at 3 years (82% vs. 68%; p < 0.02) and an HR of 0.57 (0.36; 0.89). The 2-year survival rate reported in the extension study (81%; 95%CI 77%; 85%) is in line with this result. Treatment effects remained significant and with similar hazard ratios when the analysis was adjusted for each individual prognostic factor; therefore, the imbalance in prognostic factors did not seem to have had a notable impact on the results.

No information was collected on post-relapse/progression therapies during the study and therefore the possible effects of these on survival cannot be assessed. However, it is acknowledged that there is currently no effective therapy in the relapse setting and that the prognosis remains poor. Various therapeutic options are proposed by the NCI and there is no foreseeable reason that would suggest that the two randomised arms could have had access to different therapies. Furthermore, it is known that some patients from the control arm received ch14.18 either before or after progression/relapse and this might work in favour of the control arm. Finally, the magnitude of the difference in overall survival is such that any bias in these therapies would not be expected to substantially affect the observed benefit.

A major prognostic factor in high-risk neuroblastoma is the type of response to induction chemotherapy and the benefit of the immunotherapy regimen in the most favourable setting (CR and VGPR) was questioned during the procedure. Subgroup analyses showed that most prognostic groups benefited from the immunotherapy regimen, in particular patients with CR and VGPR after induction chemotherapy, who have a more favourable prognosis than those with PR. However, there was no apparent effect on EFS/OS in patients with two favourable prognostic factors (HR around 1): those who had received a purged stem cell transplant (2-year OS of 85% [ch14.18] vs. 82% [control]) and those with hyperdiploid tumours (84% vs 86%, respectively). Given the sample sizes and the high level of missing data for the tumour biology, these results should be interpreted with caution. Finally, the small group of patients with residual

disease at MIBG (Curie score > 0) did not seem to benefit from immunotherapy and the results in patients with persistent disease are not considered convincing.

The contribution of each component of the proposed combination to the observed benefit and risks of treatment was requested during the procedure. The applicant summarised the non-clinical and clinical data available however no definite proof of clinical benefit can be ascribed to one or the other component of the combination as the definition of the dosing regimen currently proposed is the result of progressive adjustments solely based on toxicity results.

Post-hoc analysis of efficacy results were presented by antibody status for Study 302, which suggested a negative trend for EFS in subjects developing HACAs but no clear difference for OS. However amongst the patients who developed HACAs, survival outcomes did not appear worse when the antibodies were found to be neutralising. The Applicant will conduct a post-authorisation immunogenicity study to better characterise the immunogenicity profile of the commercial product as well as its impact on dinutuximab exposure (see Annex II obligation).

The efficacy of Unituxin in children aged less than 12 months has not yet been established.

2.5.4. Conclusions on the clinical efficacy

The initial indication has been modified to reflect the age group of the study population and the fact that only patients with at least partial response following induction chemotherapy were included.

2.6. Clinical safety

The Applicant has provided a safety summary of the main studies sponsored by NCI/UTC where ch14.18 was administered in combination with GM-CSF alone in two Phase I/II studies (CCG-0935 and POG-9347) and in combination with GM-CSF, IL-2, and RA in four Phase II-III clinical studies (CCG-0935A, DIV-NB-301, -302, -303, and the PK study [DIV-NB-201]); all studies were conducted in children and young adults with high-risk neuroblastoma.

Patient exposure

Overall, 984 subjects with high-risk neuroblastoma were exposed to ch14.18 in combination with GM-CSF and IL-2: 578 male (59%) and 406 female (41%) patients, with a median age of 3.8 years (range: 0.75 – 29.4; 11 children ≤ 1 year and 8 adults > 17 years) and mostly white ethnicity (75%) (see Table 30). The majority of subjects (78%) received 5 courses of ch14.18 therapy, which is the recommended regimen (see Table 34).
The safety results are presented separately for the randomised pivotal trial (with the control arm and the immunotherapy arm) and the remaining trials.

Table 30 Demographics of the safety population

	Treatment n (%) [# of Events]			
	Control group from ANBL0032 (N=108)	ch14.18 group from ANBL0032 (Randomized) (N=114)	ch14.18 from other studies (N=843)	All ch14.18 groups[1] (N=984)
Age at Enrollment (Years)				
N	108	114	843	984
Mean (SD)	4 (2.15)	4.3 (2.46)	4.5 (3.02)	4.5 (2.97)
Median	3.67	3.86	3.79	3.83
Min, Max	0.95, 13.29	0.95, 15.29	0.75, 29.4	0.75, 29.4
Categorical Age at Enrollment (Years)				
Age 1 year or less	1 (0.9%)	1 (0.9%)	10 (1.2%)	11 (1.1%)
Age > 1 to 3 years	39 (36.1%)	33 (28.9%)	290 (34.4%)	329 (33.4%)
Age > 3 to 5 years	39 (36.1%)	52 (45.6%)	307 (36.4%)	369 (37.5%)
Age > 5 to 8 years	23 (21.3%)	23 (20.2%)	159 (18.9%)	189 (19.2%)
Age > 8 to 12 years	5 (4.6%)	1 (0.9%)	55 (6.5%)	58 (5.9%)
Age > 12 to 15 years	1 (0.9%)	3 (2.6%)	11 (1.3%)	15 (1.5%)
Age > 15 to 17 years	0	1 (0.9%)	3 (0.4%)	5 (0.5%)
Age > 17 years	0	0	8 (0.9%)	8 (0.8%)
Sex				
Male	61 (56.5%)	71 (62.3%)	493 (58.5%)	578 (58.7%)
Female	47 (43.5%)	43 (37.7%)	350 (41.5%)	406 (41.3%)

Table 31: Summary of exposure to ch14.18 (safety population)

Duration of Exposure	No. of Subjects(%) (N=984)[1]
<= 1 cycle	60 (6.1%)
>1 cycle and <=2 cycles	47 (4.8%)
>2 cycles and <=3 cycles	66 (6.7%)
>3 cycles and <=4 cycles	46 (4.7%)
>4 cycles and <=5 cycles	764 (77.6%)
>5 cycles and <=6 cycles	1 (0.1%)

Adverse events

An overall summary of adverse events is presented in Table 32 and those occurring in ≥5% of the whole ch14.18 population in Table 33.

Table 32: Overall summary of Adverse Events (safety population)

	Control group from ANBL0032 (Randomized) (N=108)	ch14.18 group from ANBL0032 (Randomized) (N=114)	All ch14.18 groups (N=984)
No. of Subjects with at least one Adverse Event	91 (84.3%)	112 (98.2%)	922 (93.7%)
Total No. of Adverse Events	1023	3844	36807
No. of Subjects with at least one AdEER/SAE (ANBL0032, ANBL0931 or DIVNB201)	4/108 (3.7%)	52/114 (45.6%)	560/961 (58.3%)
No. of AdEERs/SAEs (ANBL0032, ANBL0931 or DIVNB201)	5	186	2761
No. of Subjects with at least one Grade 3 or Higher Adverse Event	69 (63.9%)	109 (95.6%)	845 (85.9%)
No. of Grade 3 or Higher Adverse Events	305	1175	8747
No. of Subjects with an Adverse Event Resulting in Death	0	2 (1.8%)	9 (0.9%)
No. of Adverse Events Resulting in Death	0	2	9
No. of Subjects with an Adverse Event that Lead to Treatment Discontinuation [1]	0	0	19/51 (37.3%)
No. of Adverse Events that Lead to Treatment Discontinuation [1]	0	0	66
No. of Subjects with an Adverse Event Related to the Study Drug	68 (63.0%)	111 (97.4%)	915 (93.0%)
No. of Adverse Events related to the Study Drug	561	3142	25584

Note: Only studies ANBL0032, ANBL0931, CCG0935A and DIVNB201 have been included for this summary.
[1] The Action Taken for an Adverse Event was collected only for the CCG0935A and DIVNB201 studies.

Table 33 Adverse Events occurring in ≥ 5% of the whole ch14.18 population

Body System/Preferred Term	Treatment n (%) [# of Events]		
	Control group from ANBL0032 (Randomized) (N=108)	ch14.18 group from ANBL0032 (Randomized) (N=114)	All ch14.18 groups[1] (N=984)
BLOOD AND LYMPHATIC SYSTEM DISORDERS	23 (21.3) [49]	59 (51.8) [181]	471 (47.9) [2108]
ANAEMIA	23 (21.3) [48]	58 (50.9) [176]	446 (45.3) [1997]
CARDIAC DISORDERS	2 (1.9) [2]	24 (21.1) [68]	186 (18.9) [956]
SINUS TACHYCARDIA	0	16 (14.0) [34]	128 (13.0) [803]
GASTROINTESTINAL DISORDERS	38 (35.2) [90]	84 (73.7) [406]	488 (49.6) [2604]
ABDOMINAL PAIN	9 (8.3) [12]	66 (57.9) [136]	323 (32.8) [820]
VOMITING	20 (18.5) [34]	49 (43.0) [99]	201 (20.4) [579]
DIARRHOEA	16 (14.8) [26]	47 (41.2) [104]	236 (24.0) [512]
NAUSEA	3 (2.8) [3]	9 (7.9) [16]	117 (11.9) [306]
CONSTIPATION	2 (1.9) [2]	7 (6.1) [12]	64 (6.5) [108]
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	32 (29.6) [56]	97 (85.1) [432]	686 (69.7) [4453]
PYREXIA	29 (26.9) [38]	81 (71.1) [221]	518 (52.6) [2173]
PAIN	6 (5.6) [14]	59 (51.8) [124]	399 (40.5) [1480]
FACE OEDEMA	0	10 (8.8) [24]	123 (12.5) [219]
FATIGUE	2 (1.9) [3]	8 (7.0) [11]	71 (7.2) [116]
OEDEMA PERIPHERAL	0	2 (1.8) [8]	58 (5.9) [91]
CHILLS	0	9 (7.9) [14]	56 (5.7) [80]
IMMUNE SYSTEM DISORDERS	9 (8.3) [33]	73 (64.0) [220]	601 (61.1) [1753]
HYPERSENSITIVITY	8 (7.4) [21]	68 (59.6) [170]	551 (56.0) [1347]
ANAPHYLACTIC REACTION	1 (0.9) [1]	28 (24.6) [41]	176 (17.9) [220]
CYTOKINE RELEASE SYNDROME	0	6 (5.3) [6]	92 (9.3) [177]
INFECTIONS AND INFESTATIONS	47 (43.5) [68]	59 (51.8) [129]	410 (41.7) [820]
DEVICE RELATED INFECTION	11 (10.2) [14]	19 (16.7) [28]	155 (15.8) [234]
INFECTION SUSCEPTIBILITY INCREASED	6 (5.6) [6]	8 (7.0) [11]	54 (5.5) [69]
INVESTIGATIONS	67 (62.0) [432]	97 (85.1) [958]	676 (68.7) [8842]
PLATELET COUNT DECREASED	44 (40.7) [140]	70 (61.4) [238]	390 (39.6) [1765]
LYMPHOCYTE COUNT DECREASED	38 (35.2) [97]	72 (63.2) [222]	335 (34.0) [1702]
ALANINE AMINOTRANSFERASE INCREASED	33 (30.6) [81]	56 (49.1) [152]	339 (34.5) [1053]
WHITE BLOOD CELL COUNT DECREASED	16 (14.8) [37]	42 (36.8) [95]	239 (24.3) [1019]
NEUTROPHIL COUNT DECREASED	17 (15.7) [23]	45 (39.5) [93]	305 (31.0) [945]
ASPARTATE AMINOTRANSFERASE INCREASED	7 (6.5) [12]	26 (22.8) [54]	258 (26.2) [680]
GAMMA-GLUTAMYLTRANSFERASE INCREASED	0	5 (4.4) [12]	98 (10.0) [289]
WEIGHT INCREASED	0	11 (9.6) [19]	95 (9.7) [286]
BLOOD CREATININE INCREASED	6 (5.6) [12]	17 (14.9) [28]	81 (8.2) [210]
BLOOD ALKALINE PHOSPHATASE INCREASED	7 (6.5) [14]	6 (5.3) [7]	67 (6.8) [129]

Body System/Preferred Term	Treatment n (%) [# of Events]		
	Control group from ANBL0032 (Randomized) (N=108)	ch14.18 group from ANBL0032 (Randomized) (N=114)	All ch14.18 groups[1] (N=984)
METABOLISM AND NUTRITION DISORDERS	29 (26.9) [115]	83 (72.8) [631]	626 (63.6) [6465]
HYPOALBUMINAEMIA	3 (2.8) [4]	35 (30.7) [109]	260 (26.4) [1322]
HYPONATRAEMIA	13 (12.0) [31]	60 (52.6) [149]	365 (37.1) [1107]
HYPOKALAEMIA	5 (4.6) [11]	42 (36.8) [80]	401 (40.8) [1075]
HYPOCALCAEMIA	0	24 (21.1) [56]	229 (23.3) [877]
HYPERTRIGLYCERIDAEMIA	12 (11.1) [18]	18 (15.8) [45]	160 (16.3) [350]
HYPERGLYCAEMIA	4 (3.7) [5]	17 (14.9) [37]	133 (13.5) [338]
HYPOPHOSPHATAEMIA	3 (2.8) [7]	25 (21.9) [37]	159 (16.2) [334]
DECREASED APPETITE	5 (4.6) [12]	17 (14.9) [36]	118 (12.0) [242]
HYPOMAGNESAEMIA	1 (0.9) [1]	13 (11.4) [26]	87 (8.8) [190]
HYPERKALAEMIA	4 (3.7) [4]	6 (5.3) [7]	84 (8.5) [136]
HYPOGLYCAEMIA	0	5 (4.4) [5]	72 (7.3) [134]
HYPERCALCAEMIA	8 (7.4) [17]	9 (7.9) [16]	70 (7.1) [120]
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	10 (9.3) [24]	41 (36.0) [72]	242 (24.6) [769]
PAIN IN EXTREMITY	4 (3.7) [7]	16 (14.0) [25]	167 (17.0) [386]
BACK PAIN	1 (0.9) [1]	12 (10.5) [15]	81 (8.2) [178]
NERVOUS SYSTEM DISORDERS	11 (10.2) [18]	35 (30.7) [63]	232 (23.6) [591]
NEURALGIA	0	11 (9.6) [22]	89 (9.0) [229]
HEADACHE	4 (3.7) [5]	8 (7.0) [8]	90 (9.1) [161]
RENAL AND URINARY DISORDERS	7 (6.5) [14]	29 (25.4) [57]	167 (17.0) [497]
PROTEINURIA	3 (2.8) [6]	15 (13.2) [25]	76 (7.7) [180]
HAEMATURIA	1 (0.9) [1]	7 (6.1) [11]	59 (6.0) [128]
URINARY RETENTION	0	7 (6.1) [10]	65 (6.6) [120]
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	7 (6.5) [23]	47 (41.2) [104]	404 (41.1) [1363]
HYPOXIA	2 (1.9) [2]	29 (25.4) [51]	185 (18.8) [385]
COUGH	2 (1.9) [5]	11 (9.6) [18]	159 (16.2) [344]
DYSPNOEA	1 (0.9) [1]	6 (5.3) [9]	114 (11.6) [165]
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	24 (22.2) [52]	65 (57.0) [181]	612 (62.2) [1987]
URTICARIA	3 (2.8) [4]	49 (43.0) [103]	485 (49.3) [1007]
PRURITUS	1 (0.9) [1]	11 (9.6) [17]	139 (14.1) [332]
DRY SKIN	16 (14.8) [29]	20 (17.5) [33]	130 (13.2) [264]
RASH PAPULAR	5 (4.6) [8]	10 (8.8) [13]	94 (9.6) [164]
VASCULAR DISORDERS	9 (8.3) [21]	82 (71.9) [273]	774 (78.7) [3552]
HYPOTENSION	3 (2.8) [4]	70 (61.4) [166]	659 (67.0) [2133]
CAPILLARY LEAK SYNDROME	1 (0.9) [1]	45 (39.5) [76]	445 (45.2) [896]
HYPERTENSION	7 (6.5) [16]	17 (14.9) [28]	112 (11.4) [497]

The most commonly reported AEs included hypotension (67%), hypersensitivity (56%), pyrexia (53%), urticaria (49%), capillary leak syndrome (45%), anaemia (45%), pain (41%), hypokalaemia (41%), decreased platelet count (40%), hyponatraemia (37%), increased ALT (35%), decreased lymphocyte count (34%), and abdominal pain (33%). When all pain-related AEs were grouped together (i.e., any PTs containing "pain" and "-algia" as well as abdominal discomfort), "pain" became the second most commonly reported AE with 66% of subjects reporting a pain related AE during clinical studies with ch14.18.

Of note, a higher proportion of subjects reported events of abdominal pain, vomiting, diarrhoea, pyrexia, decreased platelet and lymphocyte count, and hyponatraemia in the randomised study as compared to the other ch14.18 studies.

Adverse reactions are defined as those adverse events that occurred at a higher frequency in the dinutuximab, GM-CSF, IL-2 and isotretinoin-treated group compared with the isotretinoin-treated control group during the ANBL0032 randomised, controlled, pivotal study, and that have a plausible mechanistic relationship to treatment with dinutuximab. Originally reported terms have been coded to preferred terms (using the Medical Dictionary for Regulatory Activities [MedDRA]. Frequency categories described in Table 34 are defined as: very common (≥1/10); common (≥1/100 to <1/10); uncommon (≥1/1,000 to <1/100).

Table 34: Adverse reactions that have occurred during studies in high risk neuroblastoma patients receiving dinutuximab in combination with GM-CSF, IL-2, and isotretinoin.

System Organ Class	Very Common	Common	Uncommon
Infections and infestations		Device-related infection, infection susceptibility increased, bacteraemia, enterocolitis	
Blood and lymphatic system disorders	Anaemia	Febrile neutropenia	Atypical haemolytic uraemic syndrome
Immune system disorders	Anaphylactic reaction, hypersensitivity	Cytokine release syndrome	Serum sickness
Endocrine disorders			Hyperthyroidism
Metabolism and nutrition disorders	Hypokalaemia, hyponatraemia, hypocalcaemia, hypophosphataemia, hypoalbuminaemia, hyperglycaemia, decreased appetite	Hypomagnesaemia, acidosis, hypoglycaemia,	
Nervous system disorders		Neuralgia, peripheral neuropathy, headache	Posterior reversible encephalopathy syndrome
Eye disorders		Vision blurred, photophobia, mydriasis	Unequal pupils
Cardiac disorders	Tachycardia (sinusal, atrial, ventricular)		Atrial fibrillation, ventricular arrhythmia
Vascular disorders	Capillary leak syndrome, hypotension, hypertension		
Respiratory, thoracic and mediastinal disorders	Hypoxia, cough, dyspnoea	Bronchospasm, pulmonary oedema	Stridor, laryngeal oedema
Gastrointestinal disorders	Diarrhoea, vomiting, nausea	Constipation, lower gastrointestinal haemorrhage	
Skin and subcutaneous tissue disorders	Urticaria, pruritus	Maculo-papular rash	
Renal and urinary disorders		Urinary retention, proteinuria, haematuria	Renal failure
General disorders and administration site conditions	Pyrexia, pain[1], face oedema	Peripheral oedema, chills, fatigue, irritability Injection site reaction	
Investigations	Decreased platelet count, decreased lymphocyte	Increased gamma-glutamyltransferase,	Blood culture positive

System Organ Class	Very Common	Common	Uncommon
	count, decreased white blood cell count, decreased neutrophil count, increased aspartate aminotransferase, increased alanine aminotransferase	increased blood creatinine, increased weight	

[1] Includes preferred terms abdominal pain, abdominal pain upper, arthralgia, back pain, bladder pain, bone pain, chest pain, facial pain, gingival pain, musculoskeletal chest pain, myalgia, neck pain, neuralgia, oropharyngeal pain, pain, pain in extremity, and proctalgia.

As shown in Table 35, the most commonly reported severe (Grade 3 and higher) AEs that occurred across all ch14.18 dosing groups included pyrexia (37%) and laboratory abnormalities (anaemia [35%], hypokalaemia [32%], decreased lymphocyte count [30%], decreased platelet count [29%] and neutrophil count decreased [27%]).

Table 35: **Severe (≥ Grade 3) Adverse Events occurring in ≥ 5% of the whole ch14.18 population**

Body System/Preferred Term	Treatment n (%) [# of Events]		
	Control group from ANBL0032 (Randomized) (N=108)	ch14.18 group from ANBL0032 (Randomized) (N=114)	All ch14.18 groups (N=984)[1]
BLOOD AND LYMPHATIC SYSTEM DISORDERS	17 (15.7) [27]	39 (34.2) [70]	363 (36.9) [706]
ANAEMIA	17 (15.7) [26]	37 (32.5) [66]	342 (34.8) [643]
GASTROINTESTINAL DISORDERS	7 (6.5) [10]	46 (40.4) [94]	262 (26.6) [517]
ABDOMINAL PAIN		31 (27.2) [51]	148 (15.0) [240]
DIARRHOEA	1 (0.9) [3]	14 (12.3) [19]	94 (9.6) [120]
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	10 (9.3) [10]	67 (58.8) [123]	494 (50.2) [1075]
PYREXIA	6 (5.6) [6]	45 (39.5) [61]	368 (37.4) [621]
PAIN	3 (2.8) [3]	31 (27.2) [49]	207 (21.0) [404]
IMMUNE SYSTEM DISORDERS	2 (1.9) [2]	29 (25.4) [46]	218 (22.2) [306]
ANAPHYLACTIC REACTION	1 (0.9) [1]	28 (24.6) [41]	172 (17.5) [216]
HYPERSENSITIVITY	0	1 (0.9) [1]	60 (6.1) [72]
INFECTIONS AND INFESTATIONS	32 (29.6) [46]	48 (42.1) [100]	334 (33.9) [607]
DEVICE RELATED INFECTION	11 (10.2) [14]	19 (16.7) [26]	146 (14.8) [217]
INVESTIGATIONS	44 (40.7) [132]	87 (76.3) [380]	610 (62.0) [2952]
PLATELET COUNT DECREASED	26 (24.1) [58]	39 (34.2) [94]	282 (28.7) [767]
LYMPHOCYTE COUNT DECREASED	21 (19.4) [32]	58 (50.9) [123]	293 (29.8) [725]
NEUTROPHIL COUNT DECREASED	14 (13.0) [20]	38 (33.3) [67]	263 (26.7) [517]
ALANINE AMINOTRANSFERASE INCREASED	3 (2.8) [3]	22 (19.3) [30]	168 (17.1) [255]
WHITE BLOOD CELL COUNT DECREASED	9 (8.3) [14]	24 (21.1) [36]	123 (12.5) [214]
GAMMA-GLUTAMYLTRANSFERASE INCREASED	0	2 (1.8) [3]	71 (7.2) [158]
ASPARTATE AMINOTRANSFERASE INCREASED	0	11 (9.6) [12]	91 (9.2) [113]
METABOLISM AND NUTRITION DISORDERS	15 (13.9) [34]	62 (54.4) [150]	511 (51.9) [1313]
HYPOKALAEMIA	3 (2.8) [5]	35 (30.7) [48]	317 (32.2) [507]
HYPONATRAEMIA	4 (3.7) [5]	23 (20.2) [26]	210 (21.3) [293]
DECREASED APPETITE	4 (3.7) [10]	12 (10.5) [26]	63 (6.4) [116]
HYPOPHOSPHATAEMIA	0	9 (7.9) [10]	62 (6.3) [65]
HYPOALBUMINAEMIA	0	8 (7.0) [9]	51 (5.2) [61]

Body System/Preferred Term	Treatment n (%) [# of Events]		
	Control group from ANBL0032 (Randomized) (N=108)	ch14.18 group from ANBL0032 (Randomized) (N=114)	All ch14.18 groups (N=984)[1]
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	5 (4.6) [9]	23 (20.2) [38]	107 (10.9) [213]
PAIN IN EXTREMITY	2 (1.9) [3]	7 (6.1) [12]	60 (6.1) [98]
NERVOUS SYSTEM DISORDERS	3 (2.8) [3]	17 (14.9) [26]	111 (11.3) [218]
NEURALGIA	0	8 (7.0) [14]	63 (6.4) [149]
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	3 (2.8) [8]	20 (17.5) [29]	203 (20.6) [380]
HYPOXIA	1 (0.9) [1]	14 (12.3) [20]	132 (13.4) [218]
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	2 (1.9) [3]	16 (14.0) [18]	110 (11.2) [153]
URTICARIA	0	14 (12.3) [16]	88 (8.9) [118]
VASCULAR DISORDERS	1 (0.9) [6]	38 (33.3) [67]	278 (28.3) [479]
HYPOTENSION	0	22 (19.3) [30]	184 (18.7) [269]
CAPILLARY LEAK SYNDROME	0	23 (20.2) [36]	133 (13.5) [179]

Serious adverse event / deaths / other significant events

In total, 25 subjects (2%) died within 30 days of study therapy across all ch14.18 studies. The majority of deaths (19/25; 76%) were considered disease related by the investigators.

Out of the six other deaths, one death due to extensive intracranial haemorrhage occurred before the patient received any ch14.18. As for the five deaths of patients having received ch14.18, one was attributed to an IL-2 overdose and two were related to a lung infection; the last two deaths occurred during/after the first treatment course and were both characterised by sudden abdominal pain followed by collapse. The pathophysiology of the events in the last two cases remains unclear.

Serious AEs and AdEERS from the ANBL0032, ANBL0931, and DIV-NB-201 studies were combined into a single table by CTCAE version 4.0 term (Table 22). Overall, the proportion of subjects experiencing an SAE was 3.7% in the control arm, 45.6% in the randomised ch14.18 arm, and 60.6% in the all ch14.18 groups (N=961).

The most commonly reported SAEs in subjects who received ch14.18 immunotherapy included hypotension (15%), fever (14%), capillary leak syndrome (11%), catheter related infections (10%), anaphylaxis (10%), and hypokalaemia (9%).

Table 36: Serious AEs occurring in ≥1% of the whole ch14.18 population

Body System/CTCAE v4.0 Term	Treatment n (%) [# of Events]		
	Control group from ANBL0032 (Randomized) (N=108)	ch14.18 group from ANBL0032 (Randomized) (N=114)	All ch14.18 groups[1] (N=961)
BLOOD AND LYMPHATIC SYSTEM DISORDERS	0	2 (1.8) [2]	72 (7.5) [91]
ANEMIA	0	2 (1.8) [2]	64 (6.7) [77]
CARDIAC DISORDERS	0	3 (2.6) [5]	33 (3.4) [43]
SINUS TACHYCARDIA	0	2 (1.8) [2]	15 (1.6) [19]
GASTROINTESTINAL DISORDERS	1 (0.9) [2]	10 (8.8) [15]	103 (10.7) [170]
ABDOMINAL PAIN	0	4 (3.5) [5]	36 (3.7) [49]
DIARRHEA	0	3 (2.6) [3]	40 (4.2) [42]
VOMITING	1 (0.9) [1]	1 (0.9) [1]	20 (2.1) [21]
ILEUS	0	1 (0.9) [1]	10 (1.0) [11]
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	0	15 (13.2)[24]	189 (19.7) [320]
FEVER	0	7 (6.1) [9]	131 (13.6) [184]
PAIN	0	5 (4.4) [5]	42 (4.4) [54]
EDEMA FACE	0	2 (1.8) [2]	17 (1.8) [18]
CHILLS	0	1 (0.9) [1]	13 (1.4) [13]
IRRITABILITY	0	1 (0.9) [1]	12 (1.2) [12]
FATIGUE	0	1 (0.9) [1]	10 (1.0) [10]
IMMUNE SYSTEM DISORDERS	0	9 (7.9) [10]	142 (14.8) [192]
ANAPHYLAXIS	0	6 (5.3) [6]	91 (9.5) [100]
ALLERGIC REACTION	0	2 (1.8) [2]	58 (6.0) [69]
CYTOKINE RELEASE SYNDROME	0	2 (1.8) [2]	15 (1.6) [18]
INFECTIONS AND INFESTATIONS	2 (1.9) [2]	26 (22.8)[39]	245 (25.5) [374]
CATHETER RELATED INFECTION	2 (1.9) [2]	8 (7.0) [11]	95 (9.9) [131]
INFECTIONS AND INFESTATIONS - OTHER, SPECIFY INFECTION WITH NORMAL ANC OR GRADE 1 OR 2 NEUTROPHILS: BLOOD	0	10 (8.8) [13]	57 (5.9) [74]
LUNG INFECTION	0	1 (0.9) [1]	20 (2.1) [23]
SEPSIS	0	0	19 (2.0) [20]
INFECTIONS AND INFESTATIONS - OTHER, SPECIFY BLOOD	0	1 (0.9) [1]	18 (1.9) [19]
ENTEROCOLITIS INFECTIOUS	0	1 (0.9) [1]	14 (1.5) [15]
URINARY TRACT INFECTION	0	1 (0.9) [1]	10 (1.0) [14]
INVESTIGATIONS	0	10 (8.8) [12]	165 (17.2) [384]
LYMPHOCYTE COUNT DECREASED	0	4 (3.5) [4]	57 (5.9) [70]
NEUTROPHIL COUNT DECREASED	0	1 (0.9) [1]	56 (5.8) [63]
PLATELET COUNT DECREASED	0	1 (0.9) [1]	35 (3.6) [40]
ALANINE AMINOTRANSFERASE INCREASED	0	2 (1.8) [2]	32 (3.3) [38]
WHITE BLOOD CELL DECREASED	0	0	33 (3.4) [37]
ASPARTATE AMINOTRANSFERASE INCREASED	0	1 (0.9) [1]	24 (2.5) [25]
GGT INCREASED	0	0	20 (2.1) [23]
BLOOD BILIRUBIN INCREASED	0	0	16 (1.7) [19]
WEIGHT GAIN	0	0	15 (1.6) [17]
URINE OUTPUT DECREASED	0	0	11 (1.1) [15]
CREATININE INCREASED	0	2 (1.8) [2]	13 (1.4) [14]

Body System/CTCAE v4.0 Term	Treatment n (%) [# of Events]		
	Control group from ANBL0032 (Randomized) (N=108)	ch14.18 group from ANBL0032 (Randomized) (N=114)	All ch14.18 groups[1] (N=961)

METABOLISM AND NUTRITION DISORDERS	0	12 (10.5)[24]	215 (22.4) [401]
HYPOKALEMIA	0	7 (6.1) [8]	88 (9.2) [99]
HYPONATREMIA	0	0	62 (6.5) [67]
HYPOCALCEMIA	0	2 (1.8) [2]	44 (4.6) [52]
HYPOPHOSPHATEMIA	0	0	35 (3.6) [40]
HYPERCALCEMIA	0	3 (2.6) [6]	21 (2.2) [31]
HYPOALBUMINEMIA	0	2 (1.8) [2]	24 (2.5) [25]
DEHYDRATION	0	1 (0.9) [1]	17 (1.8) [18]
ANOREXIA	0	2 (1.8) [2]	17 (1.8) [17]
HYPERKALEMIA	0	1 (0.9) [1]	12 (1.2) [12]
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	0	7 (6.1) [12]	45 (4.7) [87]
PAIN IN EXTREMITY	0	3 (2.6) [5]	28 (2.9) [40]
BACK PAIN	0	1 (0.9) [1]	10 (1.0) [15]
NERVOUS SYSTEM DISORDERS	0	5 (4.4) [6]	56 (5.8) [78]
HEADACHE	0	1 (0.9) [1]	12 (1.2) [14]
SEIZURE	0	0	10 (1.0) [11]
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	0	7 (6.1) [7]	120 (12.5) [209]
HYPOXIA	0	4 (3.5) [4]	58 (6.0) [71]
BRONCHOSPASM	0	0	24 (2.5) [29]
COUGH	0	0	21 (2.2) [24]
DYSPNEA	0	0	20 (2.1) [21]
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	0	2 (1.8) [2]	56 (5.8) [68]
URTICARIA	0	0	37 (3.9) [47]
VASCULAR DISORDERS	0	11 (9.6) [18]	216 (22.5) [323]
HYPOTENSION	0	8 (7.0) [16]	140 (14.6) [176]
CAPILLARY LEAK SYNDROME	0	6 (5.3) [8]	101 (10.5) [120]
HYPERTENSION	0	0	22 (2.3) [25]

AEs occurred more frequently and with a greater severity in Courses 1, 2, and 4 as compared to Courses 3 and 5. This is illustrated in Figure 14 for the events of capillary leak syndrome, hypotension, pain, and pyrexia.

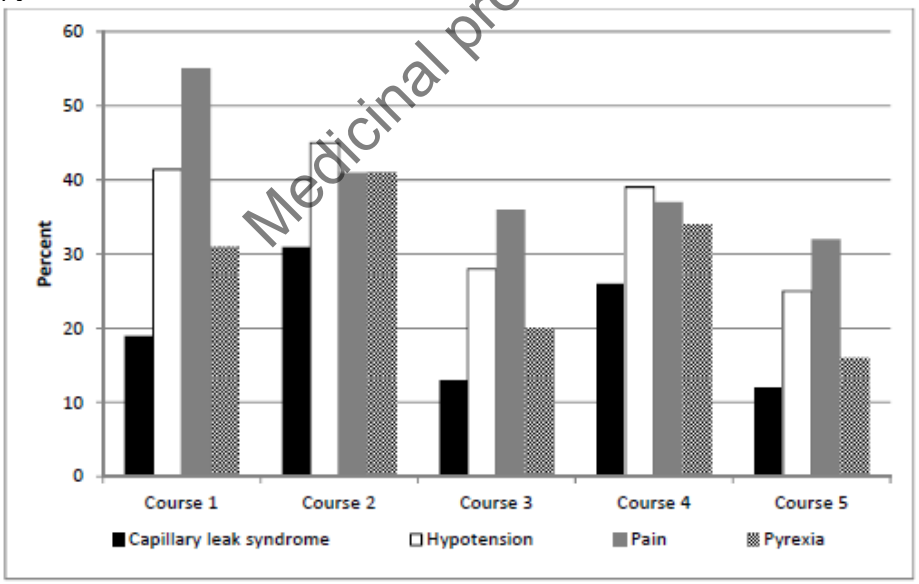


Figure 14: **Frequency of Capillary Leak Syndrome, Hypotension, Pyrexia, and Pain by treatment course (total safety population)**

Adverse events of interest

Pain-related AEs

Pain-related AEs included any PTs containing “pain” and “-algia” as well as abdominal discomfort. Overall, 66% of subjects reported a pain-related event during the course of treatment with the majority of pain-related events occurring during ch14.18 courses; 41% of the patients experienced severe pain (Grade 3-4). Pain-related events generally decreased over the course of the study suggesting improved tolerability and/or pain management over time. Concomitant medications to prevent/manage pain-related events were administered during the Phase II and III studies prior to each course of ch14.18.

Course	All ch14.18 groups Total n (%) [# of Events]
No. of Unique Subjects (N=984)	649 (66.0%)
COURSE 1 (N=984)	541 (55.0) [989]
COURSE 2 (N=924)	374 (40.5) [758]
COURSE 3 (N=877)	311 (35.5) [558]
COURSE 4 (N=811)	296 (36.5) [640]
COURSE 5 (N=766)	244 (31.9) [450]

Allergic reactions

An allergic reaction-related event was reported by 81% of the subjects; the majority were Grade 1 or 2 but 29% of the subjects reported a severe (Grade 3-4) allergic reaction. These reactions were more frequent during Courses 1, 2, and 4, which was expected as IL-2 is known to be immunogenic.

Course	All ch14.18 groups Total n (%) [# of Events]
No. of Unique Subjects (N=984)	798 (81.1%)
COURSE 1 (N=984)	527 (53.4)[838]
COURSE 2 (N=924)	561 (60.7)[976]
COURSE 3 (N=877)	385 (43.9)[575]
COURSE 4 (N=811)	456 (56.2)[788]
COURSE 5 (N=766)	306 (39.9)[447]

Allergic reactions were most commonly reported as hypersensitivity (56%), urticaria (49%), anaphylactic reaction (18%), pruritus, oedema, rash and bronchospasm. A standardized MedDRA query identified 52% of anaphylactic/anaphylactoid reactions. The most frequently reported term which contributed to this high number of subjects was hypotension, which is often associated with anaphylaxis but may also be associated with capillary leak syndrome, another commonly reported AE during immunotherapy with ch14.18.

Infection-related events

Infection-related AEs occurred in 42% of the patients. The majority of infection related events were considered Grade 3. Overall, infection-related AEs were expected in this subject population as subjects were enrolled after the completion of an ASCT and were subsequently immunosuppressed at the time of study participation. In addition, subjects received ch14.18 and cytokines via a central venous catheter, which explains the frequency of device-related infections (16%).

Other events

Other events of interest included neuropathy-related events (muscular weakness, peripheral sensory/motor neuropathy), neurologic-related disorders of the eye events (mydriasis, photophobia and vision blurred), hypoxia, dyspnoea, vomiting, diarrhoea, and hepatic dysfunction.

Peripheral sensory neuropathy was reported in 3% of patients and peripheral motor neuropathy in 2% of patients; less than 1% of patients experienced serious peripheral neuropathy.

Posterior reversible encephalopathy syndrome (PRES) has been reported in the literature as a possible ADR of anti-GD2 monoclonal antibodies. Clinical signs and symptoms include hypertension, seizures, headache, visual disturbance and altered mental status associated with oedematous changes of brain magnetic resonance imaging. Three possible cases of PRES were reported with ch14.18.

Atypical haemolytic uremic syndrome (aHUS) in the absence of documented infection and resulting in renal insufficiency, electrolyte abnormalities, anaemia, and hypertension occurred in three patients following the first treatment course. It recurred following re-challenge in one of these patients.

Laboratory findings

Clinical laboratory assessments were performed during clinical trials with ch14.18; however, data from these assessments were recorded inconsistently. Therefore, study DIV-NB-303 was used as a representative study for clinical laboratory evaluations for this summary of safety as this study included the most comprehensive collection of clinical laboratory data.

CBC values were generally below or close to the lower end of normal for expected values. In particular, neutrophil, RBC, haemoglobin, haematocrit, and platelet values were routinely below normal both prior to and after ch14.18 dosing in Courses 1-5, as expected with subjects post-ASCT.

The total percentage of eosinophils did appear to increase (approximately double) between the first and last measured values for Courses 1, 2, and 4 but not in Courses 3 or 5. This finding correlated with AE reports that were considered allergic in nature and may demonstrate a difference in the immune response for Courses 1, 2, and 4 as compared to Courses 3 and 5.

WBC and neutrophils appeared higher at the end of Courses 1, 3, and 5 as compared to Courses 2 and 4, which was likely due to the use of GM-CSF. Lymphocytes generally increased over the course of study which was expected as subjects were recovering post-ASCT. Lymphocytes appeared highest at the time of last assessment in Courses 2 and 4 as compared to Courses 1, 3, and 5 which was likely due to IL-2's ability to increase lymphocytes.

Overall, mean BUN values were within normal limits when observed over the first and last assessments during Courses 1-6. In addition, mean serum creatinine values were stable over the course of the study, indicating that renal function was maintained during the study.

In the whole safety database, the most commonly reported haematology-related AEs were anaemia, decreased platelet count, decreased lymphocyte count, decreased neutrophil count, and decreased WBC. The most commonly reported chemistry related AEs included: hypokalaemia, hyponatraemia, increased ALT, hypoalbuminaemia, and hypocalcaemia. Although the frequency of these events tended to decrease over time they were more frequent during courses 2 and 4 when ch14.18 was combined with IL-2 (see Figure 15 and Figure 16).

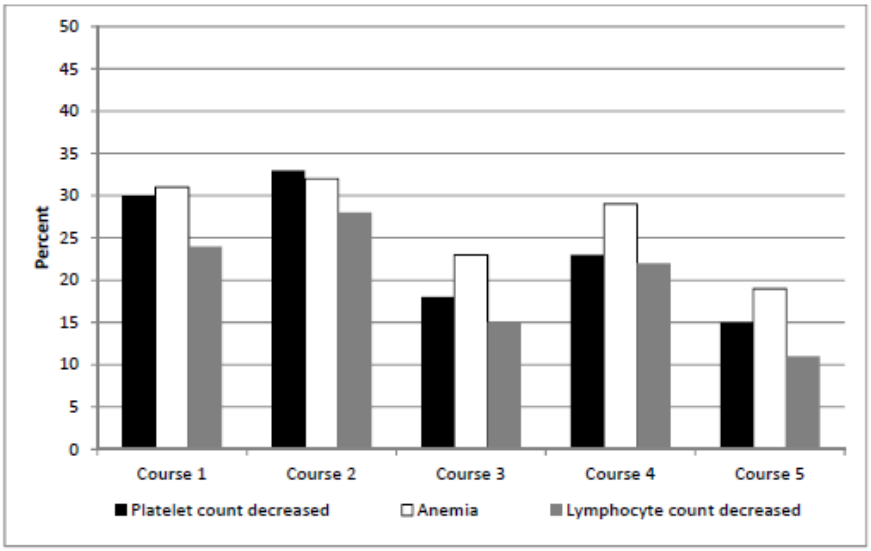


Figure 15: Frequency of most common haematology-related events by treatment course (total safety population)

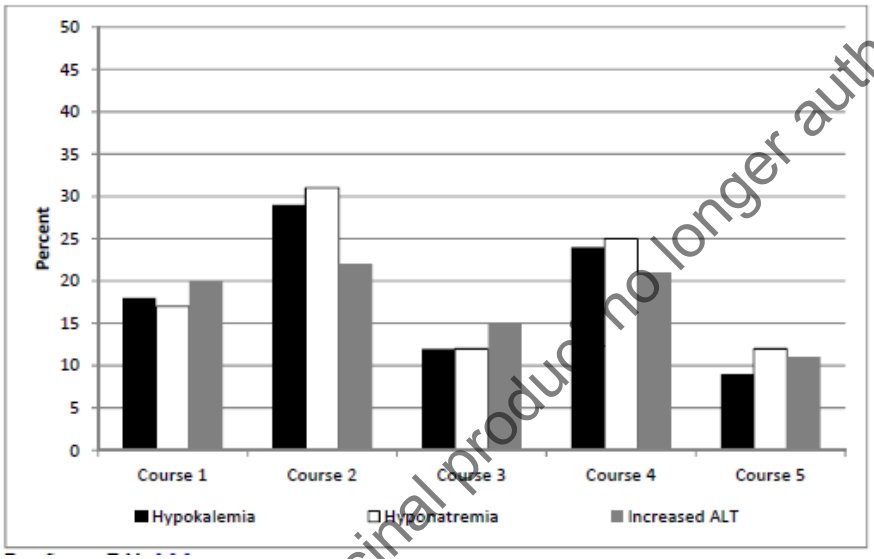


Figure 16: Frequency of most common chemistry-related events by treatment course (total safety population)

Immunological events

Human anti-chimeric antibody (HACA) assessments were conducted in the pivotal trial (DIV-NB-301) and its extensions on study Days -1, 6, 80, 90, 111, 118, and at the end of treatment, at the same time as PK measurements. Importantly, the assay used in the pivotal trial was not validated and the incidence of HACAs in this trial was much lower than in the extension studies and in the bioequivalence study, where validated assays for binding as well as for neutralising antibodies were used.

In Study 301, 7/138 subjects (5.1%) developed HACAs while overall in Studies 302 and 201, 71/409 subjects (17.4%) developed HACAs, which were neutralising in 15 subjects (3.7%). HACAs were persistent (i.e. more than one antibody-positive sample post therapy initiation) in 51 subjects (12.5%).

Safety related to drug-drug interactions and other interactions

As reflected in the protocol for the pivotal study, use of systemic corticosteroid medicinal products is not recommended due to possible interference with immune activation which is necessary for the therapeutic action of dinutuximab. It is also not recommended to use intravenous immunoglobulin after ASCT. If necessary, its use must be limited to the first 100 days after ASCT, as immunoglobulin may interfere with dinutuximab-dependent cellular cytotoxicity. Immunoglobulin must not be given within two weeks before and one week after completing each course of Unituxin.

Discontinuation due to adverse events

Out of 984 subjects, 179 (18.2%) discontinued treatment, i.e. received less than 5 ch14.18 cycles. The main reasons were progressive disease (63; 6.4%), withdrawal of consent (37; 3.8%), and toxicity (29; 3%). Discontinuation occurred at a similar rate across treatment courses (3-4%).

Dose reduction may not have been adequately recorded in all studies and may be underestimated. Nevertheless, it was about twice as frequent in the treatment courses with IL-2 (8.5%) as in the other courses (4-5%).

Post marketing experience

N/A

2.6.1. Discussion on clinical safety

The safety population exposed to the recommended dosing combination of ch14.18 with GM-CSF and IL-2 consists of 984 subjects with a median age of 3.8 years (range: 0.75 – 29.4; 11 children ≤ 1 year and 8 adults > 17 years).

In the pivotal randomised trial, most subjects (96%) exposed to the combination therapy experienced at least one severe (Grade ≥ 3) AE compared to 64% in the control arm treated with RA alone and they were almost all considered treatment-related by the investigator. The proportion of subjects experiencing AEs and severe AEs, as well as some specific AEs (digestive symptoms, pyrexia, and laboratory abnormalities) was higher in the randomised arm than in the overall exposed population. This could not be fully explained by the Applicant although part of the differences might have been related to differences in data collection and variable intervals after the ASCT.

The most commonly reported AEs included hypotension (67%), pain (66%), hypersensitivity (56%), pyrexia (53%), urticaria (49%), capillary leak syndrome (45%), haematotoxicity (anaemia [45%], decreased platelet count [40%], decreased lymphocyte count [34%]), and electrolyte disorders (hypokalaemia [41%], hyponatraemia [37%]).

More than half the subjects (61%) exposed to the combination therapy experienced SAEs. The most commonly reported SAEs included hypotension (15%), fever (14%), capillary leak syndrome (11%), catheter related infections (10%), and anaphylaxis (10%). As ch14.18 is used in combination with GM-CSF, IL-2, and isotretinoin, it is difficult to ascertain the causal relationship of each adverse reaction to a particular medicinal product.

The most frequent reactions followed a typical pattern over treatment courses; they tended to decrease over the courses with higher occurrence during Courses 1, 2, and 4 than during Courses 3 and 5. Part of this finding may be explained by preventative measures taken in individual patients but this was also expected as IL-2 (Courses 2 and 4) is associated with more significant AEs than GM-CSF.

It is noteworthy that the study protocols required prophylactic medications for the management of allergic reactions and pain. Antihistaminic medications had to be systematically administered and, when recorded, this appeared to be the case in about 70-80% of the subjects. Likewise, most patients received prophylactic analgesics, including intravenous opioids.

Severe pain (Grade 3 or 4) occurred most frequently during the first 4-day course of dinutuximab, often subsiding over time with subsequent courses. If it occurs, infusion rate should be decreased to 0.875 mg/m2/hour. Unituxin should be discontinued if pain is not adequately controlled despite infusion rate reduction and institution of maximum supportive measures (see sections 4.2, 4.4 and 4.8 of the SmPC).

Paracetamol should be administered orally 20 minutes prior to starting each dinutuximab infusion, and repeated every 4-6 hours as needed. Regular dosing every 4–6 hours is recommended when IL-2 is coadministered. If required for persistent pain, ibuprofen should be administered orally every 6 hours between doses of paracetamol. Ibuprofen should not be administered if there is evidence of thrombocytopenia, bleeding, or renal dysfunction.

An opioid, such as morphine sulphate, is recommended to be administered by intravenous infusion prior to each dinutuximab infusion and continued as an intravenous infusion during and until 2 hours after completion of the treatment. It is recommended that additional intravenous bolus doses of an opioid are administered as needed for treatment of pain up to once every 2 hours during the dinutuximab infusion. If morphine is not tolerated, then fentanyl or hydromorphone may be utilised.

Lidocaine may be administered as an intravenous infusion (2 mg/kg in 50 mL of 0.9 % sodium chloride) over 30 minutes prior to the start of each dinutuximab infusion and continued via intravenous infusion at 1 mg/kg/h up to 2 hours after completion of the treatment. Lidocaine infusion should be discontinued if the patient develops dizziness, perioral numbness, or tinnitus.

Gabapentin may be administered at the time of starting morphine premedication, at an oral dose of 10 mg/kg/day. The dose may be subsequently increased (up to a maximum of 60 mg/kg/day or 3600 mg/day) as needed for pain management.

Although most patients received prophylactic analgesics as required in the study protocols, two-thirds of the patients experienced pain and 41% experienced severe pain. Its incidence decreased over the first three treatment courses but about one third of the patients were still suffering in the last ch14.18 courses.

However, despite the significant toxicity of the combination regimen, the rate of discontinuation for adverse reactions appeared to be low (3%).

Dinutuximab is a chimeric monoclonal antibody, which implies a certain degree of immunogenicity. Based on the data currently available, 17% of the patients exposed developed HACAs, which were persistent in 12% and neutralising in 3%. These antibodies did not seem to be associated with allergic reactions but the detection of specific IgE against non-human glycans (galactose alpha-1,3-galactose and N-glycolylneuraminic acid) has not been performed. The Applicant will collect data on antibodies against non-human glycans from the ANBL0032 study and assess their impact on safety and efficacy (see RMP). In addition, the applicant will conduct a safety study to better characterise the immunogenicity of dinutuximab (see Annex II obligation).

In order to anticipate toxicities associated with ch14.18 including neuropathic pain and allergic reactions, Antihistamine premedication (e.g. hydroxyzine or diphenhydramine) should be administered by intravenous injection approximately 20 minutes before starting each dinutuximab infusion and should be repeated every 4–6 hours as required during infusion. Patients should be monitored for signs and symptoms of infusion reactions for 4 hours after the completion of the dinutuximab infusion.

Epinephrine (adrenaline) and hydrocortisone for intravenous administration should be immediately available at the bedside during administration of dinutuximab to manage life-threatening allergic reactions. It is recommended that treatment for such reactions include hydrocortisone administered by intravenous bolus, and epinephrine administered by intravenous bolus once every 3–5 minutes as necessary according to clinical response. The applicant will analyse laboratory data from patients with documented grade 4 allergic reactions to allow for improved characterisation of these reactions (see RMP).

Capillary leak syndrome is more likely when dinutuximab is co-administered with IL-2. It is therefore recommended to administer oral metolazone or intravenous furosemide every 6–12 hours as required. Supplemental oxygen, respiratory support, and albumin replacement therapy should be used as necessary according to clinical response.

In case of ADRs reported and depending of the severity of the ADR, the infusion rate should be modified or treatment discontinued (see section 4.2 of the SmPC).

In order to prevent the risk of hypotension, intravenous sodium chloride 9 mg/mL (0.9%) solution for injection (10 mL/kg) should be administered over one hour just prior to the dinutuximab infusion. If hypotension occurs, this can be repeated, or intravenous albumin or packed red blood cells can be administered as clinically indicated. It is recommended that vasopressor therapy is also administered if necessary to restore an adequate perfusion pressure.

Cases of blurred vision, photophobia, mydriasis and unequal pupils have been reported, especially with repeated courses. These changes usually resolve over time. Patients should have an ophthalmic examination before initiating therapy and be monitored for visual changes.

Cases of hepatic dysfunction have been reported and regular monitoring of liver function is recommended during dinutuximab immunotherapy.

Patients typically have a central venous catheter in situ and as a consequence of prior ASCT are likely to be immunocompromised during therapy, and therefore, at risk of developing systemic infection. Patients should have no evidence of systemic infection and any identified infection should be under control before beginning therapy.

Electrolyte abnormalities have been reported in patients who received dinutuximab. Electrolytes should therefore be monitored daily during therapy.

Haemolytic uremic syndrome in the absence of documented infection and resulting in renal insufficiency, electrolyte abnormalities, anaemia, and hypertension has been reported. Supportive measures should be instituted including control of hydration status, electrolyte abnormalities, hypertension, and anaemia.

No cases of dinutuximab overdose have been reported. In clinical trials, scheduled dinutuximab doses of up to 120 mg/m^2 (60 mg/m^2/day) have been administered with an adverse reaction profile similar to that described in section 4.8. In case of overdose, patients should be closely monitored for signs or symptoms of adverse reactions and appropriate symptomatic treatment instituted.

The safety of dinutuximab in children aged less than 12 months have not yet been established (see sections 4.2, 4.4 and 4.8 of the SmPC).

Because of the lack of data on long-term effects of dinutuximab on the central and peripheral nervous system, the prevalence of organ dysfunction, on growth and endocrine development, hearing loss, cardiac toxicity and survival data, the applicant will conduct a registry (see Annex II obligation).
From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics

2.6.2. Conclusions on the clinical safety

The safety profile of the combined treatment regimen has been reasonably characterised in the target population. The toxicity is clearly significant and includes in spite of pre-medication a high rate (81%) of allergic and infusion reactions, including hypotension, hypersensitivity, pyrexia, urticaria, anaphylactic reactions, rash, oedema, and bronchospasm. Other highly frequent adverse reactions include capillary leak syndrome, and abnormal laboratory tests (hypokalaemia, hyponatraemia, low platelet count, anaemia, and increased alanine aminotransferase).

Pain is the major ADR of anti-GD2 monoclonal antibodies, GD2 being expressed on normal nerve cells. Intravenous opioids administered before and during the infusion of ch14.18 are needed to alleviate the pain but despite prophylaxis two thirds of the children experience pain and about 40% experience severe pain.

The impact of antibodies to the product needs to be further addressed post-authorisation.

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

- Post authorisation safety study (PASS): In order to better characterise the safety and immunogenicity of the commercial product (UTC) and its impact on drug exposure, the applicant should conduct and submit the results of a safety study by Q4 2018
- Non-interventional PASS: In order to evaluate the long-term safety outcomes of dinituximab (including central and peripheral nervous system, prevalence of organ dysfunction, long-term effects on growth and endocrine development, hearing loss, cardiac toxicity and survival data in high-risk neuroblastoma patients) the applicant should conduct and submit the results of a safety registry by Q2 2029.

2.7. Pharmacovigilance

Detailed description of the pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

2.8. Risk Management Plan

The CHMP received the following PRAC Advice on the submitted Risk Management Plan:

The PRAC considered that the risk management plan version 1 could be acceptable if the applicant implements the changes to the RMP as described in the PRAC advice.

The CHMP endorsed this advice with the following change of safety concerns: the important potential risk of atypical haemolytic uraemic syndrome was added due to the update of SmPC wording (sections 4.4 and 4.8 of the SmPC) based on three serious events reported in two subjects during the ANBL0032 study with ch14.18.

The applicant implemented the changes in the RMP as requested by PRAC and CHMP.

The CHMP endorsed the Risk Management Plan version 1 (dated 20/05/2015) with the following content:

Safety concerns

Summary of safety concerns	
Important identified risks	• Infections • Haematological toxicities • Allergic conditions • Peripheral neuropathies • Neurological disorders of the eye • Hypotension • Capillary leak syndrome • Vomiting and diarrhea • Pain • Cytokine release syndrome • Hypertension
Important potential risks	• Hepatic dysfunction • Medication Errors • Off-label use in patients < 12 months of age • Arrhythmia • Immunogenicity • Atypical Haemolytic Uraemic Syndrome
Missing information	• Long term effects of treatment early in childhood • Potential harm from overdose • Drug-Drug interaction information between ch14.18 and GM-CSF and IL-2 and isotretinoin • Effects in patients with pre-existing hepatic, renal and cardiovascular impairment

Pharmacovigilance plan

Study / Activity	**Objectives**	**Safety Concerns addressed**	**Status (planned, started)**	**Date for submission of interim or final reports (planned or actual)**
Long-term Non-Interventional	• To evaluate long-term safety	Long-term effects Central and	Planned	Protocol submission: Q4

Study / Activity	Objectives	Safety Concerns addressed	Status (planned, started)	Date for submission of interim or final reports (planned or actual)
Prospective Observational Registry in Patients who received Unituxin for the Treatment of High-risk Neuroblastoma Category 1	outcomes associated with the central and peripheral nervous system and the prevalence of organ dysfunction including the long-term effects on growth and endocrine development, hearing loss and cardiac toxicity in patients who received Unituxin for high-risk neuroblastoma and have survived for > 5 years without relapse. • To evaluate event-free (EFS) and overall survival (OS) on all patients enrolled for up to five years. • To compare the late effect safety outcomes of patients treated with Unituxin and cytokines vs.patients not exposed using historical control data (e.g. literature)	peripheral nervous system Growth and endocrine development Hearing loss Cardiac toxicity		2015 First patient enrolled: Q4 2016 Enrolment complete: Q4 2028 Yearly interim results date: Beginning 1 year after 1st patient enrolled (Q4 2017). First survival data expected 5 years post-study start (~ 2021) Study completion: Q4 2029
Post-authorisation Study Category 1	• To assess the incidence rate of human-anti-chimeric antibody (HACA)	Immunogenicity	Planned	First patient enrolled: Q1 2016 Final report submission: Q4

Study / Activity	Objectives	Safety Concerns addressed	Status (planned, started)	Date for submission of interim or final reports (planned or actual)
	• To determine the incidence of neutralizing antibody in HACA positive samples • To assess the safety and tolerability of Unituxin combination in high-risk neuroblastoma patients			2018
Non-clinical toxicology study Category 3	• Measure chronic toxicity on the central and peripheral nervous system	Central and peripheral nervous system toxicity	Planned	Final report submission: May 2018
Safety and tolerability Category 3	• Assess risk of serious infusion reaction and neuropathy an the overall safety and tolerability of Unituxin • Assess variations in antibody dependent cell-mediated toxicity on safety and tolerability lots.	Infusion reaction Neuropathy Overall safety and tolerability	Planned	Final report submission: December 2017
Analysis of laboratory data for allergic conditions Category 3	• Analyse laboratory data including serum complement, IgE, tryptase, histamine, and human anti-chimeric antibody levels obtained in	Grade 4 Allergic reactions	Planned	Final report submission: March 2017

Study / Activity	Objectives	Safety Concerns addressed	Status (planned, started)	Date for submission of interim or final reports (planned or actual)
	patients with documented Grade 4 allergic reactions to allow for improved characterization of these adverse reactions and to determine if the clinical presentation and laboratory data obtained were consistent with an allergic reaction or infusion reaction			
Assay development Category 3	• Develop and validate an assay for the detection of neutralising antibodies in the presence of dinutuximab levels	N/A	Planned	Final report submission: October 2015
Neutralizing antibody study Category 3	• Assess the neutralizing anti-body response to dinutuximab with a validated assay.	Clinical impact of neutralizing antibody	Planned	Interim report: September 2016 Final report submission: June 2019
Non-human glycans study Category 3	• Collect data on antibodies against non-human glycans from the ANBL0032 study and their impact on safety and efficacy	Clinical impact of antibodies against non-human glycans	Planned	2016

Risk minimisation measures

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Infections	Subject to restricted medical prescription; provision of warnings and recommendations relating to the important identified risks in the SmPC (Sections 4.2, 4.4 and 4.8); inclusion of information from the SmPC in the carton with the drug product.	None proposed.
Haematological toxicities		
Allergic conditions		
Peripheral neuropathies		
Neurological disorders of the eye		
Hypotension		
Capillary leak syndrome		
Vomiting and diarrhea		
Pain		
Cytokine release syndrome		
Hypertension		
Hepatic dysfunction		
Medication Errors		
Off-label use in patients < 12 months of age		
Arrhythmia		
Immunogenicity		
Atypical Haemolytic Uraemic Syndrome		
Long term effects of treatment early in childhood		
Potential harm from overdose		
Drug-Drug interaction information between ch14.18 and GM-CSF and IL-2 and isotretinoin		
Effects in patients with pre-existing hepatic, renal and cardiovascular impairment		

2.9. Significance of paediatric studies

The CHMP is of the opinion that the paediatric clinical studies (DIV-NB-301, DIV-NB-302, ANBL0931 and DIV-NB-201) of dinutuximab which are contained in the agreed Paediatric Investigation Plan P/0208/2013 and completed after 26 January 2007 are significant. The assessment criteria for significance of studies as defined in Section III, Title 4.2 of the Europe Commission Communication - Guideline on the format and content of applications for agreement or modification of a paediatric investigation plan and requests for waivers or deferrals and concerning the operation of the compliance check and on criteria for assessing significant studies (2014/C 338/01) has been fulfilled, taking into account the study type of the above-mentioned study:

(1) Comparative efficacy studies (randomized/ active control or placebo): The randomized pivotal study DIV-NB-301 and its non-randomized extension DIV-NB-302 are Phase 3, open-label, multicentre clinical trials conducted in paediatric patients with high risk neuroblastoma

(2) Prospective clinical safety studies: The study ANBL0931 (DIV-NB-303) primary objective was to comprehensively define the safety profile of ch14.18 when administered with cytokines and isotretinoin (RA) in subjects with high-risk neuroblastoma after ASCT.

Furthermore study DIV-NB-201 provides meaningful pharmacokinetic information as per criteria (e) of the above mentioned Guideline which is supporting the claimed indication.

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.*

3. Benefit-Risk Balance

Benefits

Beneficial effects

In children with high-risk neuroblastoma who had responded to induction chemotherapy (at least a partial response), the immunotherapy regimen of ch14.18 combined with GM-CSF and IL-2 added to isotretinoin improved event-free survival compared to isotretinoin alone; the 2-year EFS was 65.6% vs. 48.1%, respectively; with a hazard ratio of 0.64 (95% CI 0.43, 0.97; log-rank test p = 0.033).

A sustained improvement of overall survival was observed in all analyses; the estimated 3-year OS rate was 79.5% vs. 67.3%, respectively, with a hazard ratio of 0.57 (95% CI 0.36, 0.89; log-rank test p = 0.017). In a recent update, the estimated 5-year OS rate was 74.2% vs. 57.0%, respectively, with a hazard ratio of 0.62 (95% CI 0.40, 0.96; log-rank test p = 0.030).

Most patients had a tumour classified as INSS Stage 4 and the results were similar in this subgroup to those of the whole population. Other subgroup analyses showed that most prognostic groups benefited from the immunotherapy regimen, in particular patients with CR and VGPR after induction chemotherapy, who have a more favourable prognosis than those with PR.

Patients in the control arm had worse prognosis based on their tumour biology than patients on immunotherapy but statistical analyses adjusted on various prognostic factors showed significant hazard ratios that were similar to the unadjusted estimates. Therefore, the imbalance in prognostic factors does not seem to have had a notable impact on the results.

Uncertainty in the knowledge about the beneficial effects.

The efficacy results are based on a single pivotal trial, which was prematurely discontinued following an interim analysis that did not meet the pre-defined stopping rules. However, a GCP inspection and supportive data allowed to conclude that the efficacy data were reliable and that efficacy has been established.

Subgroup analyses of EFS and OS response indicated that patients with minimal residual disease, DNA hyperploidy, and those having received a purged bone marrow may not have benefited from dinutuximab immunotherapy (see section 5.1 of the SmPC).

Finally, as the results in patients with persistent disease are not considered convincing, these patients have been excluded from the indication.

Risks

Unfavourable effects

The combination of ch14.18 to the two cytokines GM-CSF and IL-2 is associated with significant toxicity, primarily because it is highly allergenic despite pre-medication: 81% of the patients experience an allergic reaction. Other frequent adverse reactions include pain (in 66% of the patients), capillary leak syndrome, and various abnormal laboratory tests (hypokalaemia, hyponatraemia, low platelet count, anaemia, and increased alanine aminotransferase).

To alleviate pain symptoms, a known adverse reaction of anti-GD2, administration of opioids is recommended in patients.

Uncertainty in the knowledge about the unfavourable effects

There are limited safety and immunogenicity data for the ch14.18 product manufactured by UTC. More information on the safety and immunogenicity profile are needed post-authorisation and the Applicant will conduct a study to further investigate these aspects in addition to the impact on the immune response on drug exposure (see Annex II obligation).

In addition, only limited data are available regarding the long-term effects of ch14.18. A registry will be set up to investigate these effects (see Annex II obligation).

Benefit-risk balance

Importance of favourable and unfavourable effects

Patients with high-risk neuroblastoma generally require aggressive multimodal therapy including, chemotherapy, radiotherapy, autologous stem cell transplant and isotretinoin. However, despite this aggressive treatment strategy, most patients relapse or have persistent residual disease. Eradication of minimum residual disease remains therefore a major challenge and an unmet need. The immunotherapy regimen including ch14.18 demonstrates a sustained improvement of overall survival and the significant toxicity is considered acceptable in this life-threatening disease.

Benefit-risk balance

Survival was significantly improved with ch14.18 immunotherapy and isotretinoin as compared to isotretinoin alone. The immunotherapy regimen also includes GM-CSF and IL-2, precluding any firm conclusion on the real contribution of ch14.18 to the observed benefit. Therefore, the current indication has to rely on the combination that was studied.

Significant toxicities were observed with the combination regimen. The management of these toxicities requires a considerable level of medication, which is not desirable. However, despite the significant toxicity of the combination regimen, the rate of discontinuation for adverse reactions appeared to be low and preventive measures together with monitoring of patients and management of adverse events (see section 4.4 of the SmPC) mitigate the risks.

For the combined immunotherapy, the benefit in overall survival in this paediatric population with poor prognosis is statistically and clinically relevant and is considered to outweigh the toxicity of the regimen.

Discussion on the benefit-risk balance

Based on the trial data, the clinical benefit of dinutuximabhas only been shown in patients with at least partial response to induction therapy, which is reflected in the proposed indication. The contribution of each component of the recommended regimen to the efficacy results is difficult to appreciate but the combination is justified by a pharmacodynamic rationale. The results from old monotherapy trials were not considered sufficiently positive to continue the development of the product alone and cytokines were subsequently added to boost the cytotoxic effects of ch14.18. These cytokines did not work on their own in neuroblastoma.

Therapeutic options in this orphan and life-threatening condition are limited and a significant and sustained improvement in overall survival in this paediatric patient population is of clinical relevance. Despite the significant toxicity of this immunotherapy regimen, the benefits are considered to outweigh the risks.

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 Unituxin in the “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” is favourable and therefore recommends the granting of the marketing authorisation subject to the following conditions:

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 marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation. Subsequently, the marketing authorisation holder shall submit periodic safety update reports for this product in accordance with the requirements set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and published on the European medicines web-portal.

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.

If the dates for submission of a PSUR and the update of a RMP coincide, they can be submitted at the same time.

- **Obligation to complete post-authorisation measures**

The MAH shall complete, within the stated timeframe, the below measures:

Description	Due date
Non-interventional post authorisation safety study (PASS): In order to evaluate the long-term safety outcomes of dinutuximab in high-risk neuroblastoma patients (including central and peripheral nervous system, prevalence of organ dysfunction, long-term effects on growth and endocrine development, hearing loss, cardiac toxicity and survival data) the applicant should conduct and submit the results of a safety registry. The final study report should be submitted by	06/2029
Post authorisation safety study (PASS): In order to better characterise the safety and immunogenicity of Unituxin and its impact on drug exposure, the applicant should conduct and submit the results of a safety study. The final study report should be submitted by	12/2018

New Active Substance Status

Based on the CHMP review of data on the quality properties of the active substance, the CHMP considers that dinutuximab is qualified as a new active substance.

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0208/2013 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.

In accordance with Article 45(3) of Regulation (EC) No 1901/2006, significant studies in the agreed paediatric investigation plan P/0208/2013 have been completed after the entry into force of that Regulation.