CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125516Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Date: September 17, 2014
From: Whitney S. Helms, Ph.D.
Pharmacology Supervisor
Division of Hematology Oncology Toxicology for Division of Oncology Products 2
To: File for BLA # 125516
Unituxin (dinutuximab)
Re: Approvability of Pharmacology and Toxicology

On April 11, 2014 United Therapeutics Corporation (UTC) submitted Biological Licensing Application (BLA) 125516 for Unituxin (dinutuximab) for the treatment of patients with (b) (4) high-risk neuroblastoma as a component of a multi-agent, multi-modality regimen. The recommended dose of Unituxin is 17.5 mg/m^2 given daily for 4 days of a 24-28 day cycle for 5 cycles. Non-clinical studies examining the pharmacology and toxicology of dinutuximab provided to support BLA 125516 were reviewed in detail by Dubravka Kufrin, Ph.D. The findings of these studies are summarized in the "Executive Summary" of the BLA review and reflected in the product label.

Dinutuximab is a chimeric mouse/human IgG1 monoclonal antibody produced in the SP2/0 cell line that binds to the GD2 disialonganglioside. GD2 is expressed on tissues from the central nervous system and peripheral nerves as well as on many tumors of neuroectodermal origin, including neuroblastoma. The variable region of dinutuximab comes from a murine antibody, Mab14.18, originally raised in mice against a neuroblastoma cell line. The constant region consists of the human IgG1 heavy chain and kappa light chain. The resulting chimeric antibody, ch14.18, has been studied clinically over the last 20 years. Dinutuximab is the ch14.18 antibody produced by UTC's manufacturing process.

The pharmacologic characterization of dinutuximab is based on published literature detailing experiments using ch14.18 and surrogate antibodies with the same variable domain but with alternative murine constant regions. These data show that the antibody is able to bind GD2 and potentiate antibody-dependent cellular cytotoxicity (ADCC), mediated primarily by neutrophils and NK cells, as well as complement dependent cytotoxicity (CDC). In separate experiments, both GM-CSF and IL-2 were each shown to have some capacity for enhancing ADCC mediated by anti-GD2 antibodies. In the absence of complement or effector cells there was no evidence of tumor inhibition following anti-GD2 binding, though early studies did suggest that the ganglioside may play a role in cellular attachment to the extracellular matrix.

Animal studies exploring the safety of dinutuximab were conducted in Sprague Dawley rats and cynomolgus monkeys. Rats were administered dinutuximab at doses significantly higher than the clinically recommended dose for 4 weeks (4 days on, 3 days off/week) with a 6-week recovery period. The liver was identified as a target organ in this study, though only minimal histopathological and clinical chemistry changes occurred. Increases in lymphocytes, neutrophils, and NK cells were also noted in rats. The rats did appear to develop an anti-drug antibody response which affected the pharmacokinetics of dinutuximab, particularly at the lowest dose level. Dinutuximab was also administered to cynomolgus monkeys in a single-dose

cardiovascular safety study. In this study increases in heart rate were observed, consistent with tachycardia noted clinically, however, no clear changes in QTc, respiratory rate, or blood gas parameters were noted.

The major toxicity clearly associated with administration of dinutuximab clinically is neuropathic pain. Because of this finding, UTC submitted multiple publications investigating the mechanism of ch14.18-mediated pain. Overall these publications suggest that the pain is related to the pharmacologic activity of the drug: binding of the antibody to GD2 expressed on peripheral nerves and subsequent Fc domain-mediated ADCC and CDC activity. Studies in rats consistently showed decreases in mechanical pain thresholds following administration of anti-GD2 antibodies which could be prevented or reduced by preventing complement activation. In one study investigators showed that administration of an anti-GD2 antibody in rats resulted in increases in electrophysiological background activity of Aδ and C fibers, both of which are associated with the pain response. Clinical signs of toxicity in monkeys administered a single dose of dinutuximab in either the cardiovascular safety study or a dose range finding study included vomiting and reduced food consumption; these findings along with the increases in heart rate suggest that the monkey is also a relevant model for longer term studies of GD-2 mediated neuropathic pain and recovery.

Because there are approximately 20 years of clinical experience with the use of anti-GD2 antibodies in the treatment of patients with neurobalstoma, no long term toxicology studies were conducted by the Applicant to support the use of dinutuximab for this indication. The majority of clinical experience with anti-GD2 therapy for neuroblastoma is, however, in combination with other therapies, including GM-CSF and IL-2. For this reason, there are outstanding questions about the potential toxicity of dinutuximab as a single agent and whether there is a potential for longer term neuropathic effects or neuropathy following treatment. Because the significant clinical experience with ch14.18 has not resulted in a clear signal suggesting that long term neuropathic damage is likely, this concern can be addressed as a nonclinical post-marketing requirement.

Consistent with the ICH S6 guidance, genetic toxicology studies were not conducted or required for dinutuximab. Carcinogenicity studies were not required to support the licensing application for a product intended to treat advanced human cancer and are neither planned nor expected as post-marketing requirements at this time. The requirement for reproductive toxicity studies was discussed by the Applicant prior to submission of the BLA. Because the median age of the proposed patient population is 18 months and 90% of the cases occur in children under the age of 5, reproductive toxicology studies of dinutuximab were not required to support the submission of the Unituxin BLA. Unituxun is not indicated for females of reproductive potential. If UTC explores additional indications for Unituxin in the future, nonclinical studies investigating the effects of dinutuximab on embryofetal development may be required. Pregnancy Category D is recommended.

Recommendations: I concur with the conclusion of Dr. Kufrin that the pharmacology and toxicology data support the approval of BLA 125516 for UNITUXIN. There are no outstanding nonclinical issues that would prevent the approval of UNITUXIN for the treatment of patients with (b) (4) high-risk neuroblastoma. A post-marketing requirement to further

investigate the toxicologic effects of dinutuximab as a single agent following chronic administration of the antibody, particularly its effects on the nerves and neuropathic pain, is recommended.

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/s/

WHITNEY S HELMS
09/17/2014

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY / TOXICOLOGY BLA REVIEW AND EVALUATION

Application number:	125516
Supporting document/s:	1
Applicant's letter date:	April 8, 2014
CDER stamp date:	April 8, 2014
Product:	Unituxin (Dinutuximab)
Indication:	(b) (4) high-risk neuroblastoma as a component of a multi-agent, multi-modality regimen
Applicant:	United Therapeutics Corporation 55 T.W. Alexander Drive, Research Triangle Park, NC 27709
Review Division:	Division of Hematology Oncology Toxicology in support of Division of Oncology Products 2 (DOP2)
Reviewer:	Dubravka Kufrin, PhD
Supervisor/Team Leader:	Whitney Helms, PhD
Division Director:	• John K. Leighton, PhD, DABT for DHOT, OHOP • Patricia Keegan, MD for DOP2, OHOP
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1 Executive Summary

1.1 Introduction

Dinutuximab is a chimeric mouse-human monoclonal antibody (Mab), targeting the GD2 disialoganglioside. Gangliosides are carbohydrate-rich sphingolipids[1] composed of ceramide linked to an oligosaccharide chain containing hexose and N-acetylneuraminic acid (a sialic acid). Gangliosides, including GD2, are believed to play important roles in the normal function and maintenance of the nervous system. In addition to its expression on tissues from the central nervous system and peripheral nerves, GD2 is frequently expressed at high levels in tumors of neuroectodermal origin including neuroblastoma cells. United Therapeutic Corporation has submitted the current Biologic License Application (BLA) for Unituxin (dinutuximab) for the treatment of patients with (b) (4) high-risk neuroblastoma as a component of a multi-agent, multi-modality regimen. The recommended dose of Unituxin is 17.5 mg/m^2 daily for four days of a 24 or 28 day cycle.

1.2 Brief Discussion of Nonclinical Findings

Neuroblastomas are extra-cranial tumors that originate from the neuroectodermal layer, the primitive precursor cells of the peripheral (sympathetic) nervous system, and usually arise in paraspinal locations in the abdomen or chest. Neuroblastoma is one of the most common metastatic solid tumors of childhood, with a high relapse rate and poor prognosis despite even the most intensive multimodality therapies. The median age of diagnosis for children with neuroblastoma is 5 years and by this time, the disease is often widespread[2].

Dinutuximab (ch14.18) is a mouse/human chimeric antibody produced in SP2/0 hybridoma cells derived from a murine precursor, Mab14.18. The chimeric antibody is composed of the variable region heavy and light chains of the parent murine monoclonal antibody (Mab) 14.18 and a constant region consisting of the human IgG1heavy chain and kappa light chain. Investigators made the chimeric antibody in an effort to decrease the human anti-mouse antibody response, to improve the Fc-dependent functional properties of the antibody, and to increase its half-life in humans.

Both dinutuximab and its predecessor anti-GD2 antibody, Mab14.18, have been studied clinically in academic settings including the National Cancer Institute for the treatment of pediatric patients with high-risk neuroblastoma. Studies provided in this BLA describing dinutuximab binding to GD2, its mechanism of action, and its anti-cancer activity came from published reports of this early work, while tissue-cross reactivity studies with normal human, rat, and rabbit tissues as well as a safety pharmacology study in cynomolgus monkeys and a general toxicology study in Sprague-Dawley rats were specifically performed to support this application.

Multiple publications describe in vitro and in vivo studies conducted to characterize the mechanism of action of dinutuximab and include investigations of dinutuximab itself, the parent antibody Mab14.18, and a murine IgG2a isotype switch variant of the Mab14.18, 14.G2a. These papers provide evidence that dinutuximab binds to the GD2 ganglioside and is able to elicit cell lysis through both complement dependent cytotoxicity (CDC) and antibody dependent cellular cytotoxicity (ADCC). Dinutuximab was able to elicit lysis of GD2-expressing neuroblastoma cells, melanoma cells, and squamous cell lung cancer cells in the presence of human peripheral blood mononuclear cells (PBMCs) or active human complement. When used as effector cells in the presence of anti-GD2 antibodies, granulocytes, specifically neutrophils, and NK cells elicited higher levels of ADCC in GD2-expressing target cells compared to unselected PBMCs. In separate experiements, investigators demonstrated that the presence of either GM-CSF or IL-2 could enhance dinutuximab-mediated ADCC of GD2 expressing cells by effector cells. Investigators also showed that dinuximab or its predecessors were able to selectively reduce the development or growth of GD2-expressing tumor cell lines in vivo in xenograft models.

Investigators also evaluated the mechanism for the pain commonly experienced by patients on anti-GD2 antibody therapy. GD2-specific antibody binding occurred in peripheral nerves of multiple species. Studies in rats following administration of dinutuximab or other anti-GD2 antibodies consistently showed decreases in mechanical pain thresholds beginning shortly after antibody administration and persisting for up to 48 hours after the end of treatment. Further investigation in these animals showed increases in the electrophysiological background activity of Aδ and C fibers as well as decreases in motor amplitudes. Investigators were able to reduce the decreases in mechanical pain thresholds in animals by pre-administration of a C5a complement antagonist. Similarly, administration of dinutuximab to complement factor C6 deficient animals or of an anti-GD2 antibody not capable of initiating CDC resulted in a reduction of decreased pain thresholds. Pretreatment of animals with lidocaine or capsaicin could also prevent the changes in pain response following treatment with anti-GD2 antibodies. Together these data suggest that the clinically reported pain response following administration of dinutuximab is a pharmacologic consequence of dinutuximab binding to peripheral nerves and causing damage through CDC and ADCC. Several studies also showed GD2 antibody binding to myelin, suggesting an additional contributing factor to the neuropathic pain and occasional neuropathy reported clinically. While clinical signs of pain in animals can be difficult to detect, there was clear evidence of an effect of anti-GD2 antibodies on mechanical pain thresholds in rats, as well as potential signs of pain in monkeys given single doses of dinutuximab, evidenced by vomiting during or following infusion and decreased food consumption.

Dedicated safety pharmacology studies of dinutuximab were limited to a cardiovascular and respiratory safety evaluation in cynomolgus monkeys. No respiratory irregularities were reported in this study. Increases in blood pressure and heart rate along with corresponding findings of shortened PR and QT intervals in ECG evaluations were noted, though QTc intervals were unaffected. Tachycardia has also been reported clinically.

The safety of dinutuximab was evaluated in a 28-day repeat-dose toxicology study in rats. There were no deaths in this study. The liver was identified as a potential target organ with increases in liver weight, mild increases in AST, ALT, and cholesterol along with minimal microscopic findings of hepatocellular necrosis, pericentral vein/interlobular fibrosis, and centrilobular congestion. Increases in liver enzymes have been reported clinically. The majority of changes in the rats demonstrated evidence of reversibility within a 6 week recovery period. Though there were no histopathological indications of an effect of dinutuximab on peripheral nerves in this study, ch14.18 has been shown to bind to GD2 in rats. Many studies submitted to this BLA showed decreases in mechanical pain threshold in rats. For these reasons, the rat does appear to be a relevant species for toxicological evaluation of dinutuximab. Animals in this study did, however, show signs of developing a strong anti-drug antibody response, suggesting that longer term chronic toxicology studies in rats would be of limited value.

Based on the median age of the proposed patient population (90% of patients less than 5 years of age), the Agency agreed that reproductive and developmental toxicology studies would not be required to support the BLA for Unituxin for the treatment of high risk neuroblastoma. If, in the future, UTC wishes to pursue an additional indication for Unituxin, reproductive toxicology studies may be required. Unituxin is not indicated for females of reproductive potential. Based on its mechanism of action, ADCC and CDC-mediated lysis of GD2-expressing cells, and the ability of IgG1 antibodies to cross the placental barrier, dinutuximab may cause fetal harm. Pregnancy Category D is recommended.

No additional toxicology studies were conducted to support use of dinutuximab in patients with neuroblastoma. While there are outstanding questions related to the chronic toxicity of dinutuximab, particularly in regard to whether there are toxicities other than neuropathic pain that can be specifically attributed to anti-GD2 therapy and whether there is long term damage associated with use of the antibody, there is not a clear clinical signal from the 20 years of clinical experience with these products suggesting that long term neuropathic damage is likely. Thus, this concern can be addressed as a post-marketing requirement, and there are no outstanding issues from a pharmacology/toxicology perspective that would prevent the approval of dinutuximab for the treatment of the proposed patient population.

1.3 Recommendations

1.3.1 Approvability

Dinutuximab is approvable from the nonclinical perspective for the treatment of patients with (b) (4) high-risk neuroblastoma as a component of a multi-agent, multi-modality regimen.

1.3.2 Additional Non Clinical Recommendations

Because the toxicological risk assessment of dinutuximab has been based on a combination of nonclinical and clinical data, chronic toxicology studies using the drug

have not been conducted. In order to gain a clearer understanding of dinutuximab-specific toxicity, particularly of the potential for recovery of damage to peripheral nerves, a post-marketing requirement to conduct a 13-week GLP-compliant toxicology study has been requested.

1.3.3 Labeling

A separate labeling review will be provided.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional)	1363687-32-4
Generic Name	Dinutuximab
Code Name	Ch14.18
Chemical Name	Chimeric monoclonal antibody
Molecular Formula	Two heavy ((b) (4) amino acids each) and two light chains (b) (4) amino acids each) chains
Molecular Weight	~148 kDa
Structure or Biochemical Description	IgG1 (chimeric human/murine mAb)
Pharmacologic Class	chimeric monoclonal antibody directed against GD2

Figure 1: ch14.18 amino acid sequence

ch14.18 IgG1 Light Chain Sequence (Variable and Constant domains).

EIVMTQSPAT LSVSPGERAT LSCRSSQSLV HRNGNTYLHW YLQKPGQSPK
LLIHKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP
PLTFGAGTKL ELKRTVAAPS VFIFPPSDEQ LKSGTASVVC LLNNFYPREA
KVQWKVDNAL QSGNSQESVT EQDSKDSTYS LSSTLTLSKA DYEKHKVYAC
EVTHQGLSSP VTKSFNRGE C

ch14.18 IgG1 Heavy Chain Sequence (Variable and Constant domains).

EVQLLQSGPE LEKPGASVMI SCKASGSSFT GYNMNWVRQN IGKSLEWIGA
IDPYYGGTSY NQKFKGRATL TVDKSSSTAY MHLKSLTSED SAVYYCVSGM
EYWGQGTSVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV
TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TQTYICNVNH
KPSNTKVDKR VEPKSCDKTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS

RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS
VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS
REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF
FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK

(excerpted from Applicant's submission)

2.2 Relevant INDs, NDAs, BLAs and DMFs

- IND 4308- National Cancer Institute for Chimeric Monoclonal Ab (ch14.18) to the Disialoganglioside (GD2-antigen), GM-CSF, and Interleukin-2 for a treatment of metastatic melanoma, small cell lung carcinoma, soft tissue sarcomas and neuroblastoma (active from 7/18/2003)
- Master File- (b) (4)
- Master File- (b) (4)

Additional overall comment: Ch 14.18 has been used extensively in clinical studies, many of which were sponsored by the National Cancer Institute, over the past 20 years. In a randomized, open-label Phase 3 study in subjects with high-risk neuroblastoma, patients demonstrated a significant improvement in EFS and OS following treatment with ch14.18, GM-CSF, IL- 2, and isotretinoin (Yu 2010)[3].

2.3 Drug Formulation

Dinutuximab is available as a sterile, (b) (4), aqueous solution at a concentration of 3.5 mg/mL in 5 mL vials (17.5 mg/5mL). The following tables list the dinutuximab drug formulation:

Table 1-2: NCI and United Therapeutics Formulations

Formulation	Excipients	pH
National Cancer Institute	(b) (4)	
United Therapeutics	Histidine (20mM) Sodium Chloride (150mM) Polysorbate 20 (0.05%)	6.8

Table 1-1: Excipients in ch14.8 formulation

Component	Function	Quality Standard	Concentration
Histidine	(b) (4)	USP/EP	20mM (3.10g/L)
Sodium Choride		USP/EP/JP	150mM (8.77g/L)
Polysorbate 20		USP/EP	0.05% (v/v) (0.55g/L)
Hydrochloric Acid	pH Adjustment	USP/EP/JP	(b) (4)
Water for Injection	(b) (4)	USP/EP/JP	

(b) (4)

(b) (4)

Ingredients Guide for the IV route of administration.

(Excerpted from the Applicant's submission)

2.4 Comments on Novel Excipients

None.

2.5 Comments on Impurities/Degradants of Concern

The product quality team asked for input on the levels of several potential impurities identified as a result of the dinutuximab manufacturing process. These impurities are listed in Table 1 along with the Applicant's justification for these levels.

Table 1: Potential Process Impurities

Process Additive/Impurity	Batch Testing Results**	Recommended Maximum Levels	Impurity Safety Factor (Max LD_{50} Level/Testing Result)
(b) (4)			

**Three lots tested during process validation (1500365, 1500401, and 1500419)
***Per vendor documentation

Based on the batch testing results for (b) (4) (b) (4) (b) (4) and (b) (4) the levels present in the dinutuximab drug product are below the level of toxicologic concern. In each case, administration of dinutuximab at the recommended dose of 17.5 mg/m^2/day for 4 days would result in administration of no more than (b) (4) µg/day. The levels of (b) (4) would be no more than (b) (4) µg/day, well below the acceptable daily level based on a threshold of toxicological concern for genotoxic impurities of 1.5 µg. Similarly for (b) (4) the maximum daily level would be no more than (b) (4) mL and there is clinical experience with the use this (b) (4) at much higher levels. The pharmacology/toxicology team does not agree with the calculated impurity safety factors, however, as it is not appropriate to base impurity levels on LD_{50} values. The Applicant has proposed specifications for (b) (4) and (b) (4) of (b) (4) ng/mL and (b) (4) µg/mL, respectively. These levels would result in maximum doses of no more than (b) (4) µg and (b) (4) µg/day. Sufficient safety data exists in the literature to support these levels in a product used to treat patients with cancer.

2.6 Proposed Clinical Population and Dosing Regimen

Clinical population:	Patients with (b) (4) high-risk neuroblastoma as a component of a multi-agent, multi-modality regimen, including granulocyte macrophage colony-stimulating factor (GM-CSF), interleukin 2 (IL-2) and isotretionin (RA)
Dosing regimen:	The recommended dose of UNITUXIN is 17.5 mg/m^2: the drug is diluted prior to intravenous infusion (over 10-20 hours) for 4 days per course for 5 courses. UNITUXIN is administered on Days 4–7 during Courses 1, 3, and 5 (each course lasting approximately 24 days) and on Days 8–11 during Courses 2 and 4 (each course lasting approximately 28 days). (b) (4)

Table 2: Courses 1, 3, and 5 Dosing Regimen for UNITUXIN, (b) (4)

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15–24
(b) (4)															
UNITUXIN [2]				X	X	X	X								
(b) (4)															

2. UNITUXIN: 17.5 mg/m /day, administered by diluted IV infusion over 10–20 hours

(b) (4)

Table 3: Courses 2 and 4 Dosing Regimen for UNITUXIN (b) (4)

Day	1	2	3	4	5	6	7	8	9	10	11	12–14	15–28
(b) (4)													
UNITUXIN [2]								X	X	X	X		
(b) (4)													

2. UNITUXIN: 17.5 mg/m^2/day, administered by diluted IV infusion over 10-20 hours.

(b) (4)

Dose or infusion rate modifications are recommended for the following adverse reactions: allergic reactions, capillary leak syndrome, hypotension, systemic infection or sepsis, pain, peripheral neuropathy and neurological disorder of the eye.

2.7 Regulatory Background

- 20 December 2010 Granted Orphan Drug Designation
- 11 April 2012 IND 110494 is Active
- 24 October 2012 Agreement that embryofetal studies will not required
- 5 December 2013 MAA Submission to EU
- 20 December 2013 Granted Rare Pediatric Disease Status
- 11 April 2014 BLA Submitted

3 Studies Submitted

3.1 Studies Reviewed

Reference/Study number	Type of study	Administration/ test articles	Test Systems
Mujoo 1987[4]/ Mujoo 1989[5]	Tumor binding/ Antibody depended cytotoxicity (ADCC)	In vitro/murine Mab 14.18/ IP	Tumor tissues and cell lines; SK-N-AS tumor bearing athymic nu/nu BALB/c mice
Mueller 1990[6]	Binding to GD2/ Antibody depended cytotoxicity (ADCC)	In vitro/ch14.18, 14.G2a, murine Mab 14.18	M-21 melanoma cells

Reference/Study number	Type of study	Administration/ test articles	Test Systems
Barker 1991[7]	Antibody depended cytotoxicity (ADCC)	In vitro/ch14.18, murine Mab 14.18, 14.G2a	GD2 neuroblastoma cells; NMB-7, IMR-32, IMR-6, SMS-KCNR, SK-N-AS
Kendra 1999[8]	Anti-tumor activity/ Antibody depended cytotoxicity (ADCC)	In vitro/IP, SC	M-21 melanoma cell tumor-bearing CB-17 SCID/SCID mice
Chen 2000[9]	Antibody depended cytotoxicity (ADCC)	In vitro	GD2 neuroblastoma cells: SMS-KCN, SMS-LHN and LA-N-1/ GD2 neuroblastoma cells:SK-N-SH
Zeng 2005[10]	CDC	In vitro	GD2+ melanoma cells M-21 and A375/ GD2+ neuroblastoma cells: SK-N-AS, SMS-KCNR, NMB-7, CHP-134, SMS-KAN, IMR-6, Kelly
Vriesendorp 1997[11]	Effects on nervous system	IV/IP	Lewis rat, SJL mouse, NZW rabbit, beagle dog
Slart 1997[12]	Mechanism of antibody-mediated pain	IV	SD rat
Xiao 1997[13]	Mechanism of antibody-mediated pain	IV	SD rat
Sorkin 2002[14]	Mechanism of antibody-mediated pain	IV, IT	SD rat
(b) (4) **354-005c/** (b) (4) **J** (b) (4)	Effects on respiratory and cardiovascular systems	IV	Cynomolgus monkeys
(b) (4) (b) (4) **n**	Single-dose evaluation	IV	Cynomolgus monkeys
(b) (4) (b) (4)	Toxicology evaluation	IV	SD rat

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

Mab14.18 is a murine antibody that binds to GD2; it is an IgG3 antibody that was originally derived following the injection of the human LAN-1 neuroblastoma cell line into BALB/c mice. Splenocytes from these animals were fused with the SP2/0 murine myeloma cell line to make hybridomas; hybridoma 14.18 was selected for further study[1]. Ch14.18 is a chimeric murine-human monoclonal antibody. The constant regions of IgG1 heavy chain and the kappa light chain are of human origin while the variable regions are of mouse origin from the original murine antibody, Mab 14.18. Dinutuximab is produced in SP2/0 hybridoma cells.

Clinical experience with ch14.18 spans over at least 20 years. The antibody was originally developed for use in clinical trials by the NCI and is being produced by United Therapeutics, Inc. under a cooperative research and development agreement (CRADA). Based on the history of ch14.18 development and use, the majority of the preclinical studies that describe the properties of the antibody come from published references. These studies include pharmacology studies characterizing the binding and activity profiles of ch14.18, Mab 14.18, and 14.G2a[5-6], an isotype switch variant of Mab 14.18 with a murine IgG2a heavy chain. Mujoo et. al.,1989[5] and Mueller et. al.[6] reported that all three of these antibodies had similar binding affinity for GD2 and demonstrated the ability to mediate complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC).

Binding to Neuroblastoma cells and GD2

Analysis by Mujoo et. al., 1987[4] showed high binding of the IgG3 monoclonal antibody Mab14.18 to a variety of tumor cell lines of neuroectodermal origin, including eight different neuroblastoma cell lines, and several melanoma, glioblastoma and small cell lung cancer lines (Table 4). The authors also showed that Mab14.18 was binding specifically to GD2 on these cells (Figure 2). Earlier work had suggested a functional role for GD2 in helping to facilitate the attachment of neuroblastoma and melanoma cells to the extraceullar matrix[15-16]. The combination of high tumor expression of GD2 and its potential role in tumor attachment made an anti-GD2 antibody an attractive candidate for further study. Mueller et. al.[6] demonstrated that both ch14.18 and the 14.G2a antibodies derived from the parent Mab 14.18 showed similar affinity for GD2 expressing cells (K_ds of ~11.2 nM and 11.9 nM, respectively) using the M-21 melanoma cell line. Levels of antibody binding correlated with numbers of GD2 binding sites detected on various cell lines.

Table 4: ELISA reactivity of MAB 14.18 with cultured cell lines

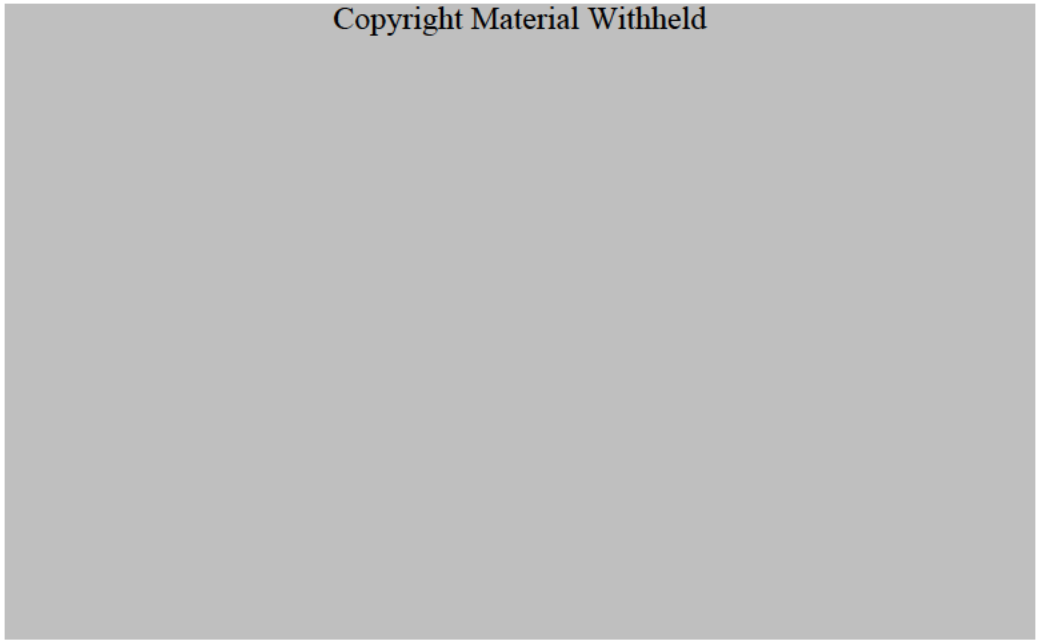
Figure 2: Mab14.18 Binds to GD2

(Table and Figure Excerpted from Mujoo et. al., 1987[3])

Based on published work by Mueller et. al,[6], Cheresh et. al.,1986[17], Zeng et. al.,[10]; Kendra et. al.[8]; Mujoo et. al., 1987[4] and Chen et. al.[9], the Applicant determined that the mechanism of action for dinutuximab mediated anti-tumor activity is a combination of antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).

Both Mujoo et. al.,1987[4] and Cheresh et. al. 1986[15] demonstrated that the Mab14.18 parent antibody was able to mediate ADCC against GD2-expressing tumor cell lines in the presence of human PBMC cells. ADCC activity correlated well with the level of GD2 expression on different cell lines.

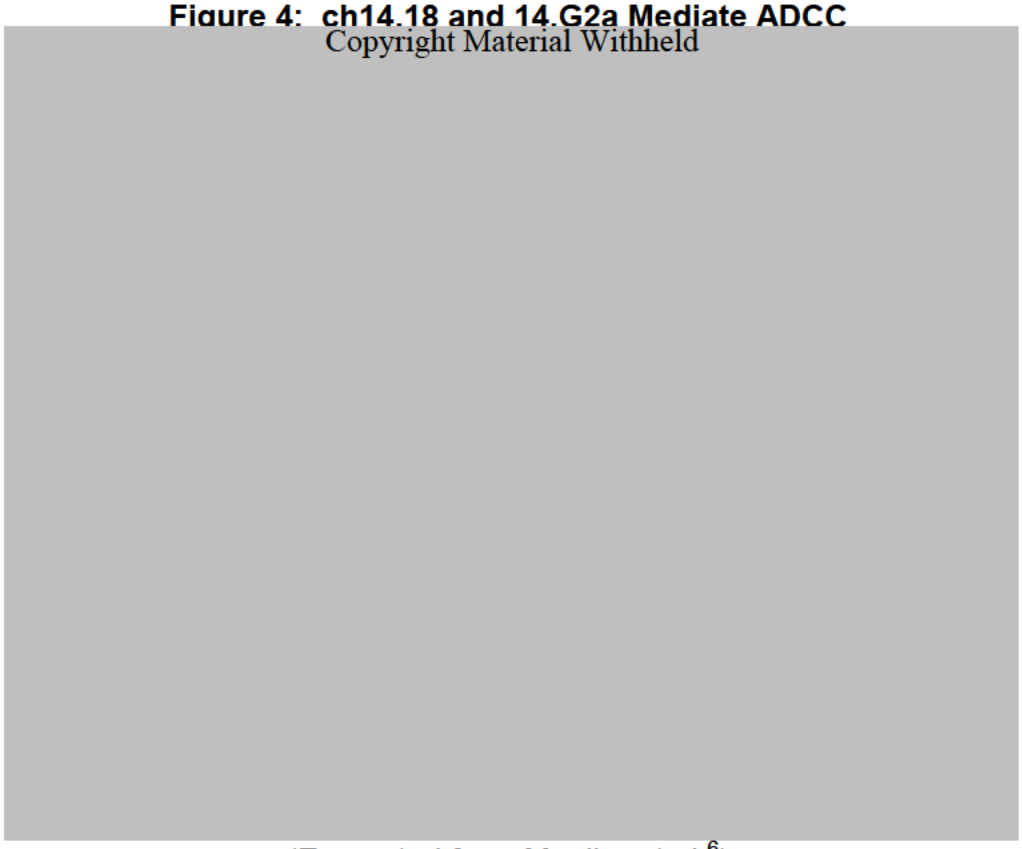
Figure 3: ADCC Activity of Parent Mab14.18



(Excerpted from Cheresh et. al., 1986[17])

The ability of ch14.18 to mediate ADCC was examined in Mueller et. al.[6] and Barker et. al.[7]. In these studies ch14.18 was more effective than murine 14.G2a in mediating the lysis of neuroblastoma cells in the presence of human effector cells (Figure 4).

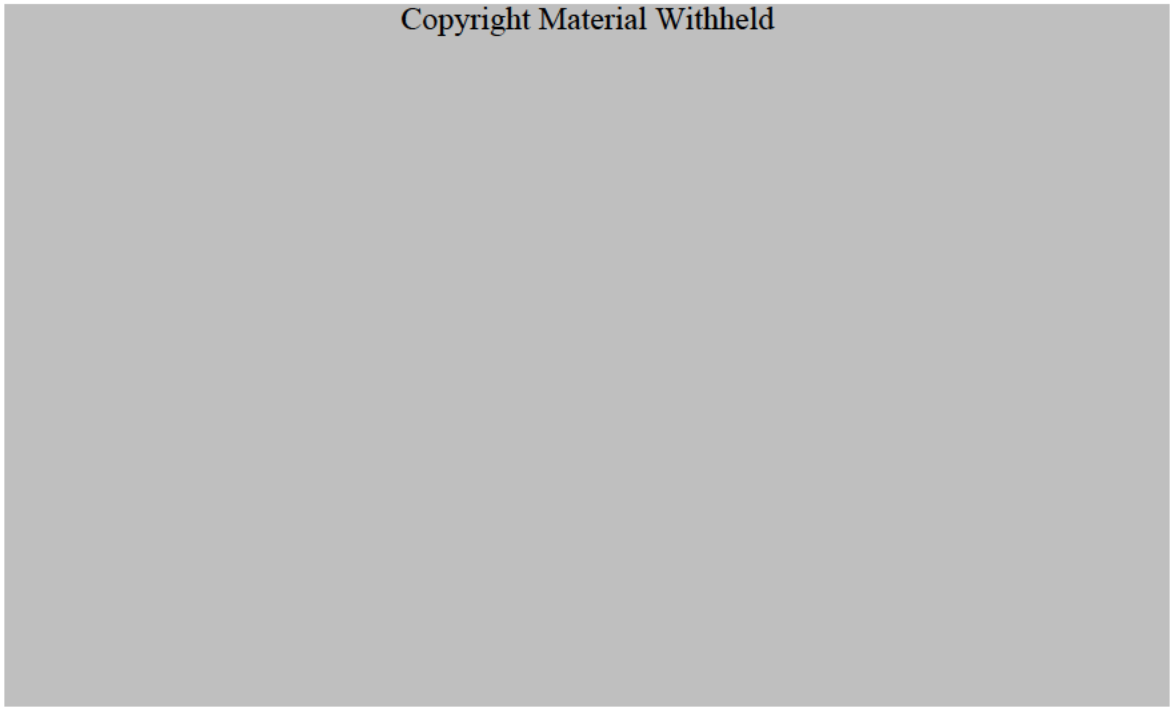
Figure 4: ch14.18 and 14.G2a Mediate ADCC



(Excerpted from Mueller et.al.[6])

To assess whether granulocytes or PBMCs are responsible for more potent ch14.18-potentiated lysis of target cells, granulocytes and PBMCs were isolated from the blood of human donors and compared for their ability to lyse the neuroblastoma cell line NMB-7 in the presence of ch14.18. The results presented in Figure 5 demonstrate that granulocytes were better than general PBMCs in mediating ch14.18-directed lysis of NMB-7 cells.

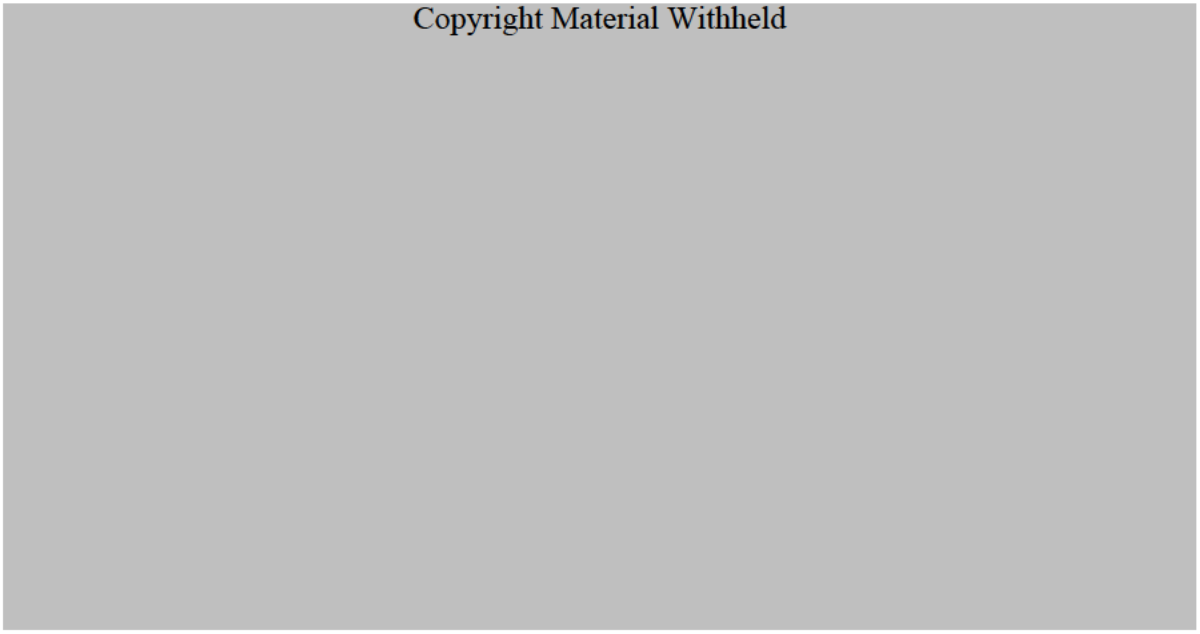
Figure 5: Granulocytes Demonstrate Potent ch18.18-mediated ADCC



(Excerpted from Barker et. al.[7])

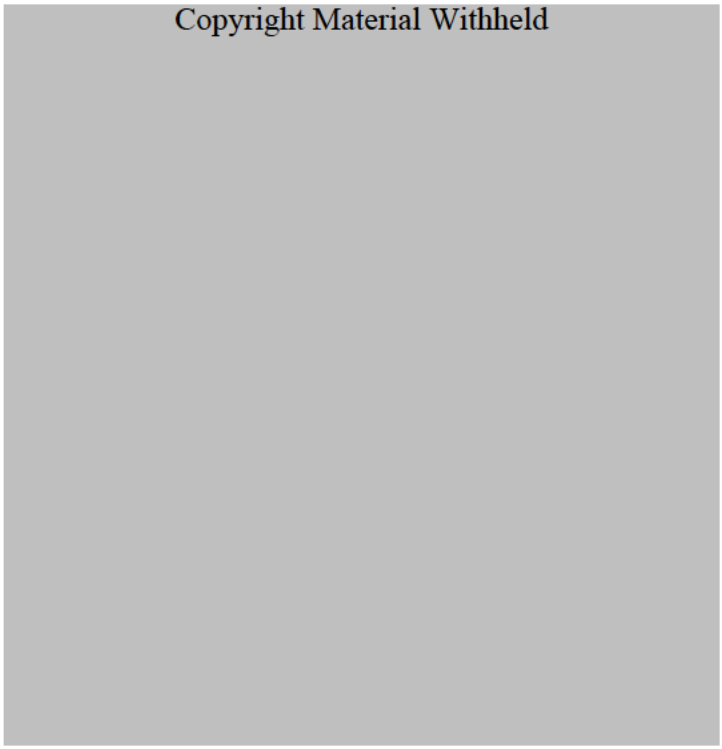
Subsequently, the authors showed further improvements in ch14.18-potentiated lysis of neuroblastoma cells by granulocytes in the presence of GM-CSF (Figure 6).

Figure 6: GM-CSF Increases ch14.18-Potentiated Tumor Cell Lysis by Granulocytes in vitro



These findings were supported by additional characterization of ch14.18 antibody-mediated cytotoxicity of neuroblastoma cells by neutrophils in Chen et. al.[9]. In a six-hour fluorescence-based assay, a dose-dependent increase in ADCC was observed with increases in ch14.18 concentration, neutrophils, or PBMCs. The presence of GM-CSF further enhanced ch14.18-potentiated ADCC by neutrophils, though the addition of the cytokine had no clear effect on ch14.18-potentiated ADCC by PBMCs. The effects of ch14.18 were dependent on GD2 expression as incubation of the antibody with a GD2-negative neuroblastoma cell line, SK-N-SH, in the presence of neutrophils did not result in tumor cell death (Figure 7).

Figure 7

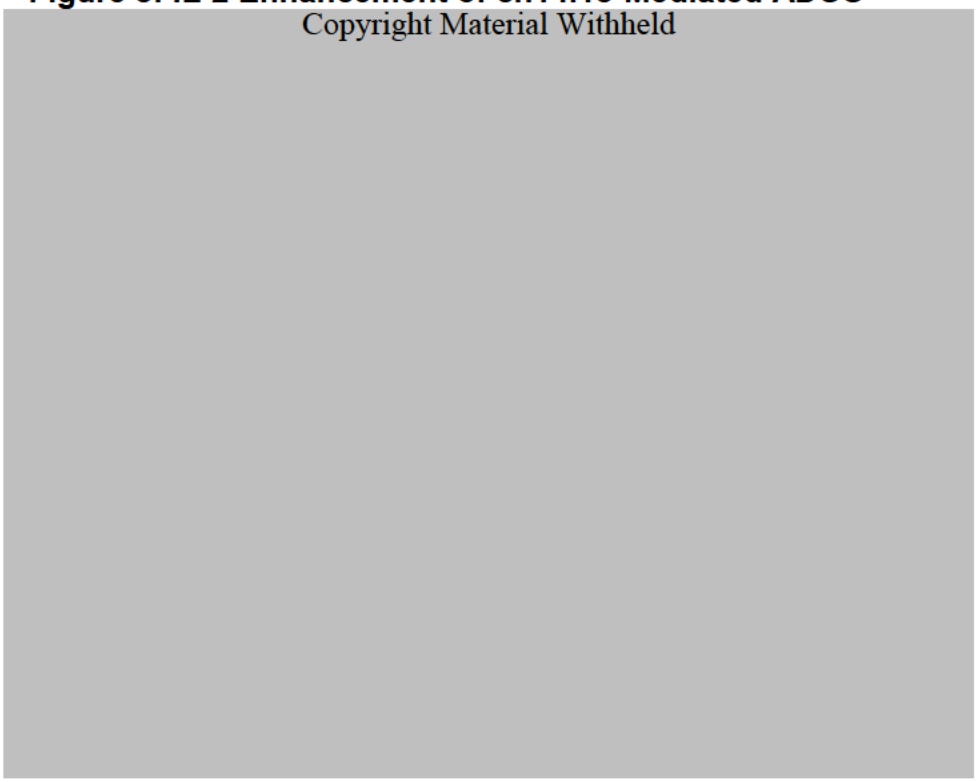


(Excerpted from Chen et. al., 2000[9])

These data suggest that ch14.18 anti-tumor activity in vivo may be dependent on high concentrations of granulocytes and neutrophils at tumor sites and that the addition of GM-CSF can improve neutrophil activity against tumors in the presence of ch14.18.

Similarly, Kendra et. al.[8] showed that the addition of IL-2 to cultures of GD2-expressing melanoma cells incubated with ch14.18 and PBMCs enhanced the level of ADCC compared to ch14.18 and PBMCs alone (Figure 8), supporting a potential role for IL-2 in enhancing the clinical activity of ch14.18.

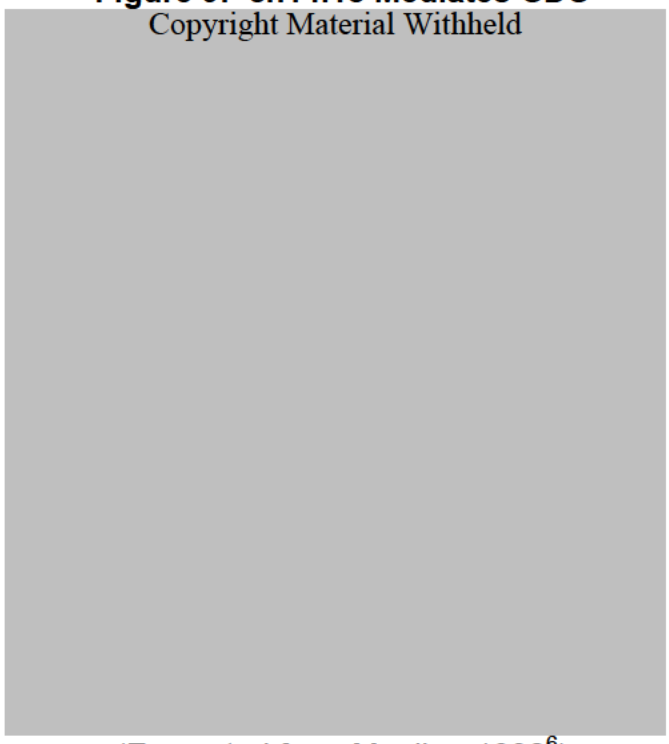
Figure 8: IL-2 Enhancement of ch14.18-Mediated ADCC



- **Complement-dependent cytotoxicity:**

To assess whether ch14.18 is also effective for the induction of complement-mediated cytolysis, GD2-expressing tumor cell lines (M21 and A375) were labeled with $(Na)_2{}^{51}CrO_4$ were incubated with ch14.18 and human serum complement. The ch14.18 antibody was able to mediate CDC (Figure 9).

Figure 9: ch14.18 Mediates CDC



(Excerpted from Mueller, 1990[6])

Zeng et. al.[10] evaluated and compared the binding specificity as well as the in vitro and in vivo activity of ch14.18 produced in several different cell lines, including SP2/0 cells. The ch14.18 antibody preparations from SP2/0, NS0 and CHO cell lines showed no differences in GD2 binding and performed equally well in eliciting CDC, though at low antibody concentrations there were small differences in their ability to elicit ADCC (FiguresFigure 10,Figure 11,Figure 12, excerpted from Zeng et. al.).

Figure 10: Binding of ch14.18 Preparations to GD2

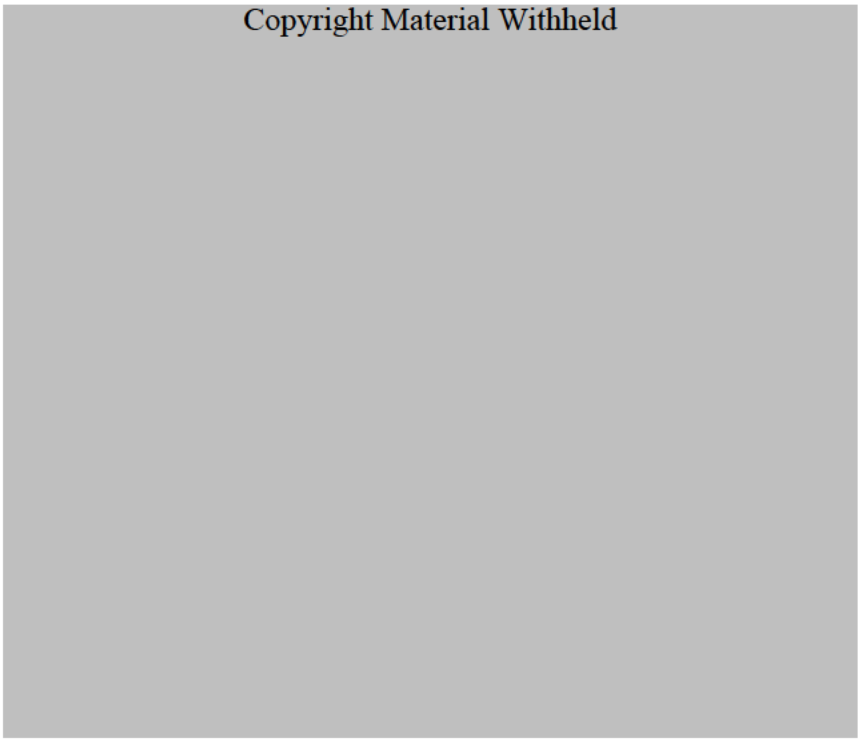


Figure 11: CDC Activity of ch14.18 Populations

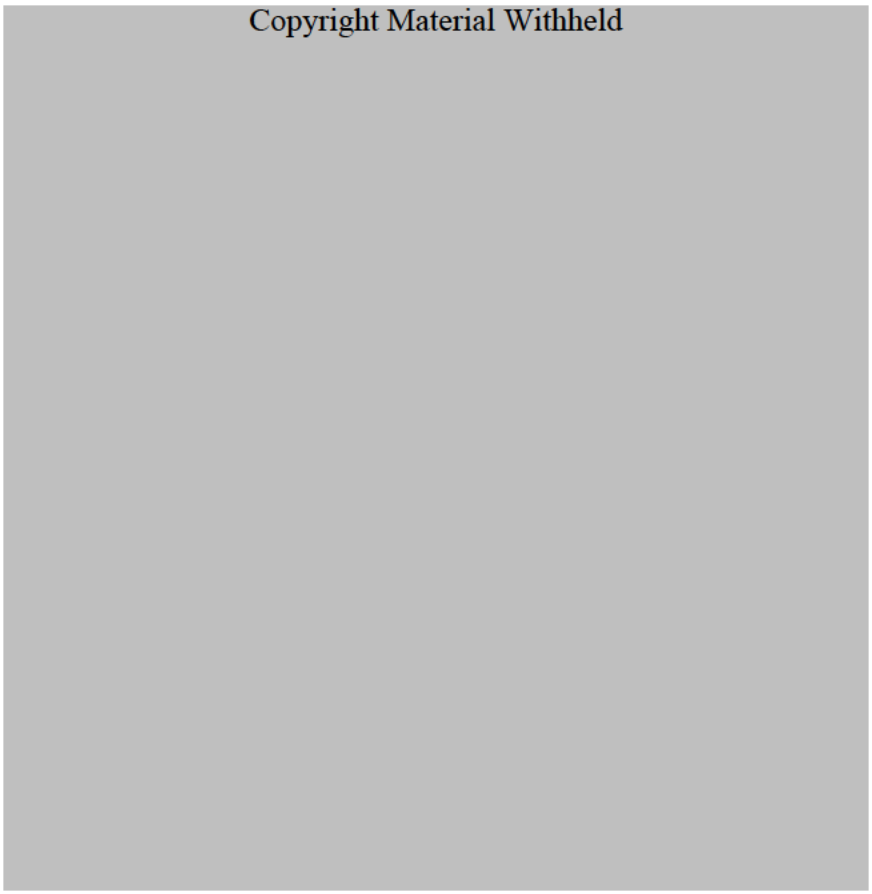
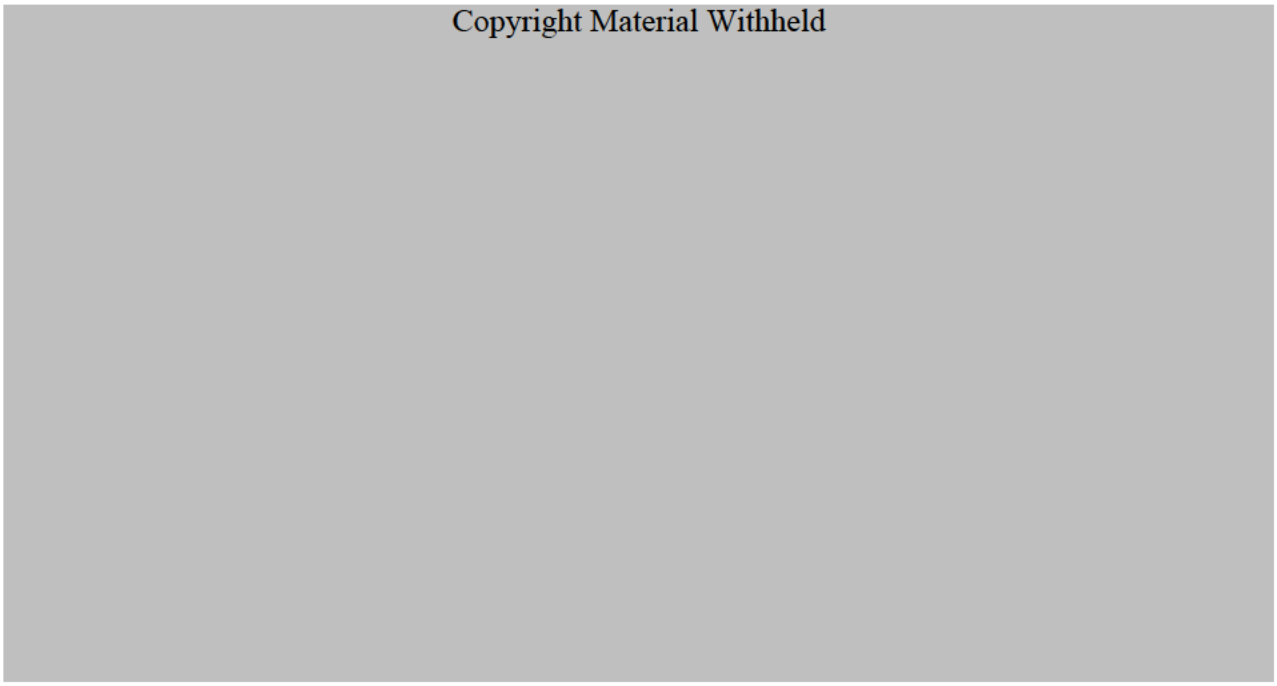
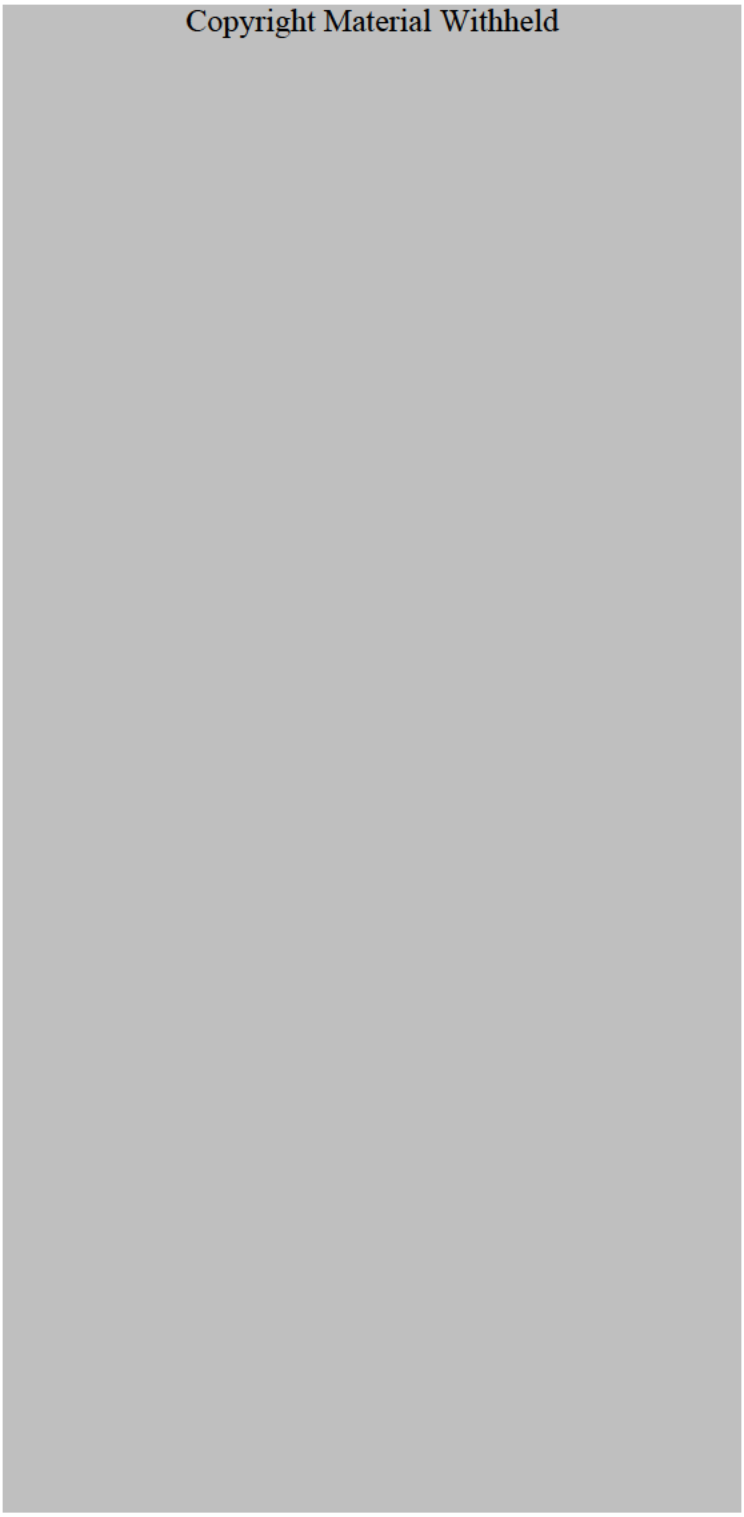


Figure 12: ADCC Against GD2-Expressing Cell with Different ch14.18 Preparations



The authors also demonstrated the in vivo activity of different ch14.18 preparations in a xenograft model of hepatic metastasis using a GD2-expressing murine neuroblastoma line, NXS2 NB, injected intravenously into the mouse tail vein. Treatment of mice with daily injections of 300 μg of ch14.18 for 5 days resulted in a reduction in hepatic metastasis. The effect of the antibody was abrogated by NK cell depletion, supporting NK-mediated ADCC as a critical part of the mechanism of action for ch14.18 in control of neuroblastoma (Figure 13).

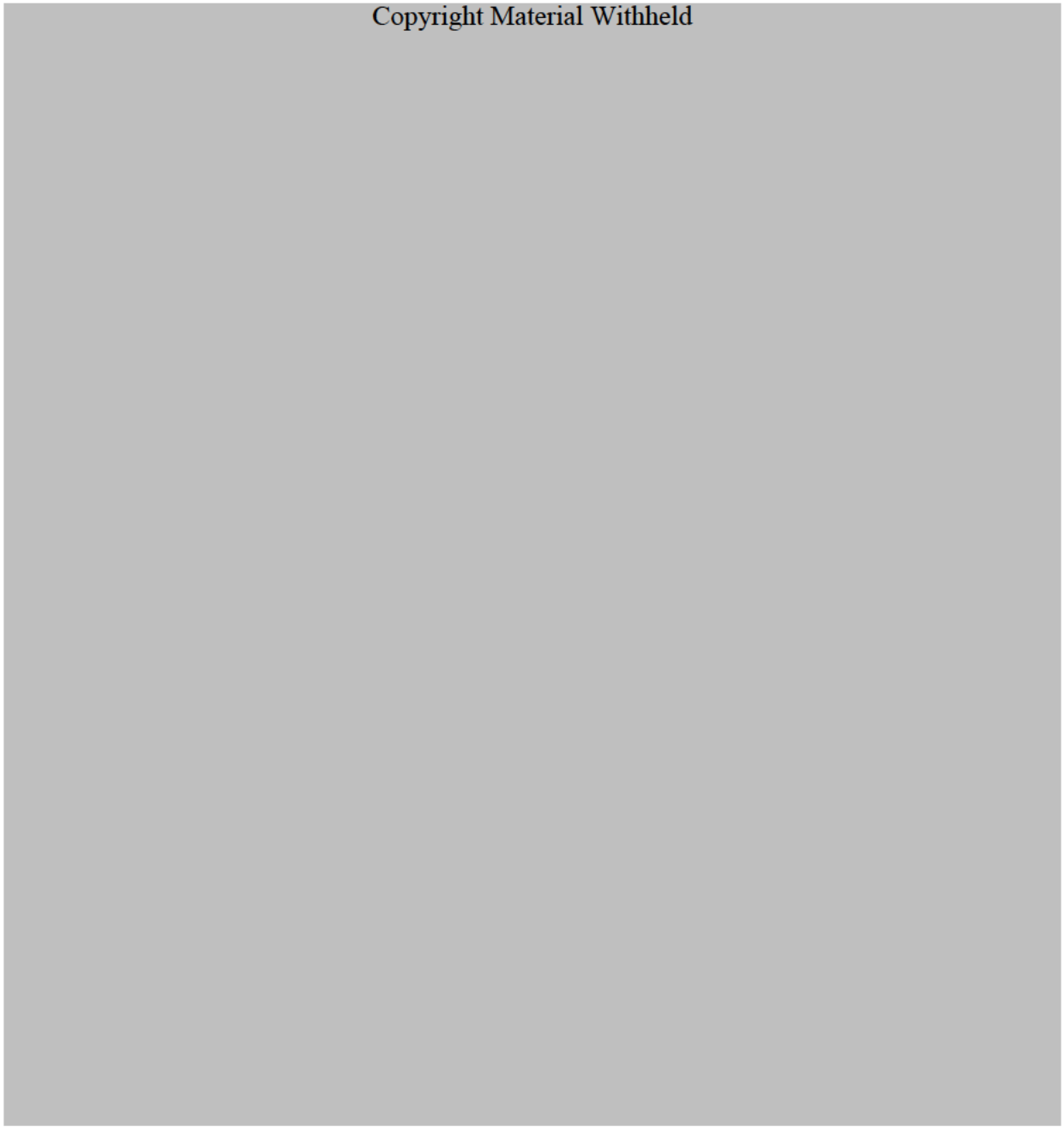
Figure 13: In vivo Activity of ch14.18 Preparations



Other authors also conducted experiments investigating the in vivo activity of ch14.18 or its predecessors. Studies in athymic (nu/nu) mice were performed by Mujoo et. al. 1987[4]. When given at either 24 hours or 9 days following subcutaneous tumor

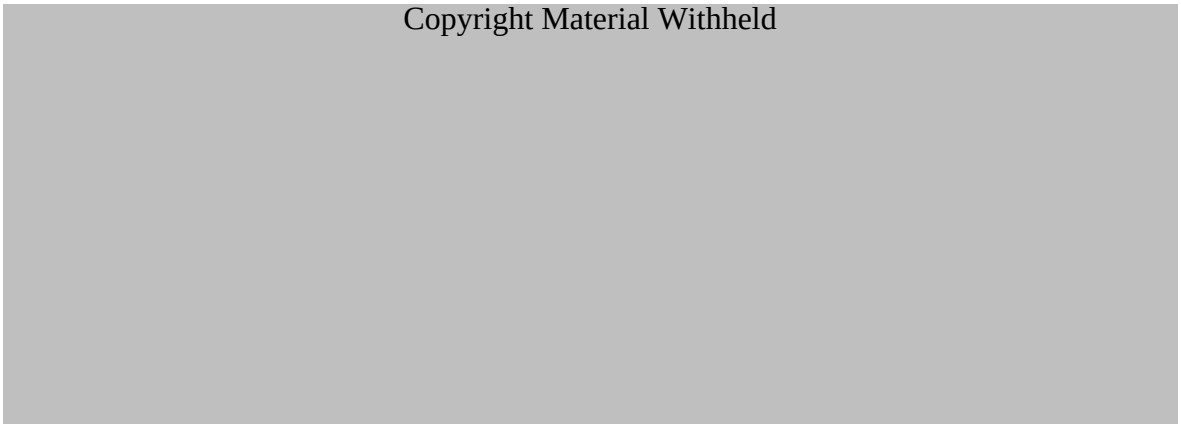
implantation, intraperitoneal (ip) administration of murine Mab14.18 at a dose of 10 mg/kg delayed the growth of a GD2+ neuroblastoma cell line. The decrease in tumor volume was statistically significant when compared to control groups administered an irrelevant antibody or PBS (Figure 14).

Figure 14: Anti-GD2 Antibody-Mediated Delay in Tumor Growth



Kendra et. al.[8] examined the in vivo activity of ch14.18. The authors injected immunodeficient female SCID mice with the M-21 GD2+ melanoma cell line and, at various timepoints, a mixture of ch14.18, human PBMCs, and recombinant human IL-2.

Binding of ch14.18 to tumors was measured by flow cytometry following sacrifice and tumor isolation and anti-tumor activity of ch14.18 was determined by measuring tumor size. The authors found that binding of ch14.18 to implanted tumors at a dose of 1 mg (50 mg/kg) increased with time, with 27% of M-21 cells from established tumors staining positive 10 hours after administration of ch14.18 and approximately 60% staining positive after 13-24 hours. Lower amounts of ch14.18 (0.1 mg) resulted in decreased tumor penetration. In longer term co-injection studies, the authors showed that while some tumor reduction occurred when implanted mice were treated with the combination of PBMCs at multiple effector:target ratios in the presence of IL-2, complete control of tumors was possible with the addition of ch14.18 (Table 5).



(Excerpted from Kendra et. al.)

4.2 Secondary Pharmacology

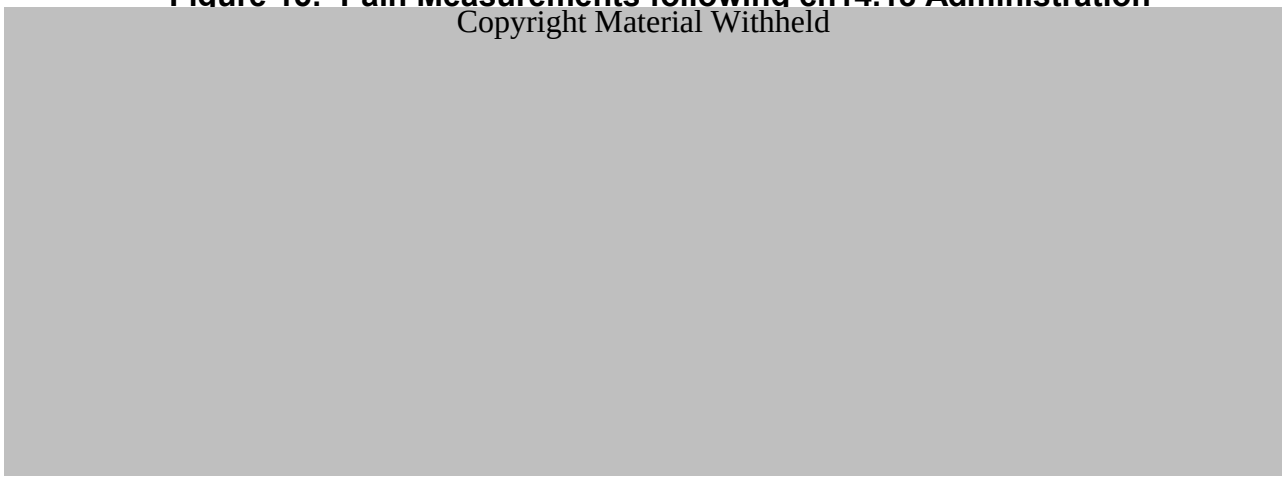
To understand the mechanism of pain observed in the clinic, several secondary pharmacology studies in animals were conducted. The GD2 ganglioside was shown to be expressed on peripheral nerves[19], suggesting that the nerves are a pharmacologically relevant target for ch14.18.

- **Evaluation of pain-related toxicities**

Slart et. al.[12] administered a single IV injection of ch14.18 to male Sprague-Dawley rats at four dose levels (0, 0.1, 1, 3, and 10 mg/kg) to study pain threshold and allodynia (pain behavior generated in response to a light tactile stimulus) in a time-dependent manner. The authors found that rats given 1, 3, or 10 mg/kg of ch14.18 had a steep, immediate drop in the mechanical pain threshold which plateaued approximately 1 hour post injection, although the decreased pain threshold was noted for the entire 2.5 hours of testing. Generally, the animals demonstrated a return to normal pain thresholds between 24 and 48 hours after ch14.18 administration. Touch evoked agitation (TEA) was used as an additional endpoint in this study; ch14.18-treated animals demonstrated statistically significant increases in TEA at all dose levels compared to saline-treated controls during the first 1.75 hours following dose administration. In some dose groups,

TEA remained elevated for several days following antibody administration, though this reaction did not show clear evidence of a dose response (Figure 15).

Figure 15: Pain Measurements following ch14.18 Administration



(Excerpted from Slart et. al., 1997)

Another study exploring the mechanism of antibody-mediated pain following administration of ch14.18 was conducted by Xiao, et. al.[13]. The authors focused on electrophysiological effects of ch14.18 on afferent nerve fibers. In this study, 4-8 male Sprague-Dawley rats were treated with ch14.18, 14.G2a, or an anti-murine melanoma control antibody at a dose of 1 mg/kg. Treatment with the 14.G2a antibody resulted in a similar allodynia response to that seen with ch14.18 within the first 15 minutes of treatment, an effect not observed with the control antibody. Limited locomotor activity was also noted for both groups of anti-GD2 antibody-treated animals, with animals not observed standing upright against the side of the cage, an observation common with untreated or control-treated rats. The authors found an increase in the background activity of Aδ and C fibers following administration of anti-GD2 antibodies, though the cause of this increase was not fully elucidated. Additionally, these authors administered lidocaine (15 mg/kg bolus injection or continuous intravenous infusion targeting a plasma level of 0.3-2.2 ug/ml) to test-article treated rats and showed that the drug reduced the electrophysiological background activity induced by anti-GD2 treatment, suggesting that lidocaine could be useful as an analgesic component of the ch14.18 treatment regimen.

Sorkin et. al., 2002[14] further examined origins of anti-GD2 antibody mediated pain in Sprague-Dawley rats following intravenous (IV) or intrathecal (IT) administration of 14.G2a. As clinical manifestations of pain following administration of ch14.18 have included reports of sensations of severe cold or heat, both mechanical and thermal allodynia were assessed in this study. Administration of 14.G2a to rats by either the IT or IV route resulted in dose-dependent decreases in the mechanical pain threshold at the lower doses tested (0.01, 0.05 and 0.1 ng) in male rats. At the highest dose level tested by IT injection (0.5 ng), there were no differences in the levels of mechanical pain threshold measured by paw lifting, although the animals reacted with whole body piloerection or piloerection with startling response, possibly indicating an experience of

high-level pain leading to lack of paw control. Administration of 14.G2a at any dose level did not result in a change in the response to thermal stimulation. Pretreatment of animals administered 14.G2a with capsaicin prevented the decrease in pain threshold.

In another paper, Sorkin et. al., 2010[18], the authors demonstrated the importance of the ability of ch14.18 to fix complement for the pharmacologic induction of pain. In the absence of complement factor C6, or with pre-administration of a C5a complement antagonist, ch14.18 allodynia was reduced or eliminated. When animals were administered a mutated ch14.18 antibody with reduced ability to initiate CDC, decreases in pain thresholds were not as steep and were of shorter duration than with ch14.18 itself. Together these data suggest that CDC may play an important role in the pain reported clinically following administration of ch14.18.

Overall the submitted studies suggest that pain associated with ch14.18 administration may be a direct result of ch14.18 binding to the peripheral nerves.

4.3 Safety Pharmacology

Study title: Effects of the anti-GD2 antibody ch14.18 on the cardiovascular and respiratory system in conscious cynomolgus monkeys

Study no.:	(b) (4) 354-005
Study report location:	Electronic submission tab 4, toxicology
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	11/19/2012
Compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Chimeric monoclonal antibody 14.18 (ch14.18); S110601; (pH 6.8; protein concentration 3.5 mg/mL)

Key Study Findings:

- Increases in blood pressure and heart rate were observed in 2/3 animals when compared to treatment of the same animals with the vehicle control
- Shortening of PR interval and QT intervals (related to increases in heart rate) were observed in 2/3 animals following treatment with ch14.18
- One animal vomited 3.5 and 6 hours after the end of the ch14.18 dosing period
- A decrease in food consumption was observed in all animals for 3-7 days after dosing with ch14.18

Methods

Dose:	0, 14 mg/kg (168 mg/m^2)
Frequency of dosing:	Once
Route of administration:	Intravenous (IV)
Dose volume:	7 mL/kg/; dosing time: 10 hours
Formulation/Vehicle:	Ch14.18/20 mM histidine, 150 mM sodium chloride, 0.05% polysorbate 20, pH 6.8
Species/Strain:	Cynomolgus monkeys, purpose bred
Number/Sex/Group:	3 males in testing group, one additional male as a control
Age:	4 to 5 years
Weight:	3.98 to 4.17 kg
Satellite groups:	none
Unique study design:	none
Deviation from study protocol:	none

Results:

Blood pressure:

- increases in systolic, diastolic and mean blood pressure were observed in one animal during infusion (5 and 10 hours after the start of dosing); magnitudes of increase ranged from 34 to 37 mmHg, when compared to measurements in the time matched control animal

Heart rate:

- increases in heart rate of 45 and 94 beats/min when compared to the time-matched control value were observed in 2 animals at different times during the infusion (5 and 10 hours after the start of infusion)

Electrocardiograms:

- shortening of the PR interval and QT intervals were observed in same two animals with blood pressure and heart rate changes at 5, 10, 12, and 24 hours after the start of dosing when compared to the time-matched control animal
- no QTc prolongations were noted
- a single premature beat was observed in one animal, at two independent intervals occurring at 10 and 12 hours after the start of dosing

No changes were noted in respiratory rate and blood gas parameters.

Clinical signs

- Decreases in food consumption were observed in all test-article administered animals between Days 3 and 7 days after dosing.
- One animal vomited 13.5 and 16 hours after the dose initiation

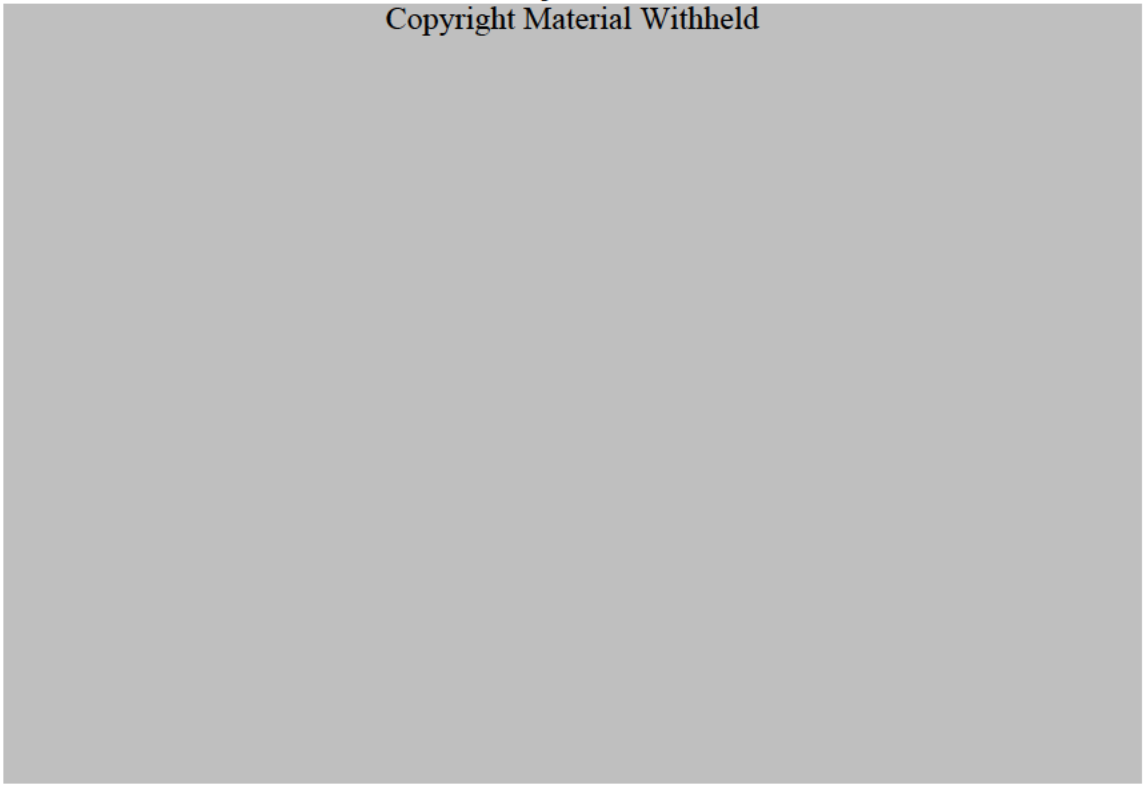
Conclusion
Infusion of ch14.18 at a dose level of 14 mg/kg (approximately 10-fold higher than the clinically recommended dose) over 10 hours resulted in changes in cardiovascular function, with increases in blood pressure and heart rate. Clinical symptoms such as vomiting and decreased food consumption were also observed. Ch14.18 had no effect on respiratory rate or blood gas parameters.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Mueller et. al.[6] studied the biodistribution and clearance of ch14.18 and 14.G2a using ^{125}I labeled antibodies intravenously injected into athymic mice bearing M21 human melanoma xenografts. The data presented in Figure 16 shows that after 24 and 96 hours the highest distribution of either antibody was in the tumor and blood, with traces detected in the skin, lung, spleen, and other organs.

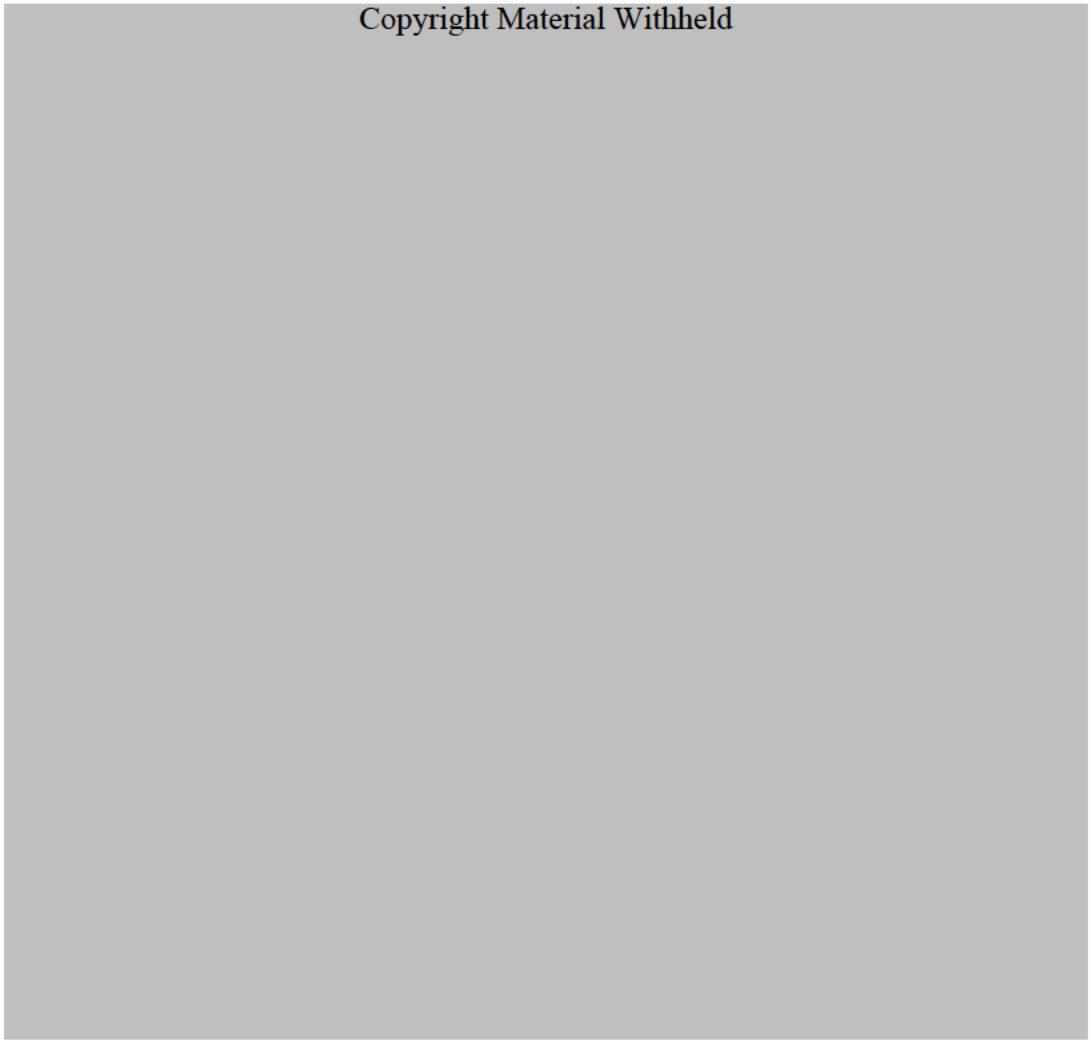
Figure 16: Clearance and Distribution of ch14.18 and 14.G2a in M21-bearing Athymic Mice



(Excerpted from Mueller et. al.,)

Additional biodistribution studies were conducted by Vriesendorp et. al.[11] in beagle dogs following administration of either radiolabelled 14.G2a (Group H) or a mix of labeled and excess unlabeled antibody (Group I). In this study, high levels of 14.G2a resulted in high

levels of specific uptake in lymph nodes. Only minimal radioactivity was observed in the nervous system of the dogs with no clear histopathological findings noted in nerves isolated from treated animals; however, low motor response amplitudes were noted in nerve conduction experiments using isolated fibers from treated animals.



(Excerpted from Vriesendorp et. al.)

Study No: 8265-980 "Validation of an ELISA method for quantification of ch14.18 in rat plasma (Conclusions only summary)

- Test facility: (b) (4)
- This validation is issued on Date 15 Nov. 2012, for test article ch14.18, Lot #S110601 (expiration date 10 Nov 2012).

Conclusions:

- **System suitability** (Calibration curve performance)-measured concentrations of 0, 10 (anchor), 25, 50, 75, 100, 200, 400, 600, 900, 1200, and 1600 (anchor)

ng/mL, with an average %AR of 81.5 between 25 and 1200 ng/mL, indicating that the targeting acceptance criteria was met

- **Inter-assay precision and accuracy**- these parameters were determined as the cumulative mean %AR for all concentration levels measured in six validation runs-criteria and found as met the specified criteria
- **Intra-assay precision and accuracy**- all control levels were less than or equal to 15.4%, therefore found as achieved the targeted acceptance criterion
- **Dilution linearity** was positive based on the 8- to 120% of the nominal concentration falling between the lower limit of quantification (LLOQ) of 25 ng/mL and upper limit of quantification (ULOQ) of 1200 ng/mL
- **Method selectivity**-based on selectivity of human plasma vs. rat plasma; the method meet the acceptance criteria because it recovered eight of ten rat plasma samples (recoveries ranged from ~85-110%)
- **Freeze/Thaw (F/T) stability**- stability controls went through 5 freeze/thaw cycles with a mean AR for the high quality control (QC) samples from 100-~109% and for the low QC from ~91-108%; the controls also met the coefficient of variation (CV) acceptance criteria
- **Bench top stability at ambient room temperature (ART)**- stability was acceptable for up to 24 hours at room temperature
- **Refrigerator stability**- established for up to 46 hours
- **Long-term stability (LTS)-** acceptable for up to 204 (per addendum) days at -60 to -80C

An addendum of this study addressed the following changes: evaluation of additional dilutions, additional stability frozen matrix to support sample storage intervals. The method has been updated to account for additional, dilution linearity and long-term stability with the updated retest date on the final product report (the same test article with the same lot number was used for this analysis). A validation study was also conducted for the detection of ch14.18 in monkey plasma (b) (4)-12-070-041). The method achieved similar results.

6 General Toxicology

6.1 Single-Dose Toxicity

Dose finding study of the anti-GD2 antibody ch14.18 in cynomolgus monkeys

Study # (b) (4)**354-004** (GLP study was done in Japan and translated to English)

Study sponsor: (b) (4)

Study initiated: 11/1/2012, with one day of acclimation and with the dosing day noted as Day 1.

Test article: chimeric mAb 14.18 from supplier United Therapeutic Corporation,

Lot # S110601, clear, colorless substance, pH 6.8, with protein concentration of 3.5 mg/mL/ placebo was 20 mM histidine, 150 mM sodium chloride, 0.05% polysorbate 20, ph 6.8

Study design:

Group	Test Article	Dose Level (mg/kg)	Dose Volume (mL/kg)	Concentration (mg/mL)	Number of Animals (Animal No.)
					Males
1	ch14.18	10.5	7	1.5	2 (1 and 2)*
2	ch14.18	21	7	3	2 (3 and 4)

*: Abnormalities (reddening of the skin in the pubic region and swelling of the foreskin) were observed in clinical signs when the first animal was dosed; however, dosing was then conducted for the second and subsequent animals because it was judged that these abnormalities would not have an impact on accomplishing the study objective.

(Excerpted from the Submission)

Dose justification: Clinical dose is 17.5 mg/m^2;
the high dose is 21 mg/kg (~252mg/m^2 converted to body surface area)[a], 10-fold the clinical dose
the low dose is the half of the high dose, 10.5 mg/kg

Dosing speed: 14 mL/kg/hr (time: 30 min/body)

Results:

10.5 mg/kg swelling of the foreskin during dosing and approximately 2 hours after the end of dosing in both animals;
Reddening of the skin in the pubic region during the dosing in one animal

21 mg/kg swelling of the foreskin observed in one animal (during dosing and for one hour after the end of dosing);
Vomiting observed in both animals at dosing and for 30 min after dosing

6.2 Repeat-Dose Toxicity

Study title: A 5 day repeat dose evaluation in dogs (Vriesendorp et. al.[11])

This study is an extension of a biodistribution study done by Vriesendorp et. al. in beagle dogs; a short summary of the toxicology findings was included in this submission.

Methods:

1. 4 dogs IV 2 mg (1.5 mCi) indium-111 labeled 14.G2a (H group)
    - 2 of the above dogs also got a total of 200 mg unlabeled 14.G2a by 2-hour infusion on Days 1-5 (I group)

[a] For antibodies, dose conversion based on BSA is uncommon as, per the CDER Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers, large proteins should be normalized to body weight. Regardless, 21 mg/kg in monkeys represents a multiple of at least 10 fold over the clinical dose in a pediatric population.

2. 4 additional dogs (2/antibody) received control antibodies ZCEO25 and B72.3 (anti- carcioembryonic antigen and anti-TAG 72, a colon cancer associated antigen)

Blood and urine samples were taken 1, 20, 40, 90, and 140 hours after the last antibody injection.
Bone marrow was sampled at 20 and 90 hours.

Sciatic nerve conduction assessments were performed just before sacrifice 6 days after the initial ^{111}In-labeled 14.G2a injection and 1 day after the last unlabeled antibody infusion. Nerve conduction studies of the sciatic nerve were performed using a TECA TE42 or Viking 2 electromyography machine with the animals under anesthesia. Percutaneous needle electrodes were used for stimulation at the ankle and sciatic notch and recording over the plantar foot muscles. Pulses of 0.1-0.5 milliseconds duration and 50-300 V were used. Distal latencies, conduction velocities and proximal to distal motor response amplitude ratios were measured.

Tissue samples from all major organs were collected and counted in a gamma counter. Histological evaluation of the nerve tissues, including brachial plexus, lumbosacral nerve roots, vagus and sciatic nerves, and myentric ganglia was performed. 14.G2a binding and tissue expression of GD2 antigen were assessed by IHC.

Results:

Sciatic nerve-conduction tissues showed significantly reduced distal motor amplitudes in animals treated at the high dose level.

Table 6: Sciatic Nerve Conduction Studies in Dogs after Radiolabelled and Unlabeled Anti-GD2 Antibody 14.G2a

(Excerpted from Vriesendorp et. al.)

1. No evidence of clinical neurotoxicity, histopathological abnormalities, or effects on nerve conduction were observed
2. 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

Based on these findings the authors hypothesized that:

- At low protein doses, anti-GD2 IgG might not cause neurologic side effects in the clinical setting

- High protein dose of anti-GD2 antibody may enhance antineoplastic effects and contribute to neurotoxicity through stimulation of normal lymphocytes with subsequent release of cytokines.

Immunochistochemistry showed 14.G2a binding to the following nervous tissues:

- CNS binding: granular layer of the cerebellum in human, dog, mouse, rabbit and rat
- Some groups of ganglion cells of myenteric ganglia and dorsal root ganglia in all above animals
- Vagus nerves of dog, sciatic nerves of rat and mouse and many sciatic nerve fibers of dogs and rabbits

Study title: A 4-week repeated intravenous dose toxicity study of the anti-GD2 antibody ch14.18 in rats with a 6-week recovery period

Study no.:	(b) (4) 354-003
Study report location:	Electronic submission tab 4, toxicology
Conducting laboratory and location:	(b) (4)
Date of study initiation:	8/23/2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Chimeric monoclonal antibody 14.18 (ch14.18); S110601; not specified

Key Study Findings

- Liver-related changes including minimal histopathological findings, increased liver weight, and mild increases in liver enzymes, primarily at doses ≥ 15 mg/kg
- Changes including hematopoiesis in the liver and spleen were observed in both sexes at 5 mg/kg and above
- Lymphocyte counts were increased in males at doses ≥ 15 mg/kg and in both sexes at 45 mg/kg
- CD3NKR-P1A+ cells (NK cells) were increased in both sexes at 15 mg/kg and above
- Histopathology: granulomas inflammation in the lung, ileum and Peyer's patch were 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

- High adrenal weight in females at 5 mg/kg and above, and increased number of germinal centers in the white pulp in the spleen in males at 45 mg/kg and in females at ≥15 mg/kg
- All changes except liver microscopy changes noted above were lowered in intensity or not found in high dose animals after 6-week recovery period.

Methods

Doses:	0, 5, 15, and 45 mg/kg/day
Frequency of dosing:	Once daily for 4 consecutive days, followed by 3 dose-free days, repeating this cycle for 4 weeks; males: Tuesday-Friday, females: Wednesday to Saturday
Route of administration:	Intravenous (IV)
Dose volume:	15 mL/kg/; speed: 15 mL/kg/hour
Formulation/Vehicle:	Ch14.18/
Species/Strain:	Rat/ Crl:CD(SD)
Number/Sex/Group:	15/sex/group, including satellite groups (as seen in the tables below)
Age:	Males:7.5 weeks; females 7 weeks
Weight:	Males:299-350g; females 208-245 g
Satellite groups:	toxicokinetics
Unique study design:	none
Deviation from study protocol:	Minimal which did not undermine study validity

Group	Test and Control Articles	Dose Level (mg/kg/day)	Dose Volume (mL/kg/day)	Concentration (mg/mL)	Necropsy	Number of Animals (Animal No.)	
						Males	Females
1	Control Article	-	15	-	ERC	5 (1 to 5)	5 (16 to 20)
					EDA	10 (6 to 15)	10 (21 to 30)
2	ch14.18	5	15	0.33	EDA	10 (31 to 40)	10 (41 to 50)
3	ch14.18	15	15	1	EDA	10 (51 to 60)	10 (61 to 70)
4	ch14.18	45	15	3	ERC	5 (71 to 75)	5 (86 to 90)
					EDA	10 (76 to 85)	10 (91 to 100)

EDA: End of the dosing period

ERC: End of the recovery period

Satellite groups: 1 control group and 3 test article groups

Group	Test and Control Articles	Dose Level (mg/kg/day)	Dose Volume (mL/kg/day)	Concentration (mg/mL)	Number of Animals (Animal No.)	
					Males	Females
5	Control Article	-	15	-	8 (101 to 108)	8 (109 to 116)
6	ch14.18	5	15	0.33	8 (117 to 124)	8 (125 to 132)
7	ch14.18	15	15	1	8 (133 to 140)	8 (141 to 148)
8	ch14.18	45	15	3	8 (149 to 156)	8 (157 to 164)

Observations and Results

Mortality-

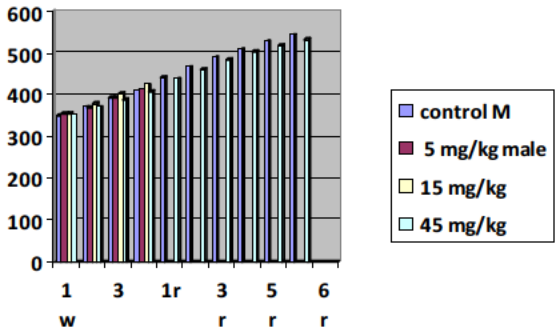
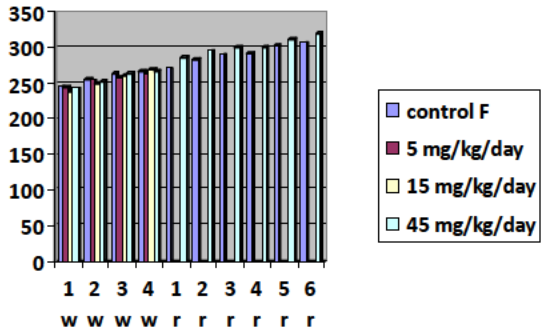
None

Clinical Signs-

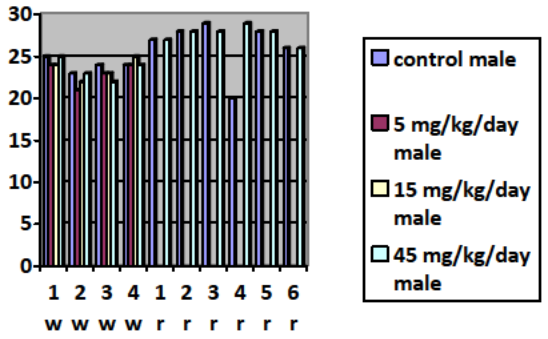
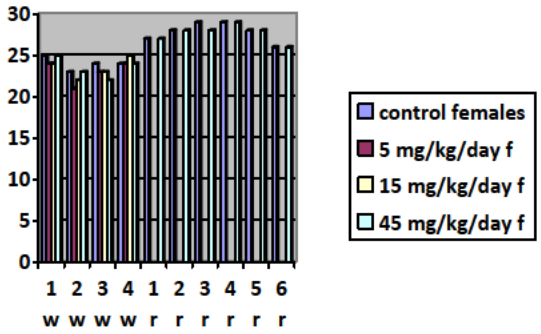
No abnormal signs noted.

Body Weights-

High-dose, (45 mg/kg) males had a minimal decrease in weight gain, when compared to control animals; this decrease was not fully recovered during one-month recovery period. The decrease in weight gain was linked to food consumption, with all dinutuximab-administered males displaying decreased consumption during the drug-administration period. Body weight in female animals was not affected by dinuxitumab administration, although food consumption decreased during the drug-administration period, specifically during second and third week.

Body weight gain in males	**Body weight gain in females**
	

Feed Consumption

Feed consumption in males	Feed consumption in females
	

Ophthalmoscopy-

No test article-related changes noted

ECG-

Not conducted

Hematology

	Control		5 mg/kg/day		15 mg/kg/day		45 mg/kg/day	
	M	F	M	F	M	F	M	F
Erythrocyte (10^6/uL)				↓7%		↓7%		
Hemoglobin c (g/dL)				↓7%		↓7%		
Hematocrit (%)				↓7%		↓7%		
Reticulocyte (%)				↑71%		↑50%		
Platelet count (10^3/uL)			↑26%	↑34%	↑17%			
Leukocyte count (10^3/uL)					↑48%		↑57%	↑53%
Neutrophil count (10^3/uL)								↑75%
Lymphocyte count (10^3/uL)					↑52%		↑46%	↑46%

	Control		5 mg/kg/day		15 mg/kg/day		45 mg/kg/day	
	M	F	M	F	M	F	M	F
Monocyte count (10^3/uL)								↑32%
Eosinophil count (10^3/uL)			↓21%	↓25%	↓13%	↓6%	↓67%	↓46%
Basophil count (10^3/uL)								↑92%
PT (s)			↑36%					
APTT (s)					↑25%			

Clinical Chemistry

	Control		5 mg/kg/day		15 mg/kg/day		45 mg/kg/day	
	M	F	M	F	M	F	M	F
Aspartate transaminase (IU/L)							↑25%	↑29%
Alanine transaminase (IU/L)				↑21%	↑31%	↑28%	↑77	↑21%
Alkaline phosphatase (IU/L)			↓16%	↑27%	↓9%	↑23%	↑15%	↑63%
Total cholesterol (mg/dL)				↑37%	↑31%	↑64%		↑78%
Total protein (g/dL)			↑6%	↑~9%	↑~9%	↑~9%	↑~9%	↑~9%
Globulin (g/dL)			↑~13%	↑20%	↑17%	↑24%	↑13%	↑26%
Albumin/Globulin			↓12%	↓17%	↓31%	↓22%	↓~8%	↓23%
Calcium (mg/dL)			Less than 5% change in all measurements					
Potassium (mEq/L)								↓12%

Urinalysis

Animals dosed at the 45 mg/kg dose level had increased positive occult blood in both sexes during the dosing period. This finding was reversible, since no occult blood was observed in high-dose recovery animals.

Gross Pathology-

No specific gross pathology findings noted

Organ Weights

Increases in total weights of organs when compared to control (first row) and the relative increases (second row).

	Control		5 mg/kg/day		15 mg/kg/day		45 mg/kg/day	
	M	F	M	F	M	F	M	F
Liver (g) Absolute					↑35%	↑31%	↑40%	↑44%
Relative (g/100gBW)			↑12%	↑15%	↑32%	↑32%	↑44%	↑46%
Spleen (g) Absolute					↑39%	↑49%	↑51%	↑41%
Relative (g/100gBW)				↑19%	↑34%	↑49%	↑51%	↑43%
Lung (g) Absolute								↑6%
Relative (g/100gBW)				↑9%				↑9%
Adrenal (g) absolute				↑18%				↑18%
Relative (g/100gBW)				↑20%				↑19%

Histopathology

Adequate Battery: yes

Peer Review: no

Histological Findings
The Applicant-submitted analysis of histological findings was excerpted from the Submission (Table 7) and further analyzed by comparison to the recorded study data.

Table 7: Rat Histopathology

Daily Dose (mg/kg)	0 (Control)		5		15		45	
Histopathology [a)]								
Number Examined	10	10	10	10	10	10	10	10
Liver								
Anisonucleosis, hepatocyte	-	-	-	± [1]	-	-	± [4], + [1]	± [7]
Basophilic change, hepatocyte, diffuse	-	-	-	± [1]	-	-	± [2], + [1]	± [4]
Congestion, centrilobular	-	-	± [2]	± [3]	± [5]	± [4], + [3]	± [5]	± [5]
Extramedullary hematopoiesis	-	-	-	± [2]	± [3]	± [2]	± [4], + [1]	± [6], + [1]
Fibrosis, pericentral vein/interlobular	-	-	-	+ [2]	-	-	± [1]	-
Increase, number, mitosis, hepatocyte	-	-	-	+ [1]	± [1]	± [1]	± [5]	± [4], + [1]
Increased number/hypertrophy, Kupffer cell	-	-	-	± [1]	± [6]	± [3]	± [4], + [1]	± [7]
Necrosis, hepatocyte, focal	-	± [1]	-	-	-	-	± [2], + [1]	-
Necrosis, single cell, hepatocyte	-	-	-	-	-	-	± [1]	-
Spleen								
Increase, extramedullary hematopoiesis	-	± [1]	-	± [1]	± [4]	± [2]	± [3], + [2]	± [3], + [1]
Increase, number, germinal center, white pulp	-	-	-	-	-	+ [1]	± [1], + [1]	± [2]
Femoral bone marrow								
Increase, cellularity, hematopoietic cell	-	-	± [2]	± [3]	± [5]	± [3]	± [6]	± [5]
Sternal bone marrow								
Increase, cellularity, hematopoietic cell	-	-	± [1]	± [3]	± [4]	± [4]	± [5]	± [5]
Lung								
Inflammation, granulomatous	-	-	-	-	-	-	+ [1]	+ [1]
Ileum								
Hemorrhage, lamina propria/muscular layer	-	-	-	-	-	-	+ [2]	-
Inflammation, granulomatous	-	-	-	-	-	-	+ [1], 2+ [1]	+ [1]
Peyer's patch (ileum)								

Findings noted after the recovery period

Daily Dose (mg/kg)	0 (Control)		5		15		45	
Number of Animals	M: 5	F: 5	M: 0	F: 0	M: 0	F: 0	M: 5	F: 5
Histopathology [a)]								
Liver								
Anisonucleosis, hepatocyte	-	-	NA	NA	NA	NA	-	± [1]
Congestion, centrilobular	-	-	NA	NA	NA	NA	± [3], + [1]	± [3], + [2]
Fibrosis, pericentral vein/interlobular	-	-	NA	NA	NA	NA	± [1]	± [1]
Adrenal								
Deletion, lipid, zona fasciculata cell	-	-	NA	NA	NA	NA	-	+ [1]
Femoral bone marrow								
Increase, cellularity, hematopoietic cell	-	-	NA	NA	NA	NA	± [1]	-
Sternal bone marrow								
Increase, cellularity, hematopoietic cell	-	-	NA	NA	NA	NA	± [1]	± [1]

-: No noteworthy findings ±: very slight +: slight NA: Not applicable
a) Numerals in square bracket represent number of animals with the finding

In addition to the findings that were listed in the Applicant-submitted tables, only liver microgranuloma of slight intensity was omitted in the summary tables. Based on the Applicant-submitted study data, 4 out of 5 control animals had liver microgranuloma, while all 5 high-dose recovery animals had the same finding noted in histopathology observations. Additional liver findings, for example centrilobular congestion, indicate that liver was a target organ of ch14.18 toxicity, therefore, increase of liver microgranuloma in the ch14.18-treated animals may be a correlating sign of liver-associated toxicity of ch14.18 administration.

Special Evaluation-

Immunophenotyping was done at the end of dosing period and at the end of recovery period. There were no changes in animals at 5 mg/kg. At 15 and 45 mg/kg, high CD3-NKR$^-$P1A$^+$ cell ratio and counts were noted in both sexes. Significant increases in CD3+ and CD3+CD4+ cell counts in males at 15 mg/kg and in CD3+CD8a+ cell count in females at 45 mg/kg were noted; however, these changes were considered to be

related to the high lymphocyte count because no changes were noted in these ratios. There were no changes noted at the end of the recovery period

Toxicokinetics

- C_0 and AUC_{0-4h} were increased almost dose proportionally up to 45 mg/kg, and there were no gender differences in these toxicokinetic parameters on Day 1 of dosing.
- There was evidence of dose accumulation during the initial 4 days of infusion
- On Day 22 of dosing (4th cycle of dosing), C_0 and AUC_{0-4h} were increased more than dose-proportionally between 5 and 45 mg/kg. Compared with Day 1 of dosing, C_0 and AUC_{0-4h} at Day 22 were markedly decreased in both sexes at 5 mg/kg, slightly decreased in females at 15 mg/kg, but slightly increased in males at 45 mg/kg.

After the 4th cycle of dosing, plasma concentrations of ch14.18 decreased rapidly at 5 mg/kg (undetectable within 48 hours), and also decreased gradually at 15 mg/kg (generally undetectable by week 3 of recovery) indicating the possible development of anti-drug antibodies in the dosed animals. Antibody concentration decreased markedly at the 45 mg/kg dose level by Day 42 of recovery in males, but the same decrease was not noted in females at this dose level.

(Excerpted from Applicant's submission)

Table 12-1 Plasma concentration (ng/mL) of ch14.18 in rats (Day 1 of dosing) Study No. (b) (4)-54-003

Sex	Dose (mg/kg/day)		Plasma concentration (ng/mL) Time after dosing					C_0 (ng/mL)	AUC_{0-4h} (ng·hr/mL)
			Pre	5 minutes	1 hour	2 hours	4 hours		
Male	0	Mean	-	0.00	-	-	-	-	-
		SD	-	0.00	-	-	-	-	-
	5	Mean	0.00	98000	106000	87700	72100	98000	358000
		SD	0.00	22500	12000	17400	29200	-	-
	15	Mean	0.00	291000	247000	230000	191000	295000	930000
		SD	0.00	50000	29000	38000	28000	-	-
	45	Mean	0.00	774000	838000	691000	537000	774000	2800000
		SD	0.00	353000	223000	116000	37000	-	-
Female	0	Mean	-	0.00	-	-	-	-	-
		SD	-	0.00	-	-	-	-	-
	5	Mean	0.00	168000	81100	51200	61200	179000	307000
		SD	0.00	149000	8500	34700	3900	-	-
	15	Mean	0.00	308000	217000	224000	228000	318000	939000
		SD	0.00	28000	128000	25000	53000	-	-
	45	Mean	11.5	1000000	797000	696000	596000	1020000	2950000
		SD	22.9	50000	40000	42000	58000	-	-

- : No data

Table 12-2 Plasma concentration (ng/mL) of ch14.18 in rats (Days 5 to 8, 15 of dosing)

Sex	Dose (mg/kg/day)		Plasma concentration (ng/mL) Day after dosing 5	6	7	8	15
Male	5	Mean	114000	77900	25300	6380	0.00
		SD	9000	46400	34100	6280	0.00
	15	Mean	400000	223000	193000	188000	54000
		SD	63000	59000	47000	39000	108000
	45	Mean	1090000	661000	629000	552000	656000
		SD	30000	62000	55000	126000	481000
Female	5	Mean	106000	96500	53300	2900	2990
		SD	20000	13600	47500	1850	5940
	15	Mean	366000	256000	222000	26500	355000
		SD	20000	27000	152000	40800	541000
	45	Mean	815000	731000	582000	265000	633000
		SD	266000	227000	257000	200000	467000

Table 12-3 Plasma concentration (ng/mL) of ch14.18 in rats (Day 22 of dosing) Study No. (b) (4)354-003

Sex	Dose (mg/kg/day)		Plasma concentration (ng/mL) Time after dosing Pre	5 minutes	1 hour	2 hours	4 hours	C_0 (ng/mL)	AUC_{0-4h} (ng·hr/mL)
Male	0	Mean	-	0.00	-	-	-	-	-
		SD	-	0.00	-	-	-	-	-
	5	Mean	0.00	1690	8850	303	2650	1690	12500
		SD	0.00	1100	17230	267	5240	-	-
	15	Mean	70900	321000	290000	167000	187000	324000	889000
		SD	120400	463000	287000	298000	195000	-	-
	45	Mean	596000	1530000	1320000	1470000	1140000	1550000	5440000
		SD	354000	780000	240000	830000	200000	-	-
Female	0	Mean	-	0.00	-	-	-	-	-
		SD	-	0.00	-	-	-	-	-
	5	Mean	0.00	24500	268	12800	22.6	36300	33200
		SD	0.00	48000	230	25600	26.4	-	-
	15	Mean	2900	161000	77900	131000	59100	171000	418000
		SD	5800	173000	128800	158000	115300	-	-
	45	Mean	285000	1090000	816000	1090000	634000	1120000	3640000
		SD	354000	640000	622000	690000	496000	-	-

- : No data

Table 12-5 Plasma concentration (ng/mL) of ch14.18 in rats (Recovery period) Study No. (b) (4)354-003

Sex	Dose (mg/kg/day)		Plasma concentration (ng/mL) Day after Recovery 1	7	14	21	28	35	42
Male	5	Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	15	Mean	72000	13500	0.00	435	0.00	26.8	0.00
		SD	144000	27000	0.00	870	0.00	53.5	0.00
	45	Mean	780000	113000	130000	28500	6100	13900	106
		SD	534000	225000	151000	57000	11550	27800	211
Female	5	Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	15	Mean	1650	280	11.7	28.3	0.00	0.00	0.00
		SD	3220	560	23.4	56.5	0.00	0.00	0.00
	45	Mean	661000	133000	121000	13600	61500	196	24600
		SD	563000	231000	242000	27100	123000	392	49200

9 Reproductive and Developmental Toxicology

No reproductive and developmental toxicology studies were conducted to support the submission of the BLA for Unituxin. The Applicant cited a review by Brodeur and Maris[1] in which neuroblastoma is defined as a pediatric cancer, with the average age of diagnosis being 18 months with about 40% of patients diagnosed by 1 year of age, 75% by 4 years and 98% by 10 years of age. Long-term survival of high-risk neuroblastoma patients is less than 40%. Based on the age of the proposed patient population, FDA agreed that traditional reproductive toxicology studies were not warranted to support the use of this product in the treatment of patients with maintenance phase of high risk neuroblastoma.

10 Special Toxicology Studies

A GLP tissue cross reactivity study conducted by (b) (4) was provided with this submission. Binding of Ch14.18 was seen in **limited juvenile and adult human tissues** in the following membranes/membrane granules and cytoplasm/cytoplasmic granules:

- **epithelium** in the kidney (tubules), skin (epidermis), and thymus (reticular); endothelium in the majority of tissues;
- **mononuclear** cells in the gastrointestinal tract (colon, esophagus, small intestine, and stomach); heart, liver (Kupffer cells probably included), lung, pancreas, skin, spleen, thymus, tonsil, and urinary bladder;
- **reticuloendothelium** in the spleen;
- **reticular** cells in GALT in the small intestine;
- **chromaffin** cells in the adrenal medulla;
- **glomerular tuft** cells in the kidney;
- **hilus** cells in the ovary; and
- **granulosa** cells in the ovaries.

  Binding to cytoplasm and/or cytoplasmic granules was seen in
  - **epithelium** in the esophagus (submucosal glands), kidney (tubules), lung (pneumocytes), prostate, skin (sweat glands), thymus (reticular), and tonsil (mucosa and crypts);
  - **myoepithelium** in the skin (sweat glands);
  - **endothelium** in the majority of tissues;
  - **stroma/stromal** cells in the majority of tissues;
  - **smooth myocytes** in the gastrointestinal tract (esophagus and stomach), prostate, urinary bladder, and uterus (endometrium);
  - **mononuclear** cells in the gastrointestinal tract (colon, esophagus, small intestine, and stomach), heart, liver (including Kupffer cells), lung, pancreas, skin, spleen, thymus, tonsil, and urinary bladder;
  - **reticular** cells in GALT in the small intestine;
  - adipocytes in the adrenal, colon, pancreas, skin, and spinal cord;
  - **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);
- **endometrium** in the heart, ovary, striated (skeletal) muscle, and urinary bladder;
- **myenteric plexus** (including neurons [ganglion cells] and reserve cells) in the gastrointestinal tract (colon, esophagus,small intestine, and stomach);
- **ganglion** cells (including neurons and reserve cells) in the prostate; neurons in the brain (cerebrum);
- **glial** cells in the brain (cerebrum) and spinal cord;
- **neutrophil** in the brain (cerebrum) and spinal cord;
- **Chromaffin** cells in the adrenal medulla; glomerular tuft cells in the kidney."

Note: The study results are consistent with known binding sites of ch14.18, although cytoplasmic binding would not be expected with clinical use of the antibody.

Conclusion: Membrane/membrane granules of kidney, GI, spleen, GALT, ovary, neurons, cerebrum, and brain were identified as ch14.18-target tissues in humans.

Study in the **Sprague-Dawley rat** tissue panel:
Membrane and cytoplasmic elements in the following:

- Epithelium in the eye (lens), testis (seminiferous tubules), and thymus (reticular cells and Hassall's corpuscles)
- Endothelium in the adrenal, bladder, Fallopian tube, gastrointestinal (GI) tract (esophagus and stomach), heart, kidney, liver, lung, ovary, placenta, skin, spleen, striated muscle, testis, thyroid, and uterus (endometrium)
- Perithelium in tandem with endothelial staining in the spleen, striated muscle, and testis
- Mononuclear leukocytes in the bladder, colon, GI tract (small intestine), lung, lymph node, spleen, and thymus
- Kupffer cells in the liver
- Ependymal cells in the cerebral cortex and spinal cord
- Luteal cells in the ovary
- Interstitial (Leydig) cells in the testis.

Cytoplasmic elements only in the following:

- Epithelium in the Fallopian tube, kidney (collecting ducts, and convoluted tubules), liver (bile ducts), lung (bronchus), skin (hair follicles), and thyroid
- Endothelium in the brain (cerebral cortex)
- Perithelium in tandem with endothelial staining in the brain (Cerebral cortex) and liver
- Cardiac myocytes in the atrium of the heart
- Neutrophils in the cerebellum, cerebral cortex, and spinal cord
- Neurons in the cerebellum (granular cell layer and Purkinje cell layer), cerebral cortex, and spinal cord
- Myenteric plexus in the GI tract (small intestine)

- Pituicytes in the pituitary
- Chondrocytes in the lung
- Stromal cells in the ovary and uterus (endometrium)
- Granulosa cells and theca cells in the ovary
- Decidual cells in the placenta

Extracellular elements in the following:

- Proteinic material (intravascular) in the brain (cerebral cortex)
- Lens fibers in the eye.

The majority of staining observed in this study was anticipated based on literature reports; however, no literature was available describing the expression of GD2 by endothelium or perithelium, cardiac muscle, chondrocytes, or reproductive tissue elements of the ovary, placenta, or testis. The staining in these tissue elements may represent previously unreported sites of GD2 expression or cross-reactivity with another epitope(s) closely related to GD2.

A Tissue Cross-reactivity study of ch14.18 in normal **New Zealand white rabbit tissues** (Testing facility (b) (4) study # 20030593)
ch14.18 produced moderate to strong staining of the positive control material (ganglioside GD2, Disialo, human brain UV-resin spot slides [GD2]) at both staining concentrations. ch14.18 did not specifically react with the negative control material (human hypercalcemia of malignancy peptide, amino acid residues 1-34, UV-resin spot slides [PTHrP 1-34]) at either staining concentration. The control article, HuIgG1, did not specifically react with either the positive or negative control materials. There also was no staining in the assay control slides.

Membrane only staining with ch14.18 was found in:

- Skeletal myocytes in the cervix
- Adipocytes in the adrenal, bladder, bone marrow, breast, Fallopian tube, GI tract (esophagus and small intestine), heart, lymph node, pancreas, salivary gland, skin, spleen, thymus, thyroid and ureter
- Decidual cells in the placenta.

Positive staining of membrane and cytoplasmic elements occurred in the following tissues:

- Epithelium in the adrenal (cortex), eye (lens), pituitary (adenohypophysis and pars intermedia), placenta (amnion and trophoblasts), testis (seminiferous tubules and rete testis), and thymus (reticular cells and Hassall's corpuscles)
- Mesothelium in the Fallopian tube, small intestine, lung (pleura), spleen, and uterus (endometrium)
- Endothelium and perithelium in tandem in the adrenal, bladder, bone marrow, brain (cerebellum and cerebral cortex), breast, colon, eye, Fallopian tube, gastrointestinal (GI) tract (esophagus, small intestine, and stomach), heart, kidney, liver, lung, lymph node, ovary, pancreas, parathyroid, peripheral nerve,

pituitary, placenta, prostate, salivary gland, skin, spinal cord, spleen, striated muscle, testis, thymus, thyroid, ureter, and uterus (cervix and endometrium
- Mononuclear leukocytes in the bladder, GI tract (small intestine), lung, lymph node, prostate, spleen, and thymus
- Hematopoietic cells in the bone marrow
- Ependymal cells in the cerebral cortex
- Stromal cells in the bladder
- Interstitial (Leydig) cells in the testis

Staining of cytoplasmic elements only occurred in the following tissues:

- Epithelium in the colon (mucosal glands), eye (conjunctiva), liver (hepatocytes), ovary (surface), skin (hair follicles), spinal cord (central canal), and ureter (translational)
- Neutrophil in the cerebellum, cerebral cortex, and spinal cord
- Neurons in the cerebellum (granular cell layer)
- Meningeal cells in the cerebellum and cerebral cortex

11 Integrated Summary and Safety Evaluation

Neuroblastoma, a highly heterogeneic malignant cancer, is the most common extracranial solid tumor in childhood, accounting for roughly 15% of all pediatric oncology deaths. It is the most frequently diagnosed neoplasm at infancy, with a median age at diagnosis of 17-22 months. Neuroblastoma accounts for more than 7% of malignancies in patients younger than 15 years. Neuroblastoma tumors can develop anywhere in the sympathetic nervous system, with more than half of all primary tumors occurring within the abdomen, and half of these arising in the adrenal medulla. Other common sites for occurrence of primary neuroblastoma include the neck, chest, abdomen and pelvis. Diagnosis is confirmed by tissue and bone marrow biopsies, MRI, CT and MIBG scans, together with blood tests.

United Therapeutics Corporation (the Applicant) has submitted a BLA for Unituxin (dinutuximab, ch14.18), a human/mouse chimeric monoclonal antibody against GD2, a disialoganglioside abundantly expressed on a variety of tumor cells of neuroectodermal origin, including neuroblastomas. Unituxin is intended for use as a component of a multi-agent, multi-modality regimen in patients with (b) (4) high-risk neuroblastoma, at a dose of 17.5 mg/m^2 for four days per course of a 24 to 28-day cycle, for 5 cycles.

Dinutuximab is the ch14.18 antibody being produced in SP2/0 cells by United Therapeutics Corporation. The manufacturing process used to produce dinutuximab by this company is derived from the process used to produce ch14.18 by the NCI during its clinical development prior to the CRADA. The ch14.18 antibody is itself derived from the IgG3 murine monoclonal antibody Mab14.18, an anti-GD2 antibody originally raised against a neuroblastoma cell line injected into BALB/c mice. Several different antibodies using the Mab14.18 variable region have been created during the course of ch14.18 development, and all of these variants have been analyzed for their

comparability to the parental protein. Accordingly, the pharmacologic characteristics of dinutuximab (ch14.18) are primarily described in academic studies published since 1986 and, except for the toxicology and safety pharmacology studies specifically conducted by the Applicant, these studies do not use the antibody produced by the Applicant's manufacturing process. According the review of the FDA product quality team, while there may be some differences in the potency of dinutuximab versus the ch14.18 antibody used in older trials in assays for ADCC, these differences are not significant enough to impact the approval of the product.

Many investigational groups have described the mechanism of action of ch14.18 as a combination of initiating complement-dependent cytotoxicity (CDC) and potentiating antibody-dependent cell mediated cytotoxicity (ADCC) of GD2 expressing cells. Mujoo et. al. 1987[4] and Mueller et. al.[6] each showed increasing levels of specific lysis of GD2-expressing cells by human PBMCs with increasing concentrations of GD2 specific antibodies (Mab14.18, ch14.18, or 14.G2a). Barker et. al.[7] and Zeng et. al.[10] went further to demonstrate that neutrophils and NK cells were key effector cells in this process. Other experiments described in this set of papers looked at the effects of the addition of cytokines to ch14.18-mediated tumor lysis. Barker et. al. (1991) showed that recombinant GM-CSF, a cytokine that stimulates neutrophil production, enhanced ch14.18-potentiated ADCC. Experiments by Kendra 1999[8] showed that ch14.18-potentiated ADCC increased with increasing ratio of effector (human PBMCs) to target cells, or with increasing ch14.18 concentration especially in the presence of IL-2 (200 units/mL).

Complement-mediated cytotoxicity was described in work by Mujoo et. al.,1987[4], in which the presence of increasing concentrations of Mab14.18 resulted in the specific in vitro lysis of neuroblastoma cell lines, in the presence of active human complement.. Mueller et. al.[6], found similar results following incubation of GD2-expressing melanoma cells lines M-21 and A375 in the presence of ch14.18 and active human complement.

Together, these data demonstrate that ch14.18 is able to mediate lysis of GD2-expressing neuroblastoma and melanoma cells through CDC and ADCC. In separate experiments both GM-CSF and IL-2 were shown to be capable of enhancing ch14.18-mediated lysis of tumor cells. In the absence of complement or effector cells, the submitted data did not indicate any direct anti-tumor effects of GD2 binding.

Secondary pharmacology studies sought to further examine pain-related consequences of ch14.18 administration in the clinic using animal models, primarily Sprague-Dawley rats. Mechanical pain threshold and allodynia (pain due to a stimulus that does not normally provoke pain, such as a light touch) were studied by Slart et. al.[12], who noted that all animals administered ch14.18 by IV injection, regardless of dose, showed a steep, immediate drop in mechanical pain threshold. A trend of a return of pain thresholds to baseline was noted within 24 to 48 hours of ch14.18 injection. These authors also studied touch evoked agitation (TEA) in the same animals and found that all ch14.18-treated animals had statistically significant increases in TEA, a finding not seen in saline control-treated animals.

A study by Xiao et. al.[13] compared the effects of ch14.18 (1 mg/kg) with the same amount of 14.G2a antibody on the pain and allodynia response in male Sprague-Dawley rats. This study showed that both antibodies caused similar decreases in pain thresholds measured by mechanical testing. This finding was corroborated by decreases in locomotion and exploratory behavior (standing upright against the side of the cage), though effects of ch14.18 appeared more pronounced than those of 14.G2a. To explore the mechanism for ch14.18-induced decreases in pain thresholds, the authors isolated afferent nerve fibers from treated and untreated animals and examined their electrophysiological characteristics. The authors found increases in the background activity of Aδ and C fibers following administration of anti-GD2 fibers, though the cause of this increase was not elucidated. As damage to these types of fibers, particularly C fibers, causes neuropathic pain, this increase in background activity may help explain the pain reported clinically following ch14.18 administration. Xiao et. al.[13] also reported that lidocaine, given as a continuous infusion to a target plasma level of 0.3-2.2 µg/ml, reduced this electrophysiologic background activity and prevented the decrease in mechanical pain threshold.

Studies described in Sorkin et. al.,2002[14] compared IV and IT administration of 14.G2a in the onset of mechanical and thermal allodynia in Sprague-Dawley rats. These studies demonstrated that the effect of lowering the mechanical pain threshold persists regardless of the route of administration, though treatment with this antibody had no effect on thermal sensitivity. Capsaicin administration prevented the decrease in mechanical pain threshold in this study. In a second paper, Sorkin et. al., 2010[18], showed that pre-administration of a C5a complement receptor antagonist prevented pain, indicating that the onset of pain associated with ch14.18 administration may be the result of activation of complement system in rats. Together, the findings described in these papers suggest that the pain associated with ch14.18 administration is due to ch14.18 binding to GD2 on peripheral nerves and subsequent damage to nerves by CDC and ADCC.

Finally, Vriesendorp et. al.[11] examined the effects of administration of 14.G2a on nerve conduction and behavior in several species including mice, rats, rabbits, and dogs. This group reported positive staining of myelin by the anti-GD2 antibody, a finding that was consistent with previous reports[19] suggesting damage to myelin following anti-GD2 binding as a contributing factor in the development of sensiromotor polyneuropathy in some patients. Vriesendorp et. al. reported no effects on clinical signs, histologic abnormalities, or electrophysiologic abnormalities following single dose administration of the anti-GD2 antibody to rodents, rabbits, or dogs. In dogs treated with multiple doses of 14.G2a, however, there was a decrease in motor amplitude.

The Applicant conducted a combined cardiovascular and respiratory safety pharmacology study in cynomolgus monkeys. A single ch14.18 dose of 14 mg/kg (~168 mg/m^2) was administered to three conscious, telemetered male monkeys on Day 3, as a 10-hour IV infusion. When compared to treatment of the same animals with the vehicle control, increases in blood pressure (one animal) and heart rate (two animals) occurred

during the 5 to 24 hour time interval. Shortening of PR interval and QT interval, related to the increase in heart rate were observed in 2 animals at 5, 10, 12, or 24 hours after the start of dosing, however, no significant changes in QTc were detected. No notable changes occurred in respiratory rate or blood gas parameters.

No specific ch14.18 tissue-distribution study was submitted, however, published studies using murine Mab 14.18 in mice or the murine IgG2a isotype switch antibody 14.G2a in athymic mice bearing human melanoma xenografts and normal, healthy dogs showed distribution of anti-GD2 antibodies to GD2-expressing tumor tissue, blood, liver, and mesenteric lymph nodes. In addition, the GLP-compliant toxicology study in rats showed that liver was a target of ch14.18 toxicity, correlating with the published findings of accumulation in this organ and suggesting that ch14.18 degradation likely occurs primarily in the liver.

Because of the historical development of ch14.18 and the Applicant's CRADA agreement to commercialize the antibody, limited formal toxicology data is available. Dinutuximab was evaluated in a one-month toxicology study in rats ((b) (4) 354-003) as well as a pilot single dose tolerability study ((b) (4) 354-004) in cynomolgus monkeys that was designed to support dosing for the evaluation of the antibody in a cardiac safety study. Only limited toxicity parameters, including body weight, food consumption and clinical observations were recorded in the monkey study. Administration of a single dose of dinutuximab at dose levels of 10.5 or 21 mg/kg to monkeys via IV infusion (30 min) resulted in vomiting within 30 minutes in the high dose monkeys and swelling of the genitals at both dose levels.

In the rat one month toxicology study, vehicle or dinutuximab at 5, 15, or 45 mg/kg (~270 mg/m^2) was administered by IV infusion over 1 hour to Sprague-Dawley rats once daily for four consecutive days, followed by three dose-free days, repeating this cycle for 4 weeks, followed by a 6-week dose-free period (5/sex of control and high-dose animals). Dose-related findings in this study included liver-specific histopathology changes (centrilobular congestion, hepatocellular necrosis, pericentral vein/interlobular fibrosis) correlating with modest increases in AST, ALT, and total cholesterol and increased liver weight at the high dose level. Other changes noted in animals during this study included non-dose dependent increases in reticulocytes and platelet counts, as well as increases in lymphocytes, neutrophils, and NK cells. All histopathology changes were slight in severity, and had recovered, or decreased in intensity during the 6-week recovery period, except for the microscopic lesions of centriolobular congestion and pericentral vein/interlobular fibrosis of the liver.

Immunohistochemistry studies in normal juvenile and adult human tissue showed ch14.18 staining of epithelia, mononuclear cells, smooth muscle cells and numerous neural tissue elements. Specific binding was also observed in tissues from the human ovary and testis, endothelium, adipocytes, and glomerular tuft cells, although there are no previously published references that identified GD2 expression in these tissues. It is not clear if the staining observed is the result of cross-reactivity of ch14.18 with another closely-related epitope similar to GD2, non-specific staining, or previously

uncharacterized sites of GD2 expression. Similar findings were obtained in additional tissue-cross reactivity studies conducted with tissues from Sprague-Dawley rats and New Zealand White rabbits with the majority of staining being consistent with literature reports; however, staining of additional tissues including endothelium, perithelium, cardiac myocytes, chondrocytes, and reproductive tissues (ovary, placenta, and testis) was also noted in the Applicant's study.

The nonclinical studies published and submitted with this BLA were not fully predictive of the clinical findings following administration of ch14.18 as a part of the proposed multi-modality treatment regimen in patients with neuroblastoma. More specifically, capillary leak syndrome, hypotension, systemic infection or sepsis, neurological disorders of the eye, and hyponatremia were not evident in animal studies; however, animals did not receive IL-2, GM-CSF, cis-retinoic acid, or radiation therapy prior to ch14.18 administration and many of the unpredicted findings have been previously associated with administration of the other drugs in the treatment regimen. While clinical signs of pain in animals can be difficult to detect, there was clear evidence of an effect of anti-GD2 antibodies on mechanical pain thresholds in rats, as well as potential signs of pain in monkeys given single doses of dinutuximab, evidenced by vomiting during or following infusion and decreased food consumption. Liver toxicity was evident in rat toxicology study, and coincided with increases in AST and ALT levels, together with cholesterol increases.

The Agency agreed that embryofetal studies were not warranted to support the marketing application for dinutuximab due to the age of patient population, although given that it is an IgG1 antibody with the potential to cross placental barrier, the extent of its risk to pregnancy is unknown. If, in the future, the Applicant pursues additional indications, reproductive toxicity studies may be required. Carcinogenicity studies were not conducted and are not required to support a marketing application for a drug intended for the treatment of advanced cancer. Genotoxicity studies were not conducted and are not warranted for a monoclonal antibody as detailed in the ICH S6 guidance for biotechnology products. From a pharmacology/toxicology perspective there are no outstanding issues that would prevent the approval of dinutuximab for the treatment of patients with (b) (4) high-risk neuroblastoma as a component of a multi-agent, multi-modality regimen. An additional chronic toxicology study is recommended as a post marketing requirement to further investigate the potential for long term neuropathy and neuropathic pain as well as to further explore the toxicity of dinutuximab as a monotherapy.

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