Multicenter double-blind, randomized, placebo-controlled trial of IDEC-C2B8 for the treatment of childhood-onset complicated nephrotic syndrome

Clinical study protocol

Clinical study protocol Number: RCRNS-01

Version: 4.0

First version：June 17,2008	Version 1.1 ：June 25, 2008
Version2.0: September 24, 2008	Version 2.1: October 20, 2008
Version 3.0: February 5, 2009	Version 3.1: September 1, 2009
Version 3.2: January 25, 2010	Version 4.0: October 20, 2010

Clinical Trial Steering Committee:
Kazumoto Iijima, Kobe University Graduate School of Medicine
Nao Tsuchida, National Center for Child Health and Development
Mayumi Sako, National Center for Child Health and Development

1 Clinical trial overview

1.1 Clinical trial diagram

This study is the multicenter double-blind, randomized, placebo-controlled study.

<table>
<tr><td colspan="5">Clinical trial period
(date consent is obtained – date of completion of the observation period)</td></tr>
<tr><td colspan="3">Screening period
(Up to 35 days following the date consent is obtained)</td><td rowspan="2">Up to 14 days</td><td colspan="2">Observation period (day 1 - day 365, 1 year)</td></tr>
<tr><td>Obtain consent</td><td>Screening</td><td>Registration / allocation</td><td>Investigational drug administration period (day 1 - date of discontinuation / completion of administration)</td><td>Post-discontinuation / completion of investigational drug administration - day 365</td></tr>
</table>

The day the first dose of the investigational drug is administered is set as “Day 1”

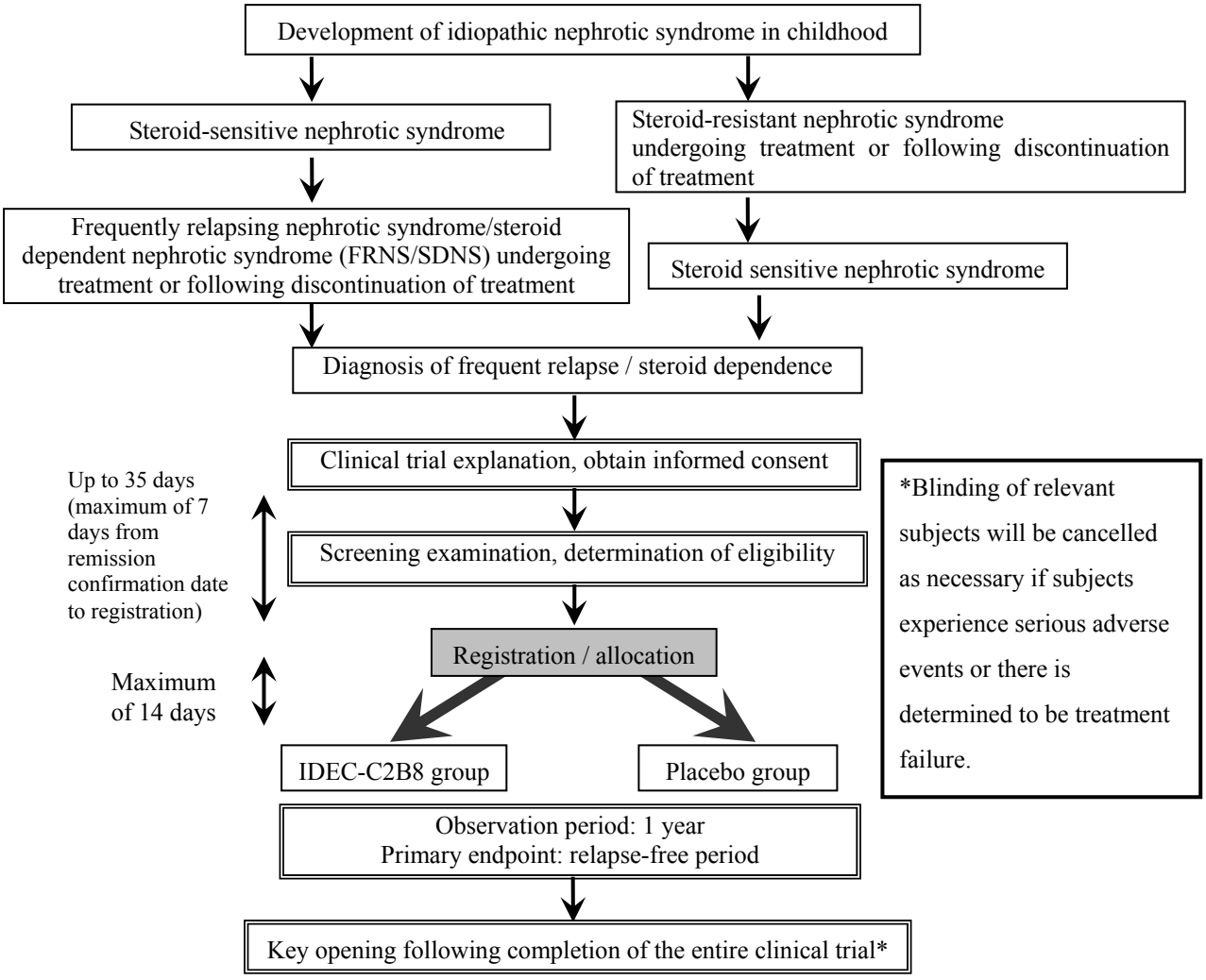


Fig. 1: Flow of the clinical trial

1.2 Purpose of the clinical trial

To verify the efficacy and evaluate the safety of 4 x 375 mg/m^2 doses of IDEC-C2B8 given at weekly intervals to childhood-onset complicated nephrotic syndrome patients with very high disease activity (5.1.3).

1.2.1 Efficacy endpoints

(1) Primary endpoint

1) Relapse-free period during the observation period (day 1 - day 365):
The period from the date of registration / allocation until the date of the occurrence of the first relapse following commencement of investigational drug administration.

(2) Secondary endpoints

1) Period until treatment failure: The period from the date of registration / allocation until the date at which treatment failure is determined.

2) Relapse rate: The number of relapses observed per person per year in each group

3) Period until occurrence of frequent relapses: The period from the date of registration / allocation until the frequent relapse occurrence date during the observation period

4) Period until steroid dependence: The period from the date of registration / allocation until the date of steroid dependence during the observation period

5) Period until the transition to steroid resistance: The period from the date of registration / allocation until the date of transition to steroid resistance during the observation period

6) Total and daily dosage of steroids: The total and daily dosage of steroids administered from the date of registration / allocation until the date of completion of the observation period

7) Change in the total and daily dosage of steroids in the 365 days before and after the date of registration / allocation: The difference of the total and daily dosage of steroids administered in the 365 days between immediately before the date of registration / allocation (not including the day of registration / allocation) and after the date of registration / allocation.

(3) Other endpoints

1) IDEC-C2B8 blood concentration
2) Number of B-cells in peripheral blood (cells/μL)
3) Peripheral blood B-cell depletion period
4) Human anti-chimeric antibody (HACA) production ratio

(4) Safety endpoints

Subjective symptoms, objective findings, physical examination (vital signs, height and weight), clinical examination (hematological examination, blood biochemical examination and urinalysis), electrocardiogram, chest X-ray

1.3 Subjects

Patients who developed idiopathic nephrotic syndrome in childhood and that fulfill the definition of "complicated nephrotic syndrome" prescribed in this trial.

1.3.1 Inclusion criteria and exclusion criteria

Refer to "8. Subject inclusion criteria and exclusion criteria"

1.4 Target number of subjects and planned clinical trial period

Target number of subjects: 60 (30 in each group)

Planned subject registration period: September 2008 – August 2011 (3 years)

Planned clinical trial period: September 2008 – August 2012 (4 years)

Clinical trial schedule

Refer to "14.1. Observation, examination, survey schedule"

	Screening period	Observation period (investigational drug administration period)				Observation period (following completion of investigational drug administration)													Clinical trial Discontinuation
Day	Within 35 days	1	8	15	22	29	57	85	113	141	169	197	225	253	281	309	337	365	
Week		1	2	3	4	5	9	13	17	21	25	29	33	37	41	45	49	53	
Obtaining informed consent	○																		
Medical examination	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Investigation drug administration		○	○	○	○														
Background survey	○																		
Concomitant drug survey	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	△
Height/weight	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	△
Blood pressure, pulse, body temperature	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	△
Pregnancy test	○																		
HIV, HCV, HBV	○																		
Electrocardiogram	○																	○	△
Chest X-ray	○																	○	△
Relapse evaluation		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	△
Adverse event evaluation		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Hematological examination	○	○	○	○	○	○	○	○	○	○	○	○		○		○		○	△
Blood biochemical examination	○	○	○	○	○	○	○	○	○	○	○	○		○		○		○	△
Immunoglobulin examination		○		○		○		○			○			○		○		○	△
Estimated glomerular filtration rate	○																	○	△
Urinalysis	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	△
B-cells/T-cells in the blood	○	○		○		○		○		○	○	○		○		○		○	○
Human anti-chimeric antibodies		◎						○			○							○	○
Drug blood concentration		◎			◎			○			○							○	○
Blood sample volume (mL)	8.5	13.5	4.5	9.5	6.5	9.5	4.5	13.5	4.5	7.5	13.5	7.5		9.5		9.5		13.5	7-13.5

2 Table of Contents

3 List of abbreviations and term definitions

3.1 Abbreviations

Abbreviation	
AIHA	Autoimmune hemolytic anemia
CTCAE	Common Terminology Criteria for Adverse Events
GEE	Generalized estimating equation
FAS	Full analysis set
HACA	Human Anti- Chimeric Antibody
INN	International non-proprietary names
ISKDC	International study of kidney disease in children
ITP	Idiopathic thrombocytopenic purpura
IU	International unit
JAN	Japanese accepted names
MedDRA	Medical dictionary for regulatory activities
PPS	Per protocol set
PML	Progressive Multifocal Leukoencephalopathy
RA	Rheumatoid arthritis
SLE	Systematic lupus erythematosus
USAN	United States adopted names

3.2 Examination items and units

Examination item	Abbreviation		Unit
Clinical examination	ALT	L-alanine aminotransferase	IU/L
	AST	Aspartate aminotransferase	IU/L
	BUN	Blood urea nitrogen	mg/dL
	CRP	C-reactive protein	mg/dL
	γ-GTP	γ-Glutamyltranspeptidase	IU/L
	HbA_{1C}	Hemoglobin A_{1C}	%
	HB antigen	Hepatitis B surface antigen	－
	HCG	Human chorionic gonadotropin	mIU/mL
	HCV antibody	Hepatitis C virus antibody	－
	HIV antibody	Human immunodeficiency virus antibody	－
	LDH	Lactate dehydrogenase	IU/L

3.3 Definitions

This clinical trial will adopt the following definitions in accordance with the definitions of the International study of kidney disease in children: ISKDC.

A. Nephrotic syndrome	The expression of urine protein-to-creatinine ratio of 1.8 or above and serum albumin of 2.5g/dL or below
B. Complicated nephrotic syndrome	A patient that fulfills any of the following from(1) to(3) is deemed to suffer from complicated nephrotic syndrome: (1) Diagnosed with frequent relapse (I) or steroid dependence (L) and once again diagnosed with frequent relapse (J) or steroid dependence (M) after completion of immunosuppressive drug therapy (cyclosporine, cyclophosphamide, mizoribine, etc.) (2) Diagnosed with frequent relapse (I) or steroid dependence (L) and once again diagnosed with frequent relapse (J) or steroid dependence (M) during immunosuppressive drug therapy (cyclosporine, cyclophosphamide, mizoribine, etc.) (3) Diagnosed with steroid resistance (O) and diagnosed with frequent relapse (J) or steroid dependence (M) during or after the completion of immunosuppressive drug therapy (cyclosporine or combination of cyclosporine and methylprednisolone, etc.)
C. Remission	Negative protein on urine dipstick in the first morning urine for 3 consecutive days.
D. Remission confirmation date	The date remission (C) is confirmed at the medical institution.
E. Steroid sensitivity	When the daily administration of prednisolone at 60 mg/m²/day leads to remission (C) within 4 weeks.
F. Relapse	Protein 2+ or above detected by urine dipstick in the first morning urine for 3 consecutive days and prednisolone treatment is required.
G. Relapse-immediately prior to the clinical trial	A relapse (F) that occurred in the 35 days prior to registration.
H. Relapse date	The first day that 2+ or above protein on urine dipstick test in the first morning urine have been, followed by the same observation for 3 consecutive days (the relapse diagnosis date is also an acceptable date with regard to the 3 most recent relapses prior to registration).
I. Frequent relapse	Two or more relapses (F) within 6 months after initial remission (C) or 4 or more relapses (F) within any 12-month period.
J. Frequent relapse-immediately prior to the clinical trial	Four or more relapses (F) within any given 12-month period in the 2 years prior to the relapse occurrence date (H) immediately prior to the

	clinical trial.
K. Frequent relapse date	The last relapse date (H) that fulfills the definition of a frequent relapse (I and J).
L. Steroid dependence	Two consecutive relapses during the reduction of steroid therapy or within 2 weeks of discontinuation of steroid therapy.
M. Steroid dependence-immediately prior to the clinical trial	Steroid dependence (L) diagnosed in the 2 years prior to the relapse date (H) immediately prior to the clinical trial.
N. Date of steroid dependence	The second relapse date (H) on which the definition of steroid dependence is met.
O. Steroid resistance	When the daily administration of prednisolone at 60 mg/m²/day does not lead to remission (C) within 4 weeks.
P. Date of transition to steroid resistance	The date on which the definition of steroid resistance (O) is met.
Q. Partial remission	The expression of serum albumin exceeding 2.5g/dL and 1+ or above protein on urine dipstick test in the first morning.
R. Nephrotic state	The expression of serum albumin of 2.5g/dL or less and 1+ or above protein on urine dipstick test in the first morning.

4 RCRNS study group

Refer to Supplementary Appendix.

5 Introduction

5.1 Background information

5.1.1 Childhood-onset nephrotic syndrome and adult-onset nephrotic syndrome

Nephrotic syndrome is a condition where protein in the glomeruli of the kidney leaks out from the blood into the urine and hypoproteinemia and systemic edema develops as a result. The pathogenesis, while not sufficiently clear, is thought to be the enhancement of protein permeability as a result of cytokines produced from T-cells acting on slit membrane.

Childhood-onset nephrotic syndrome often occurs during the age of 2 to 6 and is often discovered at the time of onset as edema in the eyelid or lower leg. Approximately 90% of cases are idiopathic nephrotic syndrome and many patients display the minimal-change type where almost no change is seen in the glomeruli in light microscope findings[1)]. The drug of choice for the treatment of childhood-onset idiopathic nephrotic syndrome is orally-administered corticosteroids (hereafter, “steroids”). Steroid sensitivity is an important prognostic factor[2)] and the ISKDC definition of frequently relapsing nephrotic syndrome and steroid-resistant nephrotic syndrome given in accordance with the reactivity to steroids is used in shared foreign and domestic diagnostic criteria.

On the other hand, while 70% of adult-onset nephrotic syndrome cases are idiopathic nephrotic syndrome, the minimal-change type is rare and only accounts for 15-20% of cases while membranous nephropathy, focal segmental glomerulosclerosis and membranoproliferative glomerulonephritis account for the majority[3)]. Histopathology is an important prognostic factor and treatment is selected in accordance with the definitive diagnosis by renal biopsy[4)]. Although the oral administration of steroids is the primary treatment for adult-onset minimal-change type idiopathic nephrotic syndrome, the steroid dosage amount is greater and period of administration longer in comparison to childhood-onset idiopathic nephrotic syndrome[5)]. There is no common foreign and domestic definition of steroid-dependent, steroid-resistant and frequently relapsing type, etc. in adult-onset nephrotic syndrome equivalent to the ISKDC definitions relating to childhood-onset nephrotic syndrome. Adult-onset nephrotic syndrome differs from childhood-onset nephrotic syndrome with respect to pathology, diagnostic methods (definitions), treatment protocol and treatment efficacy criteria, etc. and cannot be treated in the same manner.

5.1.2 Treatment of childhood-onset idiopathic nephrotic syndrome

The drug of choice for the treatment of childhood-onset idiopathic nephrotic syndrome is orally-administered steroids. Eighty to ninety% of childhood-onset idiopathic nephrotic syndrome patients suffer from steroid-sensitive nephrotic syndrome which quickly goes into remission with the administration of steroids while 10-20% of patients suffer from steroid-resistant nephrotic syndrome that does not go into remission with the administration of steroids[6].

Thirty to forty% of steroid-sensitive nephrotic syndrome cases develop frequently relapsing nephrotic syndrome where multiple relapses are experienced within a relatively short period of time or steroid-dependent nephrotic syndrome where relapses occur as a result of discontinuation of steroid treatment or a reduction in steroid dosage[7][8]. Large doses of steroids are required to treat each relapse if childhood-onset idiopathic nephrotic syndrome develops into the frequently relapsing type or steroid-dependent type, and steroid-specific adverse events become a problem in continued treatment. To avoid this, treatment with immunosuppressive drugs is carried out with the purpose of facilitating the withdrawal from steroids or a reduction in dosage. The 2005 Japanese Society for Pediatric Nephrology treatment guidelines for idiopathic nephrotic syndrome in children v.1.0 recommend cyclosporine (3-6 mg/standard body weight (kg)/day for 2 years), cyclophosphamide (2-3 mg/standard body weight (kg)/day for 8-12 weeks) and mizoribine (4 mg/standard body weight (kg)/day) as immunosuppressive drugs for frequently relapsing nephrotic syndrome[9].

Cyclosporine has a relapse inhibitory effect and is effective in facilitating the withdrawal from steroids[10][11], however the majority of patients that discontinue cyclosporine once again progress to frequently relapsing or steroid-dependent nephrotic syndrome. In addition, approximately 20% of patients do not respond to cyclosporine. Cyclophosphamide at 2-3 mg/kg/day for 8 weeks is clearly effective as a treatment for frequently relapsing nephrotic syndrome [12], however it is considered to have no effect for steroid-dependent nephrotic syndrome. There are reports stating the effectiveness of cyclophosphamide at 2 mg/kg/day for 12 weeks[13] for steroid-dependent nephrotic syndrome as well as reports to the contrary[14].

Mizoribine has demonstrated high levels of safety, however a sufficient relapse inhibitory effect has not been observed[15][16] and a large-scale prospective controlled study is necessary to evaluate the efficacy and safety of mizoribine at high doses. The withdrawal from steroids poses problems for frequently relapsing and steroid-dependent nephrotic syndrome patients in whom remission cannot be maintained with treatment utilizing known immunosuppressive drugs.

Furthermore, as a moderate dose of cyclosporine (trough level: approx. 100ng/ml) administered for 2 years or more increases the frequency of chronic cyclosporine nephrotixcity[17] and a cumulative dose of cyclophosphamide exceeding 300 mg/kg increases the frequency of gonadal failure such as aspermia, etc.[18], there are problems in long-term administration and re-administration of these immunosuppressive drugs.

The withdrawal from steroids poses problems for patients that discontinue these immunosuppressive drugs and once again progress to frequently relapsing or steroid-dependent nephrotic syndrome.

Ten to twenty% of steroid-resistant nephrotic syndrome patients undergo treatment with immunosuppressive drugs; however the possibility of end-stage renal disease is high in the case of a failure to respond to immunosuppressive drugs. The guidelines mentioned above recommend treatment with cyclosporine alone or a combination of methylprednisolone pulse therapy[9)]. Although those treatments can induce remission (a change to steroid-sensitive nephrotic syndrome), the disease can develop into the frequently relapsing or steroid-dependent nephrotic syndrome during or after the discontinuation of treatment and withdrawal from steroids is difficult.

5.1.3 Treatment of childhood-onset complicated nephrotic syndrome

Childhood-onset complicated nephrotic syndrome is not clearly defined; however is it broadly classified into the following 2 types by pediatric kidney disease specialists both in Japan and abroad amongst whom a consensus has been obtained.

i. Cases that develop into frequently relapsing or steroid-dependent nephrotic syndrome despite steroid sensitivity and in whom remission cannot be maintained with known treatments and withdrawal from steroids is impossible.
ii. Steroid-resistant cases that do not react to known treatments and progress to end-stage renal disease.

The study population of this clinical trial is childhood-onset complicated nephrotic syndrome patients with steroid sensitivity (see “i” above), which is an important prognostic factor in idiopathic nephrotic syndrome. More specifically, the study population of this clinical trial can be defined as patients that fulfill any of the following from (1) to (3).

(1) Patients diagnosed with frequently relapsing or steroid-dependent nephrotic syndrome that do not respond to known immunosuppressive drug therapy such as cyclosporine, cyclophosphamide, mizoribine, etc. (whose disease reverts back to the frequently relapsing type or steroid-dependent type during immunosuppressive drug therapy)

(2) Patients diagnosed with frequently relapsing or steroid-dependent nephrotic syndrome in whom remission can be maintained through immunosuppressive drug therapy but whose disease reverts back to the frequently relapsing type or steroid-dependent type after the discontinuation of immunosuppressive drug therapy.

(3) Patients diagnosed with steroid-resistant nephrotic syndrome in whom the administration of immunosuppressive drugs leads to remission but whose disease develops into the frequently relapsing type or steroid-dependent type during or after the discontinuation of immunosuppressive drug therapy.

The target group of this study, childhood-onset complicated nephrotic syndrome, is estimated to account for 25-40% of pediatric idiopathic nephrotic syndrome cases. Known immunosuppressive drug therapy is not able to maintain remission of childhood-onset complicated nephrotic syndrome, rendering the long-term continuous administration of steroids necessary. As a result, many patients live with this disease for a long period of time from its onset during infancy (age 2-6) through adolescence and into adulthood[19, 20]. Childhood-onset complicated nephrotic syndrome patients range widely in age from 2-year-old patients to patients in their early-twenties.

As there are patients that do not respond to the treatment (the known immunosuppressive drug therapy is not able to maintain remission) and long-term administration and re-administration is difficult due to immunosuppressive drug-specific side effects, known immunosuppressive drug therapy is not useful for the treatment of childhood-onset complicated nephrotic syndrome from both the perspective of efficacy and safety. Therefore, most of patients with complicated nephrotic syndrome are treated with long-term steroids, which can result in short stature and osteoporosis in children[21] and patients that live with this disease through adolescence and into adulthood are often administered with steroids during these periods and have particular difficulty in avoiding short stature. In addition, patients can experience compression fractures of the vertebral bones and osteonecrosis of the femoral head as a consequence of osteoporosis and be hospitalized over a long period of time resulting in a decrease in quality of life and significant impact to their everyday lives. Accordingly, the development of a safe and effective treatment for childhood-onset complicated nephrotic syndrome patients in order to improve the current situation, where there is no useful treatment, is an issue that needs to be addressed as soon as possible.

5.2 Description of the investigational drug

5.2.1 IDEC-C2B8 investigational drug

The IDEC-C2B8 investigational drug is a monoclonal antibody directed against the CD20 differentiation antigen expressed on the surface of B-cells. It is a chimeric anti-CD20 monoclonal antibody consisting of human immunoglobulin constant regions (IgG1κ) and mouse anti-CD20 antibody variable regions.
IDEC-C2B8 is considered effective against various diseases caused by B-cell abnormalities as it specifically damages CD20-positive B-cells[22)23)] and domestic approval for its use in the treatment of CD20-positive B-cell non-Hodgkin's lymphoma (B-cell lymphoma) has already been obtained. It has been approved as a therapeutic agent for B-cell lymphoma in over 100 countries around the world such as Japan, the United States and Europe. It has also been approved as a therapeutic agent for anti-TNF

therapy-resistant refractory rheumatoid arthritis in Europe and the United States. Its regimen and dosage for these diseases is indicated below.

(1) Regimen and dosage for B-cell lymphoma

A 375 mg/m² dose is administered as rituximab via a weekly infusion in adults. The maximum number of doses is 8. When administered, this drug is diluted 10-fold in isotonic sodium chloride solution or a 5% injectable glucose solution.

(2) Regimen and dosage for anti-TNF therapy-resistant refractory rheumatoid arthritis

2 x 1000 mg/body doses are administered as rituximab via infusions carried out at 2 week intervals in adults.

The mechanism of action of IDEC-C2B8 has been confirmed from in vitro tests: it eliminates B-cells in vivo by binding to B-cell surface CD20 antigens and specifically damaging B-cells through complement-dependent cytotoxic actions and antibody-dependent-cell-mediated cytotoxic actions and directly acts upon CD20 antigen-positive B-cells, inhibiting their growth and inducing apoptosis[24-28)].

5.2.2 Indications expected to be acquired through this trial

Childhood-onset nephrotic syndrome (limited to complicated nephrotic syndrome that frequently relapses despite the administration of known immunosuppressive drug therapy)

5.2.3 Non-clinical trials

Type of test	Species/Strain	Method of administration	Period of administration	Dosage (mg/kg[A])	GLP applicable	Implementation facility
Single dose toxicity study	Cynomolgus monkey	Intravenous administration	Single dose	10, 30, 100	Applicable	Bozo Research Center
Repeated dose toxicity study	Cynomolgus monkey	Intravenous administration	1 month (once a week x 4)	0, 0.25, 2.1, 16.8	Applicable	White Sands Research
Repeated dose toxicity study	Cynomolgus monkey	Intravenous administration	2 months (once a week x 8)	0, 20	Applicable	Bio-Research Lab.
Hereditory toxicity study	Not done					
Carcinogenicity study	Not done					
Reproduction toxicity study	Not done					
Local stimulus study [B] (vasostimulant)	Crab-eating monkey	Intravenous administration	2 months (once a week x 8)	0, 20	Applicable	Bio-Research Lab.
Pyrogenetic study	Japanese white rabbit	Intravenous administration	Single dose	0, 30, 50, 100	Applicable	Nippon Experimental Medical Research Center
Cross -reactivity study with human normal tissue	Human normal tissue	In vitro	-	10 μg/mL	Applicable	IMPATH Laboratories

A: Unless otherwise specified, the NOAEL dosage underlined in repeated dose toxicity studies.

B: By histopathological examination of the site of administration in 2-month repeated dose toxicity studies.

5.2.4 Effect on the fetus

As IDEC-C2B8 is a monoclonal antibody with essentially the same qualities as immunoglobulins, it is believed that it may be possible for the drug to pass through the placenta to the fetus if administered to pregnant women.

When the effects of IDEC-C2B8 administration on the fetus during the period of organogenesis in pregnancy were examined using cynomolgus monkeys, a decrease in the number of lymphoid tissue B-cells in the fetus was observed depending on the dose administered to the mother. Accordingly, it is believed that IDEC-C2B8 passes through the placenta to the fetus and damages the B-cells of the fetus. Other than the above-mentioned decrease in the number of lymphoid tissue B-cells in the fetus, no other abnormalities were observed in the mother or the fetus.

Hypoplasia of lymphoid tissue such as lymph nodes and Peyer's patches, etc. as well as reduced resistance towards exogenous antigens such as viruses, etc. is observed in B-cell knockout mice lacking B-cells from the very beginning after generation, and it is thought as a result that B-cells are required in the formation of lymphoid tissue[29-31)].

It is inferred from the above that (1) IDEC-C2B8 will pass through the placenta to the fetus and damage the B-cells of the fetus if administered to pregnant women and (2) hypoplasia of lymphatic tissue may occur if the fetus is lacking B-cells in the period of organogenesis.

However, it should be noted that there is a report of a B-cell lymphoma patient that successfully gave birth to a baby girl via caesarean section at 35 weeks after undergoing combination therapy consisting of anticancer drugs and IDEC-C2B8 at the 21st week of pregnancy[32].

5.2.5 Domestic clinical trial targeting systemic lupus erythematosus (SLE)

(1) Phase I/II clinical trial

A phase I/II clinical trial with the primary purpose of safety evaluation was conducted in order to examine the tolerability of the regimen and dosage of IDEC-C2B8 recommended in foreign countries for autoimmune diseases (2 x 1,000 mg/body doses administered at 2-week intervals) in Japanese systemic lupus erythematosus (SLE) patients.

As there has been no domestic use of IDEC-C2B8 for the treatment of autoimmune diseases, the tolerability of 4 x 500 mg/body doses given at weekly intervals (similar to the already-approved regimen and dosage for B-cell lymphoma) in SLE patients was first confirmed, after which the regimen and dosage recommended in foreign countries for autoimmune diseases (2 x 1,000 mg/body doses administered at 2-week intervals) was examined.

In terms of safety, the infusion reactions that occurred with intravenous administration of IDEC-C2B8 were all mild to moderate (grade 1-2) and within the range observed in clinical trials targeting B-cell lymphoma. The side effects thought to be caused by IDEC-C2B8 were almost all mild to moderate (grade 1-2). There were 2 cases of grade 3 infection, but both subsided with the intravenous administration of antibiotics.

In terms of efficacy, 9 of the 15 subjects' BILAG global index scores decreased from their scores prior to the commencement of the clinical trial and a decrease in their disease activity was observed.

A reduction in the number of peripheral blood B-cells was observed in all subjects and in many cases this state was maintained for more than 6 months. A decreased level of immunoglobulins in the blood resulting from a reduced number of B-cells was not observed.

From the above, the regimen and dosage of 2 x 1,000 mg/body doses administered at 2-week intervals is expected to be well-tolerated by Japanese patients and prove clinically useful and therefore it was determined as the recommended dose in confirmatory trials.

(2) Phase II/III clinical trial

A phase II/III clinical trial targeting systemic lupus erythematosus (SLE) in order to compare and contrast the safety and efficacy of the regimen and dosage of IDEC-C2B8 at 2 x 1,000 mg/body doses administered at 2-week intervals in combination with steroids with the same dose of IDEC-C2B8 administered in combination with a placebo as a steroid substitute is currently under way.

[Subjects]: SLE patients exhibiting disease activity despite the continuation of a fixed amount of steroid therapy

[Study design]: Multicenter, placebo-controlled, double-blind, randomized allocation parallel group trial

[Primary endpoint]: Proportion of effective cases (complete or partial remission)

5.3 Important safety information

5.3.1 Progressive Multifocal Leukoencephalopathy (PML)

There have been 2 cases reported overseas (1 in the United States and 1 in the United Kingdom) where commercially-available IDEC-C2B8 was used off-label in the treatment of adult SLE patients who subsequently developed progressive multifocal leukoencephalopathy (PML) and died. The 2 adult SLE patients who developed PML and died had a long SLE treatment history and had undergone immunosuppressive drug therapy multiple times prior to treatment with IDEC-C2B8. In these 2 cases, treatment with IDEC-C2B8 alone was the final treatment prior to the onset of PML and both patients developed PML and died within 12 months of their final dose of IDEC-C2B8. A relationship between IDEC-C2B8 and the onset of PML cannot be completely ruled out.

In addition, there are also reports of a subject in a United States clinical trial carried out to ensure the long-term safety of IDEC-C2B8 in the treatment of adult rheumatoid arthritis (RA) who developed PML and died. This particular patient had undergone treatment with methotrexate, multiple types of steroids and TNFα antagonists prior to treatment with IDEC-C2B8 and had a complex condition which included iatrogenic conditions as well as rheumatic conditions including Sjogren's syndrome. The subject received a total of 4 times of 2 x 1,000 mg/body doses of IDEC-C2B8, developed oropharyngeal cancer 7 months after the final dose of IDEC-C2B8 and subsequently underwent a chemotherapy regimen containing platinum and radiotherapy. The subject developed PML and died approximately 18 months after the final dose of IDEC-C2B8 and 9 months after the completion of chemotherapy and radiotherapy. Again, a relationship between IDEC-C2B8 and the onset of PML cannot be completely ruled out.

PML is a progressive demyelinating disease of the central nervous system thought to develop from the activation of the polyomavirus JC virus. The JC virus is thought to latently infect approximately 80% of healthy adults and be activated under immunosuppressive conditions, resulting in the onset of PML.

However, the mechanism leading to the activation of the JC virus is not well understood. The incidence of PML is rare; however its onset carries a poor prognosis and leads to severe disability or death.

The onset on PML has been reported in HIV-positive patients, cancer patients in an immunosuppressive state, organ transplant recipients and patients with autoimmune diseases including SLE. PML has also been reported in SLE patients with no history of treatment with IDEC-C2B8. Many SLE patients with no history of treatment with IDEC-C2B8 that develop PML had undergone a combined treatment of steroids and immunosuppressive drugs and there are also reports of SLE patients undergoing steroid therapy alone developing PML. Until now, there has been only a single report of the onset of PML in RA patients with a history of treatment of IDEC-C2B8.

The onset of PML in B-cell non-Hodgkin's lymphoma patients treated with IDEC-C2B8 has already been reported. Many of these patients received IDEC-C2B8 in combination with chemotherapy or hematopoietic stem cell transplantation.

At present, there is no standard diagnostic method for PML and PML is suspected when new neurological abnormalities are observed in patients treated with IDEC-C2B8. In such cases, a neurological specialist is consulted and brain MRI and cerebrospinal fluid examination (JC virus DNA testing), etc. are carried out.

5.3.2 Aseptic meningoencephalitis / acute pulmonary thromboembolism

It is reported that 2 of the 6 subjects who participated in a domestic clinical trial of IDEC-C2B8 re-administration to adult SLE patients experienced deterioration of their SLE condition after treatment with IDEC-C2B8 (1 case of transverse myelitis [unrecovered] and 1 case of aseptic meningoencephalitis / acute pulmonary thromboembolism [resulting in death]). The case of transverse myelitis developed approximately 3 weeks after the final dose of IDEC-C2B8 and the case of aseptic meningoencephalitis / acute pulmonary thromboembolism developed 7 months after the final dose of IDEC-C2B8. A relationship between IDEC-C2B8 and the onset of these conditions cannot be completely ruled out.

The points common to the 2 cases described above are: (1) although an effect was observed from the initial treatment with IDEC-C2B8, both subjects received repeated IDEC-C2B8 treatment due to a subsequent relapse of symptoms, (2) their condition was complicated by antiphospholipid antibody syndrome and (3) an emergence of HACA had been observed in the past. In addition, the patient that experienced transverse myelitis exhibited a strong infusion reaction when IDEC-C2B8 was re-administered.

The reason these adverse events occurred is thought to be that thrombogenic tendencies are observed in patients with antiphospholipid antibody syndrome complications and such patients are at a high risk of developing central nervous system symptoms and embolism to begin with. In addition, thrombus formation is promoted by cytokines and activated complements. It is reported that cytokines such as TNF-α and IL-6

are released when an infusion reaction occurs with the administration of IDEC-C2B8 and it is thought possible that thrombus formation was promoted in these 2 cases as a result of cytokine release.

Patients whose condition is complicated by malignant tumors or autoimmune diseases or patients with a medical history of malignant tumors or autoimmune diseases are excluded from this clinical trial from the viewpoint of ensuring the safety of subjects. In addition, subsequent IDEC-C2B8 administration will be discontinued in subjects in whom a strong infusion reaction (grade 3 or higher non-hematological toxicity) is observed with the intravenous administration of IDEC-C2B8 (infusion of the investigational drug will be discontinued at the point the grade 3 infusion reaction is observed).

5.4 Medical validity of this clinical trial

The exact pathogenesis of nephrotic syndrome is unclear, but T-cell-mediated immunological abnormalities are thought to play a role. An increase in soluble CD23 (B-cell activation marker) and soluble CD25 (T-cell activation marker) is reported in patients with nephrotic syndrome at the time of relapse[33)] and B-cells have also been implicated in the pathogenesis of nephrotic syndrome. B-cells interact with T-cells by issuing a signal that activates clonal expansion and the effector function of CD4+ T-cells, etc[34)]. Treatment with this drug aims to suppress the relapse of nephrotic syndrome by controlling B-T-cell interaction through the in-vivo elimination of B-cells.

The application of rituximab to pediatric nephrotic syndrome patients started to be examined after a report of the remission of nephrotic syndrome following the administration of rituximab for the treatment of ITP in a 16-year-old patient with refractory nephrotic syndrome (onset: age 2) complicated by ITP[35)] and a report of the remission of nephrotic syndrome following the administration of rituximab for the treatment of lymphoproliferative disease in a 12-year-old patient that received a kidney transplant as a result of underlying focal segmental glomerulosclerosis and subsequently suffered a relapse of nephrotic syndrome at the same time as the onset of post-transplant lymphoproliferative disease[36)].

Since 2004, case reports of the administration of rituximab to childhood-onset complicated nephrotic syndrome patients (a total of 8 patients, aged 7-23), whose condition remained steroid-dependent or frequently relapsing and could not withdrawal from steroids even under the administration of multiple immunosuppressive drugs, that resulted in nephrotic syndrome remission and subsequently enabled the reduction in dose or withdrawal from steroids or immunosuppressive drugs have been observed in international journals and at international conferences[35,37-41)]. In those reports, the most common dosage of rituximab administered was 4 x 375 mg/m² (maximum dose: 500 mg) and no rituximab-specific infusion reactions or other serious adverse events were observed.

There is also a report in which 4 x 375 mg/m² doses (maximum dose: 500 mg) of rituximab were

administered to 5 steroid-resistant childhood-onset complicated nephrotic syndrome patients (age: 2.8-15 years, onset: age 1-3.3 years) who remained in a nephrotic state despite treatment with multiple immunosuppressive drugs[42]. Three of these 5 patients experienced remission and the other 2 patients experienced partial remission following the administration of rituximab and a reduction in steroid and immunosuppressive drug dosage was possible. No infusion reaction or severe infections were observed in these 5 cases. Another report reveals that a pediatric steroid-resistant focal segmental glomerulosclerosis patient was administered a single 375 mg/m² (maximum dose: 500 mg) dose of rituximab after which their symptoms of proteinuria disappeared and their dosage of steroids and immunosuppressive drugs could be reduced and ultimately discontinued[43].

Finally, there is a report of a 22-year-old childhood-onset complicated nephrotic syndrome patient administered with 2 x 1000 mg doses of rituximab at an interval of 2 weeks who experienced remission of nephrotic syndrome (remission was maintained for 5 months) and in whom the dosage of steroids and immunosuppressive drugs could subsequently be reduced[44].

The following benefits are considered to be obtained from the relapse inhibitory effect of rituximab: (1) extension of the remission maintenance period, (2) withdrawal from or reduced dosage of steroids, (3) reduction or discontinuation of immunosuppressive drugs, (4) decrease in adverse reactions from steroids and immunosuppressive drugs and (5) improved patient quality of life (through shortening the period of drug treatment and reducing the patient's movement/action limitations, etc).

From the above, the IDEC-C2B8 investigational drug is expected to be an effective and safe new drug for the treatment of "childhood-onset complicated nephrotic syndrome" patients who do not respond to known treatment and for whom the long-term continuous administration of steroids is necessary, ultimately resulting in significant adverse drug reactions.

However, while there are case reports of the administration of rituximab to pediatric complicated nephrotic syndrome patients, no high-quality clinical trials have been conducted to clarify its efficacy and safety. Therefore, we have decided to launch a study team led by principal researcher Kazumoto Iijima and conduct a physician-initiated clinical trial in order to examine the efficacy and safety of IDEC-C2B8 in the treatment of childhood-onset complicated nephrotic syndrome patients.

The regimen and dosage of IDEC-C2B8 has been set at 4 x 375 mg/m² doses given at weekly intervals. The rationale for this is that 4 x 375 mg/m² doses (maximum dose: 500 mg) have been approved domestically for the treatment of adult B-cell lymphoma and safety information has been obtained, the same dosage has been used in clinical studies on pediatric autoimmune diseases where its safety and efficacy has been suggested and this is also the dosage primarily used in childhood-onset complicated nephrotic syndrome case reports.

This clinical trial has been selected to the Clinical Research Promotion Program (clinical trial plan) supported by the Japan Medical Association Center for Clinical Trials and has been planned as a trial that adheres to Good Clinical Practice (GCP).

The results obtained from this clinical trial will become an evaluation material in conjunction with foreign information regarding regimen and dosage, efficacy and safety and Zenyaku Kogyo Co., Ltd. will be requested to make an application for the drug's expanded indication. Zenyaku Kogyo Co., Ltd have agreed to provide the investigational drugs and to apply for orphan drug designation.

6 Purpose

The purpose of this clinical trial is to compare childhood-onset complicated nephrotic syndrome patients administered with 4 x 375 mg/m² doses (maximum dose: 500 mg) of IDEC-C2B8 with those administered with a placebo to evaluate the efficacy and safety of IDEC-C2B8.

6.1 Efficacy endpoints

6.1.1 Primary endpoint

(1) Relapse-free period during the observation period (day 1 - day 365):
The period from the date of registration / allocation until the date of the occurrence of the first relapse following commencement of investigational drug administration

6.1.2 Secondary endpoints

(1) Period until treatment failure:
The period from the date of registration / allocation until the date at which treatment failure is determined.

(2) Relapse rate:
The number of relapses observed per person per year in each group during the observation period

(3) Period until occurrence of frequent relapses:
The period from the date of registration until the frequent relapse date during the observation period

(4) Period until steroid dependence:
The period from the date of registration until the date of steroid dependence during the observation period

(5) Period until the transition to steroid resistance:
The period from the date of registration until the date of transition to steroid resistance during the observation period

(6) Total and daily dosage of steroids:
The total and daily dosage of steroids administered from the date of registration / allocation until the date of completion of the observation period
Daily steroid dosage (mg/day) =Total steroid dosage (mg) / observation period (days)

(7) Change in the total and daily dosage of steroids in the 365 days before and after the date of registration:

The difference of the total and daily dosage of steroids administered in the 365 days between immediately before the date of registration / allocation (not including the day of registration / allocation) and after the date of registration / allocation.

6.2 Other endpoints

(1) IDEC-C2B8 blood concentration

(2) Changes in the number of B-cells in peripheral blood

1) Number of B-cells in peripheral blood (cells/μL)
2) Peripheral blood B-cell depletion period

(3) Human anti-chimeric antibody (HACA) production ratio

6.3 Safety endpoints

Subjective symptoms, objective findings, physical examination (vital signs, height, weight), clinical examination (hematological examination, blood biochemical examination, urinalysis), electrocardiogram, chest X-ray

7 Clinical trial design

7.1 Type of clinical trial

Multicenter, double-blind, randomized, placebo-controlled trial

7.2 Clinical trial diagram

The clinical trial period for each patient will begin at the date consent is obtained and end at the date of the completion of the observation period.

<table>
<tr><td colspan="6">Clinical trial period (date consent is obtained~date of completion of the observation)</td></tr>
<tr><td colspan="3">Screening period
(date consent is obtained~up to 35 days)</td><td rowspan="2">Up to
14 days</td><td colspan="2">Observation period (Day 1~Day 365, 1 year)</td></tr>
<tr><td>Obtaining consent</td><td>Screening</td><td>Registration /allocation</td><td>Investigational drug administration period (Day 1~day of discontinuation /completion)</td><td>Post discontinuation/ completion of investigational drug administration~day 365</td></tr>
</table>

The day the first dose of investigational drug is administered is set as “Day 1”

7.3 Target number of subjects

Target number of subjects: 60 (30 in each group)

7.4 Planned clinical trial period

Planned subject registration period: September 2008 – August 2011 (3 years)

Planned clinical trial period: September 2008 – August 2012 (4 years)

[Rationale]: It is estimated that approximately 20-30 pediatric frequently relapsing nephrotic syndrome patients progress to complicated nephrotic syndrome every year. Accordingly a 3-year subject registration period and 4-year clinical trial period will be set.

7.5 Study design-related considerations

The purpose of this clinical trial is to evaluate the efficacy and safety of IDEC-C2B8 in the treatment of childhood-onset complicated nephrotic syndrome patients.

Childhood-onset complicated nephrotic syndrome is not a domestically-approved indication for

IDEC-C2B8 and no well-designed clinical trials of IDEC-C2B8 on childhood-onset complicated nephrotic syndrome patients have been conducted in Japan. The decision to conduct a multicenter double-blind, randomized, placebo-controlled clinical trial was made for the following reasons: the 4 x 375 mg/m² doses administered at weekly intervals adopted in this study have already been approved for the treatment of adult B-cell non-Hodgkin's lymphoma both domestically and abroad and safety-related information is considered to have been obtained from clinical experience despite the differing target disease. In addition, the number of childhood-onset complicated nephrotic syndrome patients is limited and the immediate development of treatment for this condition is desired.

Whether immunosuppressive drugs are used at the time of relapse immediately prior to registration, whether steroids are used at the time of relapse immediately prior to registration and the disease activity are predicted to affect the therapeutic efficacy and prognosis and will therefore be used as allocation adjustment factors. Random assignment will be carried out and the investigational drug subsequently administered. A proteinuria-reducing effect was observed up to 3 months after the initiation of administration in a report where 4 x 375 mg/m² doses (maximum dose: 500 mg) of rituximab were administered to a childhood-onset complicated steroid-resistant nephrotic syndrome patient [42)]. Based on this report, it is thought that at least 3 months is required for the expression of IDEC-C2B8 efficacy. Accordingly, immunosuppressive drugs such as cyclosporine, tacrolimus, mizoribine, azathioprine and mycophenolate mofetil that had been continuously administered to subjects for a long period of time prior to the start of this clinical trial will continue to be administered to the subject enrolled in this trial for the first 3 months after the start of administration of the investigational drug.

As steroid treatment for relapse during the study period will affect the therapeutic efficacy of the investigational drug and prognosis, their administration method will be prescribed.

As the subjects of this clinical trial are patients with high disease activity in whom remission cannot be maintained with known treatment, early and frequent relapses are concern. Accordingly, “treatment failure” has been established whereby the double-blinding will be urgently cancelled if treatment failure is determined and the principal investigators and sub-investigators will be able to select the optimal treatment for the subject. Double-blinding will also be urgently cancelled in the event a subject experiences serious adverse events after which the independent data and safety monitoring committee will make an evaluation regarding safety. The efficacy primary endpoint is the period from the date of registration / allocation until the date of the occurrence of the first relapse following commencement of investigational drug administration. A relapse is defined as when 2+ or above protein on urine dipstick in the first morning urine is observed for 3 consecutive days and prednisolone treatment is required (objective indicator). As a determination of treatment failure (1) - (3) will occur after the date of the occurrence of the first relapse following the commencement of investigational drug administration, the urgent allocation code disclosure of subjects in whom treatment failure is determined will not affect the evaluation of the efficacy primary

endpoint.

In addition, as all efficacy secondary endpoints are defined by objective measures such as relapse and steroid dose, etc. and the information up until the date of treatment failure is used for evaluation with regards to the subjects in whom treatment failure is determined, the urgent allocation code disclosure of subjects in whom treatment failure is determined will not affect the evaluation of the efficacy secondary endpoints. Furthermore, the scope of allocation code disclosure for subjects in whom treatment failure is determined is minimized in order to avoid effects from all aspects.

In terms of safety evaluation, the name and grade of adverse events have been standardized by evaluation with the Common Terminology Criteria for Adverse Events (CTCAE) v3.0 August 9, 2006 (Japanese translation: JCOG/JSCO edition, March 8, 2007).

7.5.1 Rationale for setting the study population

Patients in whom, despite steroid sensitivity, the treatment with known immunosuppressive drugs cannot prevent relapses and the long-term continuous administration of steroids (ranging from a few years to 10 years) is required, which ultimately causes serious side effects, were defined as “childhood-onset complicated nephrotic syndrome” patients and set as the study population of this clinical trial.

Steroid sensitivity is the most important prognostic factor of pediatric idiopathic nephrotic syndrome[2)] and it is assumed that patients will not progress to end-stage renal disease as long as they display steroid sensitivity. Childhood-onset complicated steroid resistant nephrotic syndrome patients do not respond to known treatments and progress to end-stage renal disease. As conditions with a different prognosis cannot be evaluated with the same endpoint, childhood-onset complicated steroid resistant nephrotic syndrome patients have been excluded from the study population.

In addition, generally, steroid-dependent nephrotic syndrome is considered a higher disease activity condition than frequently-relapsing nephrotic syndrome, however approximately 80% of Japanese frequently-relapsing nephrotic syndrome patients are also steroid-dependent. Many of the domestic clinical studies conducted on pediatric nephrotic syndrome patients so far have treated frequently-relapsing nephrotic syndrome patients and steroid-dependent nephrotic syndrome patients as the same patient population. From the above, it has been decided to consider frequently-relapsing nephrotic syndrome patients and steroid-dependent nephrotic syndrome patients as the same patient population in this study.

As a history of treatment with immunosuppressive drugs may affect the disease activity at the time of registration, the therapeutic efficacy of IDEC-C2B8 and prognosis (relapse frequency), the study population has been limited to patients undergoing treatment with the immunosuppressive drugs recommended in or in accordance with the 2005 Japanese Society for Pediatric Nephrology treatment

guidelines for idiopathic nephrotic syndrome in children v.1.0 [9].

As steroid treatment for relapses prior to registration for this clinical trial affects prognosis (relapse frequency) as well as the diagnosis of frequently-relapsing / steroid-dependent nephrotic syndrome, the study population has been limited to patients undergoing treatment prescribed by or in accordance with the International Study of Kidney Disease in Children (ISKDC).

Many childhood-onset complicated nephrotic syndrome patients live with this disease for a long period of time from its onset during infancy (age 2-6) through adolescence and into adulthood[19,20]. The age distribution of the childhood-onset complicated nephrotic syndrome patients in the 8 foreign case reports in which rituximab was administered was 7-23 years[35,37-41,44]. Based on these reports, no maximum age limit was set for the study population of this trial and the minimum age limit was set at 2 years (at the time of registration).

It is reported that almost all adult patients with B-cell lymphoma continue to maintain a normal serum immunoglobulin (IgG, IgA, IgM) concentration range after the administration of IDEC-C2B8 and, in addition, the administration of IDEC-C2B8 does not enhance their immunosuppressive state and few cases with an increase in opportunistic infections are seen as a result[45-48]. The cause of the main side effect of IDEC-C2B8, infusion reaction, is assumed to be chemical mediators and cytokines released by B-cells in peripheral blood and the severity of an infusion reaction is thought to depend more on the immune response and the number of B-cells in peripheral blood rather than the dosage amount per dose. As the immune response in children aged 2 years and older is similar to that of an adult[49,50], it is considered unlikely that they would have more frequent and more severe infusion reactions in comparison with adults.

7.5.2 Rationale for setting the primary endpoint

As childhood-onset complicated nephrotic syndrome is a high-activity disease in which relapses frequently occur, the goal of treatment is to induce remission and, at the same time, reduce the frequency of relapse and maintain remission. Accordingly, the relapse-free period (the period in which remission is maintained) was thought to be the most important efficacy evaluation index in this study.

7.5.3 Rationale for setting the screening period and observation period

It is necessary to confirm the presence/absence of HIV, HCV, HBV and other viral infections, the presence/absence of CD20-positive cells in peripheral blood and steroid sensitivity in order to evaluate the eligibility of subjects for this trial. As it takes about 7 days to search for viral infections and up to 28 days from the date of the commencement of treatment for a relapse to confirm steroid sensitivity, the screening period of this study will be set at 35 days from the date consent is obtained.

According to the foreign case reports where childhood-onset complicated nephrotic syndrome patients were

treated with rituximab, remission was maintained for 9 months or longer in 7 out of the 8 patients (1 patient had been in remission for 5 months at the time of the report)[35, 37-41,44]. In addition, the ISKDC definition of frequent relapse is relapse occurring 4 times in any 12-month period[51]. Accordingly, the observation period of this study was set at 12 months from the date investigational drug administration is commenced.

7.5.4 Rationale for setting the regimen and dosage of IDEC-C2B8

While IDEC-C2B8 eliminates B-cells in vivo, new B-cells are continuously produced from bone marrow hematopoietic stem cells and normal B-cell reconstitution is expected following treatment with this drug. Accordingly, it is necessary to select a regimen and dosage of IDEC-C2B8 that is both safe and sufficient to eliminate B-cells in vivo. Similar to a normal immunoglobulin, IDEC-C2B8 is inactive and may not result in any direct adverse reactions after the target CD20 antigen has been completely eliminated.

The 4 x 375 mg/m² doses (maximum dose: 500 mg) administered in weekly intervals set in this clinical trial is the regimen and dosage that has already been approved for adult B-cell lymphoma patients in Europe and the United States. In a domestic clinical trial on adult B-cell lymphoma patients, it was confirmed that 4 x 375 mg/m² doses administered in weekly intervals depleted the number of peripheral blood B-cells (to below the detectable limit) and this state was maintained for 6 to 9 months[45]. In the same trial, antipyretic analgesic and anti-allergic agents were administered as pre-treatment in order to alleviate the infusion reaction anticipated from administration of IDEC-C2B8. The main side effect of IDEC-C2B8 administration in the trial was infusion reaction (fever, chills, nausea, headache, pruritus, rash, cough, hypotension, lethargy and laryngeal edema) that occurred along with infusion and more than 90% of these infusion reactions were mild (grade 1 or 2)[45].

A decrease in serum immunoglobulin (IgG, IgA, IgM) concentration and resulting increase in infection has not been observed in foreign clinical studies[46-48].

In a United States clinical study where 4 x 375 mg/m² doses of rituximab were administered to SLE patients, a similar peripheral blood B-cell depletion period was observed together with therapeutic efficacy as a decrease in SLE disease activity. Once again, the main side effect of rituximab administration was infusion reaction[52].

There is no IDEC-C2B8 regimen and dosage prescribed for children. Clinical studies of rituximab targeting pediatric autoimmune disease patients such as SLE patients[53-58], idiopathic thrombocytopenic purpura patients[59-67] and autoimmune hemolytic anemia patients [68-85] have been conducted overseas and almost all of these studies have utilized the 4 x 375 mg/m² dose that has already been approved for adult B-cell lymphoma patients. It has been reported in these clinical studies of rituximab targeting pediatric autoimmune disease patients that the subjects' peripheral blood B-cells became depleted after treatment and this state was maintained for 6 months, the main side effect was again infusion reaction (grade 1, 2) and the

4 x 375 mg/m² dose was excellent in terms of tolerability.

The 4 x 375 mg/m² dose has been the main dosage observed in foreign reports of the administration of rituximab to childhood-onset complicated nephrotic syndrome patients. In 6 case reports of patients administered with this dosage (6 patients, age: 12-23 years), 5 out of 6 patients' peripheral blood B-cells became depleted and this state was maintained for 5 to 12 months. Furthermore, all 6 patients experienced a remission of nephrotic syndrome (remission was maintained for 7 to 13 months) and were able to withdrawal from or reduce their dosage of steroids / immunosuppressive drugs. No serious adverse events considered to be caused by this drug were observed in any of these patients[35, 37, 38)]. Accordingly, it is believed that the nature and severity of side effects experienced by childhood-onset complicated nephrotic syndrome patients upon the administration of a 4 x 375 mg/m² dose of IDEC-C2B8 are no different to those experienced by adult B-cell lymphoma and SLE patients.

On the other hand, remission was maintained for 10 months and no serious adverse events observed in a case report of a childhood-onset complicated nephrotic syndrome patient (1 patient, age: 14 years) administered with a single 375 mg/m² dose (maximum dose: 500 mg/ of rituximab[41)]. When 15 childhood-onset complicated nephrotic syndrome patients in Japan (age: 5-19 years) were administered with a single 375 mg/m² dose of rituximab (observation period: 3-11 months, median: 7 months), all 15 patients were able to withdrawal from or reduce their dosage of steroids and their median relapse-free period was 129 days (range: 8-270 days). Depletion of peripheral blood B-cells was observed in 13 of these 15 patients with a median depletion period of 120 days (range: 60-245 days). Infusion reactions were observed in 7 of these 15 patients, but all reactions were mild grade 1 reactions and resolved without treatment. The outcomes of these 15 patients indicate that a single 375 mg/m² dose tends to result in a shorter remission maintenance period and B-cell depletion period compared with a 4 x 375 mg/m² dose.

It is reported in both childhood-onset complicated nephrotic syndrome case reports and clinical studies on SLE patients that peripheral blood B-cells had reappeared at the time of relapse and accordingly it has been inferred that there is a relationship between peripheral blood B-cell depletion and the improvement of clinical symptoms. A high tendency towards HACA detection in low dose groups (single 100 mg/m² dose or single 375 mg/m² dose) has also been reported in a United States phase I/II clinical study on SLE patients[86)].

It is inferred that a dosage lower than the dosage approved for the use in the treatment of B-cell lymphoma cannot sufficiently eliminate peripheral blood B-cells and Genentech Inc. has recommended that a dosage sufficient to eliminate B-cells be used in clinical trials on adult autoimmune disease patients and that treatment of low-dosage groups be discontinued in ongoing clinical trials. Accordingly, it is believed that a dosage that can sufficiently eliminate B-cells from peripheral blood similar to the dosage used for the treatment of B-cell lymphoma should be used in cases of childhood-onset complicated nephrotic syndrome.

Based on the above information, this clinical trial on childhood-onset complicated nephrotic syndrome patients has also adopted a IDEC-C2B8 regimen and dosage of 4 x 375 mg/m² doses (maximum dose: 500 mg) administered in weekly intervals.

8 Subject inclusion criteria and exclusion criteria

8.1 Inclusion criteria

Potential patients must meet all of the following criteria to be eligible for enrollment in this clinical trial:

(1) Patients who fulfilled the diagnostic criteria of idiopathic nephrotic syndrome (A) patients. (The ISKDC idiopathic nephrotic syndrome diagnostic criteria is used as a reference during the patient's initial diagnosis)

(2) Patients who were aged between 1 and 18 years old at the time of onset of idiopathic nephrotic syndrome and are aged 2 years or above at the time of registration.

(3) Patients who satisfy any of the following from 1) – 3).

1) Diagnosed with frequent relapse (I) or steroid dependence (L) and once again diagnosed with frequent relapse (J) or steroid dependence (M) after completion of immunosuppressive drug therapy (cyclosporine, cyclophosphamide, mizoribine, etc.)

2) Diagnosed with frequent relapse (I) or steroid dependence (L) and once again diagnosed with frequent relapse (J) or steroid dependence (M) during immunosuppressive drug therapy (cyclosporine, cyclophosphamide, mizoribine, etc.)

3) Diagnosed with steroid resistance (O) following the onset of idiopathic nephrotic syndrome and diagnosed with frequent relapse (J) or steroid dependence (M) during or after the completion of immunosuppressive drug therapy (cyclosporine alone or combination of cyclosporine and methylprednisolone, etc.)

(4) Patients for whom the last 3 nephrotic syndrome relapse dates (H) prior to registration can be confirmed.

(5) Patients in whom steroid sensitivity is observed during treatment of relapse (G) immediately prior to registration

(6) Patients in whom 5/μL or more CD20-positive cells are observed in the peripheral blood.

(7) Patients who are able to stay in hospital overnight on all days of planned investigational drug administration.

(8) Patients who, on their own behalf (aged 20 or above) or through a legal representative (parents or legal guardians), have received an adequate explanation regarding the implementation of this clinical

trial and given their consent using a document approved by the institutional review board.

[Rationale for setting the selection criteria]

(1), (2) To limit the trial to childhood-onset idiopathic nephrotic syndrome patients.

(3) To limit the trial to patients with complicated nephrotic syndrome in line with the purpose of this trial.

(4) To facilitate efficacy evaluation.

(5) Because steroid sensitivity is the most important prognostic determinant.

(6) Because the investigational drug is a CD20 antigen-specific monoclonal antibody.

(7) To quickly discover and respond to any adverse events that occur immediately following administration.

(8) Based on article 50 of the GCP ministerial ordinance.

A. Nephrotic syndrome	Urine protein-to-creatinine ratio of 1.8 or above and serum albumin of 2.5g/dL or below
B. Complicated nephrotic syndrome	A patient that fulfills any of the following from (1) to (3) is deemed to suffer from complicated nephrotic syndrome: (1) Diagnosed with frequent relapse (I) or steroid dependence (L) and once again diagnosed with frequent relapse (J) or steroid dependence (M) after completion of immunosuppressive drug therapy (cyclosporine, cyclophosphamide, mizoribine, etc.) (2) Diagnosed with frequent relapse (I) or steroid dependence (L) and once again diagnosed with frequent relapse (J) or steroid dependence (M) during immunosuppressive drug therapy (cyclosporine, cyclophosphamide, mizoribine, etc.) (3) Diagnosed with steroid resistance (O) and diagnosed with frequent relapse (J) or steroid dependence (M) during or after the completion of immunosuppressive drug therapy (cyclosporine alone or combination of cyclosporine and methylprednisolone, etc.)
C. Remission	Negative protein on urine dipstick test in the first morning urine for 3 consecutive days.
D. Remission confirmation date	The date remission (C) is confirmed at the medical institution.
E. Steroid sensitivity	When the daily administration of prednisolone at 60 mg/m²/day leads to remission (C) within 4 weeks.
F. Relapse	Protein 2+ or above detected by urine dipstick in the first morning urine for 3 consecutive days and prednisolone treatment is required.
G. Relapse – immediately prior to	A relapse (F) that occurred in the 35 days prior to registration.

the clinical trial	
H. Relapse date	The first day that 2+ or above protein on urine dipstick test in the first morning urine, followed by the same observation for 3 consecutive days (the relapse diagnosis date is also an acceptable date with regard to the 3 most recent relapses prior to registration).
I. Frequent relapse	Two or more relapses (F) within 6 months after initial remission (C) or 4 or more relapses (F) within any 12-month period.
J. Frequent relapse – immediately prior to the clinical trial	Four or more relapses (F) within any given 12-month period in the 2 years prior to the relapse date (H) immediately prior to the clinical trial.
K. Date of diagnosis of Frequent relapse	The last relapse date (H) that fulfills the definition of a frequent relapse (I and J).
L. Steroid dependence	Two consecutive relapses (F) during the reduction of steroid therapy or within 2 weeks of discontinuation of steroid therapy.
M. Steroid dependence - immediately prior to the clinical trial	Steroid dependence (L) diagnosed in the 2 years prior to the relapse date (H) immediately prior to the clinical trial.
N. Date of diagnosis of steroid dependence	The second relapse date (H) on which the definition of steroid dependence is met.
O. Steroid resistance	The daily administration of prednisolone at 60 mg/m²/day does not lead to remission (C) within 4 weeks.
P. Date of diagnosis of transition to steroid resistance	The date on which the definition of steroid resistance (O) is met after the daily administration of prednisolone at 60 mg/m²/day for 4 weeks.

8.2 Exclusion criteria

Patients who fall under any of the following criteria will not be enrolled in this clinical trial:

(1) Patients who have been diagnosed with nephritic-nephrotic syndrome such as IgA nephropathy prior to registration or in whom secondary nephrotic syndrome is suspected.

(2) Patients with a history of treatment with immunosuppressive drugs other than those listed below following the onset of nephrotic syndrome.

1) Cyclosporine, tacrolimus
2) Cyclophosphamide
3) Mizoribine, azathioprine
4) Mycophenolate mofetil
5) Chlorambucil

(3) Patients suffering from or with a history of severe infections such as pulmonary tuberculosis and deep mycosis.

(4) Patients infected with HIV, HCV or HBV or suffering from other active viral infections.

(5) Patients inoculated with live vaccine within 4 weeks prior to registration.

(6) Patients with poorly controlled hypertension even with antihypertensive drug treatment at the time of registration. Patients that correspond to the diagnostic criteria for hypertension shown below[87,88)] at the time of registration cannot be registered for this trial.

	Hypertension	
Age	Systolic blood pressure	Diastolic blood pressure
2 years old	≧112	≧74
3-5 years old	≧116	≧76
6-9 years old	≧122	≧78
10-12 years old	≧126	≧82
13-15 years old	≧136	≧86
16-18 years old	≧142	≧92
18 years old or above	Systolic blood pressure≧140 or diastolic blood pressure ≧90	

(7) Patients with reduced renal function at the time of registration (estimated glomerular filtration rate (GFR) of less than 60mL/min/1.73m²)

The estimated GFR is calculated from height and serum creatinine levels using the following formula[89,90]:

Estimated GFR = k x height (cm) / serum creatinine level (mg/dL)

The Jaffe method is used for the serum creatinine value in the above formula.

Serum creatinine value (Jaffe method) = Serum creatinine value (EIA method) + 0.2

In addition, the below values are used for k according to age.

Age	k-value
1-12 years old	0.55
13-21 years old female	0.55
13-21 years old male	0.70

(8) Patients with severe liver dysfunction at the time of registration (if under the age of 21: GOT or GPT equal to or greater than 2.5x the upper limit of normal determined by the "Reference Intervals of Clinical Tests in Children Determined by a Latent Reference Value Extraction"[91] upper limit of normal; if aged 21 or above: GOT or GPT equal to or greater than 2.5x the upper limit of normal of each medical facility).

(9) Patients suffering from or with a medical history of angina pectoris, cardiac failure, myocardial infarction or serious arrhythmia (findings seen under grade 4 of the Common Terminology Criteria for Adverse Events (CTCAE) v3.0-August 9, 2006 [Japanese translation: JCOG/JSCO edition, March 8, 2007]).

(10) Patients suffering from or with a medical history of autoimmune diseases (Hashimoto's disease [chronic thyroiditis], Crohn's disease, ulcerative colitis, rheumatoid arthritis, idiopathic thrombocytopenic purpura, systemic lupus erythematosus, autoimmune hemolytic anemia and scleroderma, etc.) or Henoch-Schonlein purpura.

(11) Patients with malignant tumors or a medical history of malignant tumors (patients with "suspected" malignant tumors that have not been definitively diagnosed will also be excluded).

(12) Patients who have received organ transplants (excluding corneal and hair transplants).

(13) Patients with a history of drug allergy to methylprednisolone, acetaminophen and d-chlorpheniramine maleate.

(14) Patients showing the following abnormal clinical laboratory values:
Leukocytes: <2,000 /μL, neutrophils: <1,500 /μL, platelets: <50,000 /μL

(15) Patients who have been administered monoclonal antibodies (regardless of type: mouse, rat, chimeric or human) in the past.

(16) Patients who have received other investigational drugs within 6 months prior to registration or who plan to participate in other clinical trials during the period of their participation in this clinical trial.

(17) Pregnant patients or patients capable of pregnancy, and cannot agree to contraception during the period of this clinical trial (confirmation by serum HCG test is required during screening).

(18) Patients determined unfit to be subjects in this clinical trial by the investigators.

[Rationale for setting the exclusion criteria]

(1) To exclude secondary nephrotic syndrome patients.

(2) To exclude patients based on their nephrotic syndrome treatment history.

(3), (4) Because symptoms may relapse or worsen in these patients with these conditions.

(5) - (14) Because inclusion of such patients into this clinical trial is not desirable on safety reasons.

(15), (16) Because these factors may affect the safety or efficacy of the investigational drug.

(17) IDEC-C2B8 has the same qualities as an immunoglobulin. As immunoglobulins are known to pass through the placenta to the fetus, there is concern that IDEC-C2B8 may affect the fetus if administered to a pregnant woman.

(18) To exclude patients unfit to be subjects in this clinical trial.

9 Investigational drugs

The investigational drugs used in the study are a test drug and a control drug which are impossible to distinguish from each other based on their external appearance.
Zenyaku Kogyo Co., Ltd. has requested Genentech Inc. (California, USA) to manufacture the investigational drugs which will subsequently be issued to medical institutions participating in this trial.

9.1 Overview of the study drug

(1) Development code and name
Development code: IDEC-C2B8
Name: [INN] Rituximab
[JAN] (English name) Rituximab (genetic recombination)

(2) Essential substance: Chimeric anti-CD20 monoclonal antibody consisting of human immunoglobulin constant regions (IgG1κ) and mouse anti-CD20 antibody variable regions (molecular weight: 144,510 Da)

(3) Special notes: this IDEC-C2B8 test drug has been approved for use in the treatment of B-cell lymphoma and is already on the market. The commercially available product is designated a biological product.

(4) Dosage form: Injection: 1 x 50mL vial contains 500 mg of IDEC-C2B8.

(5) Additives: Polysorbate 80, sodium chloride, sodium citrate, anhydrous citric acid, pH adjusting agent.

(6) Properties: colorless to pale yellow clear or slightly turbid liquid, pH: 6.5 ± 0.3, osmotic pressure ratio: approximately 1.

(7) Storage conditions: Avoid freezing and keep in a cool (2-8°C), dark place.

9.2 Overview of the control drug (IDEC-C2B8 placebo)

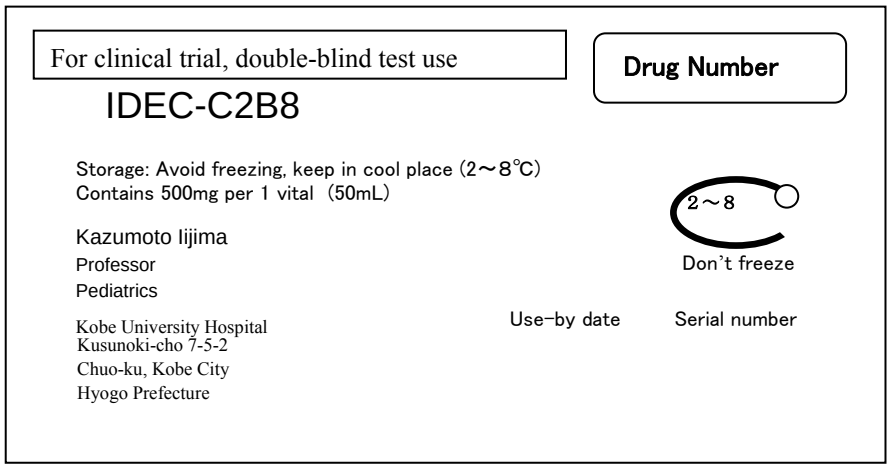
(1) IDEC-C2B8 placebo: 1 x 50mL vial that does not contain rituximab and is impossible to distinguish from IDEC-C2B8 from its external appearance.

(2) Storage conditions: Avoid freezing and keep in a cool (2-8°C), dark place.

9.3 Investigational drug display and packaging

(1) Packaging: Single patient doses (1 vial) are packaged in small boxes and 4 doses (4 small boxes) and then packed in a larger box.

(2) Display: The fact that the drug is for investigational use, the drug number, quantity of investigational drug, storage method, use-by date, name, title and address of the clinical trial steering committee chairman and the serial number, etc. are displayed on labels on the individual packing boxes and vials. A sample of the display is as shown below.



(3) Serial number and use-by date

Please refer to the "procedure manual for management of investigational drugs" for details regarding the serial number and use-by date.

9.4 Handling of investigational drugs

Please refer to the "procedure manual for management of investigational drugs" for details regarding issuance, storage, management and recovery.

10 Study plan

10.1 Clinical trial period definition

The clinical trial period is defined as the period from the date consent is obtained until the date the observation period is completed.

(1) Screening Period

From obtaining consent to registration (maximum of 35 days)

Obtain consent after diagnosing a relapse of nephrotic syndrome, and screen. Register within 7 days from confirming the remission

(2) Observation period (1 year):

The date the first dose of the investigational drug is administered (Day 1) to week 53 (Day 365)

1) Investigational drug administration period

Administration commencement date: The date the first dose of the investigational drug is administered (within 14 days from the date of registration)

Administration completion date: Day 22 in the event of complete administration of the investigational drug as prescribed, or the date the final dose of the investigational drug was administered in the event of discontinuation of administration.

10.2 Screening period (from consent to registration)

If the investigators observe a relapse of nephrotic syndrome in potential subjects of this clinical trial, they will explain the content of this clinical trial to the relevant potential subject and obtain their written consent for participation in this trial. After obtaining consent, a screening examination will be performed to verify eligibility as a subject. If eligibility is confirmed, the subject will be promptly registered for the trial.

10.2.1 Explanation and obtaining consent

Investigators will provide potential subjects with a document approved by the institutional review board that describes this clinical trial as well as give a sufficient explanation of its content. Voluntary consent to participate in this trial will be obtained in writing from subjects (22.2). When obtaining consent, sufficient time will be allowed for the potential subjects to consider the content of the above explanatory document as well as any other matters related to this trial and an opportunity will be given for them to ask questions to which sufficient answers will be provided.

(1) In the case of a patient aged 20 years or above, an explanation will be only provided to the patient themselves after which the patient will give their written consent. In the case of a patient aged under 20 years, written consent will be obtained from their legal representative (a person who exercises parental authority or a legal guardian) and the assent of the patient themselves will also be obtained wherever possible.

(2) An explanatory document (for adult use) and consent form of which comprehension by the average 16-year-old can be assumed will be created together with 2 types of explanatory documents (for child use) and assent forms of which comprehension by the average 7 to 12-year-old or 12 to 15-year old can be assumed.

(3) The explanatory document (for adult use) will be used in providing an explanation of this trial to patients aged 20 or above themselves or legal representatives (a person who exercises parental authority or a legal guardian) of patients aged under 20 years. For patients aged under 20 years, either the adult-use explanatory document or one of the 2 types of child-use explanatory documents will be selected in accordance with the relevant patient's age and level of comprehension and used in providing an explanation of this trial.

(4) Patients aged between 7 and 15 years, will be taken to provide their assent by fully understanding the content of the assent form and signing and dating a document of consent. In the case of patients aged between 7 and 11 years, assent can also be obtained orally and noted on the consent form.

(5) The sign and seal or signature of the physician providing the explanation, clinical trial collaborators (if used to supplement to explanation) and the patient themselves (aged 20 or above) or the legal representative (a person who exercises parental authority or a legal guardian) of a patient aged under 20 years as well as the date of consent will be included on the consent form. In addition, the relationship between the subject and their legal representative will be recorded.

(6) Investigators will provide a copy of the consent form to the patient themselves (aged 20 or above) or the legal representative (a person who exercises parental authority or a legal guardian) of a patient aged under 20 years and the investigator will retain the original document.

(7) If consent is obtained, the date of consent will be recorded in a "registration form". In addition, if the patient themselves (aged 20 or above) or the legal representative (a person who exercises parental authority or a legal guardian) of a patient aged under 20 years has indicated a desire to withdraw from participation in the clinical trial during the screening period or study period, the date and reason for doing so will be recorded in a "clinical trial discontinuation urgent report".

10.2.2 Screening examination and confirmation of eligibility

(1) After consent is obtained, investigators will conduct a screening examination to verify the patient's eligibility as a subject of this trial. However, if there is data present from hematological examination and blood biochemical examination conducted immediately following the last relapse (within 35 days prior to registration), this data may be used as data at the time of screening in which case re-examination will be unnecessary.

(2) Investigators will have patients or their legal representatives (a person who exercises parental authority or a legal guardian) maintain a patient diary from the day after consent is obtained for the purpose of determining steroid sensitivity.

(3) If the patient's eligibility as a subject of this trial is verified after the screening period, the patient will be registered within 7 days of the remission confirmation date following relapse.

10.2.3 Patient diary creation and notes on management

Subjects or their guardians (legal representatives) will maintain a patient diary (provisional name) from the day after consent is obtained until completion of the observation period. Patient diaries kept by subjects under the age of 20 will be checked wherever possible by their guardians (legal representative). Investigators will explain the following to subjects or their guardian (legal representative).

(1) Entries into the patient diaries are to be in block letters using black or blue ballpoint pens or ink.

(2) When making corrections, subjects should make a strike-through, etc. to ensure the content before the corrections.

(3) When urine dipstick examination on proteinuria in the first morning urine is not carried out, patients should add a diagonal line in the "date of examination" field rather than leaving a blank space in order to distinguish from an omission. If there is a reason why the urine dipstick examination was not carried out, it should be recorded in the memo field.

(4) Results of urine dipstick examination on proteinuria in the first morning urine should be recorded in the patient diary every day during the clinical trial period and the subject should take their patient diary with them on consultation days.

(5) Investigators or clinical trial collaborators will record necessary information in the physician description field of the patient diary (hospital visit dates, scheduled date of next visit, etc.)

Investigators or clinical trial collaborators will ask subjects or their guardians (legal representative) about

the situation of early-morning proteinuria examination between hospital visits and confirm the patient diary.

10.2.4 Determination of steroid sensitivity

Steroid sensitivity is defined as when treatment leads to remission (negative protein on urine dipstick test in the first morning urine is confirmed for 3 consecutive days) within 4 weeks.

Investigators will determine steroid sensitivity within 4 weeks after the start of prednisolone administration to treat the relapse. During consultation, investigators will confirm results of patients' urine dipstick testing in the first morning urine between hospital visits and conduct urinalysis (14.2) at the relevant medical institution to confirm negative protein on urine dipstick test in the first morning urine for 3 consecutive days

During consultation, investigators will ask subjects or their guardians (legal representatives) about the situation of urine dipstick results in the first morning urine and confirm the patient diary. The following circumstances will also be regarded as negative protein on urine dipstick test in the first morning urine.

(1) When the result of urine dipstick test on proteinuria in the first morning urine carried out at the relevant medical institution is (±).

(2) When the result of quantitative examination on proteinuria in the first morning urine carried out at the relevant medical institution indicates a value of less than 30 mg/dL.

(3) When the results of the patient's urine dipstick test in the first morning urine contains (±) and the first morning urinary protein/creatinine ratio is less than 0.2 in the results of examination carried out at the relevant medical institution.

10.2.5 Registration

Registration will be carried out within 7 days of the remission confirmation date following relapse.

(1) Investigators will fill out the necessary information of patients who have been confirmed eligible as subjects for this trial in a "registration form" and fax it to the data center.

<Data center>
Japan Clinical Research Support Unit (J-CRSU, NPO)
FAX: 03-5297-6259, Telephone: 03-5297-6258
Hours: Weekdays 10:00am – 5:00pm
Closed on Saturdays, Sundays, public holidays and during the new-year period
(December 30 – January 3).

(2) After the data center confirms subject eligibility from the “registration form”, they will issue a registration number and allocate the subject to the IDEC-C2B8 group or placebo group.

(3) After completion of registration and allocation, the data center will fax a “registration and allocation confirmation form” listing the registration number and drug number to the investigational drug provider and investigators of the relevant medical institution.

(4) The investigator will confirm the registration number and drug number listed on the “registration and allocation confirmation form” and then fax a “request for delivery of investigational drugs” to the investigational drug provider after filling in the necessary information. The investigator will retain the “registration and allocation confirmation form”.

<Investigational drug provider>
Zenyaku Kogyo Co., Ltd.
FAX: 03-3946-1202, hours: Weekdays 9:00am – 5:00pm

(5) The investigational drug provider will confirm the consistency of the “registration and allocation confirmation form” and “request for delivery of investigational drugs” and deliver the investigational drugs to the relevant medical institution.

10.2.6 Allocation

The data center will conduct dynamic allocation using the following allocation adjustment factors and randomly allocate subjects in order of registration to either the IDEC-C2B8 group or placebo group at an approximate ratio of 1:1. However, there is no requirement for this ratio to be preserved within each medical institution. The allocation algorithm will be determined by the person responsible for biological statistical analysis.

<Allocation adjustment factors>
Medical institution, age, treatment history (presence or absence of immunosuppressive drug administration at the relapse immediately prior to registration, presence or absence of steroid administration at the relapse immediately prior to registration), interval between the last 3 relapses

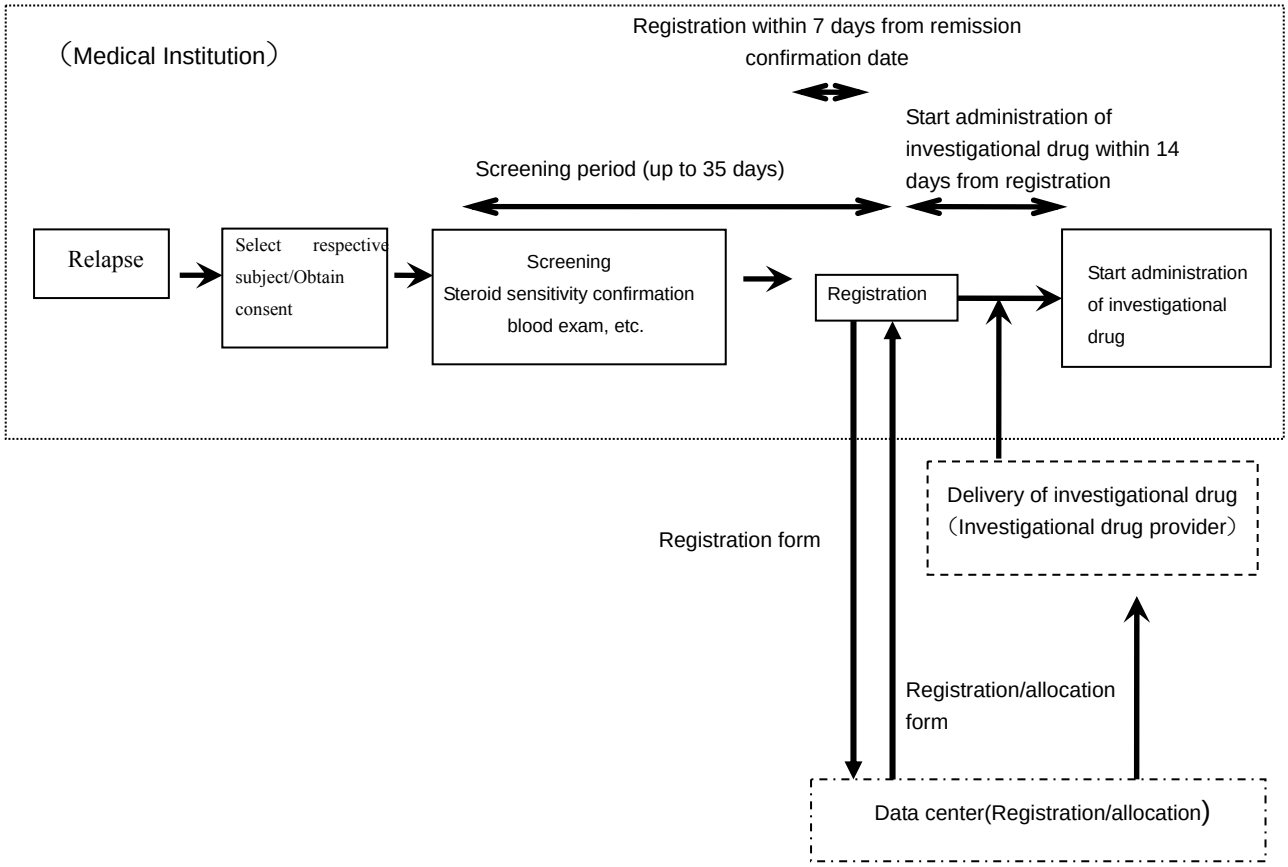


Figure 10-1. Flow up to investigational drugs administration

10.2.7 Exclusion after registration

Subjects in whom investigational drug administration was not possible within 14 days following the date of registration / allocation and subjects in whom relapse was observed prior to the administration of the first dose of the investigational drug will become ineligible as subjects for the trial and be excluded after registration. A report will be made in an “exclusion after registration urgent report” in such cases.

(1) In cases of exclusion after registration, investigators shall once again conduct a screening examination and re-confirm the eligibility of the patient after obtaining consent (10.2.2).

1) If there is data present from hematological examination and blood biochemical examination conducted following the last relapse immediately prior to re-registration (within 35 days prior to re-registration), this data may be used as data at the time of screening in which case re-examination will be unnecessary.

2) If there is data present from virus testing (HIV antibodies, HBs antigens, HBc antibodies, HCV antibody) and antinuclear antibody testing conducted within 85 days prior to re-registration, re-examination will be unnecessary.

(2) Re-registration will be carried out as soon as possible if eligibility is confirmed after screening (10.2.5).

10.3 Observation period: The date the first dose of the investigational drug is administered (Day 1) to week 53 (Day 365)

Investigators will administer the first dose of the investigational drug within 14 days after the date of registration / allocation and carry out observation, examination and survey according to the clinical trial schedule regardless of completion or discontinuation of investigational drug administration (14.1).

10.4 Criteria for the discontinuation of investigational drug administration

Investigators will discontinue the administration of investigational drugs to subjects to whom any of the following circumstances apply and conduct a "examination at the time of discontinuation of investigational drug administration" (14.6) as soon as possible at the time of discontinuation.

Subjects that do not fall under "(10.5) discontinuation of the clinical trial" will continue to be subjects in the trial even after discontinuation of investigational drug administration and the investigators will continue observation, examination and survey of these subjects according to the clinical trial schedule (14.1).

(1) If relapse is observed during the period of investigational drug administration

(2) If grade 3 or above non-hematological toxicity (excluding abnormal clinical laboratory values) is observed during infusion of the investigational drug (12.2.7)

(3) If the administration of investigational drug according the prescribed schedule is impossible.

1) If administration of the investigational drug is impossible within 24 hours after preparation (12.2.7)

2) If the dosage is changed and deviates from the prescribed dosage (12.3.1)

3) If the date of administration is changed and deviates from the prescribed administration date (12.3.2)

(4) If the subject (aged 20 years or above) or legal representative (a person who exercises parental authority or a legal guardian) requests discontinuation of the administration of the investigational drug

(5) If it becomes clear the subject was ineligible (did not fulfill the selection criteria / met the exclusion criteria) at the time of registration (including cases where it becomes clear the subject was ineligible

at the time of registration after the commencement of the clinical trial).

(6) If the investigators determine the continuation of investigational drug administration to be difficult for any other reason such as the occurrence of adverse events, etc.

10.5 Discontinuation from the clinical trial: discontinuation after the administration of the investigational drug

If investigational drug administration is discontinued in a subject after commencement and the relevant subject is also unable to adhere to the complete clinical trial schedule including efficacy and safety evaluation (14.1) for any of the following reasons from (1) to (4), they will "discontinue from the clinical trial".

(1) If the subject (aged 20 years or above) or their legal representative (a person who exercises parental authority or a legal guardian) requests discontinuation from the clinical trial or withdraws their consent for clinical trial participation.

(2) If the subject becomes unable to visit the hospital (due to a hectic schedule, moving of house, transfer to a different hospital, serious adverse events or death, etc.)

(3) If this clinical trial itself is discontinued

(4) If the investigators determine the subject's continuation of the clinical trial to be difficult for any other reason.

In the above cases (1) to (4), the investigator will immediately discontinue the subject's participation in the trial and report the time and reason for the discontinuation in a "clinical trial discontinuation urgent report". The investigator, while fully respecting the rights of the patient, will make reasonable efforts to confirm the reason for their discontinuation.

At the point of discontinuation from the trial, examinations will be carried out to ensure the safety of the subject (number of peripheral blood B-cells, HACA and IDEC-C2B8 blood concentration) (14.7). In addition, end of observation period examination items (14.4) will observed, examined and surveyed to the extent possible.

The investigators shall take appropriate measures if a patient is experiencing adverse events at the time of their discontinuation from the trial as well as keep track of that patient's outcome as far as possible by letter or telephone while the entire clinical trial is still ongoing (until the expected final observation date of the final-registered subject).

11 Blinding and key opening

11.1 Blinding

11.1.1 Type and level of blinding

This clinical trial is a placebo-controlled double-blind clinical trial. Registration and allocation will be performed using the data center's allocation program. Only the allocated drug number for each treatment group will appear to the person in charge at the data center.

The allocation codes for the allocation program will be input by the person in charge of registration and allocation, however the blinding will be protected as the allocation codes corresponding to the drug number will not be accessed other than at the time of input.

11.1.2 Procedures to ensure blinding

(1) Confirmation of investigational drug indistinguishability

Prior to the blinding of the investigational drugs, the investigational drug allocation administrator will confirm the indistinguishability of the test drug and the placebo using the formulas to be used in allocation.

(2) Maintaining blinding during the clinical trial period

In order to maintain blinding, the clinical laboratory test institution will seal the test results of the examination items related to the number of B-cells and T-cells in peripheral blood, HACA and IDEC-C2B8 blood concentration until key opening at the completion of the entire clinical trial and the medical institutions and investigators will not be informed of the test results. However, this shall not apply in cases where urgent allocation code disclosure is required or where the number of B-cells in a subject has not normalized (equal to or greater than the 5/μL reference value) at the time of their completion of the clinical trial.

In addition, the investigators will not measure the B-cells and T-cells in peripheral blood of a subject at a medical institution during their clinical trial period and, in the event these items are measured during the clinical trial period, the relevant subject will be treated as a non-evaluable case. If the investigators take measurements of a subject at a medical institution after their clinical trial period for the purpose of diagnosing the relevant subject or evaluating their treatment, the results of this examination will not be disclosed to anybody other than the relevant patient themselves or their legal representative.

(3) Allocation code creation and storage

The investigational drug allocation administrator will create "allocation codes" based on allocation table specifications and seal and retain these allocation codes until the entire clinical trial is completed and all data and determination confirmed.

(4) Urgent allocation code creation and storage

The investigational drug allocation administrator will create “urgent allocation codes” so that the investigational drug can be determined in the event of treatment failure or medical emergency. These “urgent allocation codes” will be sealed and retained by the data center.

(5) Urgent allocation code storage

Investigators will seal and retain the urgent allocation codes in line with the “procedures related to urgent allocation code disclosure” after confirming the drug group (allocation results will not be mentioned in medical records).

11.2 Entire clinical trial key opening

In order to maintain blinding, the “allocation codes” and “urgent allocation codes” will be disclosed after the entire clinical trial is completed and all data and determination secured.

11.3 Urgent disclosure of allocation codes

11.3.1 Conditions for urgent allocation code disclosure

Investigators may request the disclosure of a subject’s urgent allocation code if any of the following circumstances apply.

(1) The subject experiences a serious adverse event that leads to death or is life-threatening.

(2) The subject experiences another serious adverse event and it is determined the information is essential in considering the relevant subject’s treatment.

(3) There is determined to be treatment failure (11.4.1).

In addition, the investigational drug supplier may request the disclosure of a relevant subject’s urgent allocation code if they deem it necessary to report an adverse event to domestic or foreign regulatory authorities.

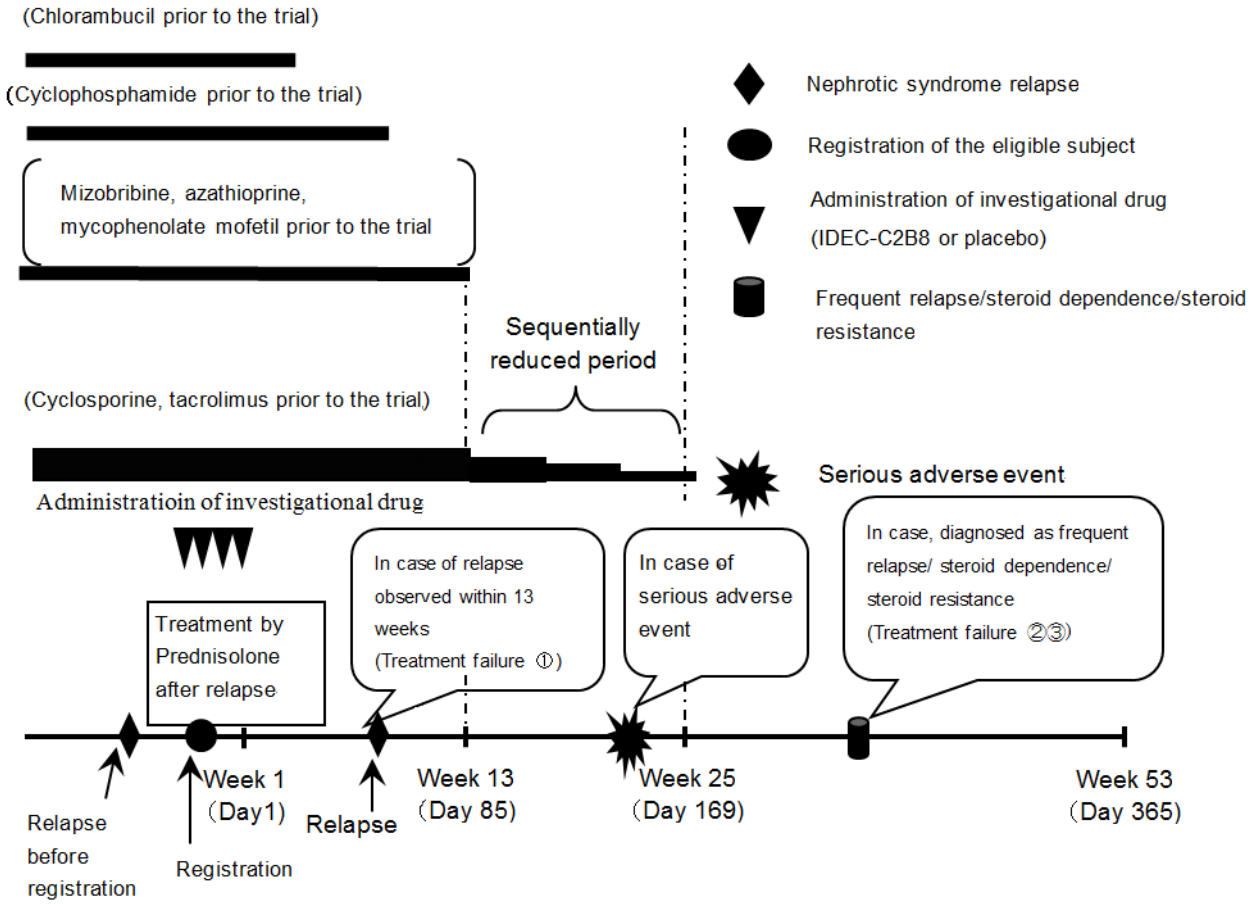


Fig. 11-1: Conditions for urgent allocation code disclosure

11.3.2 Procedure for the disclosure of urgent allocation codes

(1) If investigators deem the conditions for a subject's urgent allocation code disclosure have been met, they will list the reason for urgent allocation code disclosure and the drug number on an "investigational drug urgent allocation code disclosure request form" and fax it to the data center.

(2) Investigators will carry out an efficacy evaluation (relapse dates, number of relapses) and safety evaluation (presence/absence of serious adverse events, type of event and causality) in relation to the relevant subject, immediately fill out the necessary matters in the subject's "case report (observation period)" and fax it to the data center.

(3) The data center, upon receiving a request for urgent allocation code disclosure, will immediately contact the clinical trial steering committee after confirming and securing the safety and efficacy-related data of the relevant subject.

(4) If the clinical trial steering committee, after consultation with the independent data and safety monitoring committee as needed, deems disclosure of the urgent allocation code to be reasonable, they will request the data center to disclose the urgent allocation code of the relevant subject.

(5) The data center will then obtain the permission of the investigational drug allocation administrator and send the unopened urgent allocation code to the investigators of the relevant medical institution by post.

(6) If the data center discloses an urgent allocation code, they will keep a record of the events that led to the disclosure as well as the destination to which the urgent allocation code was sent.

(7) Investigators will unseal the urgent allocation code after confirming safety evaluation (presence/absence of non-serious adverse events, type of event and causality) in relation to the relevant subject. This protocol recommend the investigators will implement the appropriate medical care for the relevant subject after opening the urgent allocation code and confirming the subject's drug group.

(8) Investigators will record the events that led to the disclosure of the investigational drug urgent allocation code and the extent to which the disclosed result was notified in the subject's medical record (disclosure results will not be recorded in the medical record).

(9) The investigator will seal and retain the urgent allocation code in line with the "procedures related to urgent allocation code disclosure".

11.4 Handling of treatment failure

11.4.1 Treatment failure

Any of the following circumstances are defined as treatment failure in this clinical trial.

(1) If relapse occurs by week 13 (day 85)

(2) If frequent relapse or steroid dependence is diagnosed between the day following week 13 (day 86) and week 53 (day 365).

(3) If steroid resistance is diagnosed during the observation period (the date the first dose of the investigational drug is administered (Day 1) to day 365).

Relapse	Protein 2+ or above detected by urine dipstick in the first morning urine for 3 consecutive days and prednisolone treatment is required.
Relapse date	The first day that 2+ or above protein on urine dipstick test in the first morning urine, followed by the same observation for 3 consecutive days
Frequent relapse	Four relapses within the 12-month observation period (day 1 – day 365)
Frequent relapse date	The last relapse date that fulfills the definition of a frequent relapse.
Steroid dependence	When relapse occurs twice consecutively during the reduction of the prednisolone dosage or within 2 weeks after its discontinuation.
Date of steroid dependence	The second relapse date on which the definition of steroid dependence is met.
Steroid resistance	When the daily administration of prednisolone at 60 mg/m²/day does not lead to remission within 4 weeks.
Date of transition to steroid resistance	The date it is confirmed that the daily administration of prednisolone at 60 mg/m²/day does not lead to remission within 4 weeks.

11.4.2 Handling of treatment failure

An efficacy evaluation and safety evaluation will be carried out on the relevant subject at the time treatment failure is determined. Following this, the urgent allocation code will be disclosed in line with the code storage and disclosure procedures (11.3) and the following provisions followed.

(1) If treatment failure (1) or (2) is determined in a subject who has been allocated to the placebo group, one of the following provisions will be followed.

1) The relevant subject will begin the treatment deemed optimal by investigators such as the administration of new immunosuppressive drugs, and observation, examination and survey (except for B-cell/T-cell blood levels, HACA, and drug blood concentration) will be continued in line with the clinical trial schedule (14.1) until the end of the observation period (week 53, day 365).

2) The subject, with their consent or the consent of their legal representative, may be registered to a separately prescribed IDEC-C2B8 pharmacokinetic study. In this event, the relevant subject's participation in this clinical trial will be completed.

(2) If treatment failure (1) or (2) is determined in a subject who has been allocated to the IDEC-C2B8 group, the following provision will be followed.

1) As the IDEC-C2B8 has been determined to be ineffective, registration to an IDEC-C2B8 pharmacokinetic study will be impossible. The relevant subject will begin the treatment deemed optimal by investigators such as the administration of new immunosuppressive drugs, and observation, examination and survey will be continued in line with the clinical trial schedule (14.1) until the end of the observation period (week 53, day 365).

(3) If treatment failure (3) is determined in a subject who has been allocated to the placebo group, the following provision will be followed.

1) The relevant subject will begin the treatment deemed optimal by investigators such as the administration of new immunosuppressive drugs, and observation, examination and survey (except for B-cell/T-cell blood levels, HACA and, drug blood concentration) will be continued in line with the clinical trial schedule (14.1) until the end of the observation period (week 53, day 365).

(4) If treatment failure (3) is determined in a subject who has been allocated to the IDEC-C2B8 group, the following provision will be followed.

1) As the IDEC-C2B8 is determined to be ineffective, registration to an IDEC-C2B8 pharmacokinetic study will be impossible. The relevant subject will begin the treatment deemed optimal by investigators such as the administration of new immunosuppressive drugs, and observation, examination and survey will be continued in line with the clinical trial schedule (14.1) until the end of the observation period (week 53, day 365).

11.5 Evaluation of nephrotic syndrome relapse

Relapse and relapse date are defined as follows in this study.

Investigators will evaluate the occurrence of relapse based on the results of urine dipstick test on proteinuria in the first morning urine recorded in the patient diary (provisional name) observed during consultation and the results of urine dipstick test on proteinuria in the first morning urine carried out at the relevant medical institution (In the event the value of quantitative examination on proteinuria in the first morning urine carried out at the relevant medical institution was 100 mg/dL or more, it will be considered 2+ or above proteinuria by urine dipstick test[92]).

When the definition of relapse is met, a report will be made in a “relapse report”. The relapse date will be confirmed from the patient diary and recorded in the relevant subject’s medical record. In addition, the circumstances of the relapse will be reported in a “case report form (observation period)”.

Relapse	Protein 2+ or above detected by urine dipstick in the first morning urine for 3 consecutive days and prednisolone treatment is required.
Relapse date	The first day that protein 2+ or above detected by urine dipstick test in the first morning urine, followed by the same observation for 3 consecutive days.
Frequent relapse	Four relapses within the 12-month observation period (day 1–day 365).
Steroid dependence	When relapse occurs twice consecutively during the reduction of the prednisolone dosage or within 2 weeks after its discontinuation.
Steroid resistance	When the daily administration of prednisolone at 60 mg/m²/day does not lead to remission within 4 weeks.
Remission	Negative protein on urine dipstick test in the first morning urine for 3 consecutive days.
Partial remission	Serum albumin > 2.5g/dL and 1+ or above protein on urine dipstick in the first morning urine.
Nephrotic state	Serum albumin ≤ 2.5g/dL and 1+ or above protein on urine dipstick in the first morning urine.

12 Dosage regimen

The treatment protocol of this clinical trial is defined as prednisolone administration for treatment of relapse immediately prior to the clinical trial period, investigational drug administration (including pre-medication) and prednisolone administration for treatment of relapse during the clinical trial period (from the date of consent to the date of completion of the observation period).
No new treatment will be administered during the subject's clinical trial period until relapse is observed. In addition, non-prescribed administration of unused quantities of the investigational drug or commercially available rituximab will not be carried out (12.1.2).

12.1 Treatment prior to commencement of the clinical trial

12.1.1 Prednisolone administration for treatment of relapse immediately prior to registration

Prednisolone administration for treatment of relapse immediately prior to registration will be carried out as follows in accordance with the ISKDC relapse treatment method.
The prednisolone dosage will be calculated by body surface area* (the prednisolone dosage is calculated in 5 mg increments – units of 0 or greater but less than 2.5 will be rounded down to 0, units of 2.5 of greater but less than 7.5 will be rounded to 5 and a unit of 7.5 or greater will be rounded up to 10).
*Body surface area is calculated from height and standard weight based on height using the Du Bois method.
Body surface area (BSA (m²)) = Body weight (kg)$^{0.425}$ x height (cm)$^{0.725}$ x 0.007184 (Du Bois)

(1) 60 mg/m²/day in three divided doses (maximum dosage: 80 mg/day)
The administration period will be selected for each subject as follows:
In the event prednisolone was being administered when the relapse immediately prior to registration was observed: "28 days".
In the event prednisolone was not being administered when the relapse immediately prior to registration was observed: "until negative protein on urine dipstick test in the first morning urine is confirmed for 3 consecutive days". The prednisolone administration period prior to registration is included in the (1) treatment period.
The prednisolone dosage will be sequentially reduced to (2), (3) and (4)

(2) 60 mg/m² every other morning for 14 days (maximum dosage: 80 mg)

(3) 30 mg/m² every other morning for 14 days (maximum dosage: 40 mg)

(4) 15 mg/m² every other morning for 14 days (maximum dosage: 20 mg) after which prednisolone administration will be discontinued.

12.1.2 Immunosuppressive drugs administered from prior to the commencement of this clinical trial

In the event immunosuppressive drugs (cyclosporine, tacrolimus, cyclophosphamide, mizoribine, mycophenolate mofetil, chlorambucil) have been administered prior to the commencement of this clinical trial for the purpose of treating nephrotic syndrome, they will be continued to be administered in combination during the clinical trial period without any change to the regimen and dosage at the time of registration (although the dosage of cyclosporine and tacrolimus may be changed based on monitoring) and will be discontinued as follows (refer to fig. 12-1). However, this shall not apply for a reduction in dosage required as a result of side effects from these immunosuppressive drugs.

(1) Cyclosporine, tacrolimus

Cyclosporine and tacrolimus will be administered in combination at the regimen and dosage at the time of registration until day 85 (±7 days) (however the dosage may be changed based on monitoring). As a rule, the dosage will be sequentially reduced every 28 days from day 86 onwards and discontinued on day 169 (±14 days).

(2) Cyclophosphamide

The total period of cyclophosphamide administration will be 8 to 12 weeks (however this shall not apply if the investigators deem it difficult to continue administration).

(3) Mizoribine, azathioprine, mycophenolate mofetil

These drugs will be discontinued at day 85 (±7 days).

(4) Chlorambucil

The total period of chlorambucil administration will be a maximum of 8 weeks (however this shall not apply if the investigators deem it difficult to continue administration).

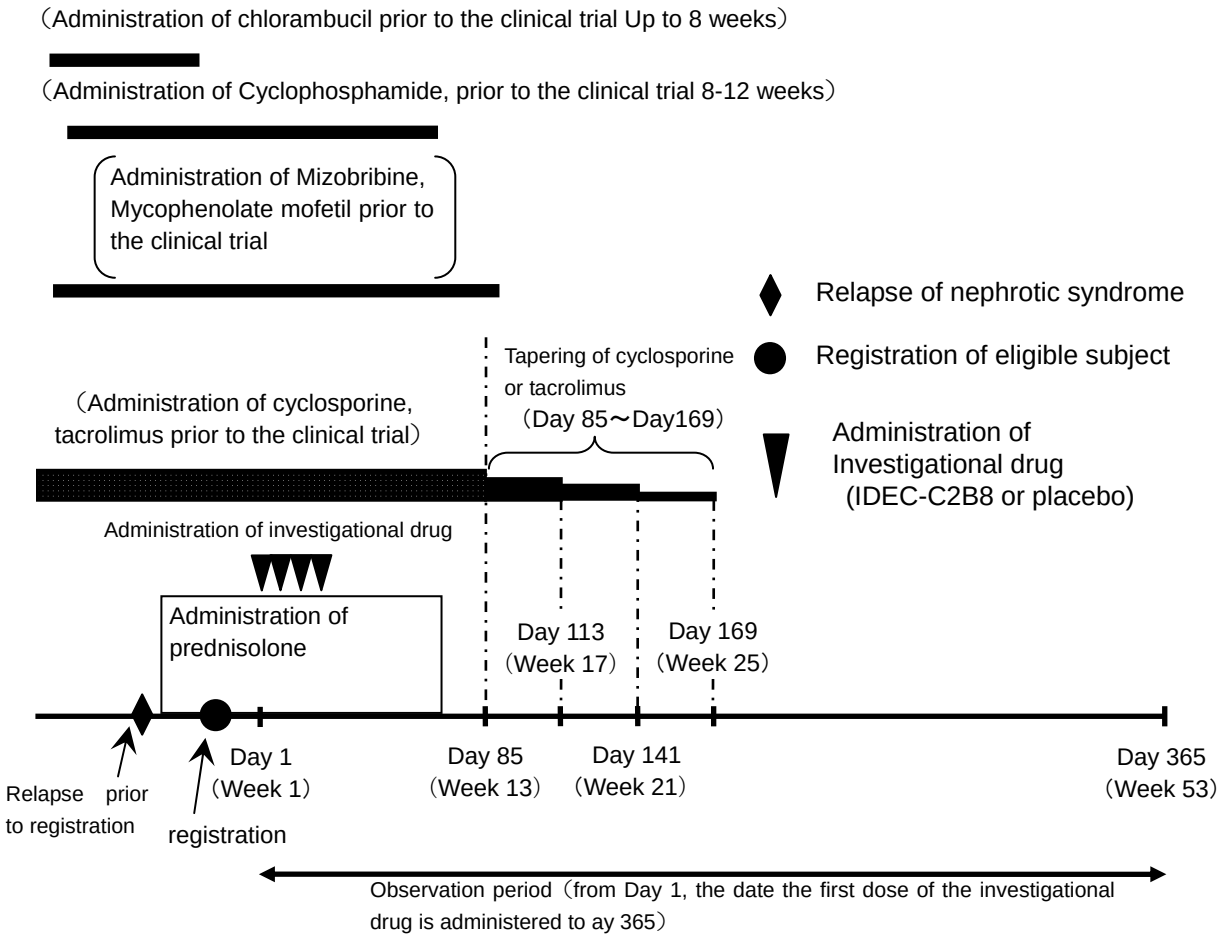


Figure 12-1 Dosage Regimen

12.2 Administration of the investigational drug

Investigators will administer the first dose of the investigational drug within 14 days after the date of registration (the date the first dose of the investigational drug is administered is set as day 1, week 1).

The investigational drug for the subject allocated to the respective groups will be administered at 4 x 375 mg/m² doses (maximum dose: 500 mg) given in weekly intervals (day 1, 8, 15, 22).

12.2.1 Considerations prior to investigational drug administration

Infusion reactions (fever, chills, nausea, headache, pruritus, rash, cough, hypotension, lethargy, flulike symptoms such as laryngeal edema, etc. and allergic-like reactions) develop frequently along with the infusion of the investigational drug. In particular, infusion reactions develop strongly at the time of the initial infusion.

The release of chemical mediators and cytokines along with the damage to B-cells caused by IDEC-C2B8 has been considered as the mechanisms of infusion reactions. Typically, infusion reactions are attenuated by temporarily suspending the infusion of IDEC-C2B8 or by administrating antihistamines or antipyretic analgesics, however there have been reports of the occurrence of myocardial infarction and arrhythmia in

patients with a history of cardiac disease and the occurrence of acute respiratory failure and bronchospasm in patients with a history of or a condition complicated by respiratory disorders and therefore great care must be taken. It is necessary to ensure emergency treatment with bronchodilators, oxygen inhalation and transfusion, etc. is available at the time of administration.

As cases of sustained falls in blood pressure as a result of IDEC-C2B8 administration in patients taking antihypertensive drugs have been reported, it is necessary to carefully administer IDEC-C2B8 while closely observing the state of the subject if said subject is taking antihypertensive drugs.

12.2.2 Investigational drug dosage calculation method and preparation

(1) The "375 mg/m² (maximum dose: 500 mg)" dosage of the investigational drug will be calculated from body surface area* at the time of registration (the dosage will be rounded to the nearest unit).
Body surface area is calculated from height and standard weight based on height using the Du Bois method.
Body surface area (BSA (m²)) = Body weight (kg)$^{0.425}$ x height (cm)$^{0.725}$ x 0.007184 (Du Bois)

(2) The investigational drug will be diluted 10-fold in Japanese Pharmacopoeia isotonic sodium chloride solution immediately prior to administration and infusion will then be completed within 24 hours at a final concentration of 1 mg/mL.

(3) Dilution of the investigational drug with 5% injectable glucose solution rather than sodium chloride solution will be acceptable for subjects with salt intake restrictions. No other drugs shall be mixed into the diluent. In addition, the preparation shall not be vigorously stirred or frothed up during dilution.

12.2.3 Investigational drug administration method

The investigational drug will be administered to subjects who are hospitalized for at least the day of administration and the following day (1 night, 2 days). Outpatient administration will be unacceptable. Change of the investigational drug administration date will be carried out in accordance with 12.3.2.

The investigational drug will be administered at 4 x 375 mg/m² doses (maximum dose: 500 mg) given in weekly intervals (day 1, 8, 15, 22).

(1) Pre-treatment with an oral antipyretic analgesic, oral antihistamine and intravenous methylprednisolone will be carried out approximately 30 minutes prior to the administration of each dose of the investigational drug with the purpose of preventing infusion reaction (12.2.4) (Fig. 12-2). It should be noted that the administration of intravenous methylprednisolone is for the purpose of suppressing HACA production.

1) Acetaminophen: oral administration
2) d-Chlorpheniramine maleate: oral administration
3) Methylprednisolone sodium succinate: intravenous injection.

(2) The IDEC-C2B8 standard infusion rate is displayed in fig. 12-2. In this clinical trial it is recommended that the investigational drug be administered at 25 mg/hr for the initial hour, 100 mg/hr for the next hour and 200 mg/hr for the remainder of the dose.
While it will also be acceptable for investigators to continue administration at a low rate (<200 mg/hr) if they so decide, administration shall be completed within 24 hours after preparation of the dose. Infusion rate changes will be carried out within ±15 minutes of the specified time. If an infusion reaction develops during infusion, the infusion rate will be decreased or the infusion temporarily suspended (12.2.7).

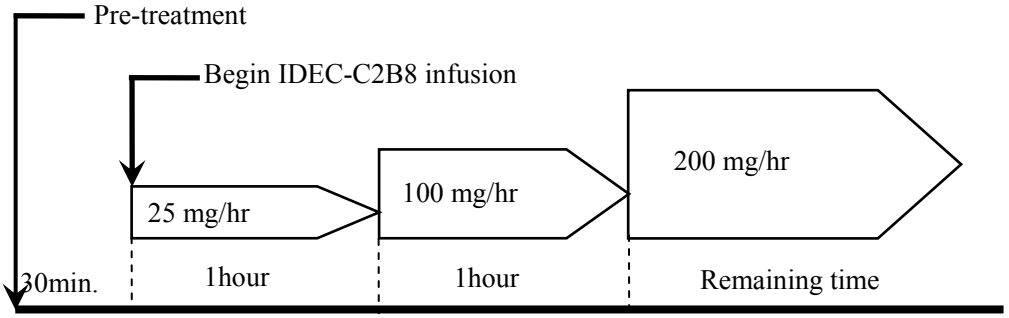


Fig. 12-2: IDEC-C2B8 standard infusion rate

(3) In clinical trials conducted on B-cell lymphoma patients, infusion reactions along with IDEC-C2B8 administration were most commonly observed during administration of the first dose where peripheral blood B-cells are destroyed rapidly, and the incidence of infusion reaction along with the second dose and beyond was less than half that of the first dose. Accordingly, it will be acceptable for administration of the second dose onwards to be commenced at an infusion rate of 100ml/hr in the case where only a mild infusion reaction (grade 1 or less, or in the case of fever, grade 2 or less) was observed at the time of administration of the previous dose.

(4) A trend of IDEC-C2B8 administration bringing about HACA production in patients when B-cell reduction is insufficient has been observed in foreign clinical studies of IDEC-C2B8 targeting patients with autoimmune disease. Accordingly, it is necessary to carefully administer IDEC-C2B8 while closely observing the state of the subject even from the second dose onwards.

12.2.4 Investigational drug administration: Pretreatment

(1) Pretreatment with an oral antipyretic analgesic, oral antihistamine and intravenous methylprednisolone will be carried out 30 minutes (±10 minutes) prior to the administration of each dose of the investigational drug with the purpose of preventing infusion reaction. Mirroring SLE clinical trials, intravenous methylprednisolone will be administered for the purpose of suppressing HACA production.

(2) Oral antipyretic analgesic: Acetaminophen (Calonal®, etc)

1) Patients whose standard weight based on height is 50kg or more or patients over the age of 16:
Acetaminophen: 1 x 300 mg (tablet)

2) Patients who do not fall under 1) or who cannot be administered tablets orally:
Target dose of 10-15 mg/kg as acetaminophen (in the case of syrup: 0.5 ml/kg, maximum dose: 300 mg)

(3) Oral antihistamine: d-Chlorpheniramine maleate (Polaramine®, etc)

1) Patients whose standard weight based on height is 50kg or more or patients over the age of 16:
1 x 2.0 mg (tablet)

2) Patients who do not fall under 1) or who cannot be administered tablets orally:
Syrup (content: 0.04%) will be administered in the following dosage

Age	Single dose	Age	Single dose
2-under 3	1.0mL	8-under12	2.5mL
3-under 5	1.5mL	12-under15	3.0mL
5-under8	2.0mL		

(4) Intravenous corticosteroid: methylprednisolone (Solu-Medrol®, etc)

1) Patients whose standard weight based on height is 50kg or more or patients over the age of 16:
Methylprednisolone 125 mg, intravenous injection

2) Patients who do not fall under 1):
The dosage for bronchial Asthma in Children, methylprednisolone 1.0 - 1.5 mg/kg

12.2.5 Observation during infusion of the first dose of the investigational drug

A particularly high possibility of infusion reaction along with infusion of the first dose of the investigational drug is expected; therefore the state of the subject will be closely observed and their vital signs (blood pressure, body temperature, pulse) and subjective and objective symptoms recorded at the

following times. The subjects will also be made to rest for at least 30 minutes after infusion.

(1) Before the commencement of infusion (-15 minutes), 30 minutes after commencement (±10 minutes), when the infusion rate is changed (±15 minutes), 1 hour after the final infusion rate change (±15 minutes), at the completion of infusion (±15 minutes), 1 hour after completion (±30 minutes)

(2) When the rate of infusion is slowed down or infusion is suspended as a result of the occurrence of adverse events or for any other reason, when the rate of infusion is increased again after having being slowed down and when infusion is resumed after having been suspended.

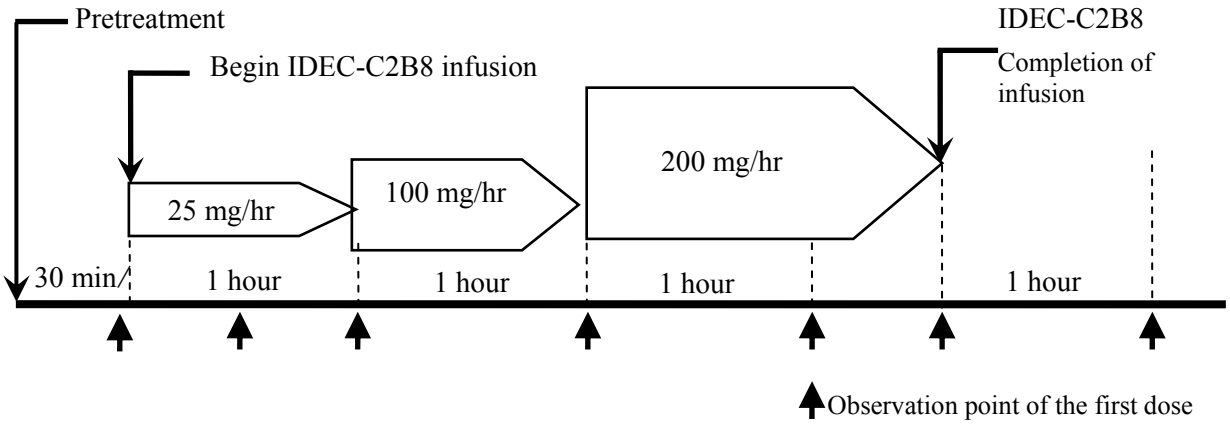


Fig. 12-3: Observation during the first dose of the investigational drug

12.2.6 Observation during infusion of the second dose and onwards of the investigational drug

In clinical trials conducted on B-cell lymphoma patients, infusion reactions were most commonly observed during administration of the first dose of IDEC-C2B8 and the incidence of infusion reaction along with the second dose and beyond was less than half that of the first dose. Accordingly, vital signs (blood pressure, body temperature, pulse), subjective and objective symptoms and the times of infusion rate change will be recorded at the following times in the case where no infusion reaction or only a mild infusion reaction (grade 1 or less, or in the case of fever, grade 2 or less) was observed at the time of administration of the previous dose. The subjects will also be made to rest for at least 30 minutes after infusion.

(1) Before the commencement of infusion (- 15 minutes), 30 minutes after commencement (± 10 minutes), 1 hour after the final infusion rate change (± 15 minutes), at the completion of infusion (± 15 minutes), 1 hour after completion (± 30 minutes)

(2) When the rate of infusion is slowed down or infusion is suspended as a result of the occurrence of adverse events or for any other reason, when the rate of infusion is increased again after having

being slowed down and when infusion is resumed after having been suspended.

12.2.7 Treatment of infusion reaction resulting from the infusion of the investigation drug

If an infusion reaction develops during infusion, the infusion rate will be decreased or infusion suspended depending on the severity of the infusion reaction as described below and supportive treatment will be administered as necessary.

(1) Grade 1:

In the event of a grade 1 infusion reaction during infusion at 25 mg/hr, the investigators will make a clinical judgment whether to continue or temporarily suspend infusion. If the infusion has been temporarily suspended, it will be resumed at 25 mg/hr after the subject's symptoms have recovered.

In the event of a grade 1 infusion reaction during infusion at 100 mg/hr, the investigators will make a clinical judgment whether to continue infusion, reduce the infusion rate to 25 mg/hr, or temporarily suspend infusion. If the infusion has been temporarily suspended, it will be resumed at 25 mg/hr after the subject's symptoms have recovered.

(2) Grade 2:

In the event of a grade 2 infusion reaction during infusion at 25 mg/hr, the infusion will be temporarily suspended. The infusion will be resumed at 25 mg/hr after the subject's symptoms have recovered to grade 1 or lower.

In the event of a grade 2 infusion reaction during infusion at 100 mg/hr, the infusion will be temporarily suspended or the infusion rate reduced to 25 mg/hr. If the infusion has been temporarily suspended, it will be resumed at 25 mg/hr after the subject's symptoms have recovered to grade 1 or lower.

Resumption of infusion after temporary suspension and increase in infusion rate following resumption or a reduction to 25 mg/hr will be determined by the clinical judgment of the investigators with reference to Fig. 12-2.

It should be noted that no provisions are set with regard to the period of time between infusion suspension and resumption; however administration of the investigational drug shall be completed within 24 hours after its preparation. Further administration of the investigational drug will be discontinued in subjects for whom administration of the investigational drug is not completed within 24 hours after its preparation (10.4).

(3) Grade 3 or above non-hematological toxicity (excluding laboratory test value abnormalities):

The infusion will be discontinued and supportive treatment will be administered as necessary. Further administration of the investigational drug to the relevant subject will be discontinued.

Supportive treatment will be administered in the form of (1) non-steroidal antipyretic analgesics (for fever, pain, etc.), (2) antihistamines (for allergic symptoms), (3) antibiotics and anti-viral agents, (4) antihypertensive drugs, vasopressors and vasodilators, (5) antiemetics, (6) stomach medicine, antidiarrheal drugs and laxatives, (7) oxygen inhalation and (8) any other medication deemed necessary by the investigators. If serum sickness-like symptoms are observed, symptomatic treatment with steroids will be carried out.

12.2.8 Investigational drug administration to subjects at high risk of infusion reaction

Subjects with a condition complicated by or a history of respiratory disorders are at a high risk of dyspnea and bronchospasm and subjects with a condition complicated by or a history of cardiac disease are at a high risk of myocardial infarction or arrhythmia. Accordingly, administration to such subjects will be carried out with great care and while frequently observing the subject's condition.

It will be acceptable to carry out infusion out at a low rate (<200 mg/hr) without increasing the infusion rate in subjects in whom a serious infusion reaction is anticipated.

12.3 Change to investigational drug dosage and administration date

12.3.1 Change to dosage

The prescribed infusion dosage may not be changed. If any dose cannot be administered as prescribed due to an infusion reaction, further administration of the investigational drug to the relevant subject will be discontinued.

12.3.2 Change to the administration date

(1) If a subject is unable to be administered with the investigational drug according to schedule due to a public holiday or their own circumstances, the administration date may be changed within ±2 days (however, if a subject is unable to be administered with the investigational drug according to schedule due to a long-term consecutive holiday such as the new-year period, the administration date may be postponed by up to 7 days). The dose following the dose for which the administration date was changed will be administered 7±2 days after the previous dose.

(2) If investigators determine that the investigational drug is unable to be administered according to schedule due to the occurrence of an adverse event, the administration date may be postponed by up to 7 days (administration will always be postponed in the case of grade 3 or above non-hematological toxicity). If the postponed date of administration falls on a public holiday, an additional 2-day (total 9 days) postponement will be possible. However, further administration of the investigational drug to the relevant subject will be discontinued if postponement exceeding 7 days as

a result of an adverse event is necessary.

12.4 Prednisolone administration for the treatment of relapse during the clinical trial period

If relapse occurs (11.5) during the clinical trial period (from the date of consent to the date of completion of the observation period), the relevant patient will be administered prednisolone as follows in accordance with the ISKDC relapse treatment method.

The prednisolone dosage will be calculated by body surface area* (the prednisolone dosage is calculated in 5 mg increments – units of 0 or greater but less than 2.5 will be rounded down to 0, units of 2.5 of greater but less than 7.5 will be rounded to 5 and a unit of 7.5 or greater will be rounded up to 10).

* Body surface area is calculated from height and standard weight based on height using the Du Bois method (appendix 1).
  Body surface area (BSA (m^2)) = Body weight $(kg)^{0.425}$ x height $(cm)^{0.725}$ x 0.007184 (Du Bois).

(1) 60 mg/m^2/day in three divided doses (maximum dosage: 80 mg/day) until negative protein on urine dipstick test in the first morning urine is confirmed for 3 consecutive days.
The prednisolone dosage will be reduced to (2) if remission is not observed after 4 weeks (steroid resistance). The investigators will make a clinical judgment regarding any subsequent reduction in dosage.

(2) 60 mg/m^2 every other morning for 14 days (maximum dosage: 80 mg)

(3) 30 mg/m^2 every other morning for 14 days (maximum dosage: 40 mg)

(4) 15 mg/m^2 every other morning for 14 days (maximum dosage: 20 mg) after which prednisolone administration will be discontinued.

13 Concomitant drugs, combination therapy and post-treatment

13.1 Concomitant drugs and combination therapy

13.1.1 Concomitant drugs and combination therapy reporting

(1) From the date of consent to the day prior to the first dose of the investigational drug:

The name, usage method (drug regimen, dosage and route of administration), period of administration and purpose of drugs and treatment used for the treatment of nephrotic syndrome and complications during this period will be reported via a "case report (screening period)".

(2) Observation period (the date the first dose of the investigational drug is administered (day 1) to day 365):

The name, usage method (drug regimen, dosage and route of administration), period of administration and purpose of drugs and treatment used for any purpose such as for the treatment of the underlying disease and adverse events during the observation period will be reported via a "case report (observation period)". All drugs continuing to be used from prior to the start of the clinical trial for the purpose of treating complications will also be recorded.

13.1.2 Prohibited concomitant drugs and combination therapy

Combination therapy with the following drugs and treatment will be prohibited during the clinical trial period (the date of consent to date of the completion of the observation period).

(1) Commercially available rituximab

(2) Immunosuppressive drugs or alkylating agents with an immunosuppressive effect except in the following cases.

1) In the case "cyclosporine, tacrolimus, cyclophosphamide, mizoribine, mycophenolate mofetil or chlorambucil" continues to be used from prior to the start of the clinical trial (12.1.2)

2) In the case treatment failure is determined (11.4.1)

(3) Plasma exchange therapy

(4) Live vaccines

(5) Other investigational drugs or domestically-unapproved drugs

13.2 Post-treatment

Treatment following the clinical trial period is not prescribed and will be left to the judgment of the investigators. The 2005 Japanese Society for Pediatric Nephrology treatment guidelines for idiopathic nephrotic syndrome in children v.1.0 [9)] recommends treatment methods for patients diagnosed with relapse, frequent relapse, steroid dependence and steroid resistance.

14 Observation, examination and survey

14.1 Observation, examination and survey schedule

During the clinical trial period, investigators will carry out observation, examination and survey in accordance with the prescribed schedule. Blood samples will be taken immediately before administration on all days of investigational drug administration.

In the event investigational drug administration is discontinued, investigators will carry out an examination at the point of discontinuation of investigational drug administration (14.6) as soon as possible and subsequently continue observation, examination and survey in accordance with the prescribed schedule (14.1).

Week 5 (day 29): a postponement “within +7 days (or within +11 days in the event there is a change to the investigational drug administration date due to an adverse event) is possible.

Week 9 (day 57) to week 13 (day 85): a change “within ±7 days” is possible.

Week 17 (day 113) to week 53 (day 365): a change “within ±14 days” is possible, however there shall be a gap of at least 14 days between the next round of observation, examination and survey.

In the event the date of a round of observation, examination and survey is changed, the change shall be limited to that round only and the following rounds of observation, examination and survey will be carried out on their prescribed dates. In the event of a change to the investigational drug administration date even within the allowable range, the reason for the date change will be reported in the special notices field of the “case report form (observation period)”.

	Screening period	Observation period (investigational drug administration period)				Observation period (following completion of investigational drug administration)													Clinical trial Discontinu-ation
Day	Within 35 days	1	8	15	22	29	57	85	113	141	169	197	225	253	281	309	337	365	
Week		1	2	3	4	5	9	13	17	21	25	29	33	37	41	45	49	53	
Obtaining informed consent	○																		
Medical examination	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Investigation drug administration		○	○	○	○														
Background survey	○																		
Concomitant drug survey	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	△
Height/weight	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	△
Blood pressure, pulse, body temperature	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	△
Pregnancy test	○																		
HIV, HCV, HBV	○																		
Electrocardiogram	○																	○	△
Chest X-ray	○																	○	△
Relapse evaluation		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	△
Adverse event evaluation		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Hematological examination	○	○	○	○	○	○	○	○	○	○	○	○		○		○		○	△
Blood biochemical examination	○	○	○	○	○	○	○	○	○	○	○	○		○		○		○	△
Immunoglobulin examination		○		○		○		○			○			○		○		○	△
Estimated glomerular filtration rate	○																	○	△
Urinalysis	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	△
B-cells/T-cells in the blood	○	○		○		○		○		○	○	○		○		○		○	○
Human anti-chimeric antibodies		◎						○			○							○	○
Drug blood concentration		◎			◎			○			○							○	○
Blood sample volume (mL)	8.5	13.5	4.5	9.5	6.5	9.5	4.5	13.5	4.5	7.5	13.5	7.5		9.5		9.5		13.5	7-13.5

◎Measured prior to investigational drug administration △Carried out to the extent possible

14.2 Screening period examination

Investigators will examine the following items during the screening period.

Item	Content	Measurement		Survey form	
		Facility	Outside facility	Registra-tion form	Screening period
Subject's background	Sex, birth date	○		○	
	Height, weight	○		○	
Date of consent		○		○	
Medical & treatment history of nephrotic syndrome	The Date of first diagnosis of the nephrotic syndrome	○		○	
	Prednisolone was administered at the relapse immediately prior to registration or not, immunosuppressant was administered at the relapse immediately prior to registration or not (if yes, name of the drug), date of most recent 3 relapses before registration	○		○	
	Prednisolone administration report (administration period, drug usage, dosage) of one year before the registration *, immunosuppressant treatment history (drug name, administration period, usage, dosage)	○			○
Anamnesis & disease complication history		○			○
Concomitant drugs/ combination therapy	Continuously used drugs for complications	○			○
Steroid sensitivity		○		○	
Vital signs	Pulse, body temperature	○			○
	Blood pressure (systolic & diastolic)	○		○	
Hematological examination	WBC, number of neutrophils, Plt	○		○	
	RBC, Hb, Ht, differential white blood count	○			○
Blood biochemical examination	AST, ALT, CRP, antinuclear antibody, serum creatinine, （estimated glomerular filtration rate ）	○		○	
	BUN, uric acid, total protein, serum albumin, Na, K, Ca, P, Cl	○			○
Number of CD20 positive cell	Number of peripheral blood B-cell	○		○	
Virus test	HIV antibody, HBs antigen, HBc antibody, HCV antibody	○		○	
Pregnancy test	Serum HCG（Necessary for females after menarche ）	○		○	
Urinalysis**	Proteinuria and hematuria on urine dipstick test in the first morning urine, quantitative examination on proteinuria and creatinine in the first morning urine	○			○
Electrocardiogram		○			○
Chest X-ray		○			○

* Only if examination of the circumstances of prednisolone administration in the year period prior to registration is possible.

** Urinalysis will be conducted on the remission confirmation date

14.3 Observation period examination: during the investigational drug administration period

14.3.1 Immediately prior to investigational drug administration

Investigators will observe the subjects and collect samples for examination immediately prior to administration of each dose of the investigational drug.

Item	Content	Measurement	
		Facility	Outside facility
Concomitant drugs/combination therapy		○	
General symptom	Subjective and objective symptoms（adverse events）	○	
Height, weight		○	
Vital sign	Blood pressure (systolic & diastolic) pulse, body temperature	○	
Relapse evaluation		○	
Hematological examination	WBC, differential white blood count, RBC, Hb, Ht, Plt		○
Blood biochemical examination	BUN, serum creatinine, uric acid, total protein, serum albumin, AST, ALT, Na, K, Ca, P, Cl, CRP		○
Immunoglobulin*	IgG, IgM, IgA		○
Number of peripheral blood B/T-cells*	B-cells are CD20 & CD19 positive cells, T-cells areCD3 positive cells		○
HACA	See Table 14.1		○
Drug blood concentration ratio	See Table 14.1		○
Urinalysis	Proteinuria and hematuria on urine dipstick test in the first morning urine	○	
	Quantitative examination on proteinuria and creatinine in the first morning urine		○

*Immunoglobulin, number of peripheral blood B/T cells: Only at immediately before the first and the third administration

14.3.2 During investigational drug infusion

Investigators will confirm the following items at the time of investigational drug infusion and record the results in the “case report form (observation period)”.

Subjects will be frequently observed for infusion reaction and the investigators will carry out appropriate measures such as lowering the rate of infusion and administering supportive treatment if an infusion reaction is observed (12.2.7).

(1) Investigational drug dosage

(2) Infusion start time (the time at which infusion of the investigational drug was actually started) and the rate of infusion

(3) In the event of infusion rate change or suspension of infusion, the time and infusion rate (the rate after change)

(4) Infusion completion time (the time at which infusion of the prescribed dose of the investigational drug was actually completed)

(5) The presence or absence of adverse events during the infusion
If adverse events such as infusion reactions are observed during the infusion, the time of the adverse event, type and severity, causal relationship with the investigational drug and the presence or absence of supportive treatment (and if supportive treatment is carried out, the type, dosage and route of administration) will be recorded in the "case report form (observation period)". If necessary, vital signs (blood pressure, pulse, body temperature) will also be recorded.

(6) If the investigational drug is discontinued, the time and reason for discontinuation

(7) If the infusion rate is changed or infusion temporarily suspended as a result of infusion reaction, etc. and subsequently resumed, the course of the events will be recorded.

14.3.3 From completion of investigational drug infusion to the following day

Particular attention will be paid to infusion reaction in the 24 hours after completion of investigational drug administration. Subjects will be made to rest for at least 30 minutes after each administration of the investigational drug. Investigators will observe subjects' general condition and measure vital signs (blood pressure, pulse and body temperature) for the first hour (±30 minutes) after completion of investigational drug infusion. If adverse events such as infusion reaction, etc. are observed, the time and details will be recorded in the "case report form (observation period)"

14.4 Observation period examination: from completion of investigational drug administration to completion of the observation period

Investigators will observe subjects on each specified hospital visit day and take samples for examination in accordance with the schedule (14.1).

Item	Content	Measurement	
		Facility	Outside Facility
Concomitant drugs/combination therapy	Drug name, dosage, route of administration, period of administration	○	
General symptom	Subjective and objective symptoms	○	
Height, weight		○	
Vital signs	Blood pressure(systolic & diastolic blood pressure), pulse, body temperature	○	
Relapse evaluation	Number of relapse, occurrence date, steroid sensitivity or resistance, presence or absence of frequent relapse or steroid dependence	○	
Evaluation of adverse events		○	
Hematological examination	WBC, differential white blood count, RBC, Hb, Plt		○
Blood biochemical examination	BUN, serum creatinine, uric acid, total protein, serum albumin, AST, ALT, Na, K, Ca, P, Cl, CRP		○
Immunoglobulin	IgG, IgM, IgA		○
Number of peripheral blood B/T- cells	B-cells are CD20 & CD19 positive cells, T-cells are CD3 positive cells		○
Drug blood concentration	See table 14.1		○
HACA	See table 14.1		○
Urinanalysis	Proteinuria and hematuria on urine dipstick test in the first morning urine	○	
	Quantitative examination on proteinuria and creatinine in the first morning urine		○
Electrocardiogram*, chest X-ray*		○	

*Electrocardiogram and chest X-rays are performed at the end of observation period only.

14.5 Observation period examination: at the time of relapse

When the definition of relapse is fulfilled (11.5), investigators will carry out observation, survey and examination on following items and report in the “Relapse Report Form”.

(1) Relapse date, relapse situation

(2) Date of relapse treatment commencement, relapse treatment situation

(3) Urinalysis (relapse confirmation date): Quantitative examination on proteinuria in the first morning urine (measurement carried out by the institution)

14.6 Examination at the time of discontinuation of investigational drug administration

In the event investigational drug administration is discontinued, investigators will carry out an examination at the point of discontinuation of investigational drug administration as soon as possible and record details in the "case report form (observation period)".

Item	Content	Measurement	
		Facility	Outside facility
Concomitant drugs/combination therapy	Drug name, dosage, route of administration, period of administration	○	
General symptoms	Subjective and objective symptoms	○	
Vital sign	Blood pressure (systolic & diastolic blood pressure), pulse, body temperature	○	
Relapse evaluation	Presence and absence of relapse, occurrence date,	○	
Evaluation of adverse events		○	
Drug blood concentration	When administration of the investigational drug is discontinued during infusion, at the time of discontinuation (within 30 min.)		○

14.7 Examination at the time of discontinuation from the clinical trial

In the event subjects discontinue from the clinical trial, investigators will examine the time and reason for discontinuation. They will also carry out an examination (number of peripheral blood B-cells, HACA and IDEC-C2B8 blood concentration) at the time of discontinuation from the trial in order to ensure subjects safety.

In addition, end of observation period examination items (14.4) will observed, examined and surveyed to the extent possible.

(1) Number of peripheral blood B-cells, HACA and IDEC-C2B8 blood concentration (central measurement by an external institution)

(2) Adverse event evaluation

14.8 Special notes concerning clinical examination, drug blood concentration examination and human anti-chimeric antibody (HACA) examination

14.8.1 Hematological examination, blood biochemical examination

These examinations will be entrusted to a contract research organization (the contract research organization's exclusive tubes will be used).

14.8.2 Immunoglobulin examination (IgG, IgM, IgA)

These examinations will be entrusted to a contract research organization (the contract research organization's exclusive tubes will be used).

14.8.3 Peripheral blood B-cells/T-cells

These examinations will be entrusted to a contract research organization (the contract research organization's exclusive tubes will be used).

14.8.4 Urinalysis

Examination of proteinuria and hematuria on urine dipstick test in the first morning urine will be conducted at each medical institution. Quantitative examination on proteinuria and creatinine in the first morning urine will be entrusted to a contract research organization (the contract research organization's exclusive tubes will be used).

14.8.5 Drug blood concentration examination

Measurement of drug blood concentration will be entrusted to a foreign contract research organization. This will be mediated by Zenyaku Kogyo Co., Ltd.

The necessary information will be entered in the prescribed "IDEC-C2B8 clinical sample transfer form (samples for drug blood concentration measurement)" which will be attached when the samples are shipped.

14.8.6 Human anti-chimeric antibody (HACA) examination

Measurement of HACA will be entrusted to a foreign contract research organization. This will be mediated by Zenyaku Kogyo Co., Ltd.

The necessary information will be entered in the prescribed "IDEC-C2B8 clinical sample transfer form (samples for HACA measurement)" which will be attached when the samples are shipped.

It should be noted that the purpose of HACA measurement is not to make a determination on whether to continue investigational drug administration, but rather to examine the relationship with safety and efficacy such as the occurrence of allergic symptoms and B-cell loss effect. Accordingly, HACA examination may be conducted as needed on patients who have a serious allergic reaction or in whom a decrease in B-cells is not brought about.

14.8.7 Sample quantity (blood sample/urine sample quantity) required for each examination and method of storage and shipping

Item	Sample quantity ml	Method of storage・shipping	Measurement	
			Facility	Outside facility
Hemotological examination	1.5	Samples will be refrigerated (at approximately 4℃)(using the contract research organization's exclusive tubes) and delivered to the contract research organization on the same day they are taken from patients		○
Blood biochemical examination	3	Serum will be separated (using the contract research organization's exclusive tubes) and the samples will be cryopreserved (-20℃ or below) until they are delivered to the contract research organization		○
Immunoglobulin examination	2	Serum will be separated (using the contract research organization's exclusive tubes) and the samples will be cryopreserved (-20℃ or below) until they are delivered to the contract research organization		○
Peripheral blood B/T-cell examination	3	The samples will be stored at room temperature (using the contract research organization's exclusive tubes) and delivered to the contract research organization on the same day they are taken from patients		○
IDEC-C2B8 drug blood concentration examination	2	Serum will be separated (using the contract research organization's exclusive tubes) and the samples will cryopreserved (-20℃ or below) until they are delivered to the contract research organization		○
Human anti-chimeric antibody (HACA) examination	2	Serum will be separated (using the contract research organization's exclusive tubes) and the samples will cryopreserved (-20℃ or below) until they are delivered to the contract research organization		○
Urinalysis-proteinuria and hematuria on urine dipstick test in the first morning urine	10	Samples will be delivered to each medical institution's laboratory on the same day they are taken from patients	○	
Urinalysis-quantitative examination of proteinuria and creatinine in the first morning urine	6	Samples will be refrigerated (at approximately 4℃) (using the contract research organization's exclusive tubes) and delivered to the contract research organization on the same day they are taken from patients		○

15 Data collection

Investigators will record the necessary information in relation to all subjects registered to this clinical trial in the following reports according to the progress of the trial up until completion of the clinical trial period and send the reports to the data center.

Type of report	Time for submission	Submission method
Registration form	Within 7 days from the most recent relapse remission date	By fax
Case report (screening period)	Immediately after commencement of investigational drug administration(day 1)	By post
Case report (observation period)	Immediately after observation on day 22, day 113, day 225 and day 365 and immediately after discontinuation from the clinical trial	By post
Case report (peripheral blood B/T-cell results report)	Immediately after receiving the peripheral blood B/T-cell result report	By post
Relapse report	Immediately after confirmation of relapse	By Fax
Exclusion after registration report	Immediately after exclusion after registration	By Fax
Clinical trial discontinuation urgent report	Immediately after discontinuation from the clinical trial	By Fax
Investigational drug urgent allocation code disclosure request form	Immediately after the conditions for urgent allocation code disclosure are met	By Fax

16 Efficacy evaluation

16.1 Primary endpoint

(1) Relapse-free period during the observation period (day 1 - day 365)

Relapse-free period during the observation period (day 1 - day 365) is defined as the period from the date of registration / allocation until the date of the occurrence of the first relapse following commencement of investigational drug administration. This relapse-free period will be terminated at the final observation date on which no relapse was confirmed for subjects who completed the observation period without experiencing relapse. Likewise, the relapse-free period will be terminated at the last day on which no relapse was confirmed prior to follow-up becoming impossible for patients who died or for whom follow-up was impossible. In the event prohibited combination treatment (13.1.2) was carried out prior to the confirmation of relapse, the relapse-free period will be terminated at the date this prohibited combination treatment was commenced.

Relapse	Protein 2+ or above on urine dipstick test in the first morning urine for 3 consecutive days and prednisolone treatment is required.
Relapse date	The first day that protein 2+ or above detected by urine dipstick test in the first morning urine, followed by the same observation for 3 consecutive days.

16.2 Secondary endpoints

(1) Period until treatment failure:

The period until treatment failure is defined as the period from the date of registration / allocation until the date at which treatment failure is determined during the observation period (day 1 – day 365). This period until treatment failure will be terminated at the final date on which no treatment failure was confirmed for subjects who completed the observation period without experiencing treatment failure. Likewise, the period until treatment failure will be terminated at the final date on which no treatment failure was confirmed prior to follow-up becoming impossible for patients who died or for whom follow-up was impossible. In the event prohibited combination treatment (13.1.2) was carried out prior to relapse, the period until treatment failure will be terminated at the date this prohibited combination treatment was commenced.

Treatment failure (1)	When relapse occurs by week 13 (Day 85)
Treatment failure (2)	When frequent relapse or steroid dependence is diagnosed between the day following week 13 (day 86) and week 53 (day 365)
Treatment failure (3)	When steroid resistance is diagnosed during the observation period [the date the first dose of the investigational drug is administered (Day 1) to day 365].

(2) Relapse rate

The number of relapses observed per person per year. This is calculated by the person-years method (number of relapses / person observation years (years)). The target period is the "observation period (day 1 – day 365)". Steroid-resistant relapses during the target period are each counted as one relapse; however relapses are not counted from the date of transition to steroid resistance. Information up until the final observation date will be used for subjects who died or for whom follow-up was impossible and information up until the date treatment failure was determined will be used for those relevant subjects.

Relapse	Protein 2+ or above detected by urine dipstick in the first morning urine for 3 consecutive days and prednisolone treatment is required.
Steroid resistance	When the daily administration of prednisolone at 60 mg/m²/day does not lead to remission within 4 weeks
Date of transition to steroid resistance	The date it is confirmed that the daily administration of prednisolone at 60 mg/m²/day does not lead to remission (C) within 4 weeks
Remission	Negative protein on urine dipstick test in the first morning urine for 3 consecutive days.

(3) Period until occurrence of frequent relapses

The period until occurrence of frequent relapses is defined as the period from the date of registration / allocation until the frequent relapse date during the observation period (day 1 – day 365). This period until occurrence of frequent relapses will be terminated at the final observation date on which no frequent relapse was confirmed for subjects who completed the observation period without experiencing frequent relapse (less than 4 relapses). Likewise, the period until occurrence of frequent relapses will be terminated at the final date on which no frequent relapse was confirmed prior to follow-up becoming impossible for patients who died or for whom follow-up was impossible. The period until occurrence of frequent relapses will be terminated at the date treatment failure was determined for those relevant subjects. In the event prohibited combination treatment (13.1.2) was carried out prior to treatment failure, the period until occurrence of frequent relapses will be terminated at the date this prohibited combination treatment was commenced.

Frequent relapse	Four relapses within the 12-month observation period (day 1 – day 365).
Frequent relapse date	The last relapse date that fulfills the definition of a frequent relapse.
Relapse date	The first day that protein 2+ or above detected by urine dipstick test in the first morning urine, followed by the same observation for 3 consecutive days.

(4) Period until steroid dependence

The period until steroid dependence is defined as the period from the date of registration / allocation until the date of steroid dependence during the observation period (day 1 – day 365). This period until steroid dependence will be terminated at the final observation date on which no steroid dependence was confirmed for subjects who completed the observation period without experiencing steroid dependence. Likewise, the period until steroid dependence will be terminated at the final date on which no steroid dependence was confirmed prior to follow-up becoming impossible for patients who died or for whom follow-up was impossible. The period until steroid dependence will be terminated at the date treatment failure was determined for those relevant subjects. In the event prohibited combination treatment (13.1.2) was carried out prior to relapse, the period until steroid dependence will be terminated at the date this prohibited combination treatment was commenced.

Steroid dependence	When relapse occurs twice consecutively during the reduction of the prednisolone dosage or within 2 weeks after its discontinuation.
Date of steroid dependence	The second relapse date on which the definition of steroid dependence is met.
Relapse date	The first day that protein 2+ or above detected by urine dipstick test in the first morning urine, followed by the same observation for 3 consecutive days.

(5) Period until the transition to steroid resistance

The period until the transition to steroid resistance is defined as the period from the date of registration / allocation until the date of transition to steroid resistance during the observation period (day 1 – day 365). This period until the transition to steroid resistance will be terminated at the final observation date on which no transition to steroid resistance was confirmed for subjects who completed the observation period without experiencing a transition to steroid resistance. Likewise, the period until the transition to steroid resistance will be terminated at the final date on which no transition to steroid resistance was confirmed prior to follow-up becoming impossible for patients who died or for whom follow-up was impossible. The period until the transition to steroid resistance will be terminated at the date treatment failure was determined for those relevant subjects. The period until the transition to steroid resistance will be terminated at the date treatment failure was determined for those relevant subjects. In the event prohibited combination treatment (13.1.2) was carried out prior to the confirmation of relapse, the period until the transition to steroid dependence will be terminated at the date this prohibited combination treatment was commenced.

Steroid resistance	When the daily administration of prednisolone at 60 mg/m²/day does not lead to remission within 4 weeks

Date of transition to steroid resistance	The date it is confirmed that the daily administration of prednisolone at 60 mg/m²/day does not lead to remission within 4 weeks.
Remission	Negative protein on urine dipstick test in the first morning urine for 3 consecutive days.

(6) Total and daily dosage of steroids

The total and daily dosage of steroids administered from the date of registration / allocation until the date of completion of the observation period.

Daily steroid dosage (mg/day) =Total steroid dosage (mg) / observation period (days)

(7) Change in the total and daily dosage of steroids in the 365 days before and after the date of registration / allocation

The difference of the total and daily dosage of steroids administered in the 365 days between immediately before the date of registration / allocation (not including the day of registration / allocation) and after the date of registration / allocation. Subjects in whom investigational drug administration is discontinued will continue to be observed and will be included in the statistics. Subjects for whom information regarding the 365 days prior to the registration / allocation was not obtained and subjects who died or for whom follow-up was impossible will be excluded.

17 Other evaluation

17.1 IDEC-C2B8 blood concentration

Changes in blood concentration.

17.2 Peripheral blood B-Cells

The relationship between the normalization of the number of B-cells in peripheral blood and relapse and the pharmacokinetic impact will be examined.

(1) Number of B-cells in peripheral blood (cells/μL)

The number of B-cells in peripheral blood at each examination

(2) Peripheral blood B-cell depletion period

The period from the date on which peripheral blood B-cell depletion (less than 5/μL) is confirmed until the date on which peripheral blood B-cell recovery (equal to or greater than 5/μL) is confirmed. The peripheral blood B-cell depletion period will be terminated at the final date on which peripheral blood B-cell depletion was confirmed prior to follow-up becoming impossible for patients who died or for whom follow-up was impossible. In addition, subjects in whom peripheral blood B-cell depletion was not confirmed during the observation period will be treated as though recovery occurred on day 0.

17.3 Human anti-chimeric antibody (HACA)

(1) Human anti-chimeric antibody (HACA) production ratio

The proportion of subjects in whom HACA production was observed during the 1-year observation period using the number of patients, regardless of eligibility or non-eligibility, who were administered the investigational drug even once as the denominator.

The presence / absence of HACA production will be examined and the relationship with adverse events evaluated.

18 Safety evaluation

18.1 Adverse events

Adverse events are any undesirable medical event (symptoms, signs, diseases, laboratory test value abnormalities) occurring in subjects following the commencement of investigational drug administration regardless of the causal relationship with the investigational drug. Adverse events where a causal relationship with the investigational drug administration cannot be ruled out will be considered to be side effects. Adverse events occurring during the observation period (day 1 – day 365) will be the target of evaluation in this clinical trial and relapse of nephrotic syndrome will not be treated as an adverse event.
Investigators will immediately take appropriate measures if an adverse event occurs.

18.2 Adverse event evaluation and criteria

Investigators will evaluate adverse events that occur during the clinical trial period and report the following items in a "case report form (observation period)". Abnormalities observed in ECG and chest X-ray examination will be reported as adverse events.
The name and severity (grade) of the adverse event will be evaluated using the Common Terminology Criteria for Adverse Events (CTCAE) v3.0. August 9, 2006 (Japanese translation: JCOG/JSCO edition, March 8, 2007).

Item	Evaluation
Name of adverse event	(In accordance with CTCAE v3.0)
Occurrence, disappearance	(Time of the occurrence and disappearance of the adverse event of the day of administration of investigational drug will be recorded)
Severity (grade) *	Grade 1, Grade 2, Grade 3, Grade 4, Grade 5 (In accordance with CTCAE v3.0)
Seriousness	0. Not serious, 1. Serious
Treatment	0. None, 1. Slow down and interruption, 2. Discontinuation of administration, 3. Medical treatment (content), 4.Others (content)
Result	1. Recovery, 2. Well, 3. Aftereffects, 4. Non-recovery, 5. Death, 6. Unknown
Relationship with the investigational drug	1. Unrelated, 2. Unlikely, 3. Possible, 4. Probable, 5. Definite

*"Grade" refers to the severity of the adverse events. Grade 1-5 displayed in CTCAE v3.0 are as follows:

Grade 1: Mild (Treatment not necessary; Abnormality found in image examination and test results)
Grade 2: Moderate (Need minimum treatment/ local treatment/ noninvasive treatment)
Grade 3: Severe (Indicates significant symptoms which need hospitalization and invasive treatment/IVR/blood transfusion/remedial endoscope/ surgery. etc.)

Grade 4: Adverse event which leads to life-threatening or disabling (Acute and life-threatening metabolic/ cardiovascular complications, etc. which needs intensive care and emergency treatment (emergency IVR/remedial endoscope/surgery etc.)

Grade 5: Adverse event which leads to death

If the grade evaluation is not consistent with the CTCAE v3.0 criteria, the reason for the inconsistency will be reported in a “case report form (observation period)”.

18.2.1 Severity of major adverse events (grade)

The major adverse events have been extracted from CTCAE v3.0 and are shown as follows.

Grade	1 Mild	2 Moderate	3 Severe	4 Life-threatening or disabling	5 Death
hypertension	Asymptomatic, transient (< 24 hrs) increase by >20mmHg(diastolic) or to>150/100 if previously WNL; intervention not indicated Pediatric: Asymptomatic, transient(<24hrs)BP increase >upper limit of normal (ULN); intervention not indicated	Recurrent or persistent (≧24 hrs) or symptomatic increase by >20mmHg (diastolic) or to >150/100 if previously WNL monotherapy may be indicated Pediatric; Reccurent or persistent (≧24hrs) BP >ULN; monotherapy may be indicated	Requiring more than one drug or more intensive therapy than previously Pediatric; Same as adult	Life-threatening consequences(e.g., hypertensive crisis Pediatrics; Same as adult	-
Hypotension	Intervention not indicated BP decrease	Brief (<24 hrs) fluid replacement or other therapy; no physiologic consequences	Sustained (≧24 hrs) therapy, resolves without persisting physiologic consequences	Shock (e.g., acidemia; impairment of vital organ function)	Death
Fever	38.0-39.0℃	＞39.0-40.0℃	＞40.0℃ for ≦24hrs	＞40.0℃ for ＞24hrs	Death
Rigors/chills	Mild	Moderate, narcotics indicated	Severe or prolonged, not responsive to narcotics	-	-
Pruritus/itching	Mild or localized	Intense or widespread	Intense or widespread and interfering with ADL	-	-
Rash (urticaria, welt, weal)	Intervention not indicated	<24hrs intervention indicated	≧24hrs intervention indicated	-	-
Adrenal insufficiency	Asymptomatic, intervention not indicated	Symptomatic, intervention indicated	Hospitalization	Life-threatening, disabling	Death
Nausea	Loss of appetite without alternation in eating habits	Oral intake decreased without significant weight loss, dehydration or malnutrition；intravenous fluid indicated<24hrs	Inadequate oral caloric or fluid intake； intravenous fluids, tube feedings or TPN indicated ≧24hrs	Life-threatening consequences	Death
Taste alternation（dysgeusia）	Altered taste but no change in diet	Altered taste with change in diet (e.g., oral supplements); unpleasant taste; loss of taste	-	-	-
Infection	Mild	Moderate	Serious	Life threatening	Death
Acidosis (metabolic or respiratory)	pH<normal, but≧7.3	-	pH<7.3	pH<7.3 with life-threatening consequences	Death
SGPT	＞ULN – 2.5×ULN	＞2.5 - 5.0×ULN	＞5.0–20.0×ULN	＞20.0×ULN	-
Amylase	＞ULN-1.25×ULN	＞1.5 - 2.0×ULN	＞2.0 – 5.0×ULN	＞5.0×ULN	-
SGOT	＞ULN – 2.5×ULN	＞2.5 - 5.0×ULN	＞5.0–20.0×ULN	＞20.0×ULN	-
Cataract	Asymptomatic, detected on examination only	Symptomatic, with moderate decrease in visual acuity (above0.5)；decreased visual function correctable with glasses	Symptomatic, with marked decrease in visual acuity (below0.5)；operative intervention indicated (e.g., cataract surgery）	-	-
Glaucoma	Elevated intraocular pressure(ELOP) with single topical agent for intervention；no visual field deficit	EIOP causing early visual fielddeficit（e.g., nasal step or arcuate deficit）；multiple topical or oral agents indicated	EIOP causing marked visualfield deficites（e.g., involving both superior and inferior visual fields）；operative intervention indicated	EIOP resulting in blindness（under 0.1）；enucleation indicated	-
Bronchospasm/wheezing	Asymptomatic	Symptomatic, not interfering with function	Symptomatic, with interfering function	Life-threatening	Death
Cough	Symptomatic, non-narcotic medication only indicated	Symptomatic, and narcotic medication indicated	Symptomatic, and significantly interfering with sleep or ADL	-	-
Laryngeal edema	Asymptmatic, weakness on clinical examination/testing only	Symptomatic, with no dyspnea	Wheeze；dyspnea；interfering with ADL	Life-threatening；tracheatomy/intubation/laryngectomy indicated	Death

18.2.2 Serious adverse events

Any of the following events are defined as serious adverse events:

(1) Death

(2) Events that could lead to death

(3) Events which necessitate hospitalization at a hospital or clinic or extension of the hospitalization period for the purpose of treatment

(This shall not include hospitalization or extension of the hospitalization period for the purpose of re-examination or follow-up)

(4) Disorder (a dysfunction that interferes with daily life)

(5) Events that could lead to disorder

(6) Events with a seriousness similar to (1) – (5)

(7) Congenital disease or abnormality in the next generation

It will be necessary to determine on medical and scientific grounds whether urgent reporting is required for other significant events that are not immediately life-threatening and do not lead to death or hospitalization but endanger the subject and require treatment so that the above outcomes are avoided. Such events should usually be considered serious events. (For example: bronchospasm requiring intensive care, blood disorders or convulsions that do not lead to hospitalization, drug abuse or drug addiction, etc.)

18.2.3 Relationship with the investigational drug

The relationship between the adverse event and the investigational drug will be given a 5-stage classification in accordance with the CTCAE v3.0 treatment of causal relationship. In the event an adverse event is adjudged as having "1 – no relationship", the reason for this determination will be listed in a "case report form (observation period)".

Code	Evaluation	Definition
5	definite	Definitely by the treatment
4	probable	Probably by the treatment
3	possible	Possibly by the treatment
2	unlikely	Unlikely by the treatment
1	no relationship (unrelated)	Not related with the treatment

Except for "1: no relationship" are referred as side effects

18.3 Urgent reporting of adverse events

18.3.1 Reporting at the time of a serious adverse event

(1) If an adverse event is adjudged as serious, the investigator will create a "serious adverse event report" and "serious adverse event contact form" and, regardless of a causal relationship between the adverse event and the investigational drug, report to the clinical trial steering committee and investigational drug supplier as well as the head of their medical institution as soon as possible (report to the clinical trial steering committee and investigational drug supplier by a sub-investigator will also be acceptable).

(2) The clinical trial steering committee will confirm the content of the serious adverse event received from the investigators and notify the investigators at other medical institutions of the relevant adverse event information.

(3) The investigators of each medical institution will confirm the content of the notification received from the clinical trial steering committee and, after consulting with the clinical trial steering committee as needed, report their opinions as investigators (including the necessity of making a report to the Minister of Health, Labour and Welfare) to the clinical trial steering committee.

(4) If it is deemed necessary by investigators to make a report to the Minister of Health, Labour and Welfare, the clinical trial steering committee will create an investigational drug side effect / infection case report –form 7 and 8* (hereafter, "form 7 and 8") and clinical trial side effect, etc. report coordination form** (hereafter, "coordination form") and report to the Minister of Health, Labour and Welfare. In addition, the clinical trial steering committee will retain a copy of "form 7 and 8" and the "coordination form".

* Prescribed by the "reporting of clinical trial side effects to the Pharmaceuticals and Medical Devices Agency" (Pharmaceutical and Food Safety Bureau Notice No. 0330001 dated March 30, 2004, amended by Notice No. 1215003 dated December 15, 2005)"

** Prescribed by the "reporting of clinical trial side effects by persons conducting their own clinical trials following the establishment of the Pharmaceuticals and Medical Devices Agency" amendment (Pharmaceutical and Food Safety Bureau Notice No. 1025005 dated October 25, 2005)"

(5) The reporting deadlines to the Minister of Health, Labour and Welfare are prescribed by article 273 of the Enforcement Regulations of the Pharmaceutical Affairs Law among other provisions and are as follows.

The starting date for the calculation of reporting deadlines will be the day the investigator became aware of the adverse event.

To be reported within 7 days	Unknown events that fall under 18.2.2: (1) and (2)
To be reported within 15 days	Known events that fall under 18.2.2: (1) and (2) and unknown events that fall under 18.2.2: (3), (4), (5) and (6)

The occurrence of an adverse event and incidence trends such as the number of occurrences, frequency of occurrence and conditions of occurrence that cannot be predicted from the investigator's brochure (including side effects already reported to the Minister of Health, Labour and Welfare and each investigator) will be assumed to be unknown and the ones that can be predicted will be assumed to be known.

(6) In the event a serious adverse event that occurred at a participating medical institution has been reported to the Minister of Health, Labour and Welfare, the investigators will report the content of "form 7 and 8" they receive from the clinical trial steering committee to the head of the relevant medical institution as soon as possible.

(7) If the investigator of the medical institution in which the relevant adverse event occurred obtains additional information concerning that event, he will make an additional report to the head of that medical institution as well as report to the clinical trial steering committee and investigational drug supplier as soon as possible. The clinical trial steering committee will pass on the additional information to the investigators of the other medical institutions. If the investigators deem this additional information to be an additional report of side effects, a withdrawn report or a new report, (4) shall apply.

18.3.2 Reporting at the time of a grade 3 adverse event

In addition to the provisions of the previous section, if a grade 3 adverse event occurs (or grade 4 in the case of hematological toxicity), investigators will create a "grade 3 adverse event report" and report to the clinical trial steering committee via the process described in 18.3.1 from the perspective of ensuring subject safety, even if the grade 3 adverse event is not deemed to be a serious adverse event.

18.4 Main side effects anticipated from the investigational drug

The main side effects anticipated from the investigational drug are extracted from the Rituxan®, rituximab (recombinant) formulation package insert as follows. Refer to the package insert for further details.

(1) Serious side effects

1) Anaphylactoid symptoms, pulmonary disorder, cardiac disorder (frequency unknown)
Hypotension, angioedema, hypoxemia, bronchospasm, pneumonia (including interstitial pneumonia and allergic pneumonia), bronchiolitis obliterans, pulmonary infiltrates, acute

respiratory distress syndrome, myocardial infarction, ventricular fibrillation and cardiogenic shock, etc. have been observed as infusion reaction symptoms.

(Important precautions): common infusion reactions (symptoms: fever, chills, nausea, headache, pain, pruritus, rash, cough, lethargy and angioedema, etc.) are reported in 90% of patients during the first administration or within 24 hours following the first administration of this drug. These symptoms are mild to moderate and usually appear when the first dose of the drug is administered. The patient's condition should be watched carefully and, if abnormalities are observed, appropriate measures should be taken (antipyretic analgesic, antihistamine administration, etc.) and the patient should be carefully observed until their symptoms recede.

2) Tumor lysis syndrome (frequency unknown)
3) Fulminant hepatitis from the hepatitis B virus, exacerbation of hepatitis (frequency unknown)
4) Liver dysfunction, jaundice (0.1 - less than 5%)
5) Mucocutaneous symptoms (frequency unknown)
6) Pancytopenia (frequency unknown), leukopenia, neutropenia (10% or above), thrombocytopenia (less than 5%)
7) Infection (frequency unknown)
8) Progressive multifocal leukoencephalopathy (PML) (frequency unknown)
9) Interstitial pneumonia (frequency unknown)
10) Cardiac dysfunction (frequency unknown)
11) Renal dysfunction (frequency unknown)
12) Gastrointestinal perforation (frequency unknown)
13) Hypotension (frequency unknown)
14) Neurological symptoms such as posterior reversible encephalopathy syndrome (frequency unknown)

(2) Other side effects (5% or above or frequency unknown)

1) Respiratory: pharyngitis, cough
2) Circulatory: increased blood pressure, tachycardia, bradycardia
3) Digestive: nausea, vomiting
4) Hypersensitivity: fever, chills, pruritus, rash, hot flashes, serum sickness
5) General condition: headache, lethargy, pain, excessive sweating, fatigue
6) Blood and coagulation: anemia, eosinophilia, fibrin degradation products [FDP, D-dimer increase]
7) Liver: AST (GOT) elevation, ALT (GPT) elevation
8) Other: CRP elevation, administered site reaction (pain, swelling, etc.), decrease in total protein, decrease in albumin, hiccups

19 Statistical Analyses

19.1 Target population for analyses

The analysis target populations used in interim analysis and final analysis are defined as follows. Details on handing the cases up until the first interim analysis are prescribed in the statistical analysis plan by the person responsible for statistical analysis. The efficacy endpoint analysis target population for final analysis will be the maximum analysis target population and analysis will be performed with reference to the analysis results of the target population conforming to the clinical trial protocol. The safety endpoint analysis target population for interim and final analysis will be the safety analysis target population.

(1) Maximum analysis target population; Full analysis set (FAS):
The subject population that received at least one dose of the investigational drug and in whom data necessary for the analysis of the primary endpoint was measured after administration.

(2) Target population conforming to the clinical trial protocol; Per Protocol Set (PPS):
The group of subjects from the FAS whom are determined from monitoring to have adhered to the clinical trial protocol without violation

(3) Safety analysis target population; Safety Analysis Set (SAS)
The subject population that received at least one dose of the investigational drug

19.2 Rationale for setting the target number of subjects

Since the subject population of this clinical trial is childhood-onset complicated nephrotic syndrome patients in whom, despite steroid sensitivity, remission cannot be maintained by the treatment with known immunosuppressive drugs and withdrawal from steroids is difficult, it is considered rare for remission to be maintained for 6 months or more. However, relapse-free periods ranging from 4 months to 13 months were achieved in previous studies where 4 x 375 mg/m² doses (maximum dose: 500 mg) of IDEC-C2B8 was administered at weekly intervals to childhood-onset complicated nephrotic syndrome patients[35-38]. In addition, the 6-month remission maintenance ratio was 0.36 and the median relapse-free period 129 days in the 15 cases where 1 x 375 mg/m² dose of IDEC-C2B8 was administered by this clinical study's principal researcher. Although all of these examples are merely case reports and are insufficient to estimate the effect of IDEC-C2B8, 30 cases for each group are required to detect a significant difference at the 5% level (2.5% one-sided) with 90% statistical power in the event the 6-month remission rate of the IDEC-C2B8 group is $p_1 = 0.4$ (equivalent to a median relapse-free period of 5 months) and the 6-month remission rate of the placebo group is $p_0 = 0.1$.

In addition, the sample size design has been set on the assumption that the integration period (in the case

relapses during the observation period will be the only event for each subject) is 0 years, the observation period is 1 year, survival time is in accordance to exponential distribution and therapeutic effect is a proportional hazard.

19.3 Analysis methods

19.3.1 Primary analysis and criterion

The purpose of primary analysis in this clinical trial is to verify whether the relapse-free period (the primary endpoint) is significantly longer in the IDEC-C2B8 group compared with the placebo group. The "relapse-free period between the 2 groups will be equal" null-hypothesis test will be performed with the log-rank test on the FAS specified in 19.1. If there are factors within the allocation adjustment factors (10.2.6) that strongly affect efficacy, stratified adjustment using these factors will be examined under blinding at the clinical conference prior to entire clinical trial key opening. As statistical significance is not of interest in the event the relapse-free period of the IDEC-C2B8 group is inferior to the placebo group, testing will be one-sided. The one-sided significance level, including interim analysis, is set at 2.5%. As the one-sided significance level consumed in interim analysis is a maximum of 0.25%, the one-sided significance level in final analysis is set at 2.5%. It will be concluded that IDEC-C2B8 is a useful treatment in the event the relapse-free period is statistically significantly longer in the IDEC-C2B8 group compared with the placebo group. The analysis results of the PPS will be used of reference only.

Estimation of the cumulative relapse curve, 50% relapse period and time-to-relapse ratio will be performed using the Kaplan-Meier method and their 95% confidence intervals will be sought using the Greenwood formula. The therapeutic effect hazard ratio of each group and its 95% confidence interval will be sought as an estimate of therapeutic effect using the Cox proportional-hazards model. Analysis will also be conducted with allocation adjustment factors, peripheral blood B-cell depletion period and disease duration, etc. as covariates.

19.3.2 Secondary endpoint (efficacy endpoint) analysis

Multiplicity adjustment will not be carried out in secondary endpoint analysis. The two-sided significance level of 5% is set.

The relapse rate for each group will be calculated by the number of relapses/observation in person-year method and comparison between the groups and configuration of the 95% confidence intervals will be performed by conducting a permutation test. Exploratory analysis will be carried out to evaluate the effectiveness of the investigational drug for repeated relapse as there are many uncertainties regarding relapse intervals and mechanisms following the administration of IDEC-C2B8. A 4-way analysis is planned using a combination of 2 baseline hazards (baseline hazard common for each relapse and baseline hazard not common for each relapse) and 2 risk interval definitions (a Total Time approach where the time for each

relapse is calculated from the date of registration / allocation and a Counting Process approach where the time for each relapse is calculated from the date of the previous relapse).

Estimation of the cumulative relapse curve and time-to-relapse ratio will be performed using the Kaplan-Meier method and the log-rank test conducted in the analysis of the period until treatment failure.

Estimation of the respective cumulative relapse curve, 50% relapse period and time-to-relapse ratio (365 days after registration / allocation, etc.) will be performed using the Kaplan-Meier method and the log-rank test conducted in the analyses of the period until occurrence of frequent relapses, period until steroid dependence and period until the transition to steroid resistance. Therapeutic effect will be estimated using the Cox proportional hazards model.

The Wilcoxon rank-sum test between groups will be performed in the analysis of the total and daily steroid dosage. In addition, the Wilcoxon rank-sum test between groups will be performed in the analysis of the cumulative dosage from day 1 at day 28, day 140 and day 252.

Change of the total and daily steroid dosage in the 365 days before and after the date of registration / allocation in each group will be compared by performing the Wilcoxon rank-sum test.

19.3.3 Other efficacy analysis

The following analysis will be carried out as part of sensitivity analysis. Details will be specified in the analysis report.

(1) Analysis from the date of the first administration of the investigational drug (day 1)
The primary endpoint (6.1.1, 16.1) and secondary endpoints (1) – (7) (6.1.2, 16.2) will be analyzed similarly using day 1 as the starting date.

(2) Analysis of the relapse rate in the 365 days prior to the date of registration / allocation and the 365 days after the date of registration / allocation
The relapse rate (relapse/year/patient) in the 365 days prior to the date of registration / allocation (control) and the 365 days after the date of registration / allocation will be compared for each group. The relapse rate is calculated as “number of relapses ÷ reported period (years)” and therefore the length of a period of the control has no relevance. A relapse ratio (R_1/R_0) will be calculated for each individual from their existing control relapse rate (R_0) and relapse rate after the date of registration / allocation (R_1), and statistical testing for the null hypothesis (“relapse ratio: $R_1/R_0 = 1$”) will be performed and 95% confidence intervals configured by conducting individual permutation tests.

(3) Analysis based on the data following completion of participation in the clinical trial

Information regarding the post-trial treatment and outcomes of subjects who joined a separate IDEC-C2B8 pharmacokinetic study after completing their participation in this clinical trial as a result of treatment failure will be collected from the relevant pharmacokinetic study. As-treated analysis of secondary efficacy endpoints (16.2) integrated into the data of this clinical trial will be performed with part of the information collected from said IDEC-C2B8 pharmacokinetic study (the information in the period up until the day corresponding with day 365 of this trial).

(4) Efficacy sensitivity analysis

In the relapse-free period (primary endpoint) and the period until treatment failure, period until occurrence of frequent relapses, period until steroid dependence and period until the transition to steroid resistance (secondary endpoints), analysis will be carried out with “the administration of prohibited combination therapy (13.1.2)” as an event. Estimation of the respective cumulative relapse ratios will be performed using the Kaplan-Meier method and the log-rank test conducted.

(5) Efficacy analysis in subjects who moved on to an IDEC-C2B8 pharmacokinetic study

Two paired relapse-free periods are observed in subjects who moved on to an IDEC-C2B8 pharmacokinetic study from this clinical trial. The log-rank test will be conducted taking into account the correlation in the individual.

19.3.4 Analyses of other endpoints

(1) Change in peripheral blood B-cells

Summary statistics will be calculated for each group at each examination period and a comparison between groups for each period carried out by performing t-tests.

The relationship between normalization in the number of peripheral blood B-cells and the presence or absence of relapse and adverse events will be evaluated by chi-square test or Fisher's exact test.

(2) Change in Human Anti-Chimeric Antibody (HACA) values

The HACA production ratio will be compared between groups at each examination period by performing Fisher's exact test. The exact confidence intervals for the binomial distribution will be used for interval estimation.

The relationship between HACA production and the presence or absence of relapse and adverse events will be evaluated by Chi-square test or Fisher's exact test.

19.3.5 Safety analysis

(1) Adverse events and side effects

The number of subjects who experienced adverse events and the adverse event occurrence ratio (number of subjects who experienced adverse events/SAS) will be classified for each treatment group, and aggregated by affected organ major classification, by adverse event (symptoms and findings) and by grade. The most severe grade of each respective adverse event observed during the clinical trial period will be used in this data.

It should be noted that even cases where treatment is discontinued (10.4) due to toxicity or a revocation of participation from the subject or their legal representative, etc. will be followed for 1 year from the date of commencement of investigational drug administration except in the cases where follow-up is impossible. In addition, events and termination will be treated in the same way even in cases where other treatments were added after the discontinuation of the trial.

The number of subjects who experienced serious adverse events and the serious adverse event occurrence ratio will also be aggregated for each group and a table will be created. In addition, the number of subjects who experienced adverse events and the occurrence ratio (%) in each group classified by adverse events (according to individual adverse event and affected organ major classification) occurring during the investigational drug administration period which caused a discontinuation of this trial will also be aggregated.

Comparison between groups will be carried out as necessary using Fisher's exact test. Aggregation of each event will be carried out over the entire clinical trial period. The adverse event terminology included in MedDRA will be used. Analysis of side effects will be carried out in the same manner.

(2) Laboratory test values

1) Laboratory test value (hematological examination, blood biochemical examination and urinalysis) measured value aggregation

Summary statistics for each group at each examination period will be calculated.

2) Laboratory test value (hematological examination, blood biochemical examination and urinalysis) abnormal value aggregation

The frequency of each laboratory test abnormal value for each group at each examination period will be aggregated.

(3) Vital signs

1) Body temperature in the 24 hours after commencement of administration will be aggregated

19.3.6 Interim analysis

(1) Interim safety analysis

No serious adverse events thought be attributable to IDEC-C2B8 have been reported in previous studies of IDEC-C2B8 therapy on childhood-onset complicated nephrotic syndrome patients (primarily with 4 x 375 mg/m² doses (maximum dose: 500 mg) administered at weekly intervals)[36,38-42], however there has been little experience in the way of administration of 4 x 375 mg/m² doses (maximum dose: 500 mg) of IDEC-C2B8 to childhood-onset complicated nephrotic syndrome patients domestically. Accordingly, interim analysis will be conducted in order to confirm safety at the point administration of the investigational drug has been completed or discontinued in 20 subjects.

(2) Interim efficacy analysis

The study population for this clinical trial is patients with very high disease activity in whom remission cannot be maintained with known immunosuppressive medication and therefore bringing about early relapse in these subjects is a concern. In the event the significant superiority of IDEC-C2B8 has been indicated while also taking into account safety considerations, interim analysis will be conducted in order to avoid the continuation of unfavorable treatment in the placebo group at the point when 30 subjects have experienced their initial relapse. 30 cases accounts for approximately half the number of the initial relapses expected to be observed throughout the entire study (assuming a placebo group relapse rate of 90%/year and a test drug group relapse rate of 20-60%/year = 50-59 cases). The items to be analyzed are: the relapse-free period (primary endpoint), relapse rate up until interim efficacy analysis (in order to observe the effect on multiple relapses), and period until the occurrence of a second relapse. The significance level for decision criterion are: 0.25% one-sided significance level for the relapse-free period which will be analyzed by conducting the log-rank test, 2.5% one-sided significance level for the relapse rate which will be analyzed by conducting the permutation test, and 2.5% one-sided significance level for the period until the occurrence of a second relapse which will be analyzed with the WLW model. If all these analysis items are statistically significant, IDEC-C2B8 will be deemed to display superiority at the point of interim analysis.

The independent data and safety monitoring committee statistician will create an interim analysis report and submit it to the independent data and safety monitoring committee. The independent data and safety monitoring committee will conduct an examination based on this report and recommend whether or not to continue the clinical trial. The clinical trial steering committee will then consult with the person responsible for statistical analysis and determine whether or not to change the clinical trial protocol and whether or not to continue the clinical trial (continue, suspend or discontinue) based on the recommendation from the independent data and safety monitoring committee.

1) In the event information of serious adverse events or grade 3 or above side effects in sequential monitoring has been reported or the frequency of adverse events is significantly higher in the IDEC-C2B8 group at the point of interim analysis, registration will be discontinued from the time of the report and whether or not to continue the trial will be discussed.

2) In the event the significant superiority of IDEC-C2B8 has been indicated at the point of interim analysis, randomized assignment will be stopped, registration continued until the IDEC-C2B8 group consists of 30 subjects and the trial continued for all subjects until the 1-year observation period of the final-registered subject is completed.

3) In the event the significant superiority of IDEC-C2B8 has not been indicated and the safety of IDEC-C2B8 not confirmed at the point of interim analysis, the trial will continue until the target number of subjects is reached.

20 Direct viewing of source documents and others

20.1 Acceptance of direct viewing

The heads of the medical institutions implementing this clinical trial and investigators, will accept investigation by monitoring and audit personnel, the institutional review boards and regulatory authorities and, upon their request, will present all source documents such as clinical trial-related records, etc. shown below for direct viewing.

Monitoring personnel will directly view the source documents such as clinical trial-related records, etc. at all medical facilities in order to confirm the content of case report forms has been described accurately and completely and is consistent with source documents. In addition, monitoring personnel will also directly view essential documents in all medical institutions to confirm that all clinical trial-related records that should be kept at the medical institutions have been accurately and completely created, and stored.

20.2 Definition of source documents

The following documents are defined as source documents.

1) Consent forms
2) Medical records (including examination forms)
3) Nursing records
4) Patient diaries
5) Laboratory data
6) Investigational drug administrative table, investigational drug invoices and investigational drug collection notes
7) Subject identification code list
8) Other records used in creating the case reports

The following items will be recorded in medical records.

1) Subject background (complications, past history and medical history)
2) Presence or absence of other drug use
3) Presence or absence of subjective symptoms and objective findings
4) Presence or absence of laboratory testing and abnormal laboratory data
5) Presence or absence of adverse events and their degree / severity and comments regarding the situation of investigational drug administration and causal relationship
6) Presence or absence of investigational drug discontinuation and comments
7) Evaluation
8) Comments of investigators

21 Quality control and quality assurance

21.1 Data quality control

The investigators will confirm that this clinical trial is being conducted in adherence to the GCP ministerial ordinance and the clinical trial protocol, etc. and carry out monitoring and quality control according to the progress of the trial in order to ensure accuracy, completeness and reliability of the data.

The investigators and monitoring personnel will explain the content of the clinical trial protocol and the procedure for the creation of case reports, etc. to the sub-investigators, etc. prior to the commencement of the clinical trial. In addition, the method of managing the investigational drug will be explained to the investigational drug administrator. During the clinical trial periods, monitoring personnel will periodically visit the medical facilities to confirm the compliance of the clinical trial protocol, the consistency of the source documents and case reports, and the presence of required documents.

21.2 Auditing

Auditors will conduct audits as part of quality assurance activities. They will evaluate whether this clinical trial has been conducted in accordance with the GCP ministerial ordinance, the clinical trial protocol, etc., both independently and separately from the quality control operations of this clinical trial.

21.3 Provision of new information

In the event investigators obtained important new information that may be relevant to the consent given by the subject (such as information related to infections, diseases, disorders or death suspected to be caused by the investigational drug or any other information about the quality, efficacy or safety of the investigational drug), the explanatory and consent documents will immediately be amended based on this information, and the approval of the institutional review board should be obtained. In addition, investigators will inform subjects currently participating in the trial or their legal representatives of the content of the amendments, confirm their intention to continue their participation in the trial and use the amended explanatory and consent documents to re-obtain consent. The specific procedures for explanation and obtaining consent will be the same as set forth in 10.2.1.

22 Ethical considerations

22.1 Ethical implementation of the clinical trial

This clinical trial will be conducted in compliance with following Japanese laws and regulations, including article 14, paragraph 3 and article 80, paragraph 2 of the Pharmaceutical Affairs Act, the Good Clinical Practices (GCP) ordinance (Ministry of Health, Labour and Welfare ordinance no. 72, March 31, 2006), the "ordinance for the partial amendment of the GCP ordinance" (Ministry of Health, Labour and Welfare ordinance no. 72, March 31, 2006), the "ordinance for the partial amendment of the GCP ordinance" (Ministry of Health, Labour and Welfare ordinance no. 24, February 29, 2008) and this clinical trial protocol, adopting the principles of the Declaration of Helsinki.

22.2 Explanation and obtaining consent

When obtaining consent, investigators will provide potential subjects with an explanatory document that explains the following matters, fully explain the content of said document and obtain consent for participation in this trial using a consent document.

22.2.1 Matters to be explained

(1) This clinical trial involves research.
(2) The purpose of the clinical trial.
(3) The clinical trial method.
(4) The planned clinical trial participation period of the subject.
(5) The number of subjects planned to participate in the clinical trial.
(6) The expected clinical benefits and risks in relation to the mental and physical health of the subject and inconvenience.
(7) The presence or absence of other treatments and the important benefits and risks expected from those treatments.
(8) The compensation and treatment the subjects can receive if their health is adversely affected in connection with this clinical trial.
(9) Participation in this trial is voluntary and subjects may refuse or withdraw their participation in this trial at any time. In addition, subjects will not receive unfavorable treatment as a result of refusal or withdrawal of participation or lose benefits as a result of non-participation.
(10) If new information that may affect subjects' intention to continue their participation in this trial is obtained, said information will immediately be conveyed to the subjects.
(11) The conditions and reasons for discontinuation of participation in this trial.
(12) Monitoring personnel, auditors, the institutional review boards of the medical facilities and regulatory authorities are able to view original medical records. In such an event, the confidential information of subjects will be protected. In addition, subjects will consent to the viewing of original

medical records by signing and sealing or simply signing the consent form.

(13) The confidential information of subjects will be protected even if the results of the clinical trial are published.

(14) The content of items where the expenses must be borne by the subjects.

(15) The content of the money, etc. paid to subjects (arrangements for the calculation of payment, etc.)

(16) The names, titles and contact details of investigators

(17) The medical institution's consultation representative to whom the subjects desire additional information about the clinical trial or their rights , or they make contact in case they suffer adverse health events.

(18) Matters to be adhered to by the subjects.

(19) The type of institutional review boards (names and establishers) that investigate and deliberate the propriety, etc. of this clinical trial and matters relating to institutional review boards including the matters of investigation and deliberation at each institutional review board.

22.3 Institutional Review Boards

Investigators will obtain approval from their institutional review board prior to conducting this clinical trial. In addition, institutional review board approval will also be necessary in the event of any amendment to the clinical trial protocol. In the event an investigator obtains any information that may affect subject safety and the implementation of this clinical trial, they will immediately report to the investigators of other medical institutions and the head of their medical institution and obtain approval for the continuation of the trial from their institutional review board.

22.4 Subject confidentiality

The following provisions will be adhered to in relation to subject confidentiality:

Case reports will be created and handled with consideration to subject confidentiality.

Subjects will be specified by their subject identification code.

Subject confidentiality will be protected in the event an institutional review board, monitoring personnel, auditors or regulatory authorities directly view source documents.

Publication of the clinical trial results and application for approval by the investigational drug supplier will be carried out with consideration to subject confidentiality.

23 Handling of data and record keeping

23.1 Case report creation and reporting

Investigators will immediately create a case report after the completion of observation and examination of each subject and submit a copy to the data center. Investigators will retain the original case reports. Case report creation and amendment will be carried out in accordance with the "guidebook on case report creation and amendment".

Investigators will create and sign and seal a "record of amendment or change to the content of a case report" in the event of major amendment or change to a case report. In addition, in the event data in a case report is inconsistent with source documents, principal investigators will create and sign and seal a "explanation of inconsistency between the content of a case report and source documents" explaining the reason for the inconsistency.

23.2 Deviation from the clinical trial protocol

In the event investigators have deviated from the clinical trial protocol in order to avoid urgent risk of the subjects or for any other unavoidable medical reason, they will record the details in a "report on deviation from the clinical trial protocol for emergency avoidance". The investigators will then immediately submit this report which contains the reason for the deviation to the head of their medical institution who will in turn submit it to the institutional review board.

Investigators will handle deviation for reasons other than emergency avoidance in accordance with the provisions of the medical institution.

23.3 Data Management

Data management personnel will manage data using a validated data management system in accordance with previously created "data management procedures".

23.4 Record keeping

The retention period for documents and records pertaining to the clinical trial is prescribed below. The investigational drug supplier will notify the medical institutions in writing if retention becomes unnecessary as a result of approval of partial changes in manufacture and sales approval having been obtained for the relevant investigational drug or discontinuation of development.

23.4.1 Investigators

Investigators will retain documents and records pertaining to the clinical trial until the later of the dates specified in 1) and 2). However, if notice is received from the investigational drug supplier stating that retention for a longer period of time is necessary, a new retention period shall be decided in consultation with the investigational drug supplier. A person will be placed in charge of retaining each record. In addition, the heads of the medical institution and the institutional review board establishers will be notified when the retention period has expired.

1) The date of approval of partial changes in manufacture and sales approval based on this clinical trial (in the event development of the investigational drug is discontinued, then 3 years after the date of the decision to discontinue development)

2) 3 years after the completion or discontinuation of this clinical trial

23.4.2 Medical institutions

The heads of medical institutions will retain documents and records pertaining to the clinical trial until the later of the dates specified in 1) and 2). However, if notice is received from the investigational drug supplier or an investigator stating that retention for a longer period of time is necessary, a new retention period shall be decided in consultation with the investigational drug supplier or investigator. A person will be placed in charge of retaining each record.

In order to ensure these documents and records are not lost or discarded during the retention period, the heads of medical institutions and the persons placed in charge of retaining documents and records will take steps such as ensuring that the documents and records can be presented upon request, etc.

1) The date of approval of partial changes in manufacture and sales approval based on this clinical trial (in the event development of the investigational drug is discontinued, then 3 years after the date of the decision to discontinue development)

2) 3 years after the completion or discontinuation of this clinical trial

23.4.3 Institutional Review Boards

The institutional review board establishers will retain records such as the board's standard operating procedures, board roster (including qualifications and affiliations of each board member), a board member occupation and affiliation list, submitted documents, summaries of meeting minutes and letters, etc. until the later of the dates specified in 1) and 2). However, if a principal investigator deems that retention for a longer period of time is necessary, a new retention period and method shall be decided in consultation with the investigator.

1) The date of approval of partial changes in manufacture and sales approval based on this clinical trial (in the event development of the investigational drug is discontinued, then the date of the decision to discontinue development. In the event notification is received that documentation relating to the clinical trial certificate of analysis will not be attached to the application for approval of partial changes in manufacture and sales approval, then the date said notification was received)

2) 3 years after the completion or discontinuation of this clinical trial

23.4.4 Investigational drug supplier

This investigational drug is designated a biological product in the indications for which it has already been approved and therefore the investigational drug supplier will retain records, etc. in accordance with legal regulations related to biological products.

24 Payment of money, other compensations, and insurance

24.1 Payment of money and other compensations

The clinical trial expenses expected in this clinical trial will be funded by the Health and Labour Sciences Research Grants Large Scale Clinical Trial Network Project.

24.2 Compensations for adverse health effects

The medical institutions will take necessary and appropriate measures related to treatment such as providing a medical care system, etc. in the event subjects suffer adverse health effects as a result of participation in this clinical trial. The payment of compensation will not be implemented in this clinical trial. In addition, if adverse health effects are deemed to have been caused intentionally or through the gross negligence of a subject or their legal representative (a person who exercises parental authority or a legal guardian), said subject or legal representative may become ineligible to receive compensation.

If liability arises as a consequence of adverse health effects caused by this clinical trial, the investigators, and medical institutions will be insured under the insurance held by the head of the Japan Medical Association Center for Clinical Trials (the policyholder) under an agreement between the investigators or the medical institutions with the Japan Medical Association Center for Clinical Trials. In addition, the investigators will be enrolled in medical professional liability insurance and the medical institutions enrolled in hospital liability insurance.

25 Arrangements regarding publication

The results of this clinical trial will, regardless of the results, be (1) submitted to medical journals and (2) presented at society meetings as the results of research collaboration. Upon publication of the results in this manner, the clinical trial steering investigators, the investigators of medical institutions that registered a certain number of eligible subjects and the person responsible for statistical analysis will be the co-authors.

The individual results of subjects registered to this clinical trial shall not be published prior to publication as results of research collaboration

26 Clinical trial protocol amendment

If the investigators and clinical trial steering committee become aware of important information necessary to properly perform the clinical trial such as matters related to the quality, efficacy and safety of the investigational drug, etc., they will consult with the other investigators and amend the clinical trial protocol as necessary and, as well as obtaining agreement between the investigators, submit the amended clinical trial protocol to the institutional review board of each medical institution for review.

If it is necessary to modify the explanation and consent documents along with the clinical trial protocol, the investigators will immediately amend the explanation and consent documents, report to the head of their medical institution and submit the amended explanation and consent documents to their institutional review board for review.

If the explanation and consent documents are amended, investigators will inform subjects currently participating in the trial or their legal representatives of the content of the amendments, confirm their intention to continue their participation in the trial and use the amended explanatory and consent documents to obtain consent once again. The specific procedures for explanation and obtaining consent will be the same as set forth in 10.2.1.

27 Clinical trial protocol change and clinical trial discontinuation and suspension

If any one of the following events occurs, the clinical trial steering committee will examine the content of the event together with the person responsible for statistical analysis and report the results of this examination to the independent data and safety monitoring committee.

The independent data and safety monitoring committee will conduct further examination if necessary and subsequently advise the clinical trial steering committee of the necessity of clinical trial protocol change and whether or not to continue the clinical trial (continue, suspend or discontinue).

The clinical trial steering committee will then consult with the person responsible for statistical analysis, determine whether or not to change the clinical trial protocol and whether or not to continue the clinical trial (continue, suspend or discontinue) based on the recommendation from the independent data and safety monitoring committee, and report their decision to the investigators and the independent data and safety monitoring committee.

In the event the clinical trial is discontinued or suspended, the investigators will immediately inform the subjects or their legal representatives (a person who exercises parental authority or a legal guardian) of the fact and carry out appropriate medical treatment and measures in relation to the subjects.

(1) If a safety-related issue arises such as the occurrence of a serious side effect, etc.
(2) If the scientific validity for the development of the investigational drug is lost
(3) If a recommendation to discontinue is received from regulatory authorities
(4) If any other circumstances arise that necessitate a change to the clinical trial protocol
(5) If any other circumstances arise that necessitate partial or complete discontinuation or suspension of the clinical trial.

If any one of the following events occurs, the investigator or head of the medical institution will suspend or discontinue the clinical trial at the relevant medical institution.

(1) If serious or continued non-compliance with the clinical trial protocol by investigators, etc. is discovered
(2) If the institutional review board makes a decision to suspend or discontinue the clinical trial

28 References

1. Robert M. Nephrotic syndrome. Nelson Textbook of Pediatrics. Philadelphia: Saunders 2007, 2190-2195.

2. Eddy AA, Symons JM. Nephrotic syndrome in childhood. Lancet 2003; 362:629-639.

3. Braunwald E, Hauser SL, Fauci AS, Longo DL, Jameson JL, Kapser DL. Nephrotic syndrome. HARISON'S Principles of Internal medicine. 15th ed, McGraw-Hill. 2001, 1584-1588.

4. Hashizume M, Shimojo F. Comprehensive review of neprotic syndrome. Clinical Diagnosis Reivew for Adult Patients. Jin To Toseki (0385-2156) 2005; 59:124-127. [Japanese]

5. Sano T. Pediatric Corticosteroids (Administration period and dosage) (Adults). "Evicence leading to the clinical practice of treating renal disorders", Bunko-do, Tokyo, Japan. 2003, 51-52 [Japanese]

6. Koskimies O, Vilska J, Rapola J, Hallman N. Long-term outcome of primary nephrotic syndrome. Arch Dis Child 1982; 57: 544-548.

7. Arbeitsgemeinschaft fur Padiatrische Nephrologie. Short versus standard prednisone therapy for initial treatment of idiopathic nephrotic syndrome in children. Lancet 1988; 1: 380-383.

8. Tarshish P, Tobin JN, Bernstein J, Edelman CM Jr. Prognostic significance of the early course of minimal change nephrotic syndrome: report of the International Study of Kidney Disease in Children. J Am Soc Nephrol 1997; 8:769-776.

9. Yoshikawa N, Honda M, Sekine K, Nakanishi K, Iijima K, Ohtomo Y, et al. The Japanese Society for Pediatric Nephrology Academic Committee: The treatment guidelines for idiopathic nephrotic syndrome in children, Version 1.0, The Journal of the Japan Pediatric Society 2005; 109:1066-1075. http://www.jspn.jp/file/pdf/0505guideline.pdf [Japanese]

10, Kitano Y, Yoshikawa N, Tanaka R, Nakamura H, Ninomiya M, Ito H. Ciclosporin treatment in children with steroid-dependent nephrotic syndrome. Pediatr Nephrol 1990; 4:474-477.

11. Tanaka R, Yoshikawa N, Kitano Y, Ito H, Nakamura H. Long-term ciclosporin treatment in children with steroid-dependent nephrotic syndrome. Pediatr Nephrol 1993; 7:249-252.

12. Barratt TM, Soothill JF. Controlled trial of cyclophosphamide in steroid-sensitive relapsing nephrotic syndrome of childhood. Lancet 1970; 2:479-482.

13. Arbeitsgemeinschaft fur Padiatrische Nephrologie Cyclophosphamide treatment of steroid dependent nephrotic syndrome: comparison of eight week with 12 week course. Arch Dis Child 1987; 62:1102-1106.

14. Ueda N, Kuno K, Ito S. Eight and 12 week courses of cyclophosphamide in nephrotic syndrome. Arch Dis Child 1990; 65:1147-1150.

15. Yoshioka K, Ohashi Y, Sakai T, Ito H, Yoshikawa N, Nakamura H, et al. A multicenter trial of mizoribine compared with placebo in children with frequently relapsing nephrotic syndrome. Kidney Int 2000; 58:317-324.

16. Nakanishi K, Yoshikawa N. Steroid sensitive nephrotic syndrome. The third annual Japanese Study Group of Renal Disese in Children meeting, May 28, 2004 Utsunomiya, Japan.[Japanese]

17. Iijima K, Hamahira K, Kobayashi A, Nakamura H, Yoshikawa N. Immunohistochemical analysis of renin activity in chronic cyclosporine nephropathy in childhood nephrotic syndrome. J Am Soc Nephrol 2000; 11:2265-2271.

18. Latta K, von Schnakenburg C, Ehrich JH.A meta-analysis of cytotoxic treatment for frequently relapsing nephrotic syndrome in children. Pediatr Nephrol 2001; 16:271-282.

19. Fakhouri F, Bocquet N, Taupin P, Presne C, Gagnadoux MF, Landais P et al. Steroid-sensitive nephrotic syndrome: from childhood to adulthood. Am J Kidney Dis 2003; 41:550-557.

20. Hayakawa H, Kabuki N, Okugawa T. Long-term prognosis of childhood-onset ideopathig nephrotic syndrome: Study of “carry-over” to adulthood. Shounika (Pediatrics). (0037-4121) 1998; 39:359-68.[Japanese]

21. Hataya H, Honda M, Cho H. Secondary osteoporosis-Steroid therapy during childhood and bone disorders including growth disorders. Clinical Calcium 1994; 9:658-660.[Japanese]

22. Anolik J, Sanz I, Looney J. B cell depletion therapy in systemic lupus erythematosus. Current Rheumatology Reports 2003; 5:350-356.

23. Looney RJ. Treating human autoimmune disease by depletion B cells. Ann Rheum Dis; 2002; 61:863-866.

24. Reff ME, Carner K, Chambers KS, Chinn PC, Leonard JE, Raab R, et al. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood 1994; 83:435-45.

25. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate *in vivo* cytoxicity against tumor targets. Nat Med 2000; 6:443-446.

26. Golay J, Zaffaroni L, Vaccari T, Lazzari M, Borleri GM, Bernasconi S, et al. Biologic response of B lymphoma cells to anti-CD20 monoclonal antibody rituximab in vitro: CD55 and CD59 regulate complement-mediated cell lysis. Blood 2000; 95:3900-3908.

27. Taji H, Kagami Y, Okada Y, Andou M, Nishi Y, Saitoh H, et al. Growth inhibition of CD20-positive B lymphoma cell lines by IDEC-C2B8 anti-CD20 monoclonal antibody. Jpn J Cancer Res 1998; 89:748-756.

28. Shan D**,** Ledbetter JA**,** Press OW**.** Apoptosis of malignant human B cells by ligation of CD20 with monoclonal antibodies. Blood 1998; 91: 1644-1652.

29. Golovkina TV, Shlomchik M, Hannum L, Chervonsky A. Organogenic Role of B Lymphocytes in Mucosal Immunity. Science 1999; 286:1965-1968.

30. Ansel KM, Ngo VN, Hyman PL, Luther SA, Fo rster R, Sedgwick JD, et al. A chemokine-driven positive feedback loop organizes lymphoid follicles. Nature 2000; 406:309-14.

31. Fu YX, Chaplin DD. Development and maturation of secondary lymphoid tissues. Annu Rev Immunol 1999; 17:399-433.

32. Herold M, Schnohr S, Bittrich H. Efficacy and safety of a combined rituximab chemotherapy during pregnancy. J Clin Oncol 2001; 19:3439.

33. Kimata H, Fujimoto M, Furusho K. Involvement of interleukin(IL)-13, but not IL-4, in spontaneous IgE and IgG4 production in nephrotic syndrome. Eur. J. Immunol 1995; 25:1497-1501.

34. Kemper MJ, Meyer-Jark T, Lilva M, Muller-Wiefel DE. Combined T- and B cell activation in childhood steroid-sensitive nephrotic syndrome. Clin Nephrol 2003; 60:242-247.

35. Benz K, Dotsch J, Rascher W, Stachel D. Change of the course of steroid-dependent nephrotic syndrome after rituximab therapy. Pediatr Nephrol 2004; 19:794–797.

36. Nozu K, Iijima K, Fujisawa M, Nakagawa A, Yoshikawa N, Matsuo M. Rituximab treatment for posttransplant lymphoproliferative disorder (PTLD) induces complete remission of recurrent nephrotic syndrome. Pediatr Nephrol 2005; 20:1660–1663.

37. Gilbert RD, Hulse E, Rigden S. Rituximab therapy for steroid-dependent minimal change nephrotic syndrome. Pediatr Nephrol 2006; 21:1698-1700.

38. Kemper MJ, Moller K, Ludwig K, Rink N, Muller-Wiefel DE. Rituximab (RTX) in the treatment of refractory steroid sensitive nephrotic syndrome. Pediatr Nephrol 2006; 21: 1628, Abs# PP25.

39. Francois H, Daugas E, Bensman A, Ronco P. Unexpected efficacy of rituximab in multirelapsing minimal change nephrotic syndrome in the Adult: First case report and pathophysiological considerations. Am J Kidney Dis 2007; 9:158-161.

40. Dallochio A, Trimoreau F, Feuilard J, Guigoinis V. A case of steroid-dependent nephrotic syndrome treated with rituximab. Pediatr Nephrol 2006; 21:375, Abs# 1615.

41. Smith GC. Is there a role for rituximab in the treatment of idiopathic childhood nephrotic syndrome? Pediatr Nephrol 2007; 22:893-898.

42. Bagga A, Sinha A, Moudgil A. Rituximab in Patients with the Steroid-Resistant Nephrotic Syndrome. N Engl J Med 2007; 356:2751-2752.

43. Nakayama M, Kamei K, Nozu K, Matsuoka K, Nakagawa A, Sako M, Iijima K. Rituximab for refractory focal segmental glomerulosclerosis. Pediatr Nephrol 2008; 23:481-485.

44. Hofstra JM, Deegens JK, Wetzels JF. Rituximab: effective treatment for severe steroid-dependent minimal change nephrotic syndrome? Nephrol Dial Transplant 2007; 22: 2100-2102.

45. Nagai H. Mechanism of adverse reaction due to rituximab and points of care for its infusion. Ketsueki/Shuyou-Ka (Hematology/Oncology) 2002; 44: 272-276.[Japanese]

46. Maloney DG, Grillo-Lopez AJ, White CA, Bodkin D, Schilder RJ, Neidhart JA, et al. IDEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. Blood 1997; 90:2188-2195.

47. McLaughlin P, Grillo-Lopez AJ, Link BK, Levy R, Czuczman MS, Williams ME, et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 1998; 16:2825-2833.

48. Davis TA, White CA, Grillo-Lopez AJ, Vela´squez WS, Link B, Maloney DG, et al. Single-agent monoclonal antibody efficacy in bulky non-Hodgkin's lymphoma: results of a phase II trial of rituximab. J Clin Oncol 1999; 17:1851-1857.

49. Gathings WE, Lawton AR, Cooper MD. Immunofluorescent studies of the development of pre-B cells, B lymphocytes and immunoglobulin isotype diversity in humans. Eur J Immunol 1977; 7:804-810.

50. Gupta S, Pahwa R, O'Reilly R, Good RA, Siegal FP. Ontogeny of lymphocyte subpopulations in human fetal liver. Proc Natl Acad Sci USA 1976; 73:912-922.

51. Arbeitsgemeinschaft fur Padiatrische Nephrologie. Effect of cytotoxic drugs in frequently renephrotic syndrome with and without steroid dependence. N Engl J Med 1982; 306:451-454.

52. Albert DA, Khan SR, Stansberry J, et al. A phase I trial of rituximab (anti-CD20) for treatment of systemic lupus erythematosus. Arthritis Rheum 2003; 48:3659.

53. Marks SD, Patey S, Brogan PA, Hasson N, Pilkington C, Woo P, et al. B lymphocyte depletion therapy in children with refractory systemic lupus erythematosus. Arthritis Rheum 2005; 52:3168-3174.

54. Leandro MJ, Cambridge G, Edwards JC, Ehrenstein MR, Isenberg DA. B-cell depletion in the treatment of patients with systemic lupus erythematosus: a longitudinal analysis of 24 patients. Rheumatology (Oxford) 2005; 44:1542-1545.

55. Willems M, Haddad E, Niaudet P, Koné-Paut I, Bensman A, Cochat P, et al. Rituximab therapy for childhood-onset systemic lupus erythematosus. J Pediatr 2006; 148: 623-627.

56. Perrotta S, Locatelli F, Manna AL, Cennamo L, Stefano PD, Nobili B. Anti-CD20 monoclonal antibody (rituximab) for life-threatening autoimmune haemolytic anaemia in a patient with systemic lupus erythematosus. Br J Haematol 2002; 116:465–467.

57. ten Cate R, Smiers FJ, Bredius RG, Lankester AC, van Suijlekom-Smit LW, Huizinga TW, Egeler RM. Anti-CD20 monoclonal antibody (rituximab) for refractory autoimmune thrombocytopenia in a girl with systemic lupus erythematosus. Rheumatology (Oxford) 2004; 43:244.

58. Edelbauer M, Jungraithmayr T, Zimmerhackl LB. Rituximab in childhood systemic lupus erythematosus refractory to conventional immunosuppression: case report. Pediatr Nephrol 2005; 20:811-813.

59. Taube T, Schmid H, Reinhard H, von Stackelberg A, Overberg US. Effect of a single dose of rituximab in chronic immune thrombocytopenic purpura in childhood. Haematologica 2005; 90:281-283.

60. Wang J, Wiley J, Luddy R, Greenberg J, Feuerstein M, Bussel J. Chronic immune thrombocytopenic purpura in children: Assessment of rituximab treatment. J Paediatr 2005; 146:217-221.

61. Bennett CM, Rogers ZR, Kinnamon DD, Bussel JB, Mahoney DH, Abshire TC, et al. Prospective phase 1/2 study of rituximab in childhood and adolescent chronic immune thrombocytopenic purpura. Blood 2006; 107:2639-2642.

62. Parodi E, Nobili B, Perrotta S, Rosaria Matarese SM, Russo G, Licciardello M, et al. Rituximab (anti-CD20 monoclonal antibody) in children with chronic refractory symptomatic immune thrombocytopenic purpura: efficacy and safety of treatment. Int J Hematol 2006; 84:48-53.

63. Bengtson KL, Skinner MA, Ware RE. Successful use of anti-CD20 (rituximab) in severe, life-threatening childhood immune thrombocytopenic purpura, J Pediatr 2003; 143: 670–673.

64. Roganovic J. Rituximab treatment in refractory idiopathic thrombocytopenic purpura in children. Eur J Pediatr 2005; 164(5):334.

65. Russo G, Licciardello M, Spina ML. Rituximab in children with idiopathic thrombocytopenic purpura: does it really work? Eur J Pediatr 2004; 163:569.

66. Pusiol A, Cesaro S, Nocerino A, Picco G, Zanesco L, Bisogno G. Successful treatment with monoclonal antibody rituximab in two children with refractory autoimmune thrombocytopenia. Eur J Pediatr 2004; 163:305–307.

67. Moschovi M, Trimis G, Pergantou H, Platokouki H, Vrachnou E, Tzortzatou-Stathopoulou F. Clinical remission following monoclonal anti-CD20 therapy in two children with chronic refractory idiopathic thrombocytopenic purpura. J Paediatr Child Health 2005; 41: 384-386.

68. Quartier P, Brethon B, Philippet P, Landman-Parker J, Le Deist F, Fischer A. Treatment of childhood autoimmune haemolytic anaemia with rituximab. Lancet 2001; 358:1511-1513.

69. Motto DG, Williams JA, Boxer LA. Rituximab for refractory childhood autoimmune hemolytic anemia. Isr Med Assoc J 2002; 4:1006-1008.

70. Zecca M, Nobili B, Ramenghi U, Perrotta S, Amendola G, Rosito P, et al. Rituximab for the treatment of refractory autoimmune hemolytic anemia in children. Blood 2003; 101: 3857-3861.

71. Zecca M, Stefano PD, Nobili B, Locatelli F. Anti-CD20 monoclonal antibody for the treatment of severe, immune-mediated, pure red cell aplasia and hemolytic anemia. Blood 2001; 97:3995-3997.

72. Hongeng S, Tardtong P, Worapongpaiboon S, Ungkanont A, Jootar S. Successful treatment of refractory autoimmune haemolytic anaemia in a post-unrelated bone marrow transplant paediatric patient with rituximab. Bone Marrow Transplant 2002; 29:871-872.

73. Nowak-Wegrzyn A, King KE, Shirey RS, Chen AR, McDonough C, Lederman HM. Fatal warm autoimmune hemolytic anemia resulting from IgM autoagglutinins in an infant with severe combined immunodeficiency, J Pediatr Hematol Oncol 2001; 23:250-252.

74. Seeliger S, Baumann M, Mohr M, Jurgens H, Frosch M, Vormoor J. Autologous peripheral blood stem cell transplantation and anti-B-cell directed immunotherapy for refractory autoimmune haemolytic anaemia. Eur J Pediatr 2001; 160:492-496.

75. McMahon C, Babu L, Hodgson A, Hayat A, Connell NO, Smith OP. Childhood refractory autoimmune haemolytic anaemia: is there a role for anti-CD20 therapy (rituximab)? Br J Haematol 2002; 117:480-483.

76. Ship A, May W, Lucas K. Anti-CD20 monoclonal antibody therapy for autoimmune hemolytic anemia following T cell-depleted, haplo-identical stem cell transplantation. Bone Marrow Transplant 2002; 29:365-366.

77. Corti P, Bonanomi S, Vallinoto C, Balduzzi A, Uderzo C, Cazzaniga G, et al. Rituximab for immune hemolytic anemia following T- and B-cell-depleted hematopoietic stem cell transplantation. Acta Haematol; 2003: 109,43-45.

78. Ng PC, Lee KK, Lo AF, Li CK, Fok TF. Anti–B-cell targeted immunotherapy for treatment of refractory autoimmune haemolytic anaemia in a young infant. Arch Dis Child 2003; 88:337-339.

79. Kerridge I, Hann I, Ethel M, Potter M, Prentice H.G. Anti-CD20 monoclonal antibody (rituximab) therapy in a child with refractory chronic autoimmune haemolytic anaemia. Intern Med J 2003; 33:544-545.

80. Raj A, Bertolone S, Cheerva A. Successful treatment of refractory autoimmune hemolytic anemia with monthly rituximab following nonmyeloablative stem cell transplantation for sickle cell disease. J Pediatr Hematol Oncol 2004; 26:312-314.

81. Bonduel M, Zelazko M, Figueroa C, Magaldi G, Rossi J, del Pozo A. Successful treatment of autoimmune hemolytic anemia with rituximab in a child with severe combined immunodeficiency following nonidentical T-cell-depleted bone marrow transplantation. Bone Marrow Transplant 2005; 35:819-821.

82. Jubinsky PT, Rashid N. Successful treatment of a patient with mixed warm and cold antibody-mediated Evans syndrome and glucose intolerance. Pediatr Blood Cancer 2005; 45:347-350.

83. McMahon C, Babu L, Hodgson A, Hayat A, Connell NO, Smith OP. Childhood refractory autoimmune haemolytic anaemia: is there a role for anti-CD20 therapy (rituximab)? Br J Haematol 2002; 117:480-483.

84. El-Hallak M, Binstadt B, Leichtner A, Bennett C, Neufeld E, Fuhlbrigge R, et al. Clinical Effects and Safety of Rituximab for Treatment of Refractory Pediatric Autoimmune Diseases. J Pediatr 2007; 150:376-382.

85. Rao A, Kelly M, Musselman M, Ramadas J, Wilson D, Grossman W, Shenoy S. Safety, efficacy, and immune reconstitution after rituximab therapy in pediatric patients with chronic or refractory hematologic autoimmune cytopenias. Pediatr Blood Cancer 2008; 50:822-825.

86. Looney RJ, Anolik JH, Campbell D, Felgar RE, Young F, Arend LJ, SLoand JA, Rosenblatt J, Sanz I. B cell depletion as a novel treatment for systemic lupus erythematosus: a phase I/II dose-escalation trial of rituximab. Arthritis Rheum 2004; 50:2580-2589.

87. Task force on blood pressure control in children. Report of the second task force on blood pressure control in children-1987. Pediatrics 1987; 79:1-25.

88. The Joint National Committee on the Detection, Evaluation, and Treatment of High Blood Pressure The sixth report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure. Arch Intern Med 1997; 157:2413-2446.

89. Schwartz GJ, Haycock GB, Edelmann CM Jr, Spitzer A. A simple estimate of glomerular filtration rate

in children derived from body length and plasma creatinine. Pediatrics 1976; 58:259-263.

90. Schwartz GJ, Gauthier B. A simple estimate of glomerular filtration rate in adolescent boys. J Pediatr 1985; 106:522-526.

91. Tanaka T, Yamashita A, Ichihara K. Reference Intervals of Clinical Tests in Children Determined by a Latent Reference Value Extraction Method. The Journal of the Japan Pediatric Society 2008; 112:1117-1132. [Japanese]

92. The Japan Society of Nephrology, Committee on determining uric protein. Report from committee on glomerular filtration rate (GFR) and uric protein. The Japanese Journal of Nephrology 2001; 43:1-19. [Japanese]