SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 13-090540- -00000-0000

Fecha: 2013-04-08 12:01:15 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 379 FASENACIONALII
Act. 411 PRESENTACION Folios: 4

Industria y Comercio

SUPERINTENDENCIA

DELEGATURA DE PROPIEDAD INDUSTRIAL

DIRECCIÓN DE NUEVAS CREACIONES

SOLICITUD DE PATENTE DE INVENCIÓN

TITULO: AGENTES DE UNIÓN A CD33.

SOLICITANTE: **BOEHRINGER INGELHEIM INTERNATIONAL GMBH**

DIRECCIÓN: **Binger Strasse. 173, 55216 Ingelheim am Rhein Alemania**

APODERADO: CARLOS R. OLARTE

BOGOTÁ 05 de Abril de 2013

P2013/000046

OLARTEMOURE

OLARTE MOURE & ASOCI

Industria y Comercio
SUPERINTENDENCIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 13-090540- -00000-0000

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PETITORIO - FORMULARIO ÚNICO DE SOLICITUD
PATENTE PCT
ENTRADA EN FASE NACIONAL **P2013/000046**

SOLICITUD DE:

(1)

[X] Patente de Invención. [ ] Patente de Modelo de Utilidad.

[ ] Capítulo I. [X] Capítulo II.

(2) **SOLICITANTE (71)**

Nombre: BOEHRINGER INGELHEIM INTERNATIONAL GMBH
Dirección: Corporate Patents, Binger Str. 173, 55216 Ingelheim am Rhein
Nacionalidad o Domicilio: Alemania
Lugar de Constitución: Alemania
Teléfono: _____ **Fax:** _____
E-mail: _____

IDENTIFICACIÓN
C.C. [ ] **NIT** [ ]
C.E. [ ] **Otro** [ ]
Cual _____
Número _____

REPRESENTANTE O APODERADO

Nombre: CARLOS R. OLARTE
Dirección: Carrera 5 No. 34-03 Bogotá, Colombia
Teléfono: 601-7700 **Fax:** 601-7799
E-mail: office@olartemoure.com

IDENTIFICACIÓN
C.C. [X] **NIT** [ ]
C.E. [ ] **Otro** [ ]
Cual _____
Número 79.782.747Bta
TP 74.295

INVENTOR (ES) (72)

Nombre: VER PAGINA ADJUNTA.
Dirección: VER PAGINA ADJUNTA.
Nacionalidad o Domicilio: VER PAGINA ADJUNTA.

(5) **PCT (86)**

Solicitud Internacional No. PCT/EP2011/067339 Fecha Octubre 04, 2011

Publicación Internacional No. WO 2012/045752 A1 Fecha Abril 12, 2012

(6) **Título (54)** **AGENTES DE UNIÓN A CD33.**

Clasificación Internacional (51) C07K 16/28; A61K 39/395.

Prioridad SI [X] NO [ ]

(33) País de Origen	(32) Fecha	(31) Número de Solicitud
EP	Octubre 04, 2010	10186468.4

(9) **Comprobante de pago No.** 13- **Fecha** _____

(10) **ANEXOS**

X	Comprobante de pago de la tasa de presentación de la solicitud.
X	Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.
X	Poderes, si fuere el caso.
	Documento de cesión del inventor al solicitante o a su causante.
	Declaraciones. Declaración bajo la Regla 4.17 (ii) del PCT.
X	Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos).
X	Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos).
X	Copia del examen preliminar internacional efectuado por la Autoridad Internacional de Examen Preliminar (IPEA).
	Traducción del examen preliminar internacional efectuado por la IPEA.
	De ser el caso, copia del contrato de acceso.
	De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.
	De ser el caso, certificado de depósito del material biológico.
	Arte final 12 x 12.
	De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionadas parcial o totalmente con la invención de esta solicitud.
	De ser el caso, modificaciones efectuadas a la solicitud internacional.
X	Otros. Búsqueda Internacional

(11) **FIGURA CARACTERÍSTICA:**

(12) Solicito la concesión de la patente,

NOMBRE: CARLOS R. OLARTE FIRMA:

C.C. 79.782.747 Btá T.P. 74.295

LISTA DE INVENTORES

PCT/EP2011/067339

Renate KONOPITZKY
Boehringer Ingelheim GmbH
Corporate Patents
173 Binger Strasse
Ingelheim am Rhein 55216
Alemania
Nacionalidad: Austriaca

Eric BORGES
Boehringer Ingelheim GmbH
Corporate Patents
173 Binger Strasse
Ingelheim am Rhein 55216
Alemania
Nacionalidad: Alemana

Paul ADAM
Boehringer Ingelheim GmbH
Corporate Patents
173 Binger Strasse
Ingelheim am Rhein 55216
Alemania
Nacionalidad: Británica

Karl-Heinz HEIDER
Boehringer Ingelheim GmbH
Corporate Patents
173 Binger Strasse
Ingelheim am Rhein 55216
Alemania
Nacionalidad: Alemana

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
NIT : 800.176.089-2
- / -

Industria y Comercio
SUPERINTENDENCIA

RECIBO DE CAJA

No. 13 - 0032226

Bogotá D.C., Abril 08 de 2013 - 11:50:13

RECIBIDO DE : OLARTE MOURE & ASOCIADOS NI 830.122.870

*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR.PAGO
CONSIGNACION	BANCO DE BOGOTA	062754387	181981796	05/04/2013	9.515.000.00

*** Conceptos Pagados ***

CANT.	RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
1	50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE INVENCION	500.000.00	500.000.00
				$500.000.00

SON: **QUINIENTOS MIL PESOS MONEDA CORRIENTE***

Responsable: _____
-*****-

Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 13-090540- -00000-0000
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Tra. 11 PATENTEIYII Eve: 379 FASENACIONALII
Act. 411 PRESENTACION Folios: 4

PATENTE DE INVENCIÓN ☐ MODELO DE

- ☐ Indicación que se solicita una patente.
- ☐ Datos de identificación del solicitante o de la persona que presenta la solicitud
- ☐ Descripción de la invención
- ☐ Dibujos de ser estos pertinentes
- ☐ Comprobante de pago de las tasas establecidas (De ser el caso formato de descuento)

Completa ☐ Incompleta ☐

PATENTE DE INVENCIÓN PCT ☑ MODELO DE UTILIDAD PCT ☐ Art.33 Decisión 486/00, Circular Única

- ☑ Indicación que se solicita una PCT
- ☑ Copia de la solicitud en español, tal como fue presentada Inicialmente (capítulo descriptivo, reivindicatorio, resumen)
- ☑ Dibujos de ser estos pertinentes
- ☑ Comprobante de pago de las tasas establecidas (de ser el caso formato de descuento)

Completa ☐ Incompleta ☐

DISEÑO INDUSTRIAL ☐ (Art. 119 Decisión 486/00)

- ☐ Indicación que se solicita Diseño industrial
- ☐ Datos de identificación del solicitante o de la persona que presenta la solicitud
- ☐ Representación gráfica y fotográfica del Diseño industrial o muestra del material que incorpora el diseño
- ☐ Comprobante de pago de las tasas establecidas

Completa ☐ Incompleta ☐

ESQUEMA DE TRAZADO ☐ (Art. 92 Decisión 486/00)

- ☐ Indicación que se solicita un esquema de trazado
- ☐ Datos de identificación del solicitante o de la persona que presenta la solicitud
- ☐ Representación gráfica de un esquema de trazado
- ☐ Comprobante de pago de las tasas establecidas

Completa ☐ Incompleta ☐

Industria y Comercio SUPERINTENDENCIA	PLANILLA DE RADICACIÓN - DIGITALIZACIÓN DIARIA ***RELACIÓN DE ANEXOS***	
Dependencia:	Fecha: _____/ _____/ _____/ AAAA MM DD	Recorrido:
Tipo de Radicación: Entrada: ☐ Salida: ☐ Traslado: ☐ Convenio: ☐		
Radicador:		Planilla:

ID	*No. Radicación*	*Consecutivo*	*Item*
1	1 - [illegible]		
2	13-90540-0		
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16			
17			

Item	*Unidad de Conservación*
1	Bolsa
2	Caja
3	Libro
4	Sobre
5	Tomo
6	Otro
Item	*Soporte Documental*
7	CD
8	Disquete
9	DVD
10	Folleto
11	Foto
12	Periódico
13	Plano
14	Publicación Periódica
15	USB
10	Otro

Observaciones:

Anexan CD

TESTIMONIO

ESCRITURA: 001416
MINUTA: 000657

K: 007735

DELEGACION DE PODER
QUE OTORGA
BOEHRINGER INGELHEIM INTERNATIONAL GMBH
REPRESENTADO POR
JANNE IVETTE LUQUE CARDENAS
A FAVOR DE
CARLOS REINALDO OLARTE

&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&

I N T R O D U C C I O N ==

EN LA CIUDAD DE LIMA AL VEINTISEIS DE JULIO DEL DOS MIL DIEZ, ANTE MI; MARIO GINO BENVENUTO MURGUIA, NOTARIO DE ESTA CAPITAL. ==

C O M P A R E C E N: ==

JANNE IVETTE LUQUE CARDENAS, QUIEN MANIFIESTA SER DE NACIONALIDAD: PERUANA, DE ESTADO CIVIL CASADA, DE PROFESION U OCUPACIÓN: ABOGADA Y DOMICILIAR EN AVENIDA REPUBLICA DE PANAMA 3531, OFICINA 1203, DISTRITO DE SAN ISIDRO PROVINCIA Y DEPARTAMENTO DE LIMA, DEBIDAMENTE IDENTIFICADA CON DOCUMENTO NACIONAL DE IDENTIDAD NUMERO 29562321, QUIEN PROCEDE EN REPRESENTACION DE **BOEHRINGER INGELHEIM INTERNATIONAL GMBH**, CUYAS FACULTADES CONSTAN EN EL PODER OTORGADO CON FECHA QUINCE DE JULIO DEL DOS MIL DIEZ, DEBIDAMENTE LEGALIZADO POR EL MINISTERIO DE RELACIONES ESTERIORES CON FECHA VEINTITRES DE JULIO DEL DOS MIL DIEZ, QUE SE INSERTA. ==== LA COMPARECIENTE ES INTELIGENTE EN EL IDIOMA CASTELLANO, QUIEN SE OBLIGA CON CAPACIDAD, LIBERTAD Y CONOCIMIENTO SUFICIENTE DE CONFORMIDAD CON EL EXAMEN QUE LE HE EFECTUADO DE LO QUE DOY FE; Y ME ENTREGO UNA MINUTA QUE ARCHIVO EN SU LEGAJO RESPECTIVO BAJO EL NUMERO DE ORDEN CORRESPONDIENTE Y CUYO TENOR LITERAL ES COMO SIGUE: ==

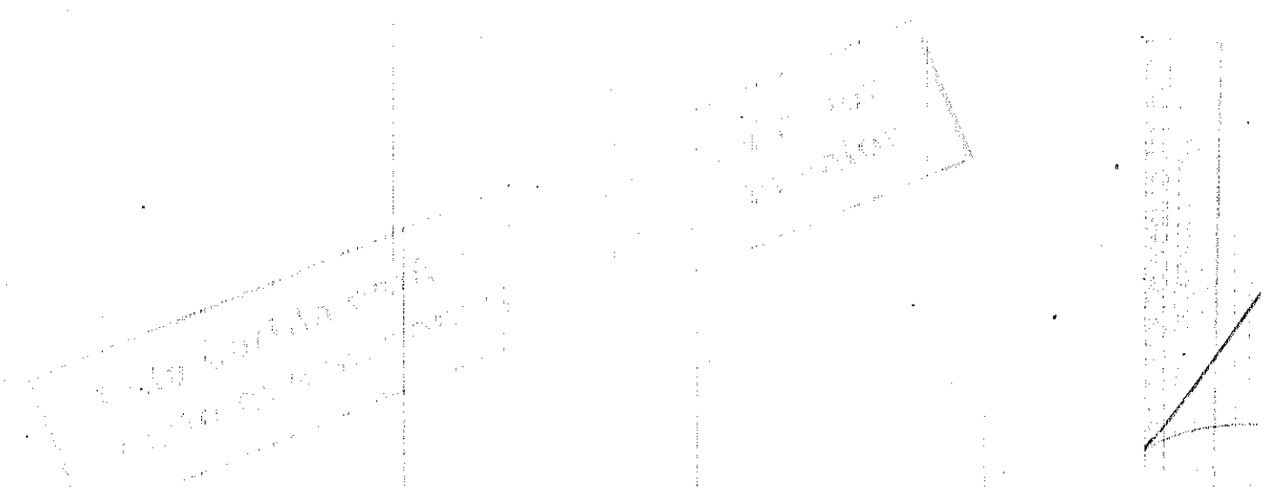
M I N U T A: ==

SEÑOR NOTARIO: ==

SÍRVASE EXTENDER EN SU REGISTRO DE ESCRITURAS PÚBLICAS UNA DE **DELEGACION DE PODER** QUE OTORGA LA SEÑORA **IVETTE LUQUE CARDENAS**, IDENTIFICADA CON DNI N° 29562321, DE NACIONALIDAD PERUANA, ESTADO CIVIL CASADA, OCUPACIÓN ABOGADO Y CON DOMICILIO EN AV. REPÚBLICA DE PANAMÁ N° 3531, OFICINA 1203, SAN ISIDRO, QUIEN ACTÚA EN REPRESENTACIÓN DE **BOEHRINGER INGELHEIM INTERNATIONAL GMBH**, UNA EMPRESA CONSTITUIDA Y EXISTENTE BAJO LAS LEYES DE ALEMANIA, CON DOMICILIO EN BINGER STRASSE 173, 55216 INGELHEIM AM RHEIN, ALEMANIA, SEGÚN PODER OTORGADO CON FECHA 13 DE OCTUBRE DE 2009, Y A QUIEN EN ADELANTE SE LE DENOMINARÁ **EL PODERDANTE**; A FAVOR DEL SEÑOR **CARLOS REINALDO OLARTE**, IDENTIFICADO CON CÉDULA N° 79.782.747, A QUIEN EN ADELANTE SE LE DENOMINARÁ **EL APODERADO**; EN LOS SIGUIENTES TÉRMINOS Y CONDICIONES: ==

PRIMERO: CON FECHA 15 DE JULIO DE 2010, LA EMPRESA **BOEHRINGER INGELHEIM INTERNATIONAL GMBH**, UNA EMPRESA CONSTITUIDA Y EXISTENTE BAJO LAS LEYES DE ALEMANIA, CON DOMICILIO EN BINGER STRASSE 173, 55216 INGELHEIM AM RHEIN, ALEMANIA, OTORGÓ PODER A FAVOR DE **EL PODERDANTE**, EL CUAL USTED SEÑOR NOTARIO SE SERVIRÁ INSERTAR. ==

SEGUNDO: POR EL PRESENTE DOCUMENTO, **EL PODERDANTE** DELEGA PODER A FAVOR DE **EL APODERADO** PARA QUE REPRESENTE EN LA REPÚBLICA DE COLOMBIA A LA SOCIEDAD **BOEHRINGER INGELHEIM INTERNATIONAL GMBH**, TAL COMO CONSTA EN EL PODER ADJUNTO, ANTE CUALQUIER AUTORIDAD ADMINISTRATIVA O JUDICIAL, INCLUYENDO LA SECRETARÍA Y TRIBUNAL DE JUSTICIA DE LA COMUNIDAD ANDINA, PARA EL INICIO, TRAMITACIÓN, OBTENCIÓN, DEFENSA Y HACER RESPETAR TODAS Y CUALQUIERA DE LAS PATENTES DE INVENCIÓN, MODELOS DE UTILIDAD, DISEÑOS INDUSTRIALES, MARCAS, LEMAS COMERCIALES, NOMBRES COMERCIALES, PROTECCIÓN DE DATOS DE PRUEBA, PROTECCIÓN DE SECRETOS EMPRESARIALES, DERECHOS DE AUTOR, REGISTRO DE NOMBRES DE DOMINIO, O SEGUIMIENTO DE PROCEDIMIENTOS EN MATERIAS TALES COMO PROPIEDAD INTELECTUAL. ==



TESTIMONIO

EL APODERADO ACTUANDO EN FORMA INDIVIDUAL PODRÁ DEMANDAR, RECONVENIR, CONTESTAR DEMANDAS Y RECONVENCIONES, PRESENTAR TODO TIPO DE SOLICITUDES, RECURSOS, RESPUESTAS U OPOSICIONES, PRESENTAR TODO TIPO DE RECURSOS DE IMPUGNACIÓN, PRESENTAR DECLARACIONES, JURADAS O NO, PRESENTAR CUALQUIER OTRO RECURSO O REMEDIO PROCESAL PERMITIDO POR LEY, PAGAR IMPUESTOS Y ANUALIDADES, SOLICITAR TESTIMONIOS, RENUNCIAR A LOS DERECHOS DE REGISTRO SOBRE UN ELEMENTO DE LA PROPIEDAD INDUSTRIAL, DESISTIRSE DEL PROCESO Y DE LA PRETENSIÓN, ALLANARSE A LA PRETENSIÓN, CONCILIAR, TRANSIGIR, SOMETER A ARBITRAJE LAS PRETENSIONES CONTROVERTIDAS EN EL PROCESO, SOLICITAR Y EJECUTAR MEDIDAS CAUTELARES ASÍ COMO VISITAS INSPECTIVAS, RATIFICAR LOS ACTOS JURÍDICOS PROCESALES SUSCRITOS CON ANTERIORIDAD AL OTORGAMIENTO Y/O PRESENTACIÓN DEL PRESENTE PODER, SUSTITUIR O DELEGAR LA REPRESENTACIÓN, REVOCAR LOS PODERES OTORGADOS, RECIBIR NOTIFICACIONES EN NOMBRE DE LA COMPAÑÍA Y LOS DEMÁS ACTOS QUE EXPRESE LA LEY. ==========
AGREGUE USTED SEÑOR NOTARIO, LAS CLÁUSULAS DE LEY, E INSERTE EL PODER AL QUE SE HA HECHO REFERENCIA. =======
LIMA, 21 DE JULIO DE 2010. ==========
FIRMADO.- UNA FIRMA ILEGIBLE.- ==========
ABOGADO QUE AUTORIZA.- UN SELLO QUE DICE.- JURGEN STROBACH FLORIAN.- ABOGADO.- C.A.L: 30548.- UNA FIRMA ILEGIBLE.-
INSERTO UNO: TRADUCCION OFICIAL ==========
MARIO GINO BENVENUTO MURGUIA, ABOGADO NOTARIO DE LIMA, CERTIFICO QUE HE TENIDO A LA VISTA **LA TRADUCCION OFICIAL DEL PODER ESPECIAL,** DEBIDAMENTE LEGALIZADO POR EL MINISTERIO DE RELACIONES EXTERIORES CON FECHA VEINTITRES DE JULIO DEL DOS MIL DIEZ, CUYO TENOR LITERAL ES COMO SIGUE: ==========

TRADUCCIÓN OFICIAL

TRA. N° TOI.0077.10

Primera página del documento original:

PODER ESPECIAL de fecha 15 de julio de 2010 redactado en idioma castellano e inglés

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Segunda página del documento original:

UR. Nr. 1446/2010

Por la presente se certifica que las firmas que anteceden de:

- Dr. Michael Kompter, nacido el 11 de mayo de 1955, con domicilio en 55216 Ingelheim am Rhein, Binger Straße 173.
- Dr. Christian Hauke, nacido el 31 de agosto de 1947, con domicilio en 55216 Ingelheim am Rhein, Binger Straße 173.

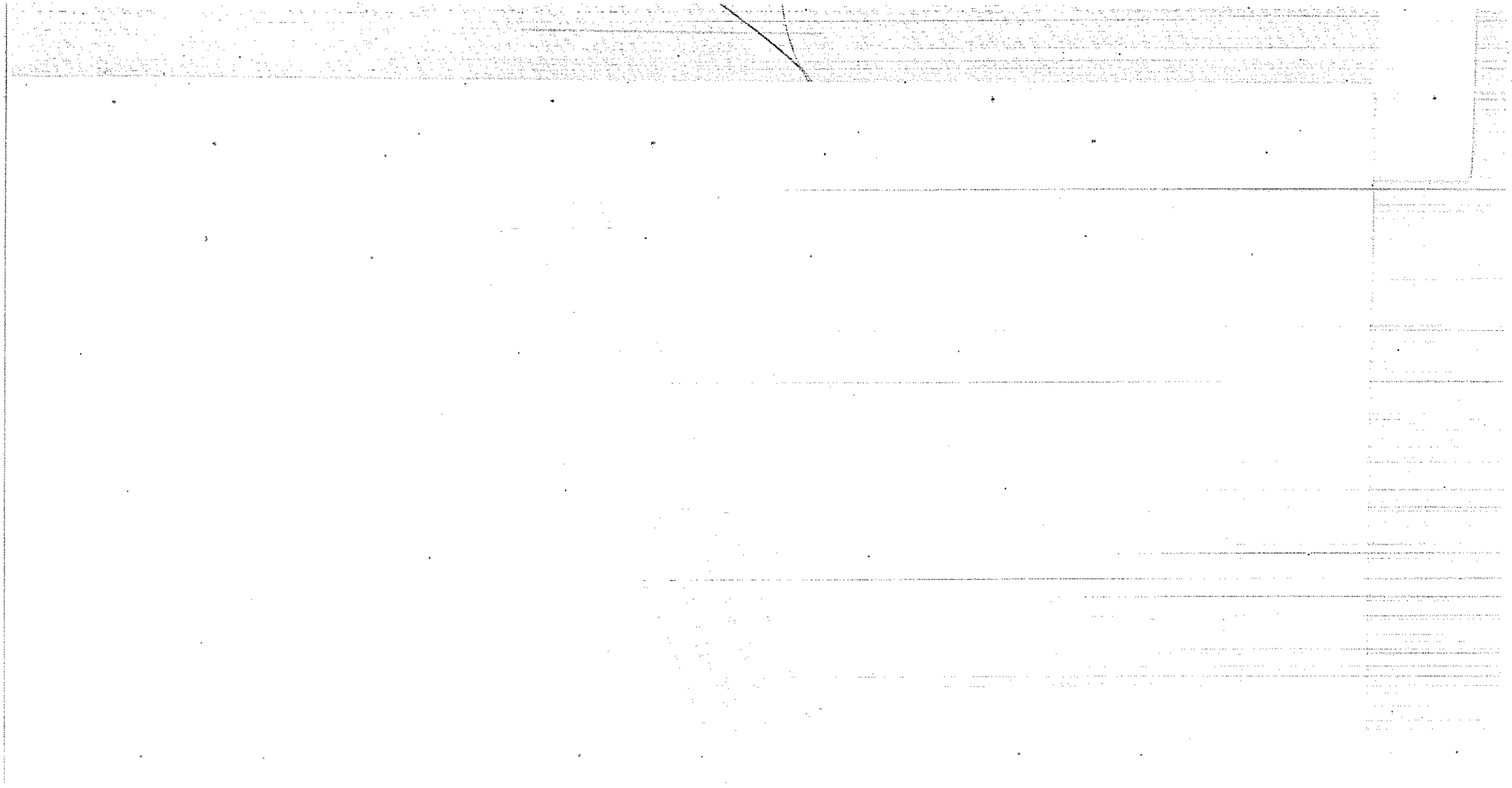
a quienes he conocido en persona,
han sido estampadas en mi presencia

Asimismo, certifico que Boehringer Ingelheim International GmbH es una sociedad debidamente constituida bajo las leyes de la República Federal de

Mary Ann Montenegro Medina
Traductora Pública Juramentada
Registro JVT N° 105

1

Estados Unidos 888 - Lima 11 - Perú - Telfs. (511) 460-1659 • (511) 463-1157 • Fax: (511) 463-4546
maemm@terra.com.pe



TESTIMONIO

TRA. N° TOI.0077.10

Alemania, con domicilio en Binger Strasse 173, 55216 Ingelheim am Rhein, Alemania.

Dicha sociedad se encuentra inscrita en el Registro Mercantil del Juzgado de Distrito de Bingen, bajo el número HR B 1063 desde el 14 de mayo de 1968. Dicha inscripción ha sido transferida por ley al Registro Mercantil del Juzgado de Distrito de Mainz, donde se encuentra inscrita bajo el número HR B 21063 desde el 28 de diciembre de 2005.

Asimismo, certifico que los doctores Michael Kompter y Christian Hauke, han sido autorizados para firmar el documento que antecede y actualmente se encuentran debidamente autorizados para firmar a nombre de Boehringer Ingelheim International GmbH en calidad de apoderados.

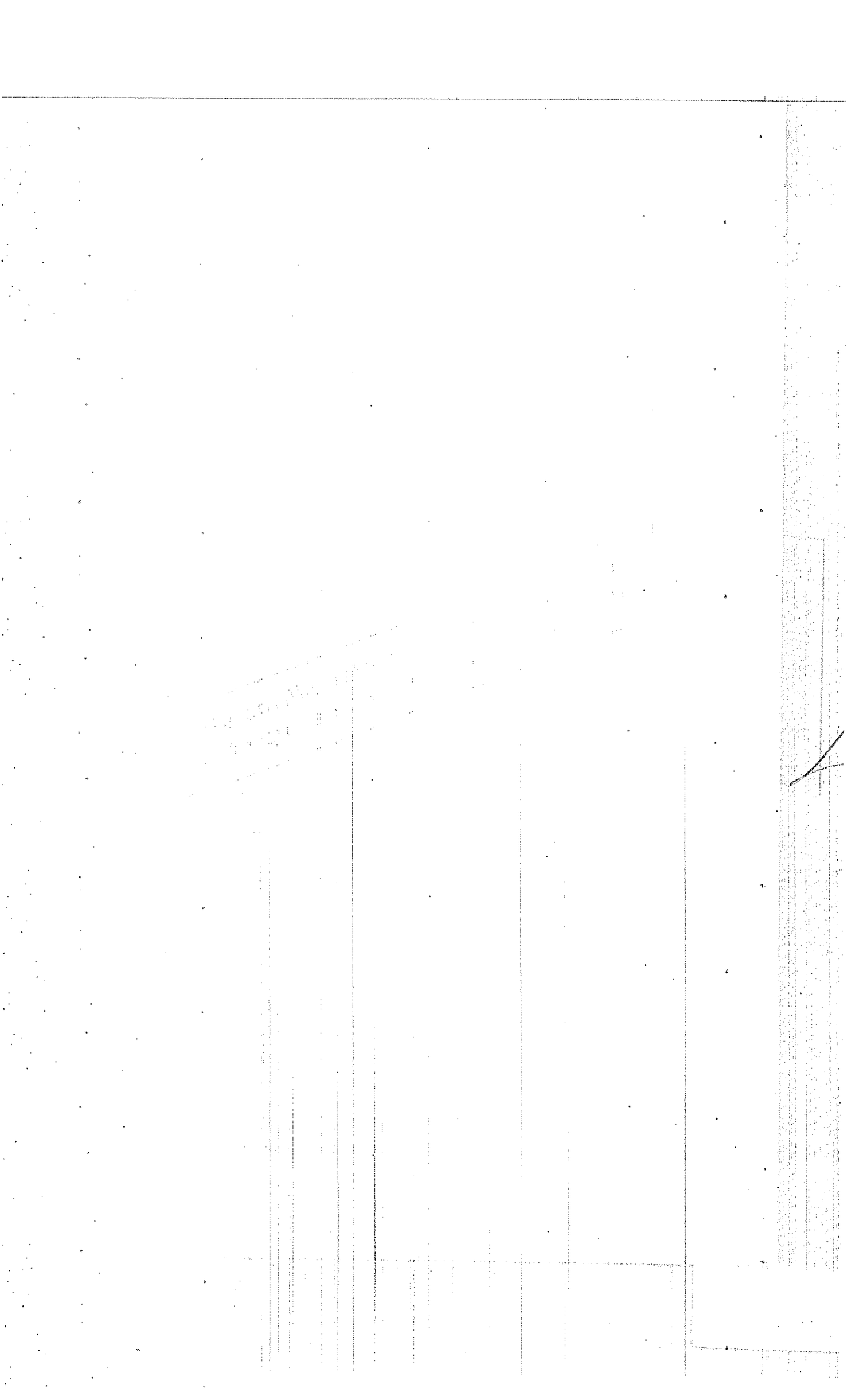
Ingelheim am Rhein, 15 de julio de 2010

(firmado ilegible)
Notario Público

Mary Ann Monteagudo Medina
Traductora Pública Juramentada
Registro JVT N° 105

Estados Unidos 888 - Lima 11 - Perú - Tells.: (511) 460-1859 • (511) 463-1167 • Fax: (511) 463-4548
maemm@terra.com.pe

Av. Juan de Arona N° 545 Altura Cdra. 31 de la Av. Arequipa – San Isidro
Central: 719 – 1667 / 719 – 1668 / 719 – 1669 / Telefax.: 717 – 1031
www.notariabenvenuto.com / e-mail: notario@notariabenvenuto.com



TESTIMONIO

TRA. N° TOI.0077.10

Reverso de la segunda página del documento original:

(sello en idioma extranjero distinto al inglés, de fecha 16 de julio de 2010)

(sello en idioma castellano del Consulado del Perú, de fecha 20 de julio de 2010)

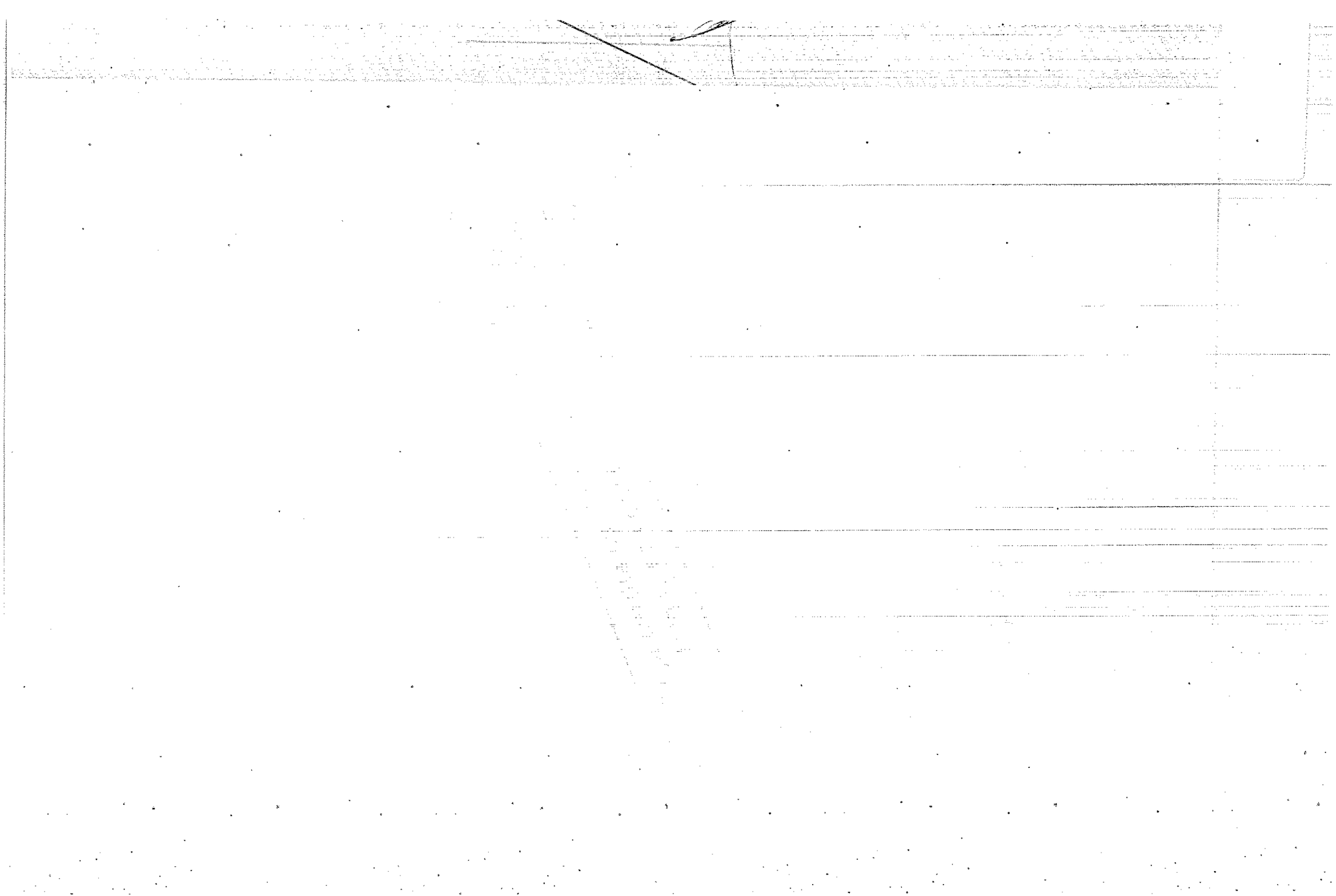
(sello) MINISTERIO DE RELACIONES EXTERIORES
DIRECCIÓN GENERAL DE POLÍTICA CONSULAR
Legalización N° 092720
Se legaliza la firma que antecede del Sr.(a) Alfredo Arecco-Sablich sin juzgar el contenido del documento
Lima, 23 de julio de 2010
(fdo.) Maribel del Rosario LUYO JAVIER
Departamento de Legalizaciones
Dirección de Trámites Consulares/sello

La infrascrita, Traductora Pública Juramentada, certifica que la presente traducción es fiel y correcta del texto original en idioma Castellano..... adjunto. Esta traducción no debe interpretarse como reconocimiento de la autenticidad del documento traducido.
En fe de lo cual, firmo y sello en.....Lima.....
a los...26....días del mes de...julio.....de 2010..

MINISTERIO DE RELACIONES EXTERIORES
DIRECCIÓN GENERAL DE POLÍTICA CONSULAR
Legalización N°
Se legaliza la firma que antecede del Sr.(a)
SIN JUZGAR EL CONTENIDO DEL DOCUMENTO
Julia Adela Moreano de Dueñas
Departamento de Legalizaciones
Dirección de Trámites Consulares
LIMA 27 JUL. 2010

Mary Ahn Montesinos Medina
Traductora Pública Juramentada
Registro JVT N° 105

Estados Unidos 888 - Lima 11 - Perú - Telfs.: (511) 460-1859 • (511) 463-1167 • Fax: (511) 463-4548
maemm@terra.com.pe



TESTIMONIO

FERNÁNDEZ - DÁVILA & BUENO
ABOGADOS

PODER ESPECIAL

Conste por el presente documento el Poder Especial que otorga *[insertar nombre de poderdante]*, domiciliada en *[insertar domicilio]* constituida de acuerdo a las leyes de *[insertar país de constitución]*, representada por su *[insertar cargo en la cía.]* Sr.(a) *[insertar nombre del representante de la cía.]*, a favor de FERNÁNDEZ-DÁVILA ABOGADOS SAC, CARLOS FERNÁNDEZ DAVILA WINKLER, ROSA BUENO MERA, JULIETA DEL PILAR TIJERO MARTINO, DIEGO CORDOVA FLORES, IVETTE LUQUE CARDENAS y/o CLARISA SILES VASCONES, domiciliados en Av. República de Panamá 3531, Oficina 1203, San Isidro, Lima, Perú, para que la representen en la República de Perú, República de Bolivia, República de Chile, República de Colombia, República de Ecuador y República Bolivariana de Venezuela, ante cualquier autoridad administrativa o judicial, incluyendo la Secretaría y Tribunal de Justicia de la Comunidad Andina, para el inicio, tramitación, obtención, defensa y hacer respetar todas y cualquiera de las patentes de invención, modelos de utilidad, diseños industriales, marcas, lemas comerciales, nombres comerciales, protección de datos de prueba, protección de secretos empresariales, derechos de autor, registro de nombres de dominio, o seguimiento de procedimientos en materias tales como Propiedad Intelectual.

Los apoderados podrán ejercer el presente poder actuando en forma individual e indistinta, y gozarán de las siguientes facultades, tales como demandar, reconvenir, contestar demandas y reconvenciones, presentar todo tipo de solicitudes, recursos, respuestas u oposiciones, presentar todo tipo de recursos de impugnación, presentar declaraciones, juradas o no, presentar cualquier otro recurso o remedio procesal permitido por ley, pagar impuestos y anualidades, solicitar testimonios renunciar a los derechos de registro sobre un elemento de la propiedad industrial, desistirse del proceso y de la pretensión, allanarse a la pretensión, conciliar, transigir, someter a arbitraje las pretensiones controvertidas en el proceso, solicitar y ejecutar medidas cautelares así como visitas inspectivas, ratificar los actos jurídicos procesales suscritos con anterioridad al otorgamiento y/o presentación del presente poder, sustituir o delegar la representación procesal, revocar los poderes otorgados, recibir notificaciones en nombre de la compañía y los demás actos que exprese la ley.

Suscrito en..... el ..... de ..... de 2010

ESTE PODER REQUIERE DE LEGALIZACIÓN NOTARIAL (PRECISANDO QUE EL REPRESENTANTE TIENE LAS FACULTADES PARA OTROGARLO Y QUE ESTUVO PRESENTE AL MOMENTO DE HACERLO) Y DE CONSUL PERUANO (CUANDO ES OTORGADO EN EL EXTRANJERO)

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By means of this Special Power of Attorney *Boehringer Ingelheim International GmbH* with address in *Binger Strasse 173, 55216 Ingelheim am Rhein, Germany* and incorporated under the laws of *Germany* duly represented by its *Authorized Signatories* Dr. Michael KOMPTER and Dr. Christian HAUKE, grants to FERNÁNDEZ-DÁVILA ABOGADOS SAC, CARLOS FERNÁNDEZ DAVILA WINKLER, ROSA BUENO MERA, JULIETA DEL PILAR TIJERO MARTINO, DIEGO CORDOVA FLORES, IVETTE LUQUE CARDENAS and/or CLARISA SILES VASCONES, with address at Av. República de Panamá 3531, Oficina 1203, San Isidro, Lima, Perú, the powers to represent in the Republic of Perú, the Republic of Bolivia, Republic of Chile, Republic of Colombia, Republic of Ecuador and the Bolivarian Republic of Venezuela before any administrative or judicial authority of any class, including the Andean Secretariat and the Andean Court of Justice to initiate, prosecution, obtain, defend and enforce any and all of the company's Patents for Invention, Utility Models, Industrial Designs, Trademarks, Trade Names, Commercial Emblems, Test Data Protection, Protection of Trade Secrets, Copyrights, Registration of Domain names and handle proceedings related to Intellectual Property.

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Executed in Ingelheim, on 15th July of 2010

BOEHRINGER INGELHEIM INTERNATIONAL GMBH

ppa.

Dr. Michael KOMPTER
Authorized Signatory

ppa.

Dr. Christian HAUKE
Authorized Signatory

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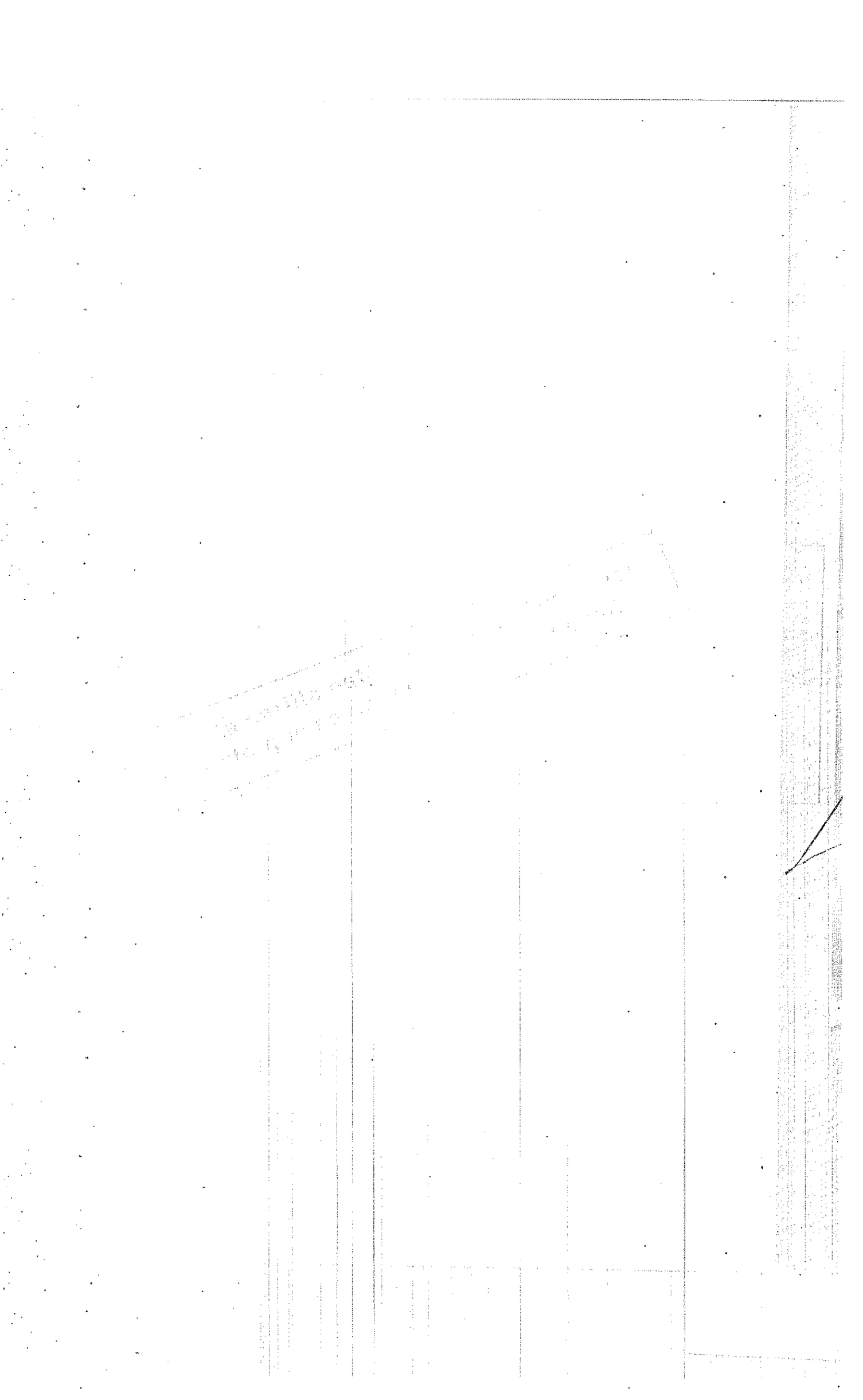

TESTIMONIO

UR.Nr. 1446/2010

It is herewith certified that above signatures of:

- Dr. Michael **K o m p t e r**, born 11th day of May 1955, with its business address at 55216 Ingelheim am Rhein, Binger Straße 173,
- Dr. Christian **H a u k e**, born 31st day of August 1947, with its business address at 55216 Ingelheim am Rhein, Binger Straße 173,

personally known,
were duly given in my presence.

It is herewith certified that **Boehringer Ingelheim International GmbH** is a corporation duly organized and existing under the laws of the Federal Republic of Germany having its principal place of business at Binger Strasse 173, 55216 Ingelheim am Rhein, Germany.

It had been entered into the Commercial Register of the District Court of Bingen under HR B 1063 as of 14th day of May 1968. This registration was transferred by law to the Commercial Register of the District Court of Mainz, where it has been registered under HR B 21063 since 28th day of December 2005.

Furthermore, I certify that Dr. Michael Kompter and Dr. Christian Hauke were authorized to sign the foregoing document and still are duly authorized to sign on behalf as procurists of **Boehringer Ingelheim International GmbH**.

Ingelheim am Rhein, the 15th day of July 2010/Ge

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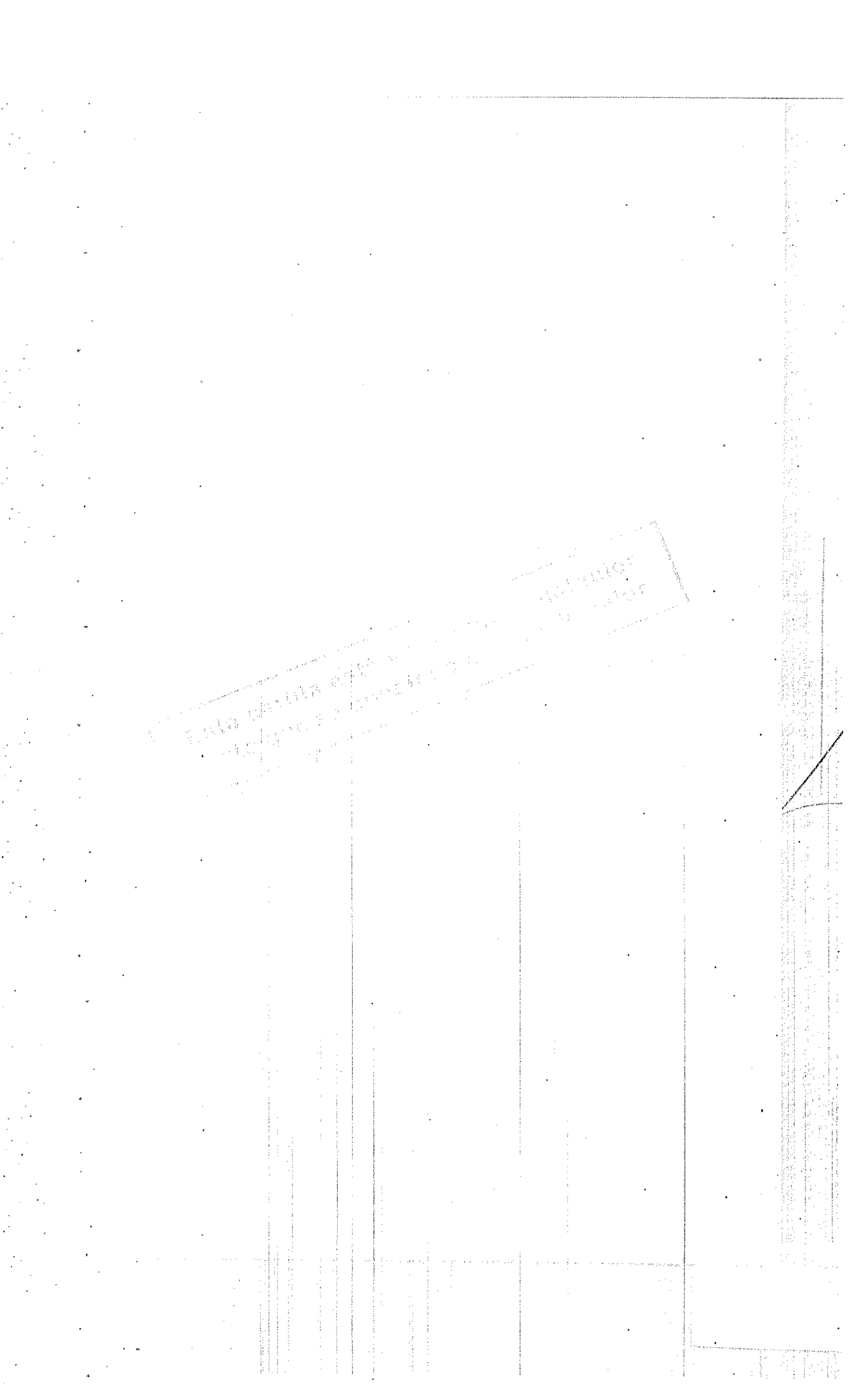

TESTIMONIO

AL DORSO: ==========

910 E 1

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Notars Horst Heintskill in Ingelheim am Rhein
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Präsidentin des Landgerichts 2 Mainz

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Gebühr für die Erteilung
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Wert: 50.000,00 €
JVKostO v. 26.7.1957
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Gebühr: 33,00 €
Mainz, 16.07.10
Heppes-Schwarz
Amtsinspektorin

Präsidentin des Landgerichts 2 Mainz

Mainz, den 16. Juli 2010
Die Präsidentin des Landgerichts
In Vertretung
Dr. Matthias Friedrich
Vorsitzender Richter am Landgericht

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FRANCFORT DEL MENO

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Nº de Actuación: 1471
Nº de Tarifa: 22 a
Derechos percibidos: S/C: 80.00
e: 72.00

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Cónsul General del Perú

CONSULADO GENERAL DEL PERU FRANCFORT DEL MENO

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INSERTO. ==========

DE CONFORMIDAD CON LA LEY DEL NOTARIADO, ARTICULO 59, INCISO D), TRANSCRIBO: ==========

CÓDIGO PROCESAL CIVIL: "ARTÍCULO 74º.- FACULTADES GENERALES.- LA REPRESENTACIÓN JUDICIAL CONFIERE AL REPRESENTANTE LAS ATRIBUCIONES Y POTESTADES GENERALES QUE CORRESPONDEN AL REPRESENTADO, SALVO AQUELLAS PARA LAS QUE LA LEY EXIGE FACULTADES EXPRESAS. LA REPRESENTACIÓN SE ENTIENDE OTORGADA PARA TODO EL PROCESO, INCLUSO PARA LA EJECUCIÓN DE LA SENTENCIA Y EL COBRO DE COSTAS Y COSTOS, LEGITIMANDO AL REPRESENTANTE PARA SU INTERVENCIÓN EN EL PROCESO Y REALIZACIÓN DE TODOS LOS ACTOS DEL MISMO, SALVO AQUELLOS QUE REQUIERAN LA INTERVENCIÓN PERSONAL Y DIRECTA DEL REPRESENTADO." ==========

CÓDIGO PROCESAL CIVIL: "ARTÍCULO 75º.- FACULTADES ESPECIALES.- SE REQUIERE EL OTORGAMIENTO DE FACULTADES ESPECIALES PARA REALIZAR TODOS LOS ACTOS DE DISPOSICIÓN DE DERECHOS SUSTANTIVOS Y PARA DEMANDAR, RECONVENIR, CONTESTAR DEMANDAS Y RECONVENCIONES, DESISTIRSE DEL PROCESO Y DE LA PRETENSIÓN, ALLANARSE A LA PRETENSIÓN, CONCILIAR, TRANSIGIR, SOMETER A ARBITRAJE LAS PRETENSIONES CONTROVERTIDAS EN EL PROCESO, SUSTITUIR O DELEGAR LA REPRESENTACIÓN PROCESAL Y PARA LOS DEMÁS ACTOS QUE EXPRESE LA LEY. EL OTORGAMIENTO DE FACULTADES ESPECIALES SE RIGE POR EL PRINCIPIO DE LITERALIDAD. NO SE PRESUME LA EXISTENCIA DE FACULTADES ESPECIALES NO CONFERIDAS EXPLÍCITAMENTE." ==========

CONCLUSION: ==========

FORMALIZADO EL INSTRUMENTO LA COMPARECIENTE LE DIO LECTURA, DESPUES DE LO CUAL SE AFIRMA Y RATIFICA EN SU CONTENIDO, DECLARANDO QUE SE TRATA DE UN ACTO VALIDO Y NO SIMULADO, MANIFESTANDO IGUALMENTE CONOCER LOS ANTECEDENTES Y/O TITULOS QUE SE ORIGINAN POR EL PRESENTE INSTRUMENTO Y DECLARANDO RECONOCER COMO SUYA LA FIRMA DE LA MINUTA QUE LA ORIGINA. ==========

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LA PRESENTE ESCRITURA PUBLICA SE INICIA EN LA FOJA DE PAPEL NOTARIAL CON NUMERO DE SERIE B Nº0706090 Y CONCLUYE EN LA FOJA DE PAPEL NOTARIAL CON NUMERO DE SERIE B Nº0706093(VUELTA), DE LO QUE DOY FE. ==========

FIRMADO: JANNE IVETTE LUQUE CARDENAS.- UNA HUELLA DIGITAL.- UNA FIRMA ILEGIBLE.- FIRMÓ EL VEINTISEIS DE JULIO DEL AÑO DOS MIL DIEZ.- ==========

EL PROCESO DE FIRMAS CONCLUYO EL: VEINTISEIS DE JULIO DEL DOS MIL DIEZ.- ==========

FIRMADO: DR. MARIO GINO BENVENUTO MURGUIA. ABOGADO - NOTARIO DE LIMA. ==========

ES TRANSCRIPCION DE LA ESCRITURA PUBLICA QUE CORRE EN EL REGISTRO CON FECHA VEINTISEIS DIAS DEL MES DE JULIO DEL AÑO DOS MIL DIEZ DE FOJAS 004590 A FOJAS 004593(VUELTA), Y A SOLICITUD DE PARTE INTERESADA EXPIDO EL PRESENTE, DE ACUERDO A LEY EL QUE RUBRICO EN CADA UNA DE SUS HOJAS, SELLO, SIGNO Y FIRMO EN LIMA, VEINTISEIS DE JULIO DEL AÑO DOS MIL DIEZ. ==========

MARIO GINO BENVENUTO MURGUIA
ABOGADO
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No. 1793

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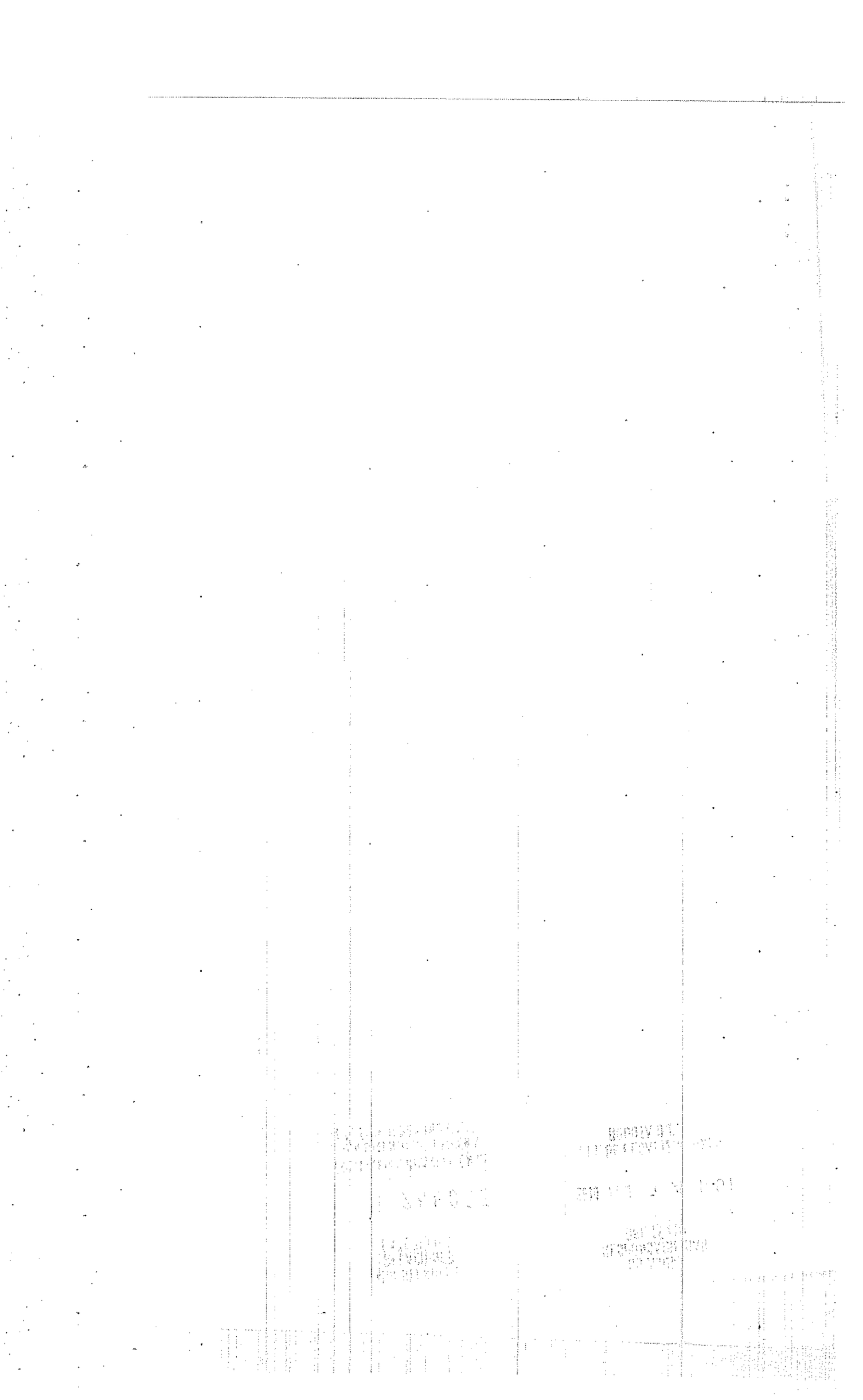

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

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WO 2012/045752 A1

(54) **Title**: CD33 BINDING AGENTS

(57) **Abstract**: The present invention relates to immunotherapies that are based on myeloid cell depletion. In particular, the present invention relates to CD33 binding agents for use in such therapies, e.g. in the treatment of myeloid cell malignancies and myelodysplastic syndrome (MDS).

CD33 binding agents

Field of the invention

The present invention relates to immunotherapies that are based on myeloid cell depletion. In particular, the present invention relates to CD33 binding agents for use in such therapies, e.g. in the treatment of myeloid cell malignancies and myelodysplastic syndrome (MDS).

Background of the invention

In the early 1980s CD33 was identified as a marker of myeloid leukemias (Andrews et al., Blood 62, 24-132, 1983). CD33 is a cell-surface antigen specifically expressed on myeloid cells including myeloid leukemia cells. It is the smallest member of the siglec (sialic acid-binding Ig-related lectins) family. CD33 is expressed on early multilineage hematopoietic progenitor cells and myelomonocytic precursors. It is absent from pluripotent hematopoietic stem cells (Andrews et al., Journal of Experimental Medicine 169, 1721-1731, 1989). It is downregulated on mature granulocytes but retained on macrophages, monocytes and dendritic cells (Andrews et al., Blood 62, 24-132, 1983). Besides myelomonocytic cells, CD33 has also been found to be expressed on human mast cells and blood basophils (Valent et al., Blood 15; 73(7):1778-85, 1989). Monoclonal antibodies directed against CD33 are used in diagnosis of leukemia as well as for therapeutic targeting and *in vitro* purging of bone marrow for autologous transplantation in acute myeloid leukemia (AML) (Duzkale et al., Biol Blood Marrow Transplant. 9(6):364-72, 2003). Initial efforts in therapeutic targeting focused on the development of immunotoxins using an anti-CD33 antibody conjugated to the toxin ricin. Since CD33 rapidly internalizes upon antibody binding (Audran et al., J Immunol Methods. 188(1):147-54, 1995) the immunotoxin approach was obvious.

CD33 is a 67 KD transmembrane glycoprotein. The sialic acid-binding extracellular domain of CD33 is involved in cell-cell adhesion. The intracellular immunoreceptor tyrosine-based inhibitory motifs (ITIM) confer inhibitory signals to the cell, affecting proliferation and cell survival. The actual signalling pathways of CD33 are poorly understood but are assumed to involve the ITIM and ITIM-like motifs and the recruitment of tyrosine phosphatases (von Gunten et al., Ann. N.Y. Acad. Sci. 1143: 61–82, 2008). A murine CD33 orthologue has been defined but its functional comparability to human CD33 was questioned (Brinkman-Van der Linden et al., Mol Cell Biol., 23(12): 4199-206, 2003). The functional role of human CD33 on normal and malignant leukocytes remains unknown.

Several publications have described CD33 as a stable cell surface marker on primary AML and CML cells expressed by 70-100% of tested patients (Plesa et al., Cancer 112(3), 572-80, 2007; Hauswirt et al., Eur J Clin Invest. Jan 73-82, 2007; Scheinberg et al., Leukemia Vol. 3, 440-445, 1989). CD33 is expressed on malignant myeloid blast cells, which represent the majority of malignant cells in peripheral blood and bone marrow of leukemia patients, and on leukemic stem cells, a relatively small number of less differentiated cells in the bone marrow which are characterized by their capacity for self-renewal and the maintenance of the leukemic clonal hierarchy. Depletion of leukemic stem cells is regarded the key mechanism for sustained tumor free survival. The CD33 targeting immunotoxin Mylotarg®, a humanized IgG_4 antibody conjugated to the toxin chaliceamicin is used for the treatment of AML patients by delivering its toxic payload to CD33 positive AML cells (Amadori et al., Cancer Treat Rev. 34(1):49-60, 2008). Lintuzumab (SGN-33, HuM195), a "naked" CD33 specific humanized monoclonal antibody was evaluated in phase II clinical trials for the treatment of AML and MDS with initial clinical signs of efficacy from a phase I dose escalation study and tolerable adverse events being reported (Raza et al. Abstract #983, 14th EHA Congress, June 4-7, 2009).

Targeting AML cell lines with CD33 specific HuM195 *in vitro* reduces TNF-α induced secretion of inflammatory cytokines like IL-8, MCP-1 and RANTES (Sutherland et al., Mabs 1:5, 481-490, 2009). The relevance of this effect for AML

therapy is unknown but modulating the cytokine milieu of the tumor microenvironment may contribute to the therapeutic efficacy of the antibody. In addition, the antibody induces antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cell-mediated phagocytosis (ADCP) of AML cell lines *in vitro* (Sutherland et al., Mabs 1:5, 481-490, 2009). ADCC is considered to be a decisive mechanism for anti-tumor activity of antibodies in hematological malignancies. Data from clinical trials with the CD20-specific monoclonal antibody Rituximab have demonstrated the significance of effector cell mediated mechanisms for treatment of B-cell malignancies with respect to response to antibody treatment (Weng and Levy, J Clin Oncol. 21 (21): 3940-7, 2003).

In conclusion, it has been shown that CD33 antigen is expressed on normal cells of the myelomonocytic lineage and frequently expressed on tumor cells in myeloid leukemias. In a phase I trial with an antibody against CD33 (lintuzumab) first signs of efficacy were observed without severe adverse events. However, clinical development of lintuzumab was discontinued after results from a phase II trial in combination with chemotherapy did not yield the expected improvement in efficacy. Therefore, there is a clear need for the development of improved CD33-targeting treatment modalities.

In view of the prior art there is a need for providing a further improved therapy for myeloid cell malignancies and MDS, particularly for acute myeloid leukemia.

In particular there is a need for providing further improved antagonistic binding agents to CD33 for treating cancer, in particular AML.

Summary of the invention

The present invention provides novel CD33 binding agents binding to human CD33 and that are defined by

a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from SeqID No:1-14,

CDR2 has an amino acid sequence selected from SeqID No:14-28, CDR3 has an amino acid sequence selected from SeqID No:29-42, CDR4 has an amino acid sequence selected from SeqID No:43-56, CDR5 has an amino acid sequence selected from SeqID No:57-70, CDR6 has an amino acid sequence selected from SeqID No:71-84, or

b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (SeqID No: 141) of human CD33.

The present invention further provides CD33 binding agents wherein the internalization kinetics of the CD33 binding agents are such that at least 30%, preferably 40%, of the initial amount of the antibody remain on the cell surface of HL60 cells at a time of 4 hours after incubation.

The present invention further provides CD33 binding agents, wherein the heavy chain variable region comprises an amino acid sequence selected from SeqID No:85-98 and the light chain variable region comprises an amino acid sequence selected from SeqID No:99-112.

The present invention further provides CD33 binding agents, wherein the heavy chain having an amino acid sequence selected from SeqID No:113-126 and the light chain having an amino acid sequence selected from SeqID No: 127-140.

The present invention further provides CD33 binding agents having mutations in the F_c domain that increase ADCC.

Further preferred embodiments are outlined in the following specification and in the claims.

The CD33 binding agents according to the present invention have been found to have high affinity to human CD33 and further to have favourable internalization kinetics, which is characterized by a long presence of the CD33 binding agents when bound to CD33 on the surface of the target cells, which translates into

favourable ADCC activity.

The inventors have also found that the CD33 binding agents according to the present invention bind to a different epitope of the extracellular domain of CD33 compared to Lintuzumab. Without wishing to be bound by any particular theory this is believed to be the reason for the different internalization kinetics of the CD33 binding agents according to the present invention and Lintuzumab.

Description of the Figures

Figures 1-3 show the internalization of exemplary CD33 binding agents according to the invention in comparison to lintuzumab on HL60 cells.
Figure 4 shows the internalization rate on HL60 cells of two exemplary antibodies according to the invention in comparison to lintuzumab.
Figure 5 shows the ADCC performance on HL60 cells of two exemplary antibodies according to the invention in comparison to lintuzumab.

Detailed description of the invention

CD33 Binding Agents

The term "binding agent" as used herein means a protein or peptide that specifically binds to a target antigen. A binding agent can be, for example, an antibody, a derivative of such an antibody, or other agent that specifically binds to the target antigen. A binding agent can also be a protein comprising an Fv region or a portion thereof (e.g., a V_H or V_L or a CDR(s) of an antibody that specifically binds to the target antigen). In a preferred embodiment herein the binding agent is an antibody.

The term "CD33 binding agent" as used herein refers to a binding agent that specifically binds to CD33, typically a portion of the extracellular domain of human CD33.

The term "antibody" as used herein refers to (a) immunoglobulin polypeptides and immunologically active portions of immunoglobulin polypeptides (i.e., polypeptides of the immunoglobulin family, or fragments thereof, that contain an antigen binding site that immunospecifically binds to a specific target antigen), or (b) conservatively substituted derivatives of such immunoglobulin polypeptides or fragments that immunospecifically bind to the target antigen. Antibodies are generally described in, for example, Harlow and Lane, *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 1988). The term "antibody" refers to intact monoclonal antibodies, polyclonal antibodies, monospecific antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments that exhibit the desired biological activity (e.g., antigen-binding). The antibody can be of any type or class (e.g., IgG, IgE, IgM, IgD, and IgA) or sub-class (e.g., IgGl, lgG2, IgG3, IgG4, IgAl and IgA2), preferably of the IgG class, more preferably an IgG1.

An "intact" antibody is one which comprises an antigen-binding variable region as well as a light chain constant domain and heavy chain constant domains as appropriate for the antibody class. The constant domains may be native sequence constant domains (e.g., human native sequence constant domains) or amino acid sequence variants thereof.

An "antibody fragment" comprises a portion of an antibody, including the antigen-binding or variable region or a portion thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments, V_H and V_L antigen binding fragments, diabodies, triabodies, tetrabodies, single-chain antibody, scFv, scFv-Fc, a SMTP, and multispecific antibodies formed from antibody fragment(s).

"Heavy chain variable region" or "V_H" means the part of the heavy chain comprising the CDR1, CDR2 and CDR3 and surrounding framework regions.

"Light chain variable region" or "V_L" means the part of the light chain comprising

the CDR4, CDR5 and CDR6 and surrounding framework regions.

"CDR" means the hypervariable regions of the heavy and light chains, which determine the complementarity / binding specifity of an antibody or antibody fragment. The order of the CDRs in the present application is purely numerically.

"Epitope" herein means a part of an antigen, which is recognized by an antibody or antibody fragment. In particular this term refers to parts of CD33, which can be recognized by an antibody.

"mAbs" as used herein refers to monoclonal antibodies.

An antibody may have one or more "effector functions" which refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions include CIq binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody- dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor; BCR), etc.

"Single-chain Fv" or "scFv" antibody fragments comprise the V_H and V_L domains of an antibody, wherein these domains are present in a single polypeptide chain. The Fv polypeptide typically further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for antigen-binding. For a review of scFv, see Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore, *eds.*, Springer-Verlag, New York, pp. 269-315 (1994).

A binding agent such as an antibody that is "directed to," "which binds" or that "specifically binds" an antigen of interest (i.e., a target antigen) is one capable of binding that antigen with sufficient affinity such that the binding agent is useful in targeting a cell expressing the antigen. Typically, the binding agent binds with an

affinity of at least about $1x10^7$ M^{-1}, and binds to the predetermined antigen with an affinity that is at least two-fold greater than its affinity for binding to a non-specific antigen (e.g., BSA, casein) other than the predetermined antigen or a closely-related antigen.

An "antibody derivative" as used herein refers to an antibody, as defined above, that is modified by covalent attachment of a heterologous molecule such as, e.g., by attachment of a heterologous polypeptide, or by glycosylation, deglycosylation, acetylation or phosphorylation or other modification not normally associated with the antibody. In some embodiments, the heterologous molecule is not a therapeutic agent. In some embodiments, the heterologous molecule does not exhibit a cytostatic or cytotoxic effect by itself.

A further comprehensive reference for all terms and procedures used herein is Sambrook at al., *Molecular Cloning*, Cold Spring Harbor Laboratory Press; 3rd edition (January 15, 2001).

The CD33 binding agents specifically bind to a receptor, CD33, associated with a given target cell population. CD33 is a member of the sialoadhesion family that is expressed on cells of the hematopoietic lineage, including myeloid precursors, monocytes, macrophages, dendritic cells, and mast cells. CD33 is also expressed on tumor cells associated with myeloproliferative or mast cell proliferative diseases, including acute myeloid leukemia and myeloplastic syndromes, and on leukemic stem cells. Antibodies targeting CD33 and their uses have been generally described (see, e.g., Pierelli et al., 1993, *Br. J. Haematol.* 84:24-30; Matutes et al., 1985, *Hemaiol. Oncol.* 3: 179- 186; Taussig et al., 2005, *Blood* 106:4086- 4092; Florian et al., 2006, *Leuk. & Lymph.* 47:207-222).

In some embodiments, the CD33 binding agent is an antibody (e.g., a monoclonal antibody). Useful monoclonal antibodies can be homogeneous populations of antibodies to a CD33 (e.g., the extracellular domain of human CD33). A monoclonal antibody (mAb) can be prepared by using any technique known in the

art. These include, but are not limited to the hybridoma technique originally described by Köhler and Milstein (1975, *Nature* 256:495- 497), the human B cell hybridoma technique (Kozbor et al., 1983, *Immunology Today* 4:72), and the EBV-hybridoma technique (Cole et al., 1985, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA and IgD and any subclass thereof. The hybridoma producing a monoclonal antibody may be cultivated *in vitro* or *in vivo*.

Useful monoclonal antibodies include, but are not limited to, human monoclonal antibodies, humanized monoclonal antibodies, chimeric monoclonal antibodies and functionally active antibody fragments of any of these.

Useful CD33 antibodies include antibodies that can achieve a therapeutic effect by various mechanisms known in the art, such as antibody-dependent cell-mediated cytotoxicity (ADCC), anti-dependent cell phagocytosis (ADCP) and/or complement dependent cytotoxicity (CDC). For example the antibody can mediate ADCC by interacting with immune effector cells such as NK cells, monocytes, and macrophages.

Recombinant antibodies, such as chimeric and humanized monoclonal antibodies, comprise both human and non-human portions, which can be made using standard recombinant DNA techniques. (See, e.g., Cabilly et al., U.S. Patent No. 4,816,567; and Boss et al., U.S. Patent No. 4,816,397; both of which are incorporated herein by reference in their entirety). "Humanized antibodies" are antibody molecules from non-human species having one or more complementarity determining regions (CDRs) from the non-human species and a framework region from a human immunoglobulin molecule. (See, e.g., Queen, U.S. Patent No. 5,585,089, which is incorporated herein by reference in its entirety.) Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in International Publication No. WO 87/02671; European Patent Publication No. 0184187; European Patent

Publication No. 0171496; European Patent Publication No. 0173494; International Publication No. WO 86/01533; U.S. Patent No. 4,816,567; European Patent Publication No. 012023; Berter et al., 1988, Science 240: 1041-1043; Liu et al., 1987, Proc. Natl. Acad Set. USA 84:3439-3443; Liu et al., 1987, J. Immunol. 139:3521-3526; Sun el al., 1987, Proc. Natl. Acad. Sci. USA 84:214-218; Nishimura et al., 1987, Cancer. Res. 47:999-1005; Wood et al. 1985, Nature 314:446-449; Shaw et al, 1988, J. Natl. Cancer Inst. 80: 1553-1559; Morrison, 1985, Science 229: 1202-1207; Oi et al., 1986, BioTechniques 4:214; U.S. Patent No. 5,225,539; Jones et al., 1986, Nature 321:552-525; Verhoeyan et al., 1988, Science 239:1534; and Beidler et al., 1988, J. Immunol. 141:4053-4060: each of which is incorporated herein by reference in its entirety.

Human monoclonal antibodies may be made by any of numerous techniques known in the art (see, e.g., Teng el al., 1983, Proc. Natl. Acad. Sci. USA. 80: 7308-7312; Kozbor et al, 1983, Immunology Today 4:72-79; Olsson at al., 1982, Meth. Enzymol. 92:3-16; and U.S. Patent Nos. 5,939,598 and 5,770,429).

Fully human antibodies can be produced using transgenic mice that are incapable of expressing endogenous immunoglobulin heavy and light chains genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a CD33 polypeptide. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see, e.g., Lonberg and Huszar, 1995, Int. Rev. Immunol. 13:65-93. For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., U.S. Patent Nos. 5,625,126; 5,633,425; 5,569,825; 5,661,016; and 5,545,806. Other human antibodies can be obtained commercially from, for example, Medarex

(Princeton, NJ), which are obtained by immunizing mice.

Fully human antibodies that recognize a selected epitope also can be generated using a technique referred to as "guided selection". In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a fully human antibody recognizing the same epitope. (See, e.g., Jespers el al, 1994, Biotechnology 12:899-903). Human antibodies also can be produced using various techniques known in the art, including phage display libraries (see, e.g., Hoogenboom and Winter, 1991, J. MoI. Biol 227:381; Marks et al, 1991, J MoI Biol 222:581 ; Quan and Carter, 2002, The rise of monoclonal antibodies as therapeutics, In Anti-IgE and Allergic Disease, Jardieu and Fick Jr., eds., Marcel Dekker, New York, NY, Chapter 20, pp. 427-469).

Useful antibody fragments include, but are not limited to, $F(ab')_2$ fragments, Fab' fragments, Fab fragments, Fvs, single chain antibodies (SCAs) (e.g., as described in U.S. Patent No. 4,946,778: Bird, 1988, Science 242:423-42; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al, 1989, Nature 334: 544-54), scFv, scFv-Fc, FvdsFv, minibodies, diabodies, triabodies, tetrabodies, SMIPs (see, e.g., Published U.S. Patent Application No. 2005-0238646) and any other molecule comprising one or more CDRs and that has the same specificity as the antibody.

In other embodiments, the antibody is a fusion protein of an antibody, or a functionally active fragment thereof, joined to another protein. For example, an antibody or antibody fragment can be fused via a covalent bond (e.g., a peptide bond), at either the N-terminus or the C-terminus to an amino acid sequence of another protein (or portion thereof, typically at least a 10, 20 or 50 amino acid portion of the protein) that is not the antibody or an antibody fragment. In some embodiments, the antibody or fragment thereof can be covalently linked to the other protein at the C-terminus of the variable domain or a constant domain.

Antibodies can be modified, e.g., by the covalent attachment of any type of

molecule as long as such covalent attachment permits the antibody to retain its antigen-binding immunospecificity. For example, a derivative of an antibody can be one that has been further modified, e.g., by glycosylation, de-glycosylation, acetylation, pegylation, phosphorylation, amidation, derealization by known protecting/blocking groups, proteolytic cleavage, linkage to another protein, etc. Any of numerous chemical modifications can be carried out by known techniques, including, but not limited to, specific chemical cleavage, acetylation, formylation, metabolic synthesis in the presence of tunicamycin, or the like. Additionally, the derivative can contain one or more unnatural amino acids.

In specific embodiments, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. (See, e.g., U.S. Patent Publication Nos. 2006-0003412 and 2006-0008882). Amino acid sequence variants of the antibodies are prepared by introducing appropriate nucleotide changes into the antibody nucleic acid, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletions, insertions, and/or substitutions is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the antibody, such as changing the number or position of glycosylation sites.

A useful method for identification of certain residues or regions of the antibody that are favored locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989, Science 244: 1081-1085). Here, a residue or group of target residues are identified (e.g., charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (typically alanine or polyalanine) to affect the interaction of the amino acids with antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se need not be

predetermined. For example, to analyze the performance of a mutation at a given site, alanine scanning or random mutagenesis is conducted at the target codon or region and the expressed antibody variants are screened for the desired activity.

Amino acid sequence insertions can include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide.

Another type of antibody is an amino acid substitution variant of an antibody. Such variants have at least one amino acid residue in the antibody molecule replaced by a different residue. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but framework region alterations are also contemplated.

Substantial modifications in the biological properties of the antibody can be accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally-occurring residues are divided into groups based on common side-chain properties:

(1) hydrophobic: norleucine, met, ala, val, leu, ile;

(2) neutral hydrophilic: cys, ser, thr;

(3) acidic: asp, glu;

(4) basic: asn, gin, his, lys, arg;

(5) residues that influence chain orientation: gly, pro; and

(6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

It is desirable to modify the antibody with respect to effector function, e.g., so as to enhance antibody-dependent cell-mediated cyotoxicity (ADCC), anti-dependent cell phagocytosis (ADCP) and/or complement dependent cytotoxicity (CDC) of the antibody. In particular, the ADCC activity can be enhanced by introducing amino acid mutations in the constant region of the antibody (Lazar et al., PNAS 103, 11, 4005–4010, 2006). This may be achieved by introducing one or more amino acid substitutions in an Fc region of the antibody, see, e.g., Published U.S. Patent Application No. 2006-0160996. Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased CDC and ADCC. (See, e.g., Caron et al., 1992, J Exp. Med 176:1191-1195; and Shopes, 1992, J. Immunol. 148:2918-2922.) Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff et al., 1993, Cancer Research 53:2560-2565. Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. (See, e.g., Stevenson et al.. 1989, Anti-Cancer Drug Design 3:219-230.)

A variety of modifications of the Fc region have been suggested in the art, both in the scientific literature and in patent documents, e.g. in EP 0307434, WO 9304173, WO 9734631, WO 9744362, WO 9805787, WO 9943713, WO 9951642, WO 9958572, WO 02060919, WO 03074679, WO 2004016750, WO 2004029207, WO 2004063351, WO 2004074455, WO 2004035752, WO 2004099249, WO 2005077981, WO 2005092925, WO 2006019447, WO 2006031994,

WO 2006047350, WO 2006053301, WO 2006088494 and WO 2007041635.

In preferred embodiments, the antibodies of the invention are Fc variants with amino acid substitutions at positions 332 and/or 239 and/or 236. In preferred embodiments, the antibodies of the invention have mutations in the Fc domain selected from the group of

i) a single substitution at position 332, preferably I332E;
ii) a combination of substitutions at positions 239 and 332, preferably S239D/I332E;
iii) a combination of substitutions at positions 236 and 332, preferably G236A/ I332E;
iv) a combination of substitutions at positions 236, 239 and, 332, preferably G236A /S239D/I332E.

In this context it is particularly preferred to introduce mutations in the F_c domain at one or more positions selected from amino acids at positions 332 and/or 239 and/or 236 according to the Kabat EU numbering index. Particular preferred are substitutions at positions 239 and 332, especially S239D/I332E.

The Fc variants in the antibodies of the present invention are defined according to the amino acid modifications that compose them. Thus, for example, I332E is an Fc variant with the substitution I332E relative to the parent Fc polypeptide. Likewise, S239D/I332E defines an Fc variant with the substitutions S239D and I332E and S239D/I332E/G236A defines an Fc variant with the substitutions S239D, I332E, and G236A relative to the parent Fc polypeptide.

To increase the serum half life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in U.S. Patent No. 5,739,277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (e.g., IgG_1, IgG_2, IgG_3, or IgG_4) that is responsible for increasing the in

vivo serum half-life of the IgG molecule.

Antibodies may be glycosylated at conserved positions in their constant regions (see, e.g., Jefferis and Lund, 1997, Chem. Immunol. 65:11 1-128; Wright and Morrison, 1997, TibTECH 15:26-32). The oligosaccharide side chains of the immunoglobulins can affect the protein's function (see, e.g., Boyd et al., 1996, MoI. Immunol. 32: 1311 -1318; Wittwe and Howard, 1990, Biochem. 29: 4175-4180), and the intramolecular interaction between portions of the glycoprotein which can affect the conformation and presented three-dimensional surface of the glycoprotein (see, e.g., Jefferis and Lund, supra; Wyss and Wagner, 1996, Current Opin. Biotech. 7:409-416). Oligosaccharides may also serve to target a given glycoprotein to certain molecules based upon specific recognition structures. For example, it has been reported that in a galactosylated IgG, the oligosaccharide moiety 'flips' out of the inter-C_H2 space and terminal N-acetylglucosamine residues become available to bind mannose binding protein (see, e.g., Malhotra et al. 1995, Nature Med. 1 :237-243). Removal by glycopeptidase of the oligosaccharides from CAMPATH-1H (a recombinant humanized murine monoclonal IgG1 antibody which recognizes the CDw52 antigen of human lymphocytes) produced in Chinese Hamster Ovary (CHO) cells resulted in a complete reduction in complement mediated lysis (CMCL or CDC) (Boyd et al., 1996, MoI. Immunol. 32:131, 1-1318), while selective removal of sialic acid residues using neuraminidase resulted in no loss of CMCL. Glycosylation of antibodies has also been reported to affect ADCC. In particular, CHO cells with tetracycline-regulated expression of β(1.4)-N-acetylglucosaminyltransferase III (GnTIII), a glycosyltransferase catalyzing formation of bisecting GIcNAc, was reported to have improved ADCC activity (see, e.g., Umana et al., 1999, Nature Biotech. 17: 176-180).

Glycosylation of antibodies is typically either N-linked or O-Iinked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition

sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalaciosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Glycosylation variants of antibodies are variants in which the glycosylation pattern of an antibody is altered. By altering is meant deleting one or more carbohydrate moieties found in the antibody, adding one or more carbohydrate moieties to the antibody, changing the composition of glycosylation (i.e., glycosylation pattern), the extent of glycosylation, or the like.

Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites). Similarly, removal of glycosylation sites can be accomplished by amino acid alteration within the native glycosylation sites of the antibody.

The amino acid sequence is usually altered by altering the underlying nucleic acid sequence. These methods include, but are not limited to, isolation from a natural source (in the case of naturally-occurring amino acid sequence variants) or preparation by oligonucleoiide-mediated (or site-directed) mutagenesis, PCR mutagenesis, or cassette mutagenesis of an earlier prepared variant or a non-variant version of the antibody.

The glycosylation (including glycosylation pattern) of antibodies may also be altered without altering the amino acid sequence or the underlying nucleotide sequence. Glycosylation largely depends on the host cell used to express the antibody. Since the cell type used for expression of recombinant glycoproteins,

e.g., antibodies, as potential therapeutics is rarely the native cell, significant variations in the glycosylation pattern of the antibodies can be expected. (See, e.g., Hse et al., 1997, Biol. Chem. 272:9062-9070.) In addition to the choice of host cells, factors which affect glycosylation during recombinant production of antibodies include growth mode, media formulation, culture density, oxygenation, pH, purification schemes, and the like. Various methods have been proposed to alter the glycosylation pattern achieved in a particular host organism, including introducing or overexpressing certain enzymes involved in oligosaccharide production (see, e.g., U.S. Patent Nos. 5,047,335; 5,510,261; and 5,278,299). Glycosylation, or certain types of glycosylation, can be enzymatically removed from the glycoprotein, for example using endoglycosidase H (Endo H). In addition, the recombinant host cell can be genetically engineered, e.g., made defective in processing certain types of polysaccharides. These and similar techniques are well known in the art.

The glycosylation structure of antibodies can be readily analyzed by conventional techniques of carbohydrate analysis, including lectin chromatography, NMR, mass spectrometry, HPLC, GPC, monosaccharide compositional analysis, sequential enzymatic digestion, and HPAEC-PAD, which uses high pH anion exchange chromatography to separate oligosaccharides based on charge. Methods for releasing oligosaccharides for analytical purposes are also known, and include, without limitation, enzymatic treatment (commonly performed using peptide-N-glycosidase F/endo-β-galactosidase), elimination using harsh alkaline environment to release mainly O-linked structures, and chemical methods using anhydrous hydrazine to release both N- and O-linked oligosaccharides.

The antibodies also can have modifications (e.g., substitutions, deletions or additions) in amino acid residues that interact with Fc receptors. In particular, antibodies can have modifications in amino acid residues identified as involved in the interaction between the anti-Fc domain and the FcRn receptor (see, e.g., International Publication No. WO 97/34631).

In its broadest aspect the present invention relates to CD33 binding agents binding to human CD33 and that are defined by

a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from SeqID No:1-14, CDR2 has an amino acid sequence selected from SeqID No:15-28, CDR3 has an amino acid sequence selected from SeqID No:29-42, CDR4 has an amino acid sequence selected from SeqID No:43-56, CDR5 has an amino acid sequence selected from SeqID No:57-70, CDR6 has an amino acid sequence selected from SeqID No:71-84, or

b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (SeqID No: 141) of human CD33.

The present invention further provides CD33 binding agents wherein the internalization kinetics of the CD33 binding agents are such that at least 30% of the initial amount of the antibody remain on the cell surface of HL60 cells at a time of 4 hours after incubation.

It has been found that the CD33 binding agents according to the present invention bind to a different epitope than lintuzumab, see example 4 herein. Both epitopes (SeqID No: 141 and SeqID No: 142) are non-overlapping. It is believed that the different epitopes on the exatracellular domain of CD33, which are recognized by the CD33 binding agents according to the present invention and by lintuzumab, are the reason for the different internalization behaviour and ADCC performance of the CD33 binding agents according to the present invention and lintuzumab (cf. examples 2 and 3 herein).

In a preferred embodiment at least 40% of the initial amount of the CD33 binding agents remain on the cell surface at a time of 4 hours after incubation.

In a preferred embodiment the heavy chain variable region comprises an amino acid sequence selected from SeqID No:85-98 and the light chain variable region

comprises an amino acid sequence selected from SeqID No:99-112.

In a preferred embodiment the CD33 binding agent has a heavy chain having an amino acid sequence selected from SeqID No:113-126 and a light chain having an amino acid sequence selected from SeqID No:127-140.

In a preferred embodiment the CD33 binding agent has an affinity to both human CD33 and cynomolgus CD33 with a K_D of equal to or less than 10 nM.

In a preferred embodiment the CD33 binding agent is humanized.

In a preferred embodiment the CD33 binding agent is fully human.

In a preferred embodiment the CD33 binding agent further comprises an effector function.

In a preferred embodiment the effector function is mediated by an F_c domain.

In a preferred embodiment the CD33 binding agent comprises one or more mutations in the F_c domain that modulate the function of the F_c domain.

In a preferred embodiment the modulation of the function of the F_c domain is an increase of ADCC by at least 10%, preferably 50% or 100%.

Particularly preferred CD33 binding agents according to the present invention are listed in table 1:

No	Clone ID#	SeqID CDR1	SeqID CDR2	SeqID CDR3	SeqID CDR4	SeqID CDR5	SeqID CDR6	SeqID V_H	SeqID V_L	SeqID heavy chain	SeqID light chain
1	280-03-08	1	15	29	43	57	71	85	99	113	127
2	280-21-09	2	16	30	44	58	72	86	100	114	128
3	280-29-12	3	17	31	45	59	73	87	101	115	129
4	280-31-01	4	18	32	46	60	74	88	102	116	130
5	280-31-01 (mut)	5	19	33	47	61	75	89	103	117	131
6	280-34-02	6	20	34	48	62	76	90	104	118	132
7	280-50-01	7	21	35	49	63	77	91	105	119	133
8	280-50-01 (mut)	8	22	36	50	64	78	92	106	120	134
9	280-61-07	9	23	37	51	65	79	93	107	121	135
10	283-03-03	10	24	38	52	66	80	94	108	122	136
11	283-05-01	11	25	39	53	67	81	95	109	123	137
12	283-07-03	12	26	40	54	68	82	96	110	124	138
13	283-11-03	13	27	41	55	69	83	97	111	125	139
14	283-14-01	14	28	42	56	70	84	98	112	126	140

Table 1

Treatment of Cancer

Several publications have described CD33 as a cell surface marker on primary AML and CML cells which is expressed on malignant cells of 70% to 100% of patients (Scheinberg et al., 1989; Hauswirt et al., 2007; Plesa et al., 2007; Webber et al., 2008). CD33 is expressed on malignant myeloid blast cells, which represent the majority of malignant cells in peripheral blood and bone marrow of leukemia patients, and on leukemic stem cells, a relatively small number of less differentiated cells in the bone marrow which are characterized by their capacity for self-renewal and the maintenance of the leukemic clonal hierarchy. The clinical feasibility of targeting CD33 using antibodies has been demonstrated by Mylotarg® (Gemtuzumab ozogamizin), an antibody-calicheamycin conjugate which was approved for the treatment of relapsed AML patients not eligible for other treatment options. An alternative approach directed towards CD33 is the development of lintuzumab (SGN-33, HuM195), a humanized IgG1 monoclonal antibody which has shown early signs of efficacy in clinical phase I trials (Raza et al., 2009). Taken together, there is a wealth of data, both preclinical and clinical, that underscore the relevance and feasibility of CD33 targeting for the treatment of AML and other CD33-positive malignancies.

Acute myeloid leukemia (AML) is a malignancy of the myeloid lineage of white blood cells. This hematological neoplasia is a blood and bone marrow disease that, if left untreated, is typically fatal within weeks to months. There is an estimated prevalence of 30000 cases of AML in the US and 47000 in the European Union (10 year prevalence data confirmed by Mattson-Jack, 2010). AML is the most prevalent form of adult acute leukemia (about 90%), comprising about 33% of new leukemia cases. The median age of patients diagnosed with AML is 67 years. AML accounts for about 1.2% of cancer deaths in the United States.

AML causes non-specific symptoms such as weight loss, fatigue, fever, and night sweats. AML is diagnosed by blood tests, bone marrow examination, and laboratory tests to determine the AML subtype and to determine treatment decisions.

Therapy for AML is highly depending on the age and performance status of the patient. Patients who can tolerate intensive induction (and subsequent consolidation

and maintenance) chemotherapy will be treated intensely with a combination of cytotoxic drugs. These patients have a likelihood of achieving a complete response of approximately 75%. In this patient population the therapeutic goal is a cure. Still, relapse of AML occurs in about half of the patients within one year after achievement of a complete response. Long-term cure rates are in the 30% range.

However, higher age at diagnosis or existence of co-morbidities does not allow administration of intensive induction therapy leading to a palliative treatment goal. Therefore, remission rates decline significantly in older patients with AML. The median survival of older patients with AML is less than 6 months.

In one aspect, the CD33 binding agents are useful for treating cancer, such as by delaying progression of a cancer and/or reducing cancer-associated cachexia, or preventing or delaying recurrence of a hematological malignancy (e.g., leukemia), in a mammal, preferably a human patient. The CD33 binding agent can be administered alone or co-administered with another therapeutic agent. In some embodiments, the CD33 binding agent is co-administered with a standard of care chemotherapeutic. The CD33 binding agent can be administered in an unconjugated form (i.e., not conjugated to a cytotoxin) or as a conjugate.

In this subsection, a "patient" is a human or other mammal who is undergoing treatment for, or has been diagnosed as having, cancer.

In some embodiments, the CD33 binding agents are useful for delaying progression of a cancer and/or reducing cancer-associated cachexia in a patient by administering to the patient in need thereof an effective dosage of the CD33 binding agent. Without being bound to a particular mechanism, the CD33 binding agent binds to effector or accessory cells of the myeloid or monocytic lineages (e.g., monocytes, macrophages, dendritic cells, and neutrophils), thereby inhibiting or reducing the production of various cytokines, chemokines and growth factors from the effector or accessory cells and/or the tumor cells. These cytokines, chemokines and growth factors, which can promote the growth and proliferation of

tumor cells and/or contribute to cancer cachexia, include, but are not limited to, interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-8 (IL-8), interferon-γ (IFN-γ), vascular endothelial growth factor (VEGF), leukemia inhibitory factor (LlF), monocyte chemoattraclant protein-1 (MCP-1), RANTES, interleukin-10 (IL-10), interleukin-12 (IL-12), matrix metalloproteinase 2 (MMP2), IP-10 and/or macrophage inflammatory protein 1α (MIP1α). The CD33 binding agents also can reduce the migration of macrophages to the site of the tumor cells.

In some embodiments, administration of an effective dosage of a CD33 binding agent to a patient reduces the levels of at least one cytokine, chemokine or growth factor, which cytokine, chemokine or growth factor can promote the growth and proliferation of tumor cells, promote the migration of non-malignant effector cells, such as tumor associated macrophages (TAMS), to the vicinity of the tumor site and/or contribute to cancer cachexia. In specific embodiments, the cytokine, chemokine or growth factor is for example interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-8 (IL-8), interferon-γ (IFN-γ), vascular endothelial growth factor (VEGF), leukemia inhibitory factor (LIF), monocyte chemoattractant protein-1 (MCP-1), RANTES, interleukin-10 (IL-10), interleukin-12 (IL-12), matrix metalloproteinase 2 (MMP2), IP-10 and/or macrophage inflammatory protein 1α (MIP1α).

In another embodiment, a method is provided for delaying progression of a cancer by administering to a patient an effective regimen of a CD33 binding agent that can specifically bind to CD33. As a result of administration of the CD33 binding agent, the progression of the cancer is delayed, such as by reducing the growth or proliferation of the tumor cells, decreasing metastasis, reducing the level of at least one cytokine, chemokine or growth factor, reducing non-malignant effector cells in the vicinity of the tumor cells, or the like.

In another embodiment, a method is provided for reducing the tumor burden in a patient by administering to the patient an effective regimen of a CD33 binding

agent that can specifically bind to CD33. As a result of administration of the CD33 binding agent, the tumor burden in the patient is arrested or reduced, such as by reducing the size or mass of the tumor, reducing the level of at least one cytokine, chemokine or growth factor, reducing non- malignant effector cells in the vicinity of the tumor cells, inhibiting the migration of macrophages in the vicinity of the tumor cells, reducing the number of non-malignant effector cells (e.g., TAMS or macrophages) in the tumor, or the like.

In another embodiment, a method is provided for reducing the tumor burden or delaying progression of a cancer in a patient by administering to the patient an effective regimen of a CD33 binding agent that can specifically bind to CD33. As a result of administration of the CD33 binding agent, the tumor burden in the patient is arrested or reduced, such as by recruiting immune effector cells like NK cells or macrophages or monocytes, which can destroy tumor cells by immune mediated mechanisms.

Antibody dependent cellular cytotoxicity (ADCC) is an immune effector cell mediated mechanism which may contribute to anti-tumor activity of monoclonal antibodies (Weiner GJ. Monoclonal antibody mechanisms of action in cancer. Immunol Res. 2007,39(1-3):271-8). The relevance of ADCC for anti-tumor efficacy has been demonstrated in preclinical models, e.g. in mouse tumor models (e.g. Clynes RA, Towers TL, Presta LG, Ravetch JV.Inhibitory Fc receptors modulate in vivo cytoxicity against tumor targets. Nat Med. 2000 Apr;6(4):443-6). Data from clinical trials support the relevance of ADCC for clinical efficacy of therapeutic antibodies (e.g. Weng WK, Levy R Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. J Clin Oncol. 2003 Nov 1;21(21):3940-7. Epub 2003 Sep 15). Interactions of monoclonal antibodies with Fc receptors on immune cells contribute to ADCC. The Fc of antibodies can be modified in order to display enhanced affinity to Fc receptors (e.g. Presta LG Engineering of therapeutic antibodies to minimize immunogenicity and optimize function. Adv Drug Deliv Rev. 2006 Aug 7;58(5-6):640-56. Epub 2006 May 23). Such enhanced affinity to

Fc receptors results in increased ADCC activity which may lead to increased anti tumor efficacy in patients.

In the various embodiments described in this section, the CD33 binding agent can be used to treat a CD33 positive cancer (i.e., a cancer comprised of cancer cells that over express CD33 on their cell surface, or that express CD33 at levels considered acceptable for therapy with CD33 antibodies). The CD33 binding agent also can be used to treat a cancer that does not overexpress CD33 on the non-malignant effector cells relative to normal tissue of the same type. The cancer can be, for example, a non- hematological malignancy or a hematological malignancy. In specific examples, the hematological malignancy can be CD33-positive and can be, for example, acute lymphoid leukemia, acute myeloid leukemia, chronic myelomonocytic leukemia, an erythrocytic leukemia, acute megakaryoblastic leukemia, histiocytic lymphoma, a myeloid sarcoma, a mast cell proliferative disorder or myelodysplastic syndrome (MDS). In some embodiments, the hematological malignancy is a CD33-positive malignancy, such as acute myeloid leukemia or myelodysplastic syndrome (MDS).

In the various embodiments described in this section, the CD33 binding agent can be an unconjugated anti-CD33 antibody. For example, the antibody can be a fully human, humanized or chimeric antibody, such as a chimeric or humanized M 195 antibody. The antibody also can be another antibody, such as an antibody that competes with M 195 antibody for specific binding to CD33. The antibody also can bind to the same epitope as M 195 antibody or to a different epitope.

In other embodiments, the CD33 binding agent can be bound (i.e., conjugated) to a cytotoxin. The cytotoxin can be, for example, a peptide toxin, such as saporin, ricin, chlorotoxin, pseudomonas exotoxin, pseudomonas endotoxin or diphtheria toxin. The cytotoxin also can be a chemical (i.e., non-peptide-based) toxin, such as a calicheamicin, doxorubicin, a camptothecin, daunorubicin, or other DNA binding agents. The cytotoxin also can be an auristatin, a maytansinoid, a dolastatin, or other microtubule blocking agents.

A CD33 binding agent that is an anti-CD33 antibody can be administered to the patient intravenously or subcutaneously at a dose of 0.1 mg/kg or less to about 25 mg/kg, preferably 1.0 mg/kg to about 10 mg/kg. A CD33 binding agent that is an anti-CD33 antibody fragment or other CD33 binding protein can be administered in a dosage equivalent to a dose of 0.1 mg/kg to about 25 mg/kg. 1.0 mg/kg to about 10 mg/kg of intact antibody. The CD33 binding agent can be administered intravenously or subcutaneously to the patient on a schedule that is, for example, daily, weekly, biweekly, tri-weekly (i.e., every three weeks) or monthly, or a combination thereof, to the patient. The CD33 binding agent can be administered for a period of at least one month, at least two months, at least three months, at least four months, at least five month, at least six months, or more, as needed. In some embodiments, a treatment phase (supra) of the CD33 binding agent is followed by a maintenance phase, in which doses of the CD33 binding agent are administered less frequently than during the treatment phase. For example, maintenance doses can be administered weekly, biweekly, tri-weekly or monthly, for a period of 1-6 months. The dosages in the maintenance phase can be the same as the dosages in the treatment phase.

Treatment of A Hematological Malignancy in Remission

In another aspect, methods are provided of preventing or delaying recurrence of a hematological malignancy (e.g., leukemia) in a patient by administering to the patient in remission from the hematological malignancy an effective dosage of a CD33 binding agent, resulting in preventing or delaying recurrence of the underlying hematological malignancy. The CD33 binding agent specifically binds to CD33 on the surface of the hematological malignancy (i.e., leukemic cell) and/or to non-malignant effector cells.

In this disclosure, a "patient" is typically a human who is undergoing treatment for, or has been diagnosed as having, hematological malignancy. In some embodiments, the hematological malignancy is a CD33-positive hematological

malignancy. Hematological malignancies include, but are not limited to, leukemias (e.g., acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), chronic myelomonocytic leukemia, hairy cell leukemia). Related blood disorders include, but are not limited to, myelodysplastic syndrome (MDS), myelofibrosis, myeloproliferative disease (e.g., polycythemia vera (PV, PCV or PRV), essential thrombocytosis (ET)), and amyloid due to light-chain disease.

The term "CD33-positive hematological malignancy" refers to a hematological malignancy characterized by the expression of CD33 on the surface of the malignant cells. CD33-positive hematological malignancies include, but are not limited to, acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic myelomonocytic leukemia, thrombocyte leukemia, a myelodysplastic syndrome, a myeloproliferative disorder, refractory anemia, a preleukemia syndrome, a lymphoid leukemia, or an undifferentiated leukemia.

In some embodiments, the methods include administering to a patient in remission from a CD33 positive hematological malignancy an effective regimen of a CD33 binding agent, whereby the recurrence of the hematological malignancy is prevented or delayed. In some embodiments, the patient lacks detectable cells of the hematological malignancy. As used herein, a 'lack of detectable cells" is determined by standard diagnostic or prognostic methods. A patient in remission from AML typically exhibits resolution of abnormal clinical features, return to normal blood counts and normal hematopoiesis in the bone marrow with <5% blast cells, a neutrophil count of >1.000-1,500, a platelet count of >100,000, and disappearance of the leukemic clone. See, e.g., The Merck Manual, Sec. 11, Ch. 138 (17th ed. 1997): Estey, 2001, Cancer 92(5): 1059-1073.

The CD33 binding agent can be, for example, an antibody that specifically binds to CD33 and the hematological malignancy can be acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic myelomonocytic leukemia, thymoid leukemia, a myelodysplastic syndrome, a myeloproliferative disorder, refractory

anemia, a preleukemia syndrome, a lymphoid leukemia, or an undifferentiated leukemia.

In some embodiments, the patient in remission from the hematological malignancy has not undergone a bone marrow transplant. In other embodiments, the patient in remission from the hematological malignancy has undergone a bone marrow transplant. The bone marrow transplant can be either an autologous or an allogeneic bone marrow transplant.

The following cancer types are especially suitable to be treated by the antibodies according to the present invention:

Blood-borne cancers, including, but not limited to: acute lymphoblastic leukemia "ALL", acute lymphoblastic B-cell leukemia, acute lymphoblastic T-cell leukemia, acute myeloblastic leukemia "AML", acute promyelocytic leukemia "APL", acute monoblastic leukemia, acute erythroleukemic leukemia, acute megakaryoblaslic leukemia, acute myelomonocytic leukemia, acule nonlymphocytic leukemia, acute undifferentiated leukemia, chronic myelocytic leukemia "CML", chronic lymphocytic leukemia "CLL", hairy cell leukemia, multiple myeloma.

Acute and chronic leukemias, which are suitable to be treated by CD33 binding agents include: lymphoblastic, myelogenous, lymphocytic, myelocytic leukemias and thrombocytic leukemia. Further myelodysplasia syndrome, myeloproliferative disorder, refractory anemia, preleukemia syndrome, lymphoid leukemia, or undifferentiated leukemia can be treated by CD33 binding agents.

Combinations with other Active Substances

Depending on the disorder to be treated, the CD33 binding agents of the invention may be used on its own or in combination with one or more additional therapeutic agents, in particular selected from DNA damaging, DNA demethylating or tubulin binding agents or therapeutically active compounds that inhibit angiogenesis,

signal transduction pathways or mitotic checkpoints in cancer cells or have immunomodulatory function (IMIDs).

The additional therapeutic agent may be administered simultaneously with, optionally as a component of the same pharmaceutical preparation, or before or after administration of the CD33 binding agent.

In certain embodiments, the additional therapeutic agent may be, without limitation, one or more inhibitors selected from the group of inhibitors of EGFR family, VEGFR family, VEGF, IGF-1R, Insulin receptors, AuroraA, AuroraB, PLK and PI3 kinase, FGFR, PDGFR, Raf, KSP or PDK1.

Further examples of additional therapeutic agents are inhibitors of CDKs, Akt, Src, Bcr-Abl, cKit, cMet/HGF, Her2, Her3, c-Myc, Flt3, HSP90, hedgehog antagonists, inhibitors of JAK/STAT, Mek, mTor, NFkappaB, the proteasome, Rho, an inhibitor of Wnt signaling or Notch signaling or an ubiquitination pathway inhibitor.

Further examples of additional therapeutic agents are inhibitors of DNA polymerase, topoisomerase II, multityrosine kinase inhibitors, CXCR4 antagonists, IL3RA inhibitors, RAR antagonists, KIR inhibitors, immunotherapeutic vaccines, TUB inhibitors, Hsp70 inducers, IAP family inhibitors, DNA methyltransferase inhibitors, TNF inhibitors, ErbB1 receptor tyrosine kinase inhibitors, multikinase inhibitors, JAK2 inhibitors, RR inhibitors, apoptosis inducers, HGPRTase inhibitors, histamine H2 receptor antagonists and CD25 receptor agnosists.

Examples for Aurora inhibitors are, without limitation, PHA-739358, AZD-1152, AT-9283, CYC-116, R-763, VX-667, MLN-8045, PF-3814735, SNS-314, VX-689, GSK-1070916, TTP-607, PHA-680626, MLN-8237, BI847325 and ENMD-2076.

Examples for PLK inhibitor are GSK-461364, BI2536 and BI6727.

Examples for raf inhibitors are BAY-73-4506 (also a VEGFR inhibitor), PLX-4032, RAF-265 (also a VEGFR inhibitor), sorafenib (also a VEGFR inhibitor), XL-281, Nevavar (also an inhibitor of the VEGFR) and PLX4032.

Examples for KSP inhibitors are ispinesib, ARRY-520, AZD-4877, CK-1122697, GSK-246053A, GSK-923295, MK-0731, SB-743921, LY-2523355, and EMD-534085.

Examples for a src and/or bcr-abl inhibitors are dasatinib, AZD-0530, bosutinib, XL-228 (also an IGF-1R inhibitor), nilotinib (also a PDGFR and cKit inhibitor), imatinib (also a cKit inhibitor), NS-187, KX2-391, AP-24534 (also an inhibitor of EGFR, FGFR, Tie2, Flt3), KM-80 and LS-104 (also an inhibitor of Flt3, Jak2).

An example for a PDK1 inhibitor is AR-12.

An example for a Rho inhibitor is BA-210.

Examples for PI3 kinase inhibitors are PX-866, PX-867, BEZ-235 (also an mTor inhibitor), XL-147, and XL-765 (also an mTor inhibitor), BGT-226, CDC-0941.

Examples for inhibitors of cMet or HGF are XL-184 (also an inhibitor of VEGFR, cKit, Flt3), PF-2341066, MK-2461, XL-880 (also an inhibitor of VEGFR), MGCD-265 (also an inhibitor of VEGFR, Ron, Tie2), SU-11274, PHA-665752, AMG-102, AV-299, ARQ-197, MetMAb, CGEN-241, BMS-777607, JNJ-38877605, PF-4217903, SGX-126, CEP-17940, AMG-458, INCB-028060, and E-7050.

An example for a c-Myc inhibitor is CX-3543.

Examples for Flt3 inhibitors are AC-220 (also an inhibitor of cKit and PDGFR), KW-2449, LS-104 (also an inhibitor of bcr-abl and Jak2), MC-2002, SB-1317, lestaurtinib (also an inhibitor of VEGFR, PDGFR, PKC), TG-101348 (also an

inhibitor of JAK2), XL-999 (also an inhibitor of cKit, FGFR, PDGFR and VEGFR), sunitinib (also an inhibitor of PDGFR, VEGFR and cKit), and tandutinib (also an inhibitor of PDGFR, and cKit).

Examples for HSP90 inhibitors are, tanespimycin, alvespimycin, IPI-504, STA-9090, MEDI-561, AUY-922, CNF-2024, and SNX-5422.

Examples for JAK/STAT inhibitors are CYT-997 (also interacting with tubulin), TG-101348 (also an inhibitor of Flt3), and XL-019.

Examples for Mek inhibitors are ARRY-142886, AS-703026, PD-325901, AZD-8330, ARRY-704, RDEA-119, and XL-518.

Examples for mTor inhibitors are temsirolimus, deforolimus (which also acts as a VEGF inhibitor), everolimus (a VEGF inhibitor in addition), XL-765 (also a PI3 kinase inhibitor), and BEZ-235 (also a PI3 kinase inhibitor).

Examples for Akt inhibitors are perifosine, GSK-690693, RX-0201, and triciribine.

Examples for cKit inhibitors are masitinib, OSI-930 (also acts as a VEGFR inhibitor), AC-220 (also an inhibitor of Flt3 and PDGFR), tandutinib (also an inhibitor of Flt3 and PDGFR), axitinib (also an inhibitor of VEGFR and PDGFR), sunitinib (also an inhibitor of Flt3, PDGFR, VEGFR), and XL-820 (also acts as a VEGFR- and PDGFR inhibitor), imatinib (also a bcr-abl inhibitor), nilotinib (also an inhibitor of bcr-abl and PDGFR).

Examples for hedgehog antagonists are IPI-609, CUR-61414, GDC-0449, IPI-926, and XL-139.

Examples for CDK inhibitors are seliciclib, AT-7519, P-276, ZK-CDK (also inhibiting VEGFR2 and PDGFR), PD-332991, R-547, SNS-032, PHA-690509, PHA-848125, and SCH-727965.

Examples for proteasome inhibitors are bortezomib, carfilzomib, and NPI-0052 (also an inhibitor of NFkappaB).

Examples for proteasome inhibitors/NFkappaB pathway inhibitors are bortezomib, carfilzomib, NPI-0052, CEP-18770, MLN-2238, PR-047, PR-957, AVE-8680, and SPC-839.

An example for an inhibitor of the ubiquitination pathway is HBX-41108.

Examples for demethylating agends are 5-azacitidine and decitabine.

Examples for anti-angiogenic agents are inhibitors of the FGFR, PDGFR and VEGF(R), and thalidomides, such agents being selected from, without limitation, bevacizumab, motesanib, CDP-791, SU-14813, telatinib, KRN-951, ZK-CDK (also an inhibitor of CDK), ABT-869, BMS-690514, RAF-265, IMC-KDR, IMC-18F1, IMiDs, thalidomide, CC-4047, lenalidomide, ENMD-0995, IMC-D11, Ki-23057, brivanib, cediranib, 1B3, CP-868596, IMC-3G3, R-1530 (also an inhibitor of Flt3), sunitinib (also an inhibitor of cKit and Flt3), axitinib (also an inhibitor of cKit), lestaurtinib (also an inhibitor of Flt3 and PKC), vatalanib, tandutinib (also an inhibitor of Flt3 and cKit), pazopanib, PF-337210, aflibercept, E-7080, CHIR-258, sorafenib tosylate (also an inhibitor of Raf), vandetanib, CP-547632, OSI-930, AEE-788 (also an inhibitor of EGFR and Her2), BAY-57-9352 (also an inhibitor of Raf), BAY-73-4506 (also an inhibitor of Raf), XL-880 (also an inhibitor of cMet), XL-647 (also an inhibitor of EGFR and EphB4), XL-820 (also an inhibitor of cKit), nilotinib (also an inhibitor of cKit and brc-abl), CYT-116, PTC-299, BMS-584622, CEP-11981, dovitinib, CY-2401401, ENMD-2976 and BIBF1120.

The additional therapeutic agent may also be selected from EGFR inhibitors, it may be a small molecule EGFR inhibitor or an anti-EGFR antibody. Examples for anti-EGFR antibodies, without limitation, are cetuximab, panitumumab, nimotuzumab, zalutumumab; examples for small molecule EGFR inhibitors are gefitinib,

erlotinib, vandetanib (also an inhibitor of the VEGFR) and afatinib (also an inhibitor of Her2). Another example for an EGFR modulator is the EGF fusion toxin.

Further EGFR and/or Her2 inhibitors useful for combination with an CD33 binding agent of the invention are lapatinib, trastuzumab, pertuzumab, XL-647, neratinib, BMS-599626 ARRY-334543, AV-412, mAB-806, BMS-690514, JNJ-26483327, AEE-788 (also an inhibitor of VEGFR), AZD-8931, ARRY-380 ARRY-333786, IMC-11F8, Zemab, TAK-285, AZD-4769, and afatinib (dual inhibitor of Her2 and EGFR).

DNA polymerase inhibitors useful in the combination with an CD33 binding agent of the invention are Ara-C/cytarabine, Clolar/ clofarabine.

A DNA methyltransferase inhibitor useful in the combination with an CD33 binding agent of the invention is Vidaza/azacitidine.

An apoptosis inducer useful in the combination with an CD33 binding agent of the invention is Trisenox/arsenice trioxide.

Topoisomerase II inhibitors useful in the combination with an CD33 binding agent of the invention are idarubicin, daunorubicin and mitoxantrone.

A RAR antagonist useful in the combination with an CD33 binding agent of the invention is Vesanoid/tretinoin.

A HGPRTase inhibitor useful in the combination with an CD33 binding agent of the invention is Mercapto/mercaptopurine.

A histamine H2 receptor antagonist useful in the combination with an CD33 binding agent of the invention is Ceplene/histamine dihydrochloride.

A CD25 receptor agonist useful in the combination with an CD33 binding agent of the invention is IL-2.

The additional drug may also be selected from agents that target the IGF-1R and insulin receptor pathways. Such agents include antibodies that bind to IGF-1R (e.g. CP-751871, AMG-479, IMC-A12, MK-0646, AVE-1642, R-1507, BIIB-022, SCH-717454, rhu Mab IGFR and novel chemical entities that target the kinase domain of the IGF1-R (e.g. OSI-906 or BMS-554417, XL-228, BMS-754807).

Other agents that may be advantageously combined in a therapy with the CD33 binding agent of the invention are molecules targeting CD20, including CD20 specific antibodies like rituximab, LY-2469298, ocrelizumab, MEDI-552, IMMU-106, GA-101 (= R7159), XmAb-0367, ofatumumab, radiolabbeled CD20 antibodies,like tositumumab and ibritumomab tiuxetan or other CD20 directed proteins, like the SMIP Tru015, PRO-131921, FBT-A05, veltuzumab, R-7159.

CD33 binding agents may be combined with inhibitors of other surface antigens expressed on leukocytes, in particular antibodies or antibody-like molecules, e.g. anti-CD2 (siplizumab), anti-CD4 (zanolimumab), anti-CD19 (MT-103, MDX-1342, SAR-3419, XmAb-5574), anti-CD22 (epratuzumab), anti-CD23 (lumiliximab), anti-CD30 (iratumumab), anti-CD32B (MGA-321), anti-CD38 (HuMax-CD38), anti-CD40 (SGN40), anti-CD52 (alemtuzumab), anti-CD80 (galiximab).

Other agents to be combined with CD33 binding agents are immunotoxins like BL-22 (an anti-CD22 immunotoxin), inotuzumab ozogamicin (an anti-CD23 antibody-calicheamicin conjugate), RFT5.dgA (anti-CD25 Ricin toxin A-chain), SGN-35 (an anti-CD30-auristatin E conjugate), and gemtuzumab ozogamicin (an anti-CD33 calicheamicin conjugate), MDX-1411 (anti-CD70 conjugate), or radiolabelled antibodies like ^{90}Y-epratuzumab (anti-CD22 radioimmunoconjugate).

In addition, CD33 binding agents may be combined with immunomodulators,

agents, e.g. antibodies, that induce apoptosis or modify signal transduction pathways like the TRAIL receptor modulators mapatumumab (a TRAIL-1 receptor agonist), lexatumumab (a TRAIL-2 receptor agonist), tigatuzumab, Apomab, AMG-951 and AMG-655; an anti-HLA-DR antibody (like 1D09C3), an anti-CD74, an osteoclast differentiation factor ligand inhibitor (like denosumab), a BAFF antagonist (like AMG-623a) or an agonist of a Toll-like receptor (e.g. TLR-4 or TLR-9).

Other drugs that may be used in combination with the CD33 binding agents of the present invention are selected from, but not limited to hormones, hormonal analogues and antihormonals (e.g. tamoxifen, toremifene, raloxifene, fulvestrant, megestrol acetate, flutamide, nilutamide, bicalutamide, cyproterone acetate, finasteride, buserelin acetate, fludrocortinsone, fluoxymesterone, medroxyprogesterone, hydroxyprogesterone caproate, diethylstilbestrol, testosterone propionate, fluoxymesterone/equivalents, octreotide, arzoxifene, pasireotide, vapreotide, adrenocorticosteroids/ antagonists, prednisone, dexamethasone, ainoglutethimide), aromatase inhibitors (e.g. anastrozole, letrozole, liarozole, exemestane, atamestane, formestane), LHRH agonists and antagonists (e.g. goserelin acetate, leuprolide, abarelix, cetrorelix, deslorelin, histrelin, triptorelin), antimetabolites (e.g. antifolates like methotrexate, trimetrexate, pemetrexed, pyrimidine analogues like 5-fluorouracil, fluorodeoxyuridine, capecitabine, decitabine, nelarabine, 5-azacytidine, and gemcitabine, purine and adenosine analogues such as mercaptopurine, thioguanine, azathioprine, cladribine and pentostatin, cytarabine, fludarabine, clofarabine); antitumor antibiotics (e.g. anthracyclines like doxorubicin, daunorubicin, epirubicin and idarubicin, mitomycin-C, bleomycin dactinomycin, plicamycin, splicamycin, actimomycin D, mitoxantrone, mitoxantroneidarubicin, pixantrone, streptozocin, aphidicolin); platinum derivatives (e.g. cisplatin, oxaliplatin, carboplatin, lobaplatin, satraplatin); alkylating agents (e.g. estramustine, semustine, mechlorethamine, melphalan, chlorambucil, busulphan, dacarbazine, cyclophosphamide, ifosfamide, hydroxyurea, temozolomide, nitrosoureas such as carmustine and lomustine, thiotepa); antimitotic agents (e.g. vinca alkaloids like vinblastine, vindesine,

vinorelbine, vinflunine and vincristine; and taxanes like paclitaxel, docetaxel and their formulations, larotaxel; simotaxel, and epothilones like ixabepilone, patupilone, ZK-EPO); topoisomerase inhibitors (e.g. epipodophyllotoxins like etoposide and etopophos, teniposide, amsacrine, topotecan, irinotecan, banoxantrone, camptothecin) and miscellaneous chemotherapeutics such as retinoic acid derivatives, amifostine, anagrelide, interferon alpha, interferon beta, interferon gamma, interleukin-2, procarbazine, N-methylhydrazine, mitotane, and porfimer, bexarotene, celecoxib, ethylenemine/methyl-melamine, thriethyienemelamine, triethylene thiophosphoramide, hexamethylmelamine, and enzymes L-asparaginase, L-arginase and metronidazole, misonidazole, desmethylmisonidazole, pimonidazole, etanidazole, nimorazole, RSU 1069, EO9, RB 6145, SR4233, nicotinamide, 5-bromodeozyuridine, 5-iododeoxyuridine, bromodeoxycytidine, erythrohydroxynonyl-adenine, anthracenedione, GRN-163L (a competitive telomerase template antagonist), SDX-101 (a PPAR agonist), talabostat (a DPP inhibitor), forodesine (a PNP inhibitor), atacicept (a soluble receptor targeting TNF family members BLyS and APRIL), TNF-alpha neutralizing agents (Enbrel, Humira, Remicade), XL-844 (a CHK1/2 inhibitor), VNP-40101M (a DNA alkylating agent), SPC-2996 (an antisense bcl2 inhibitor), obatoclax (a bcl2 inhibitor), enzastaurin (a PKC beta modulator), vorinistat (an HDAC inhibitor), romidepsin (an HDAC inhibitor), AT-101 (a Bcl-2/Bcl-xL inhibitor), plitidepsin (a multi-actioned depsipeptide), SL-11047 (a polyamine metabolism modulators).

The CD33 binding agents of the invention may also be used in combination with other therapies including surgery, stem cell transplantation, radiotherapy, endocrine therapy, biologic response modifiers, hyperthermia and cryotherapy and agents to attenuate any adverse effect (e.g. antiemetics), G-CSF, GM-CSF, photosensitizers such as hematoporphyrin derivatives, Photofrin, benzoporphyrin derivatives, Npe6, tin etioporphyrin, pheoboride-a bacteriochlorophyll-a, naphthalocyanines, phthalocyanines, zinc phthalocyanines.

Pharmaceutical Compositions and Methods of Administration

The CD33 binding agents can be in any form that allows for the composition to be administered to a patient. For example, the composition can be in the form of a solid or liquid. The preferred mode of application is parenteral, by infusion or injection (intraveneous, intramuscular, subcutaneous, intraperitoneal, intradermal), but other modes of application such as by inhalation, transdermal, intranasal, buccal, oral and intra- tumor may also be applicable. Parenteral administration includes subcutaneous injections, intravenous, intramuscular, intrastemal injection or infusion techniques. In one aspect, the compositions are administered parenterally. In yet another aspect, the compounds are administered intravenously.

Pharmaceutical compositions can be formulated so as to allow a compound to be bioavailable upon administration of the composition to a patient. Compositions can take the form of one or more dosage units, where, for example, a container of a compound in aerosol form can hold a plurality of dosage units.

Materials used in preparing the pharmaceutical compositions can be non-toxic in the amounts used. It will be evident to those of ordinary skill in the art that the optimal dosage of the active ingredient(s) in the pharmaceutical composition will depend on a variety of factors. Relevant factors include, without limitation, the type of patient (e.g., human), the particular form of the compound, the manner of administration, and the composition employed.

The pharmaceutically acceptable carrier or vehicle can be particulate, so that the compositions are, for example, in powder form. The carrier(s) can be liquid, with the compositions being, for example, an injectable liquid. The composition can be in the form of a liquid, e.g., for parenteral injection. In a composition for administration by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent can also be included.

The liquid compositions, whether they are solutions, suspensions or other like form, can also include one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono- or digylcerides which can serve as the solvent or suspending medium, polyethylene glycols, glycerin, cyclodextrin, propylene glycol or other solvents; stabilizers such as amino acids; surfactants such as polysorbates; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacelic acid; buffers such as acetates, citrates or phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose. A parenteral composition can be enclosed in ampoule, a disposable syringe or a multiple-dose vial made of glass, plastic or other material. Physiological saline is an exemplary adjuvant. An injectable composition is preferably sterile.

The CD33 binding agents may also be dried (freeze-dried, spray-dried, spray-freeze dried, dried by near or supercritical gases, vacuum dried, air-dried), precipitated or crystallized or entrapped in microcapsules that are prepared, for example, by coacervation techniques or by interfacial polymerization using, for example, hydroxymethylcellulose or gelatin and poly-(methylmethacylate), respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), in macroemulsions or precipitated or immobilized onto carriers or surfaces, for example by pcmc technology (protein coated microcrystals). Such techniques are disclosed in Remington: The Science and Practice of Pharmacy, 21st edition, Hendrickson R. Ed.

The amount of the composition that is effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, in vitro or in vivo assays can optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the compositions will also depend on the route of

administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances.

The compositions comprise an effective amount of a drug(s) or agent(s) such that a suitable dosage will be obtained. Typically, this amount is at least about 0.01% of a drug or agent by weight of the composition. When intended for oral administration, this amount can be varied to range from about 0.1% to about 80% by weight of the composition. In one aspect, oral compositions can comprise from about 4% to about 50% of the compound by weight of the composition. In yet another aspect, present compositions are prepared so that a parenteral dosage unit contains from about 0.01% to about 2% by weight of the compound.

For intravenous administration, the composition can comprise from about 1 to about 50 mg of a drug or agent per kg of the patient's body weight. In one aspect, the composition can include from about I, 1.5 or 2.5 to about 50 mg of a drug or agent per kg of the patient's body weight. In another aspect, the amount administered will be in the range from about 1, 1.5 or 2.5 to about 25 mg/kg of body weight of a drug or agent.

In some embodiments, the dosage administered to a patient is less than 0.1 mg/kg to about 50 mg/kg of the patient's body weight. (For conversion to mg/mm^2, a BSA of 1.8 m^2 and a body weight of 80 kg can be used.)

As discussed herein, a CD33 binding agent can be administered intravenously or subcutaneously to the patient on a schedule that is, for example, daily, weekly, biweekly, tri-weekly or monthly to the patient. For example, a CD33 binding agent can be administered weekly, for a period of 2 to 10 weeks, typically 3-6 weeks. In some embodiments, the dosage regimen of the CD33 binding agent maintains a blood serum concentration of antibody at least 5 μg/ml or at least 10 μg/ml during the dosage cycle. The CD33 binding agent can be administered, for example, from 1-8, or more cycles. In some embodiments, a CD33 binding agent is

administered chronically to a subject.

By way of example, the invention includes a method of treating a cancer, such as myeloid leukemia, by administering 0.1 mg/kg to 50 mg/kg, for instance about 1.5-8 or 2.5-8 mg/kg, of an anti-CD33 antibody according to the invention weekly. This treatment can be usually be continued for about 1-3 months, typically about two months. In an embodiment, the dosing schedule is maintained until a reduction in blasts is noted. For example, dosing can be continued up to about 6 months. This treatment can be followed by a less frequent dosing schedule, involving for instance biweekly doses (or twice per month). This dosing schedule can be maintained 1, 2, 3, 4, 5, 6 months or more to maintain a reduction in blasts and/or a remission.

In some embodiments, a prophylactic agent can be administered with a CD33 binding agent to minimize infusion reactions. Suitable prophylactic agents include, for example, methyl prednisolone, diphenyldramine, acetaminophen or other suitable agent. The prophylactic agent can be administered prior to or at about the same time as the CD33 binding agent.

The drug(s) or agent(s) or compositions can be administered by any convenient route, for example, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.). Administration can be systemic or local. Various delivery systems are known, e.g., encapsulation in liposomes, microparticles, microcapsules, capsules, etc., and can be used to administer a compound. In certain embodiments, more than one drug or agent or composition is administered to a patient.

It can be desirable to administer one or more drugs or agents or compositions locally to the area in need of treatment, as appropriate for the drug or agent. This can be achieved, for example, and not by way of limitation, by local infusion during surgery; topical application, e.g., in conjunction with a wound dressing after surgery; by injection: by means of a catheter; by means of a suppository; or by

means of an implant, the implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of a cancer, tumor or neoplastic or pre-neoplastic tissue.

The drug(s) or agent(s) or compositions can be delivered in a controlled release system, such as a pump or various polymeric materials. In yet another embodiment, a controlled-release system can be placed in proximity of the target of the drug(s) or agent(s) or compositions, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, vol. 2, pp. 115-138, 1984). Other controlled- release systems discussed in the review by Langer (1990, Science 249: 1527-1533) can be used.

The drugs or agents are formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to animals, particularly human beings, as appropriate for the drug or agent. Typically, the carriers or vehicles for intravenous administration are sterile isotonic aqueous buffer solutions. Where necessary, the compositions can also include a solubilizing agent. Compositions for intravenous administration can optionally comprise a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water-free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where drug or agent is to be administered by infusion, it can be dispensed, for example, with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the drug or agent is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients can be mixed prior to administration.

Compositions of therapeutic agents also can be administered according to accepted dosage forms in the form of tablets, lozenges, aqueous or oily suspensions, granules, powders, emulsions, capsules, syrups, or elixirs, for example. Orally

administered compositions can contain one or more optional agents, for example, sweetening agents such as fructose, aspartame or saccharin; flavoring agents such as peppermint, oil of wintergreen, or cherry; coloring agents; and preserving agents, to provide a pharmaceutically palatable preparation. Moreover, where in tablet or pill form, the compositions can be coated to delay disintegration and absorption in the gastrointestinal tract thereby providing a sustained action over an extended period of time. Selectively permeable membranes surrounding an osmotically active driving compound are also suitable for orally administered drugs or agents. In these later platforms, fluid from the environment surrounding the capsule is imbibed by the driving compound, which swells to displace the agent or agent composition through an aperture. These delivery platforms can provide an essentially zero order delivery profile as opposed to the spiked profiles of immediate release formulations. A time-delay material such as glycerol monostearate or glycerol stearate can also be used.

The composition can include various materials that modify the physical form of a solid or liquid dosage unit. For example, the composition can include materials that form a coating shell around the active ingredients. The materials that form the coating shell are typically inert, and can be selected from, for example, sugar, shellac, and other enteric coating agents. Alternatively, the active ingredients can be encased in a gelatin capsule.

The compositions can be administered to a patient in need thereof at a frequency, or over a period of time, that is determined by the attending physician. The compositions can be administered over a period of 1 day, 2 days, 3 days, 5 days, 7 days, 10 days, 14 days, 21 days, 28 days, one month, two months, or longer periods of time. It is understood that the compositions can be administered for any period of time between 1 day and two months or longer.

Production of Antibodies

Antibodies can be produced using any method useful for the synthesis of antibodies, in particular, such as by recombinant expression or chemical synthesis.

Recombinant expression of antibodies, or fragments or derivatives thereof, typically involves construction of a nucleic acid that encodes the antibody. If the nucleotide sequence of the antibody is known, a nucleic acid encoding the antibody or a polypeptide thereof maybe assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier at al., 1994, BioTechniques 17: 242), which involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides, e.g., by PCR.

Alternatively, a nucleic acid molecule encoding an antibody or a polypeptide thereof can be generated from a suitable source. If a clone containing the nucleic acid encoding the particular antibody is not available, but the sequence of the antibody is known, a nucleic acid encoding the antibody can be obtained from a suitable source (e.g., an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the immunoglobulin) by, e.g., PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence.

If an antibody that specifically recognizes a particular antigen is not commercially available (or a source for a cDNA library for cloning a nucleic acid encoding such an immunoglobulin is not available), antibodies specific for a particular antigen can be generated by any method known in the art, for example, by immunizing a patient, or suitable animal model such as a rabbit or mouse, to generate polyclonal antibodies or, more preferably, by generating monoclonal antibodies, e.g., as described by Kohler and Milstein (1975, Nature 256:495-497) or, as described by Kozbor et al. (1983, Immunology Today 4:72) or Cole et al. (1985, in Monoclonal

Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Alternatively, a clone encoding at least the Fab portion of the antibody can be obtained by screening Fab expression libraries (e.g., as described in Huse et al., 1989, Science 246, 1275-1281) for clones of Fab fragments that bind the specific antigen or by screening antibody libraries (see, e.g., Clackson et al, 1991, Nature 352:624; Hane et al, 1997, Proc. Natl. Acad. Sci. USA 94:4937).

Once a nucleic acid sequence encoding at least the variable domain of the antibody is obtained, it can be introduced into a vector containing the nucleotide sequence encoding the constant regions of the antibody (see, e.g., International Publication No. WO 86/05807; WO 89/01036; and U.S. Patent No. 5,122,464). Vectors containing the complete light or heavy chain that allow for the expression of a complete antibody molecule are available. Then, the nucleic acid encoding the antibody can be used to introduce the nucleotide substitution(s) or deletion(s) necessary to substitute (or delete) the one or more variable region cysteine residues participating in an intrachain disulfide bond with an amino acid residue that does not contain a sulfhydyl group. Such modifications can be carried out by any method known in the art for the introduction of specific mutations or deletions in a nucleotide sequence, for example, but not limited to, chemical mutagenesis and in vitro site directed mutagenesis (see, e.g., Hutchinson et al, 1978, J. Biol. Chem. 253:6551).

In addition, techniques have been developed for the production of "chimeric antibodies" (see, e.g., Morrison et al., 1984, Proc. Natl. Acad. Sci. USA 81: 851-855; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454). A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region, e.g., humanized antibodies.

Once a nucleic acid sequence encoding an antibody has been obtained, the vector for the production of the antibody can be produced by recombinant DNA

technology using techniques known in the art. Methods that are known to those skilled in the art can be used to construct expression vectors containing the antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. See, for example, the techniques described in Sambrook et al (1990, Molecular Cloning, A Laboratory Manual. 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.; and Sambrook et al., 2001; Molecular Cloning, A Laboratory Manual, 3rd Ed., Cold Spring Harbor Publish., Cold Spring Harbor, N.Y.) and Ausubel et al. (eds., 1993-2006, Current Protocols in Molecular Biology, John Wiley & Sons, N.Y.).

An expression vector comprising the nucleotide sequence of an antibody or the nucleotide sequence of an antibody can be transferred to a host cell by conventional techniques (e.g., electroporation, liposomal transaction, calcium phosphate precipitation or transduction), and the resulting cells are then cultured by conventional techniques to produce the antibody. In specific embodiments, the expression of the antibody is regulated by a constitutive, an inducible or a tissue-specific promoter.

The host cells used to express the recombinant antibody can be either bacterial cells such as Escherichia coli, or, preferably, eukaryotic cells, especially for the expression of recombinant immunoglobulin molecules. In particular, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector containing the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for immunoglobulins (see, e.g., Foecking et al., 1986, Gene 45:101; Cockett el al., 1990, BioTechnology 8:2). The CHO cell line can be, for example, DG44 or CHO-S. In another example, an antibody can be expressed using the CHEF system. (See, e.g., U.S. Patent No. 5,888,809.)

A variety of other host-expression vector systems can be utilized to express antibodies. Such host-expression systems represent vehicles by which the coding sequences of the antibody can be produced and subsequently purified, but also represent cells that can, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody immunoglobulin molecule in situ. These include, but are not limited to, microorganisms such as bacteria (e.g., *E. coli* and B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing immunoglobulin coding sequences; yeast (e.g., Saccharomyces pichia) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the immunoglobulin coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g. , cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV)) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, CHO-S, BH, 293, 293T or 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors can be advantageously selected depending upon the use intended for the antibody being expressed. For example, when a large quantity of such a protein is to be produced, vectors that direct the expression of high levels of fusion protein products that are readily purified might be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al, 1983, EMBO J. 2:1791-94), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye and Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke and Schuster, 1989, J. Biol. Chem. 24:5503-5509); and the like. pGEX vectors can also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from

lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) or the analogous virus from Drosophila melanogaster can be used as a vector to express foreign genes. The virus grows in Spodopiera frugipenta cells. The antibody coding sequence can be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems can be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest can be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene can then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) results in a recombinant virus that is viable and capable of expressing the immunoglobulin molecule in infected hosts. (See, e.g., Logan and Shenk, 1984, Proc. Natl. Acad. Sci. USA 81:355-359). Specific initiation signals can also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon is operably related to the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see, e.g., Bittner et al., 1987, Methods in Enzymol. 153:51-544).

In addition, a host cell strain can be chosen to modulate the expression of the inserted sequences, or modify and process the gene product in the specific fashion

desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products can be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-lranslational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells that possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product can be used. Such mammalian host cells include, but are not limited to, CHO (e.g., DG44 or CHO-S), VERY, BH, HeIa, COS, MDCK, 293, 293T, 3T3, WI38, BT483, Hs578T, HTB2, BT20 and T47D, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines that stably express an antibody can be engineered. Rather than using expression vectors that contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells can be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci that in turn can be cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines which express the antibody. Such engineered cell lines can be particularly useful in screening and evaluation of tumor antigens that interact directly or indirectly with the antibody.

A number of selection systems can be used. For example, the herpes simplex virus thymidine kinase (see, e.g., Wigler el al., 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (see, e.g., Szybalska and Szybalski, 1992, Proc. Natl Acad. Sci. USA 48:202), and adenine phosphoribosyltransferase (see, e.g., Lowy et al., 1980, Cell 22:817) genes can be employed in tk-, hgprt- or aprt- cells,

respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: DHFR, which confers resistance to methotrexate (see e.g., Wigler el al., 1980, Proc. Natl. Acad Sci. USA 77:3567-70; O'Hare et al., 1981, Proc. Natl. Acad. Sci. USA 78: 1527-31); gpt, which confers resistance to mycophenolic acid (see, e.g., Mulligan and Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072-76); neo, which confers resistance to the aminoglycoside G-418 (see, e.g., Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Arm. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932; Morgan and Anderson, 1993, Ann. Rev. Biochem. 62: 191-217; and May, 1993, TIB TECH 11(5): 155- 215) and hygro, which confers resistance to hygromycin (see, e.g., Santerre et al., 1984, Gene 30:147-50). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds., 1993-2006, Current Protocols in Molecular Biology. John Wiley & Sons, NY; Kriegler, 1990, Gene Transfer and Expression. A laboratory Manual, Stockton Press, NY; and in Chapters 12 and 13, Dracopoli et al. (eds., 1994, Current Protocols in Human Genetics, John Wiley & Sons, NY; and Colberre-Garapin et al, 1981, 7. MoI. Biol. 150:1-14).

The expression levels of an antibody can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol. 3., Academic Press, New York, 1987). When a marker in the vector system expressing an antibody is amplifiable, an increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the nucleotide sequence of the antibody, production of the antibody will also increase (see, e.g., Crouse et al., 1983, Mol. Cell. Biol. 3:257-66).

The host cell can be co-transfected with two expression vectors, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors can contain the same or different selectable markers that enable equal expression of heavy and light chain

polypeptides. Alternatively, a single vector can be used to encode both heavy and light chain polypeptides. In such situations, the light chain is typically placed before the heavy chain to avoid an excess of toxic free heavy chain (see, e.g., Proudfoot, 1986, Nature 322:562-65; Kohler, 1980, Proc. Natl. Acad. Sci. USA 77:2197-9). The coding sequences for the heavy and light chains can comprise cDNA or genomic DNA.

Once the antibody has been recombinantly expressed, it can be purified using any suitable method for purification of an antibody, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

A comprehensive reference for all steps used for making the monoclonal antibodies according to the present invention is Yokoyama et al., "Production of Monoclonal Antibodies", Current Protocols in Immunology, Unit 2.5, 2006.

Examples

Example 1: Affinitity to CD33

The CD33 binding agents have a K_D to both human and cynomolgus CD33 of Affinities of 10 nM or less on the cell line HL60 and HEK293-cynomolgus CD33, respectively.

Fourteen CD33 binding agents (fully human monoclonal antibodies as listed in table 1 as No. 1-14, respectively) to human and cynomolgus CD33 were determined by FACS Scatchard analysis as described in Brockhoff et al., (Cytometry. 1994 Sep 1;17(1):75-83) on CD33 expressing cells (AML-derived HL60 cell line, recombinant HEK293-cynomolgusCD33 cell line). Briefly, dilutions of a CD33 binding agent were prepared in a 96 well plate starting with 100-400 nM in the first well (80 µl), followed by 11 dilution steps (1:2, 40 +

40 µl). 50 µl of CD33 binding agent dilutions are added to FACS tubes, 150 µl cells ($0,8x10^6$/ ml=$1,2x10^5$ cells/tube) are added to each FACS tube. Cells were gently mixed and incubated for 1 h on ice. Thereafter 50 µl FITC conjugated secondary antibody (concentration 15 µg/ml; mouse mAb anti-human IgG) was added, mixed, and incubated for 30 min. on ice. 4 ml PBS ph7.2 containing 0.02% acid were added thereafter, cells were pelleted and resuspended in 300 µl PBS pH 7.2 and subjected to FACS analysis using a BD FACS Canto. All experimental steps were performed on wet ice, all CD33 binding agent dilutions were made in PBS/ 0,5% BSA + 0.02% acid. FACS calibration was performed using Quantum FITC MESF (Premix) Beads (Bangs Laboratories). All samples were measured using the same FACS parameters. The ratio of bound IgG versus free IgG was calculated from MFI values at different CD33 binding agent concentrations and displayed as Scatchard blot. A regression line was drawn through the resulting data points, the slope of this line corresponds to the negative value of the association constant. The results are listed in table 2.

No	Clone ID #	Human CD33 K_D (nM)	Cynomolgus CD33 K_D (nM)
1	280-03-08	0.3	0.03
2	280-21-09	0.4	0.3
3	280-29-12	0.5	0.6
4	280-31-01	0.4	0.3
5	280-31-01(mut)	1	0.5
6	280-34-02	0.3	0.5
7	280-50-01	0.3	0.5
8	280-50-01(mut)	0.4	1.7
9	280-61-07	0.3	0.4
10	283-03-03	0.3	1.9
11	283-05-01	0.2	1.1
12	283-07-03	0.3	2.1
13	283-11-03	0.3	0.6
14	283-14-01	3.2	2.3

Table 2

Example 2: Internalization kinetics

Internalization of antibody refers to the reduction of the amount of antibody/ antigen complexes on the cell surface of the target cell after incubation with antibody. Internalization tests were performed with the CD33 expressing HL60 cell line. Cells were incubated with a fixed amount of a CD33 binding agent (10 µg/ml of fully human monoclonal antibodies as listed in table 1 as No. 1-14) for defined time periods (0h, 1h, 4h, 24h) at 37° C to allow internalization of the antibody/antigen complex. At the indicated time points acid was added to the incubation mix to prevent further internalization. Thereafter a fixed amount of CD33 binding agent was added to saturate all CD33 antigen sites on the cell surface. The total amount of CD33 binding agent bound to the cell surface was determined by FACS analysis utilizing a FITC conjugated anti-human IgG secondary antibody. The time point 0h was used to determine the initial level of CD33 antibody/antigen complexes on the surface and was defined as 100%. The results are listed in table 3 and illustrated in Figures 1-3.

No	Clone ID #	Remaining CD33 antibody/antigen complexes on the cells surface after 4h incubation with antibody (%)
1	280-03-08	49
2	280-21-09	41
3	280-29-12	49
4	280-31-01	44
5	280-31-01(mut)	52
6	280-34-02	44
7	280-50-01	53
8	280-50-01(mut)	55
9	280-61-07	47
10	283-03-03	45
11	283-05-01	51
12	283-07-03	48
13	283-11-03	46
14	283-14-01	47

Table 3

Lintuzumab was included as a reference antibody. Lintuzumab/CD33 complexes rapidly internalized upon binding of lintuzumab which was in accordance to published data. After a 4h-incubation period only about 20% of the initial amount of CD33/lintuzumab complexes was left on the cell surface. It could be demonstrated that, unexpectedly, internalization of all 14 CD33 binding agents according to the present invention was decelerated compared to lintuzumab.

Example 3: ADCC activity

Decelerated internalization rate translates into increased ADCC activity *in vitro*. To assess the effect of decelerated internalization on the ADCC activity of the CD33 binding agents (fully human monoclonal antibodies as listed in table 1 as No. 1-14), target cells (HL60) were incubated with a CD33 binding agent for the 0, 1, 4 and 24h. Subsequently an ADCC assay was performed with IL-2 stimulated PBMC as effector cells and antibody coated HL60 cells as target cells. For all experiments a mAb concentration of 30 µg/ml was used. The co-cultivation of effector cells with target cells in presence of CD33 binding agent was performed in quadruplicates or triplicates in 96-well round-bottom microtiter plates in a final volume of 200 µl assay medium per well consisting of 10% human serum and 1% BSA in RPMI in 1:1 ratio. First effector cells (freshly isolated PBMC cells in 100 µl 10% human serum in RPMI per well) were plated, followed by target cells and CD33 binding agent solution (diluted in 50 µl 1% BSA in RPMI). As a control, effector cells were cultivated in assay medium alone (effector cell control) and target cells were cultivated either in assay medium alone (spontaneous lysis) or in assay medium supplemented with 1% Triton X-100 (maximal lysis). The co-culture was incubated at 37°C in a humid CO_2 incubator for 3 hours. At the end of the incubation cells were removed from the culture medium by centrifugation (200 x g, i.e. 1000 rpm; 10 min) at room temperature. Cell free supernatants (100 µl/well) are transferred into corresponding wells of a 96-well flat-bottom plate. To determine the LDH activity in these supernatants 100 µl reaction mixture (freshly mixed 250µl catalyst with 11.25 ml dye solution) are added to each well and incubated 30 min at room temperature in the dark. Then the absorbance is measured as described below.

Cytotoxicity Detection Kit (LDH; Roche 11 644 793 001) was used to measure ADCC activity. The detection of cytotoxicity is based on the measurement of LDH enzyme activity released from plasma membrane-damaged cells. LDH released into the culture supernatants reduces the tetrazolium salt from the kit to formazan. The absorption maximum of formazan dye is measured at 490 nm against a reference wavelength of 650 nm in an ELISA plate reader. To determine the percentage cell mediated cytotoxicity the average absorbance of the quadruplicates or triplicates was calculated and the background was subtracted. These corrected values were substituted into the following equation to calculate ADCC (%):
(effector/target cell mix-effector cell control-spontaneous release) divided by (maximal release-spontaneous release)

ADCC activity at timepoint 0h (no antibody pre-incubation of target cells) was defined as 100% ADCC activity. ADCC activity for various timepoints of antibody pre-incubation was calculated relative to timepoint 0h and displayed as relative cytotoxicity (%).

Decelerated internalization of the CD33 binding agents compared to lintuzumab resulted in increased ADCC activity compared to lintuzumab. In conclusion, decelerated internalization leads to increased ADCC activity. Internalization for the described CD33 binding agents is inversely correlated to ADCC activity of the CD33 binding agents which is indicative for advantages with respect to clinical activity of such CD33 binding agents. The results are illustrated in Figure 4 for the internalization kinetics in this experiment and in Figure 5 for the ADCC activity.

Example 4: Epitope mapping

The binding epitope of CD33 binding agents described herein relative to the epitope of lintuzumab was determined by Hydrogen Exchange Mass Spectrometry (HXMS).

This method determined the susceptibility of the amide backbone hydrogens of CD33 protein to exchange with D_2O. The experiments were conducted with recombinant CD33 protein alone and CD33 protein with added CD33 binding agent / lintuzumab (in the following in this example referred to as "antibody / antibodies"). Regions of the CD33 protein showing significant protection from

exchange due to binding of antibody were thus identified. Resolution of the method is determined by the peptides produced by digestion with pepsin, e.g. the resulting amino acid sequence may be larger than the actual epitope of the antibody. These CD33 derived peptides were identified by additional control experiments with unexchanged samples employing standard accurate mass and HPLC MS/MS technologies.

For the protein + antibody sample, CD33 protein and antibody were incubated for 15 minutes at room temperature. The final molar ratio antibody/CD33 was 2:1. Using a LEAP robot system (exchange plate kept at 25°C, sample/quench plate kept at 4°C), 8µl of sample was added to 80 µl of exchange buffer (10mM NaH_2PO_4 in D_2O, pH=7 or 10mM NaH_2PO_4 in H_2O, pH=7), mixed, and allowed to exchange for various times (15, 60, 120, 240, and 600 seconds) at 25°C. 80µl of this solution was then transferred to 80µl of quench buffer (1M urea, 0.1M TCEP-HCl) at 4°C and mixed. 90µl of this solution was then transferred to 10µl of pepsin (4 mg/ml) at 4°C and mixed. After 2 minutes, 60µl of this solution was injected onto a Michrom C18 trap cartridge. The cartridge was washed with H_2O + 0.1%TFA for 2 minutes at 100µl/min. A valve was then switched and the cartridge eluted onto a Phenomenex Jupiter C5 column, 1.0 x 50mm, 5µm, 300A. Mobile Phase A was water/acetonitrile/TFA (99/0.95/0.05) and Mobile Phase B was acetonitrile/water/TFA (95/4.95/0.05). Flow rate was 100µl/min. Gradient was: 0 minutes (0%B), 6 minutes (40%B), 7 minutes (40%B), 8 minutes (90%B), 10 minutes (90% B), 11 minutes (0%B). The LEAP system precools the mobile phase to 4°C and also maintains the trap column and analytical column at 4°C. For the MS experiments (used to quantitate exchange with the D_2O buffer), a single scan method from 300-2000 for 14 minutes was used at resolution 60,000. For the MS/MS experiments (used to ID peptides with the H_2O exchange buffer), a method with 7 scans was used for 14 minutes. The first scan was a full range scan from 300-2000 at 30,000 resolution. Subsequent scans were CID scans of the 6 most intense ions from scan #1. Isolation width was 1.5amu, collision energy was 35V, activation time was 30msec. Pepsin peptides were identified using fragmentation data and the program Proteome Discoverer (Thermo). Identified peptides were analyzed using an in-house program which calculates the average mass for exchanged peptides.

All CD33 binding agents protected the identical peptide fragment with the amino acid sequence FFHPIPYYDKNSPVHGYW (SeqID No: 141) (Table 4). The sequence of CD33 protected by the CD33 binding agents described herein are different and non-overlapping to the peptide sequence of CD33 protected by binding of Lintuzumab (MDPNFWLQVQE, SeqID No: 142). In silico modeling using the crystal structure of SIGLEC-5, a SIGLEC family member homologous to CD33, revealed binding epitopes of all antibodies in the proximal domain of the protein, where the binding epitope of Lintuzumab is different to those of the CD33 binding agents described herein. In conclusion, the CD33 binding agents described in this patent application bind to a different epitope than Lintuzumab.

No	Clone ID #	CD33 Epitope
1	280-03-08	FFHPIPYYDKNSPVHGYW
2	280-21-09	FFHPIPYYDKNSPVHGYW
3	280-29-12	FFHPIPYYDKNSPVHGYW
4	280-31-01	FFHPIPYYDKNSPVHGYW
5	280-31-01 (mut)	FFHPIPYYDKNSPVHGYW
6	280-34-02	FFHPIPYYDKNSPVHGYW
7	280-50-01	FFHPIPYYDKNSPVHGYW
8	280-50-01 (mut)	FFHPIPYYDKNSPVHGYW
9	280-61-07	FFHPIPYYDKNSPVHGYW
10	283-03-03	FFHPIPYYDKNSPVHGYW
11	283-05-01	FFHPIPYYDKNSPVHGYW
12	283-07-03	FFHPIPYYDKNSPVHGYW
13	283-11-03	FFHPIPYYDKNSPVHGYW
14	283-14-01	FFHPIPYYDKNSPVHGYW
15	Lintuzumab	MDPNFWLQVQE

Table 4

Claims

1. A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by

a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from SeqID No:1-14, CDR2 has an amino acid sequence selected from SeqID No:15-28, CDR3 has an amino acid sequence selected from SeqID No:29-42, CDR4 has an amino acid sequence selected from SeqID No:43-56, CDR5 has an amino acid sequence selected from SeqID No:57-70, CDR6 has an amino acid sequence selected from SeqID No:71-84, or

b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (SeqID No: 141) of human CD33.

2. A CD33 binding agent according to claim 1, wherein the epitope is determined by Hydrogen Exchange Mass Spectroscopy.

3. A CD33 binding agent according to any of the preceding claims, wherein the internalization kinetics of the CD33 binding agent are such that at least 30% of the initial amount of the CD33 binding agent remains on the cell surface of HL60 cells at a time of 4 hours after incubation.

4. A CD33 binding agent according to to any of the preceding claims, wherein the internalization kinetics of the CD33 binding agents are such that at least 40% of the initial amount of the CD33 binding agents remain on the cell surface at a time of 4 hours after incubation.

5. A CD33 binding agent according to any of the preceding claims, wherein the heavy chain variable region comprises an amino acid sequence selected from SeqID No:85-98 and the light chain variable region comprises an

amino acid sequence selected from SeqID No:99-112.

6. A CD33 binding agent according to any of the preceding claims, wherein the CD33 binding agent has a heavy chain having an amino acid sequence selected from SeqID No: 113-126 and a light chain having an amino acid sequence selected from SeqID No: 127-140.

7. A CD33 binding agent according to any of the preceding claims, wherein the CD33 binding agent has an affinity to both human CD33 and cynomolgus CD33 with a K_D of equal to or less than 10 nM.

8. A CD33 binding agent according to any of the preceding claims, wherein the CD33 binding agent is humanized.

9. A CD33 binding agent according to any of the preceding claims, wherein the CD33 binding agent is fully human.

10. A CD33 binding agent according to any of the preceding claims, wherein the CD33 binding agent further comprises an effector function.

11. A CD33 binding agent according to claim 10, wherein the effector function is mediated by an F_c domain.

12. A CD33 binding agent according to claim 10, wherein the CD33 binding agent comprises one or more mutations in the F_c domain that modulate the function of the F_c domain.

13. A CD33 binding agent according to claim 12, wherein the modulation of the function of the Fc domain is an increase of ADCC by at least 10%, preferably 50% and more preferably 100%.

14. A CD33 binding agent according to any of claim 12-13, wherein the mutations in the F_c domain are located at one or more positions selected from amino acids at positions 332 and/or 239 and/or 236 according to the Kabat EU numbering index.

15. A CD33 binding agent according to any of claim 12-14, wherein the mutations in the F_c domain are a combination of substitutions at positions 239 and 332, preferably S239D/I332E.

16. A CD33 binding agent selected from
an antibody comprising a CDR1 of SeqID No: 1, a CDR2 of SeqID No: 15, a CDR3 of SeqID No: 29, a CDR4 of SeqID No: 43, a CDR5 of SeqID No: 57 and a CDR6 of SeqID No: 71,
an antibody comprising a CDR1 of SeqID No: 2, a CDR2 of SeqID No: 16, a CDR3 of SeqID No: 30, a CDR4 of SeqID No: 44, a CDR5 of SeqID No: 58 and a CDR6 of SeqID No: 72,
an antibody comprising a CDR1 of SeqID No: 3, a CDR2 of SeqID No: 17, a CDR3 of SeqID No: 31, a CDR4 of SeqID No: 45, a CDR5 of SeqID No: 59 and a CDR6 of SeqID No: 73,
an antibody comprising a CDR1 of SeqID No: 4, a CDR2 of SeqID No: 18, a CDR3 of SeqID No: 32, a CDR4 of SeqID No: 46, a CDR5 of SeqID No: 60 and a CDR6 of SeqID No: 74,
an antibody comprising a CDR1 of SeqID No: 5, a CDR2 of SeqID No: 19, a CDR3 of SeqID No: 33, a CDR4 of SeqID No: 47, a CDR5 of SeqID No: 61 and a CDR6 of SeqID No: 75,
an antibody comprising a CDR1 of SeqID No: 6, a CDR2 of SeqID No: 20, a CDR3 of SeqID No: 34, a CDR4 of SeqID No: 48, a CDR5 of SeqID No: 62 and a CDR6 of SeqID No: 76,
an antibody comprising a CDR1 of SeqID No: 7, a CDR2 of SeqID No: 21, a CDR3 of SeqID No: 35, a CDR4 of SeqID No: 49, a CDR5 of SeqID No: 63 and a CDR6 of SeqID No: 77,
an antibody comprising a CDR1 of SeqID No: 8, a CDR2 of SeqID No: 22,

a CDR3 of SeqID No: 36, a CDR4 of SeqID No: 50, a CDR5 of SeqID No: 64 and a CDR6 of SeqID No: 78,
an antibody comprising a CDR1 of SeqID No: 9, a CDR2 of SeqID No: 23, a CDR3 of SeqID No: 37, a CDR4 of SeqID No: 51, a CDR5 of SeqID No: 65 and a CDR6 of SeqID No: 79,
an antibody comprising a CDR1 of SeqID No: 10, a CDR2 of SeqID No: 24, a CDR3 of SeqID No: 38, a CDR4 of SeqID No: 52, a CDR5 of SeqID No: 66 and a CDR6 of SeqID No: 80,
an antibody comprising a CDR1 of SeqID No: 11, a CDR2 of SeqID No: 25, a CDR3 of SeqID No: 39, a CDR4 of SeqID No: 53, a CDR5 of SeqID No: 67 and a CDR6 of SeqID No: 81,
an antibody comprising a CDR1 of SeqID No: 12, a CDR2 of SeqID No: 26, a CDR3 of SeqID No: 40, a CDR4 of SeqID No: 54, a CDR5 of SeqID No: 68 and a CDR6 of SeqID No: 82,
an antibody comprising a CDR1 of SeqID No: 13, a CDR2 of SeqID No: 27, a CDR3 of SeqID No: 41, a CDR4 of SeqID No: 55, a CDR5 of SeqID No: 69 and a CDR6 of SeqID No: 83,
an antibody comprising a CDR1 of SeqID No: 14, a CDR2 of SeqID No: 28, a CDR3 of SeqID No: 42, a CDR4 of SeqID No: 56, a CDR5 of SeqID No: 70 and a CDR6 of SeqID No: 84.

17. A CD33 binding agent selected from
an antibody comprising a heavy chain variable region of SeqID No: 85 and a light chain variable region of SeqID No: 99,
an antibody comprising a heavy chain variable region of SeqID No: 86 and a light chain variable region of SeqID No: 100,
an antibody comprising a heavy chain variable region of SeqID No: 87 and a light chain variable region of SeqID No: 101,
an antibody comprising a heavy chain variable region of SeqID No: 88 and a light chain variable region of SeqID No: 102,
an antibody comprising a heavy chain variable region of SeqID No: 89 and a light chain variable region of SeqID No: 103,

an antibody comprising a heavy chain variable region of SeqID No: 90 and a light chain variable region of SeqID No: 104,
an antibody comprising a heavy chain variable region of SeqID No: 91 and a light chain variable region of SeqID No: 105,
an antibody comprising a heavy chain variable region of SeqID No: 92 and a light chain variable region of SeqID No: 106,
an antibody comprising a heavy chain variable region of SeqID No: 93 and a light chain variable region of SeqID No: 107,
an antibody comprising a heavy chain variable region of SeqID No: 94 and a light chain variable region of SeqID No: 108,
an antibody comprising a heavy chain variable region of SeqID No: 95 and a light chain variable region of SeqID No: 109,
an antibody comprising a heavy chain variable region of SeqID No: 96 and a light chain variable region of SeqID No: 110,
an antibody comprising a heavy chain variable region of SeqID No: 97 and a light chain variable region of SeqID No: 111,
an antibody comprising a heavy chain variable region of SeqID No: 98 and a light chain variable region of SeqID No: 113,

18. A CD33 binding agent selected from
an antibody comprising a heavy chain of SeqID No: 113 and a light chain of SeqID No: 127,
an antibody comprising a heavy chain of SeqID No: 114 and a light chain of SeqID No: 128,
an antibody comprising a heavy chain of SeqID No: 115 and a light chain of SeqID No: 129,
an antibody comprising a heavy chain of SeqID No: 116 and a light chain of SeqID No: 130,
an antibody comprising a heavy chain of SeqID No: 117 and a light chain of SeqID No: 131,
an antibody comprising a heavy chain of SeqID No: 118 and a light chain of SeqID No: 132,

an antibody comprising a heavy chain of SeqID No: 119 and a light chain of SeqID No: 133,
an antibody comprising a heavy chain of SeqID No: 120 and a light chain of SeqID No: 134,
an antibody comprising a heavy chain of SeqID No: 121 and a light chain of SeqID No: 135,
an antibody comprising a heavy chain of SeqID No: 122 and a light chain of SeqID No: 136,
an antibody comprising a heavy chain of SeqID No: 123 and a light chain of SeqID No: 137,
an antibody comprising a heavy chain of SeqID No: 124 and a light chain of SeqID No: 138,
an antibody comprising a heavy chain of SeqID No: 125 and a light chain of SeqID No: 139,
an antibody comprising a heavy chain of SeqID No: 126 and a light chain of SeqID No: 140.

19. A DNA molecule comprising a region encoding the heavy chain variable region of a CD33 binding agent according to any of the preceding claims.

20. A DNA molecule comprising a region encoding the light chain variable region of a CD33 binding agent according to any of claims 1-18.

21. An expression vector containing a DNA molecule of claim 19 or 20.

22. A host cell carrying one or more vectors of claim 21.

23. A method for producing a CD33 binding agent of any one of claims 1-18, comprising transfecting a host cell with one or more vectors of claim 21, culturing the host cell and recovering and purifying the antibody molecule.

24. A pharmaceutical composition comprising, as the active ingredient, one or more CD33 binding agents according to any one of claims 1 to 18, and at least a physiologically acceptable carrier.

25. The pharmaceutical composition of claim 24, further comprising one or more additional therapeutic agents.

26. The pharmaceutical composition of any one of claims 24-25 for depleting cells that express CD33 on their surface.

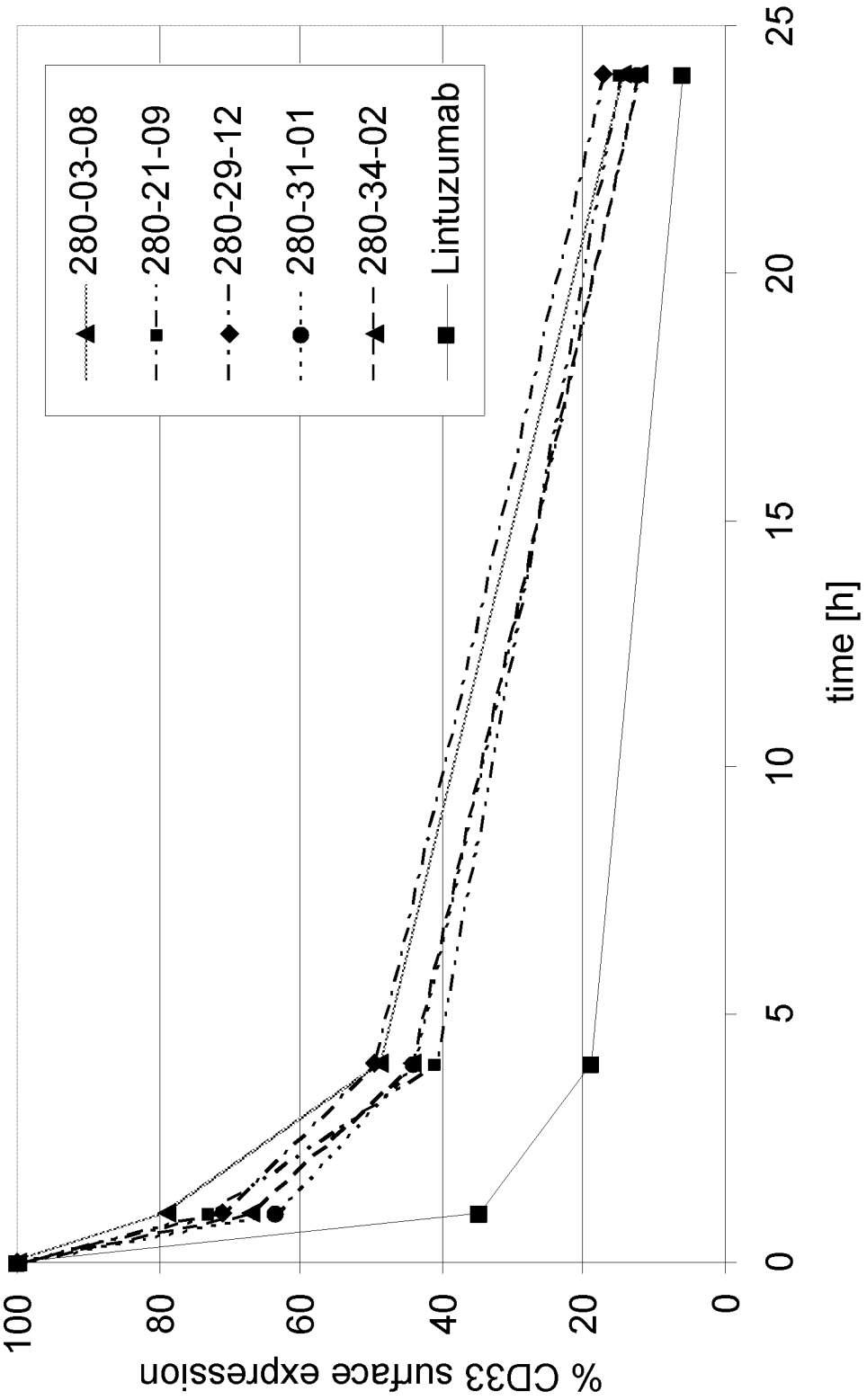
27. The pharmaceutical composition of any one of claims 24-25 for the treatment of myeloid cell malignancies and myelodysplastic syndrome (MDS).

28. The pharmaceutical composition of claim 27, wherein said myeloid malignancy is selected from acute myeloid leukemia and chronic myeloid leukemia.

29. The pharmaceutical composition of any one of claims 24-25 for the treatment of autoimmune and inflammatory diseases that involve myeloid cells in their pathology.

30. The method of depleting CD33 expressing myeloid cells in a patient, comprising administering to said patient one or more CD33 binding agents according to any one of claims 1-18 or a pharmaceutical composition according to any of claims 24-25.

Figure 1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280-29-12
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280-34-02
Lintuzumab
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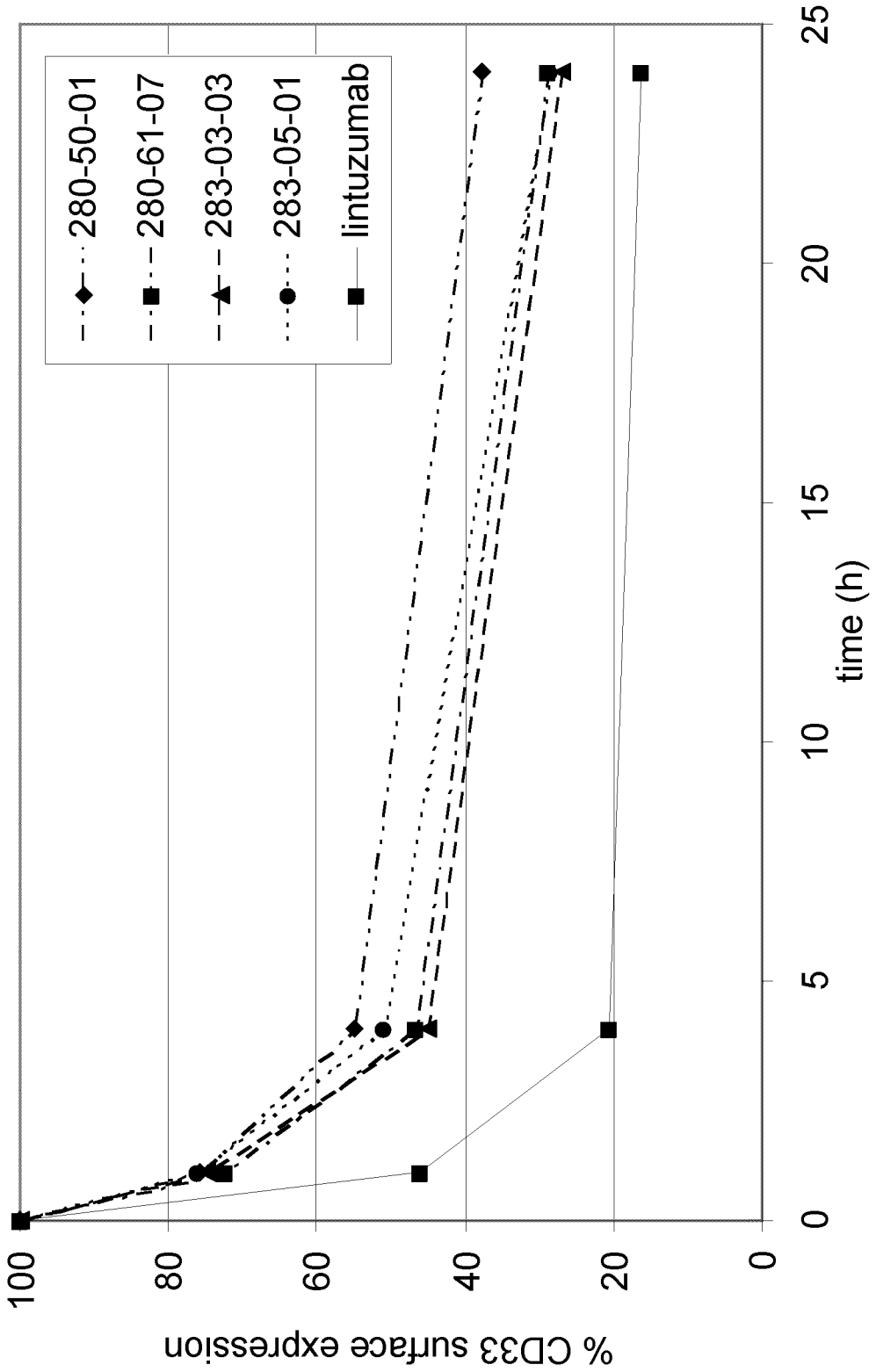
80
60
40
20
0
0
5
10
15
20
25
time [h]

2/5

Figure 2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% CD33 surface expression
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time (h)
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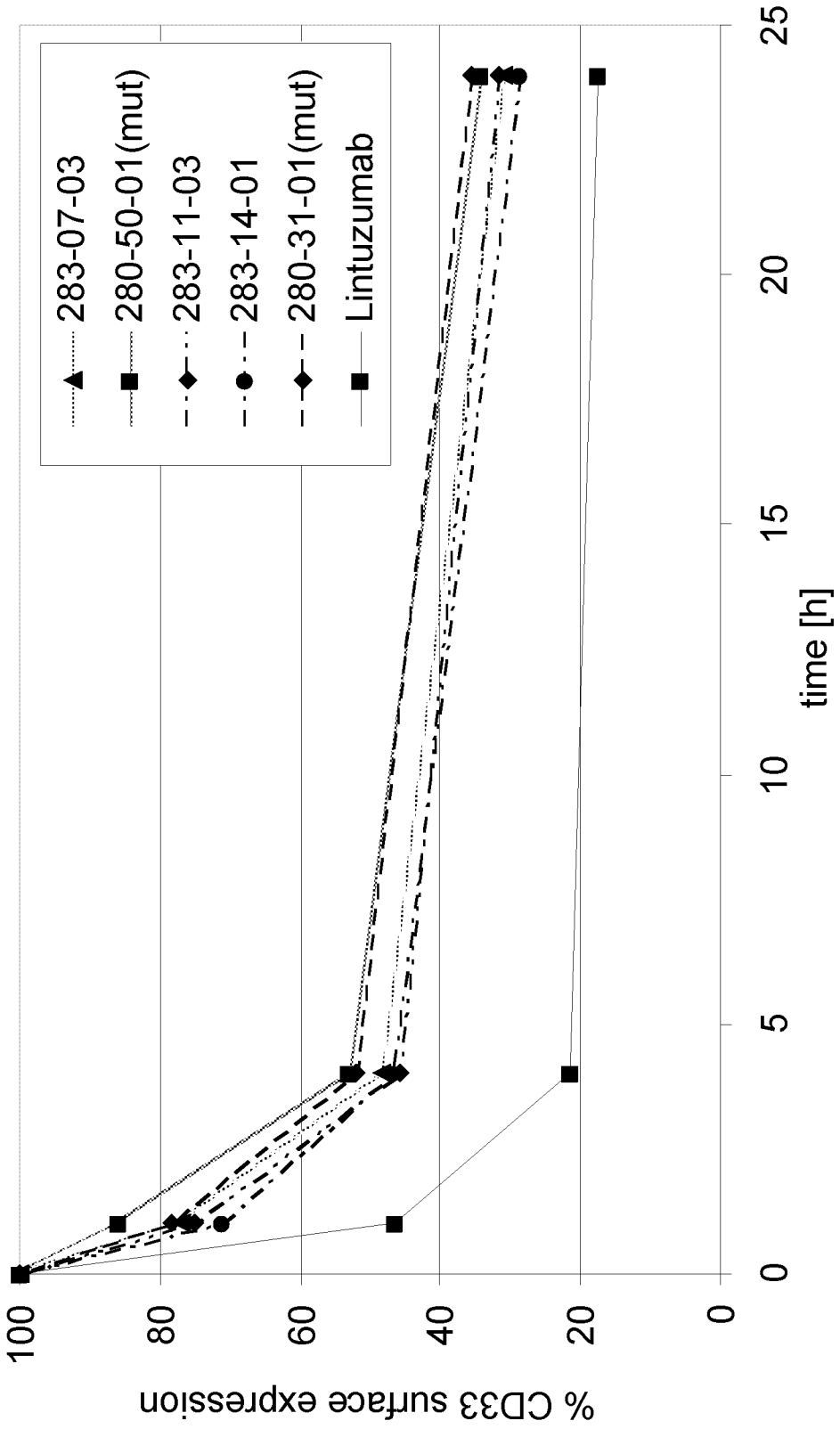
5
10
15
20
25

3/5

Figure 3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280-50-01(mut)
283-11-03
283-14-01
280-31-01(mut)
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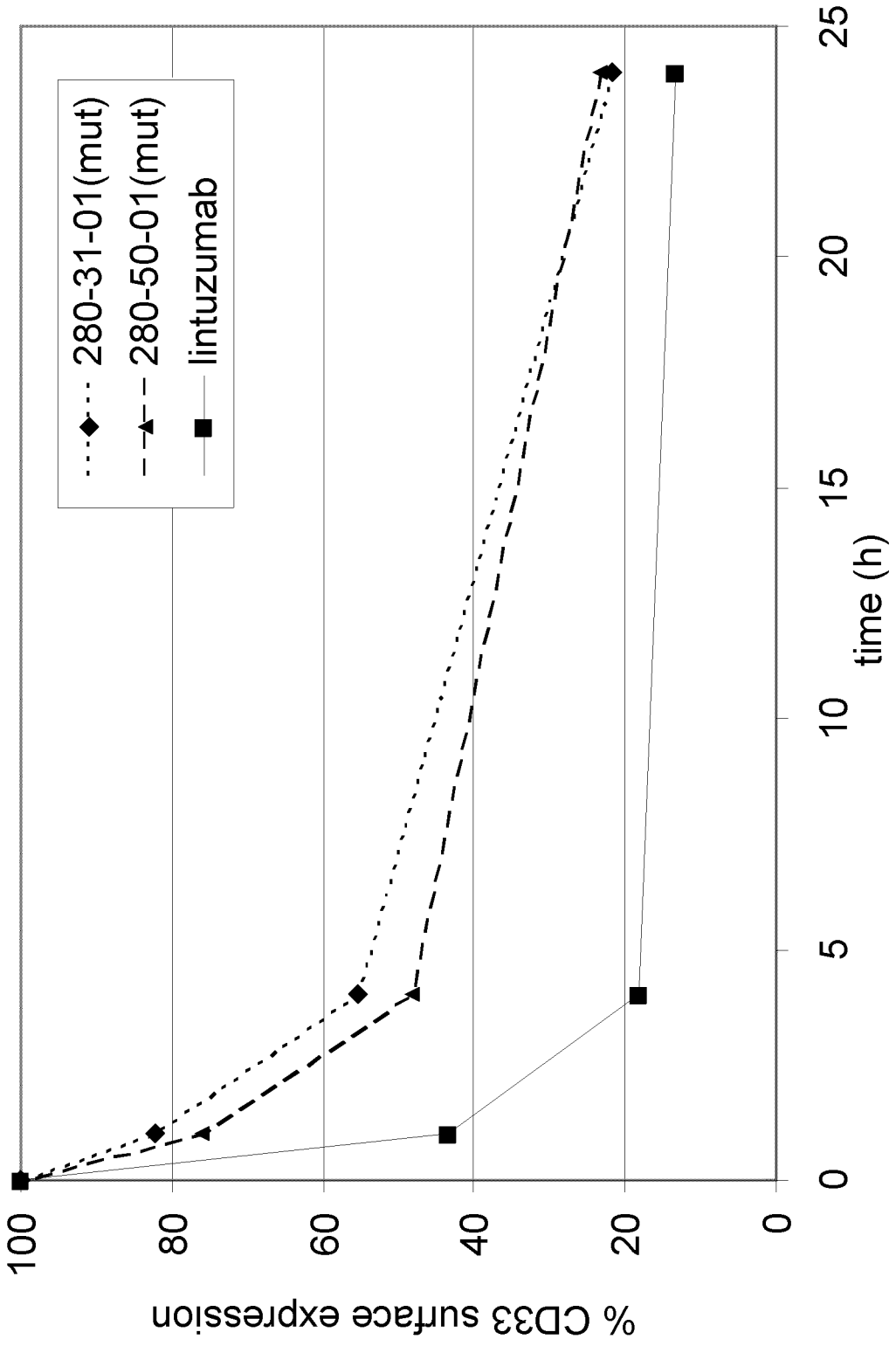
20
40
60
80
100
time [h]
0
5
10
15
20
25

Figure 4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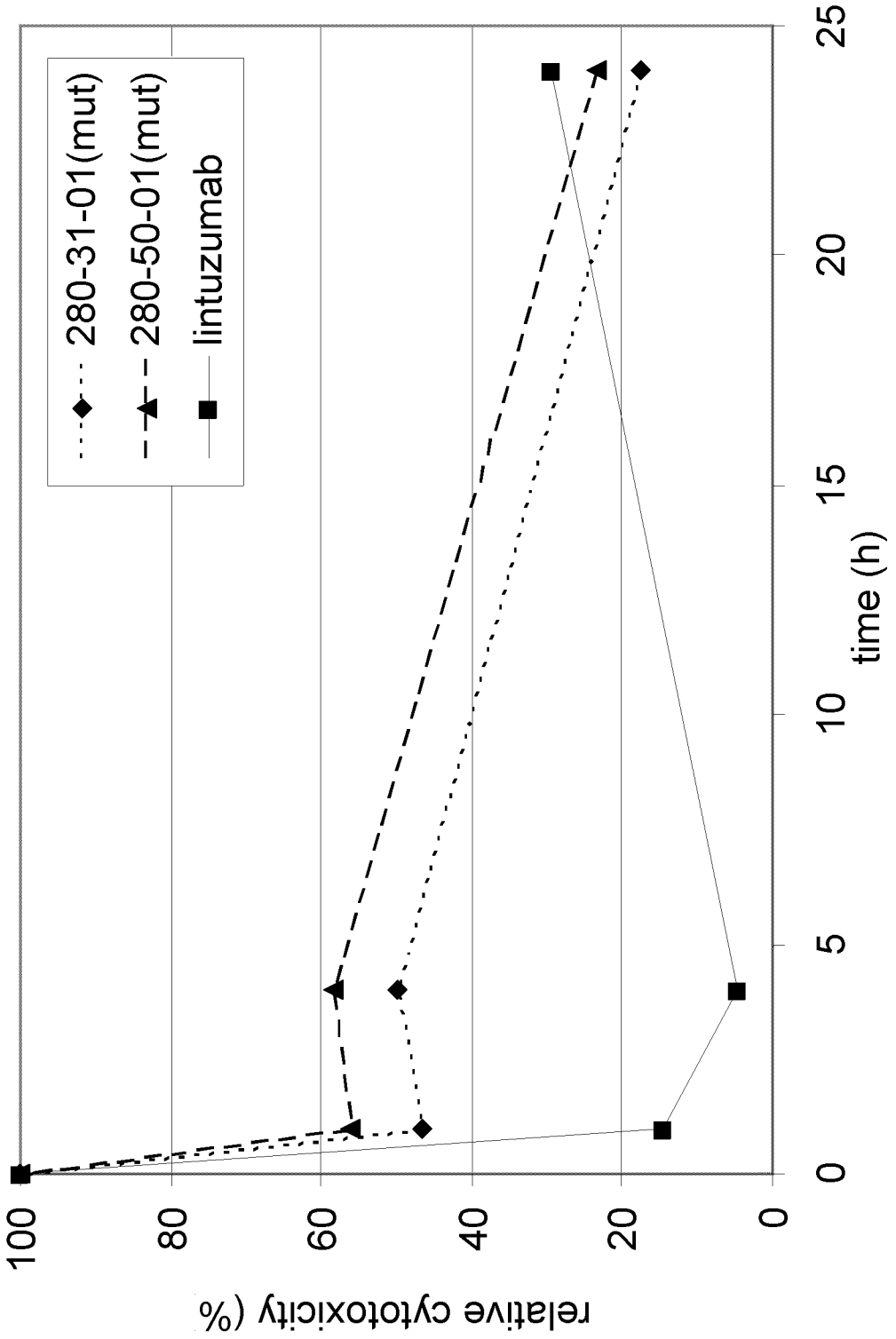
100
80
60
40
20
0
0
5
10
15
20
25
time (h)
280-31-01(mut)
280-50-01(mut)
lintuzumab

Figure 5


280-31-01(mut)
280-50-01(mut)
lintuzumab
relative cytotoxicity (%
100
80
60
40
20
0
0
5
10
15
20
25
time (h)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/067339

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61K39/395
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CARON PHILIP C ET AL: "Biological and immunological features of humanized M195 (anti-CD33) monoclonal antibodies", CANCER RESEARCH, vol. 52, no. 24, 1992, pages 6761-6767, XP009142412, ISSN: 0008-5472 the whole document	1-30

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 January 2012	30/01/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Sirim, Pinar

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CARON P C ET AL: "MURINE AND HUMANIZED CONSTRUCTS OF MONOCLONAL ANTIBODY M195 (ANTI-CD33) FOR THE THERAPY OF ACUTE MYELOGENOUS LEUKEMIA", CANCER, AMERICAN CANCER SOCIETY, PHILADELPHIA, PA, US, vol. 73, no. 3, SUPPL, 1 January 1994 (1994-01-01), pages 1049-1056, XP009036638, ISSN: 0008-543X the whole document	1-30
X	CARON P C ET AL: "ENGINEERED HUMANIZED DIMERIC FORMS OF IGG ARE MORE EFFECTIVE ANTIBODIES", THE JOURNAL OF EXPERIMENTAL MEDICINE, ROCKEFELLER UNIVERSITY PRESS, US, vol. 176, no. 4, 1 January 1992 (1992-01-01), pages 1191-1195, XP000914982, ISSN: 0022-1007, DOI: DOI:10.1084/JEM.176.4.1191 the whole document	1-30
A	KUEGLER MARKUS ET AL: "A recombinant trispecific single-chain Fv derivative directed against CD123 and CD33 mediates effective elimination of acute myeloid leukaemia cells by dual targeting", BRITISH JOURNAL OF HAEMATOLOGY, WILEY-BLACKWELL PUBLISHING LTD, GB, vol. 150, no. 5, 1 September 2010 (2010-09-01), pages 574-586, XP009142424, ISSN: 0007-1048, DOI: DOI:10.1111/J.1365-2141.2010.08300.X [retrieved on 2010-07-16] the whole document	1-30
A	SINGER HEIKO ET AL: "Effective Elimination of Acute Myeloid Leukemic Cells by Recombinant Bispecific Antibody Derivatives Directed Against CD33 and CD16", JOURNAL OF IMMUNOTHERAPY, LIPPINCOTT WILLIAMS & WILKINS, HAGERSTOWN, MD, US, vol. 33, no. 6, 1 July 2010 (2010-07-01), pages 599-608, XP009142457, ISSN: 1524-9557, DOI: DOI:10.1097/CJI.0B013E3181DDA225 the whole document	1-30

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LINENBERGER MICHAEL L ET AL: "Antibody-directed therapies for hematological malignancies", TRENDS IN MOLECULAR MEDICINE, ELSEVIER CURRENT TRENDS, GB, vol. 8, no. 2, 1 February 2002 (2002-02-01), pages 69-76, XP009142419, ISSN: 1471-4914 the whole document	1-30
A	WO 2008/058021 A2 (SEATTLE GENETICS INC [US]; DRACHMAN JONATHAN G [US]; SUTHERLAND MAY KU) 15 May 2008 (2008-05-15) the whole document	1-30
A	KORVER W ET AL: "Monoclonal antibodies against IREM-1: potential for targeted therapy of AML", LEUKEMIA, MACMILLAN PRESS LTD, US, vol. 23, no. 9, 1 September 2009 (2009-09-01), pages 1587-1597, XP009142413, ISSN: 0887-6924 the whole document	1-30
A	LAZAR GREG A ET AL: "Engineered antibody Fc variants with enhanced effector function", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES (PNAS), NATIONAL ACADEMY OF SCIENCE, US, vol. 103, no. 11, 14 March 2006 (2006-03-14), pages 4005-4010, XP002590010, ISSN: 0027-8424, DOI: DOI:10.1073/PNAS.0508123103 the whole document	1-30
A	CARTER P J: "POTENT ANTIBODY THERAPEUTICS BY DESIGN", NATURE REVIEWS. IMMUNOLOGY, NATURE PUBLISHING GROUP, GB, vol. 6, 7 April 2006 (2006-04-07), pages 343-357, XP007901440, ISSN: 1474-1733, DOI: DOI:10.1038/NRI1837 the whole document	1-30

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MAN SUNG CO: "CHIMERIC AND HUMANIZED ANTIBODIES WITH SPECIFICITY FOR THE CD33 ANTIGEN1", JOURNAL OF IMMUNOLOGY, AMERICAN ASSOCIATION OF IMMUNOLOGISTS, US, vol. 148, no. 4, 15 February 1992 (1992-02-15), pages 1149-1154, XP000368716, ISSN: 0022-1767 the whole document -----	1-30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2011/067339

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:1, CDR2 has an amino acid sequence selected from SeqID No:15, CDR3 has an amino acid sequence selected from SeqID No:29, CDR4 has an amino acid sequence selected from SeqID No: 43, CDR5 has an amino acid sequence selected from SeqID No:57, CDR6 has an amino acid sequence selected from SeqID No:71, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

---

2. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:2, CDR2 has an amino acid sequence selected from Seq ID No:16, CDR3 has an amino acid sequence selected from Seq ID No:30, CDR4 has an amino acid sequence selected from Seq ID No:44, CDR5 has an amino acid sequence selected from Seq ID No:58, CDR6 has an amino acid sequence selected from Seq ID No:72, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

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3. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:3, CDR2 has an amino acid sequence selected from Seq ID No:17, CDR3 has an amino acid sequence selected from Seq ID No:31, CDR4 has an amino acid sequence selected from Seq ID No:45, CDR5 has an amino acid sequence selected from Seq ID No:59, CDR6 has an amino acid sequence selected from Seq ID No:73, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

---

4. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:4, CDR2 has an amino acid sequence selected from Seq ID No:18, CDR3 has an amino acid sequence selected from Seq ID No:32, CDR4 has an amino acid sequence selected from Seq ID No:46, CDR5 has an amino acid sequence selected from Seq ID No:60, CDR6 has an amino acid sequence selected from Seq ID No:74, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

---

5. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:5, CDR2 has an amino acid sequence selected from Seq ID No:19, CDR3 has an amino acid sequence selected from Seq ID No:33, CDR4 has an amino acid sequence selected from Seq ID No:47, CDR5 has an amino acid sequence selected from Seq ID No:61, CDR6 has an amino acid sequence selected from Seq ID No:75, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

---

6. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:6, CDR2 has an amino acid sequence selected from Seq ID No:20, CDR3 has an amino acid sequence selected from Seq ID No:34, CDR4 has an amino acid sequence selected from Seq ID No:48, CDR5 has an amino acid sequence selected from Seq ID No:62, CDR6 has an amino acid sequence selected from Seq ID No:76, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

---

7. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1,

CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:7, CDR2 has an amino acid sequence selected from Seq ID No:21, CDR3 has an amino acid sequence selected from Seq ID No:35, CDR4 has an amino acid sequence selected from Seq ID No:49, CDR5 has an amino acid sequence selected from Seq ID No:63, CDR6 has an amino acid sequence selected from Seq ID No:77,or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

---

8. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:8, CDR2 has an amino acid sequence selected from Seq ID No:22, CDR3 has an amino acid sequence selected from Seq ID No:36, CDR4 has an amino acid sequence selected from Seq ID No:50, CDR5 has an amino acid sequence selected from Seq ID No:64, CDR6 has an amino acid sequence selected from Seq ID No:78, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

---

9. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:9, CDR2 has an amino acid sequence selected from Seq ID No:23, CDR3 has an amino acid sequence selected from Seq ID No:37, CDR4 has an amino acid sequence selected from Seq ID No:51, CDR5 has an amino acid sequence selected from Seq ID No:65, CDR6 has an amino acid sequence selected from Seq ID No:79, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

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10. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:10, CDR2 has an amino acid sequence selected from Seq ID No:24, CDR3 has an amino acid sequence

selected from Seq ID No:38, CDR4 has an amino acid sequence selected from Seq ID No:52, CDR5 has an amino acid sequence selected from Seq ID No:66, CDR6 has an amino acid sequence selected from Seq ID No:80, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

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11. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:11 or 13, CDR2 has an amino acid sequence selected from Seq ID No:25 or 27, CDR3 has an amino acid sequence selected from Seq ID No:39 or 41, CDR4 has an amino acid sequence selected from Seq ID No:53 or 55, CDR5 has an amino acid sequence selected from Seq ID No:67 or 69, CDR6 has an amino acid sequence selected from Seq ID No:81 or 83, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

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12. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:12, CDR2 has an amino acid sequence selected from Seq ID No:26, CDR3 has an amino acid sequence selected from Seq ID No:40, CDR4 has an amino acid sequence selected from Seq ID No:54, CDR5 has an amino acid sequence selected from Seq ID No:68, CDR6 has an amino acid sequence selected from Seq ID No:82,or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

---

13. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:14, CDR2 has an amino acid sequence selected from Seq ID No:28, CDR3 has an amino acid sequence selected from Seq ID No:42, CDR4 has an amino acid sequence selected from Seq ID No:56, CDR5 has an amino acid sequence selected from Seq ID No:70, CDR6 has an amino acid sequence selected from Seq ID No:84, or b) recognizing an epitope

within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2011/067339

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008058021 A2	15-05-2008	AU 2007317333 A1	15-05-2008
		CA 2667808 A1	15-05-2008
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		JP 2010509234 A	25-03-2010
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Agentes de unión a CD33

Campo de la invención

La presente invención se refiere a inmunoterapias que están basadas en la disminución del número de células mieloides. En particular, la presente invención se refiere a agentes de unión a CD33 para su uso en dichas terapias, por ejemplo para el tratamiento de malignidades de células mieloides y el síndrome mielodisplásico (MDS).

Antecedentes de la invención

A principios de los años 80, el CD33 se identificó como un marcador de leucemias mieloides (Andrews et al., Blood, 62, 24-132,1983). El CD33 es un antígeno de la superficie celular que se expresa de modo específico sobre células mieloides, incluyendo células de leucemia mieloide. Es el miembro más pequeño de la familia SIGLEC (lecitinas relacionadas con Ig de unión al ácido siálico). El CD33 se expresa en células progenitoras hematopoyéticas de múltiples linajes tempranas y en precursores mielomonocíticos. Está ausente de células precursoras hematopoyéticas pluripotenciales (Andrews et al., Journal of Experimental Medicine, 169, 1721-1731, 1989). Está infrarregulado sobre granulocitos maduros pero se mantiene sobre macrófagos, monocitos y células dendríticas (Andrews et al., Blood, 62, 24-132,1983). Además de las células mielomonocíticas, también se ha descubierto que el CD33 se expresa sobre células cebadas y basófilos sanguíneos humanos (Valent et al., Blood, 15, 73(7):1778-85, 1989). Se emplean anticuerpos monoclonales dirigidos contra CD33 para el diagnóstico de la leucemia, así como para el transporte dirigido terapéutico y la purgación *in vitro* de médula ósea para el transplante autólogo en la leucemia mieloide aguda (AML) (Duzkale et al., Biol. Blood Marrow Transplant, 9(6):364-372, 2003). Los esfuerzos iniciales en el transporte dirigido terapéutico se centraron en el desarrollo de inmunotoxinas empleando un anticuerpo anti-CD33 conjugado con la toxina ricina. Puesto que el CD33 se internaliza con rapidez después de la unión del anticuerpo (Audran et al., J. Immunol. Methods., 188(1):147-154,1995), el enfoque de la inmunotoxina era obvio.

CD33 es una glicoproteína transmembrana de 67 KD. El dominio extracelular de unión al ácido siálico de CD33 está implicado en la adhesión de célula a célula. Los motivos inhibidores basados en tirosina del inmunorreceptor intracelular (ITIM)

confieren señales inhibidoras a la célula, afectando a la proliferación y a la supervivencia celular. La vías reales de señalización del CD33 no están bien comprendidas pero se supone que implican al ITIM y a motivos similares a ITIM y al reclutamiento de tirosina fosfatasas (von Gunten et al., Ann. N.Y. Acad. Sci., 1143: 61–82, 2008). Se ha definido un ortólogo de CD33 murino pero se ha cuestionado su comparabilidad funcional con el CD33 humano (Brinkman-Van der Linden et al., Mol. Cell. Biol., 23(12): 4199-4206, 2003). El papel funcional del CD33 humano en leucocitos humanos y malignos sigue siendo desconocido.

Varias publicaciones han descrito al CD33 como un marcador de la superficie celular estable sobre células de AML y CML primarias expresado por 70-100% de los pacientes ensayados (Plesa et al., Cancer, 112(3), 572-580, 2007; Hauswirt et al., Eur. J. Clin. Invest., enero, 73-82, 2007; Scheinberg et al., Leukemia, vol. 3, 440-445, 1989). El CD33 se expresa sobre células blásticas mieloides malignas, que representan la mayoría de las células malignas en la sangre periférica y en la médula ósea de pacientes con leucemia, y sobre células precursores leucémicas, un número relativamente pequeño de células menos diferenciadas en la médula ósea que se caracterizan por su capacidad para la autorrenovación y el mantenimiento de la jerarquía clonal leucémica. Se considera la disminución del número de células precursoras leucémicas como un mecanismo clave para una supervivencia sostenida sin tumores. La inmunotoxina dirigida a CD33 Mylotarg®, un anticuerpo IgG_4 humanizado conjugado con la toxina calicheamicina, se emplea para el tratamiento de pacientes con AML mediante el transporte de su carga útil tóxica a células de AML positivas a CD33 (Amadori et al., Cancer Treat. Rev., 34(1):49-60, 2008). El lintuzumab (SGN-33, HuM195), un anticuerpo monoclonal humanizado específico de CD33 “desnudo”, se evaluó en ensayos clínicos de fase II para el tratamiento de AML y MDS con señales clínicas iniciales de eficacia procedentes de un estudio de escalada de dosis de fase I, y se han indicado acontecimientos adversos tolerables (Raza et al., resumen nº 983, 14th EHA Congress, 4-7 de junio, 2009).

El transporte dirigido a líneas celulares de AML con HuM195 específico de CD33 *in vitro* reduce la secreción inducida por TNF-α de citoquinas inflamatorias, tales como IL-8, MCP-1 y RANTES (Sutherland et al., Mabs, 1:5, 481-490, 2009). La importancia de este efecto para la terapia de la AML es desconocida, pero la modulación del medio de citoquinas del microentorno del tumor puede contribuir a la eficacia terapéutica del anticuerpo. Además, el anticuerpo induce una citotoxicidad mediada por células dependiente de anticuerpos (ADCC) y una fagocitosis mediada

por células dependiente de anticuerpos (ADCP) de líneas celulares de AML *in vitro* (Sutherland et al., Mabs, 1:5, 481-490, 2009). Se considera que la ADCC es un mecanismo decisivo para la actividad antitumoral de anticuerpos en malignidades hematológicas. Los datos de ensayos clínicos con el anticuerpo monoclonal específico de CD20 rituximab han demostrado la importancia de los mecanismos mediados por células efectoras para el tratamiento de malignidades de células B con respecto a la respuesta a un tratamiento con anticuerpos (Weng y Levy, J. Clin. Oncol., 21 (21): 3940-3947, 2003).

En conclusión, se ha demostrado que el antígeno CD33 se expresa sobre células normales del linaje mielomonocítico y con frecuencia se expresa sobre células tumorales en leucemias mieloides. En un ensayo de fase I con un anticuerpo contra CD33 (lintuzumab) se observaron las primeras señales de eficacia sin graves efectos adversos. Sin embargo, el desarrollo clínico del lintuzumab se detuvo después de los resultados de un ensayo de fase II en combinación con quimioterapia, que no produjeron la mejora esperada en la eficacia. Por tanto, existe una necesidad clara de desarrollar modalidades de tratamiento mejoradas dirigidas a CD33.

En vista de la técnica anterior, es necesario proporcionar una terapia aún mejor para las malignidades de células mieloides y MDS, en particular para la leucemia mieloide aguda.

En particular, es necesario proporcionar agentes de unión antagonistas aún mejores para CD33 para tratar el cáncer, en particular la AML.

Sumario de la invención

La presente invención proporciona nuevos agentes de unión a CD33 que se unen al CD33 humano y que se definen porque:

a) tienen una región variable de cadena pesada que comprende CDR1, CDR2 y CDR3, y una región variable de cadena ligera que comprende CDR4, CDR5 y CDR6, en los que CDR1 tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:1-14, CDR2 tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:14-28, CDR3 tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:29-42, CDR4 tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:43-56, CDR5 tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:57-70, CDR6 tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:71-84, o

b) reconocen un epítopo dentro de la secuencia de aminoácidos FFHPIPYYDKNSPVHGYW (SEQ ID NO:141) del CD33 humano.

La presente invención proporciona además agentes de unión a CD33, en los que la cinética de internalización de los agentes de unión a CD33 es tal que al menos 30%, preferiblemente 40% de la cantidad inicial del anticuerpo permanece sobre la superficie celular de células HL60 a las 4 horas después de la incubación.

La presente invención proporciona además agentes de unión a CD33, en los que la región variable de cadena pesada comprende una secuencia de aminoácidos seleccionada de SEQ ID NO:85-98, y la región variable de cadena ligera comprende una secuencia de aminoácidos seleccionada de SEQ ID NO:99-112.

La presente invención proporciona además agentes de unión a CD33, en los que la región variable de cadena pesada tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:113-126, y la región variable de cadena ligera tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:127-140.

La presente invención proporciona además agentes de unión a CD33 que tienen mutaciones en el dominio F_c que aumentan la ADCC.

Otras realizaciones preferidas se indican en la siguiente memoria descriptiva y en las reivindicaciones.

Se ha descubierto que los agentes de unión a CD33 según la presente invención tienen una alta afinidad por el CD33 humano y además tienen una cinética de internalización favorable, que se caracteriza por una larga presencia de los agentes de unión a CD33 cuando están unidos al CD33 sobre la superficie de las células diana, lo cual se traduce en una actividad ADCC favorable.

Los inventores también han descubierto que los agentes de unión a CD33 según la presente invención se unen a un epitopo diferente del dominio extracelular de CD33 comparados con el lintuzumab. Sin querer limitarse por ninguna teoría concreta, se cree que ésta es la razón de la diferente cinética de internalización de los agentes de unión a CD33 según la presente invención y el lintuzumab.

Descripción de las figuras

Las figuras 1-3 muestran la internalización de ejemplos de agentes de unión a CD33 según la invención, en comparación con el lintuzumab en células HL60.

La figura 4 muestra la velocidad de internalización en células HL60 de dos ejemplos de anticuerpos según la invención, en comparación con el lintuzumab.

La figura 5 muestra la actuación de ADCC en células HL60 de dos ejemplos de anticuerpos según la invención, en comparación con el lintuzumab.

Descripción detallada de la invención

Agentes de unión a CD33

La expresión "agente de unión", tal como se emplea en la presente, significa una proteína o un péptido que se une de modo específico con un antígeno diana. Un agente de unión puede ser, por ejemplo, un anticuerpo, un derivado de dicho anticuerpo, u otro agente que se une de modo específico al antígeno diana. Un agente de unión también puede ser una proteína que comprende una región Fv o una porción de ésta (por ejemplo, una V_H o V_L o uno o más CDR de un anticuerpo que se une de modo específico al antígeno diana). En una realización preferida en la presente, el agente de unión es un anticuerpo.

La expresión "agente de unión a CD33", tal como se emplea en la presente, se refiere a un agente de unión que se une de modo específico al CD33, generalmente a una porción del dominio extracelular del CD33 humano.

El término "anticuerpo", tal como se emplea en la presente, se refiere a (a) polipéptidos de inmunoglobulinas y porciones inmunológicamente activas de los polipéptidos de inmunoglobulinas (es decir, polipéptidos de la familia de las inmunoglobulinas o sus fragmentos, que contienen un sitio de unión al antígeno que se une de modo inmunoespecífico a un antígeno diana específico); o (b) derivados sustituidos de modo conservativo de dichos polipéptidos de inmunoglobulinas o sus fragmentos que se unen de modo inmunoespecífico al antígeno diana. Los anticuerpos se describen en general por ejemplo en Harlow y Lane, *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 1988). El término "anticuerpo" se refiere a anticuerpos monoclonales intactos, anticuerpos policlonales intactos, anticuerpos monoespecíficos intactos, anticuerpos multiespecíficos (por ejemplo, anticuerpos biespecíficos) intactos, y fragmentos de anticuerpos que muestren la actividad biológica deseada (por ejemplo, de unión al antígeno). El anticuerpo puede ser de cualquier tipo o clase (por ejemplo, IgG, IgE, IgM, IgD, e IgA) o subclase (por ejemplo, IgG1, IgG2, IgG3, IgG4, IgA1, e IgA2), preferiblemente de clase IgG, más preferiblemente una IgG1.

Un anticuerpo "intacto" es aquel que comprende una región variable de unión al

antígeno, así como un dominio constante de cadena ligera y los dominios constantes de cadena pesada, según sea apropiado para la clase de anticuerpo. Los dominios constantes pueden ser dominios constantes de secuencia nativa (por ejemplo, dominios constantes de secuencia nativa humana) o sus variantes de secuencia de aminoácidos.

Un "fragmento de anticuerpo" comprende una porción de un anticuerpo, incluyendo la región variable o de unión al antígeno, o una porción de éstas. Los ejemplos de fragmentos de anticuerpos incluyen fragmentos Fab, Fab', $F(ab')_2$, y Fv, fragmentos de unión al antígeno de V_H y V_L, diacuerpos, triacuerpos, tetracuerpos, anticuerpos monocatenarios, scFv, scFv-Fc, un SMTP, y anticuerpos multiespecíficos formados a partir de un fragmento o de fragmentos de anticuerpos.

La “región variable de cadena pesada” o “V_H” significa la parte de la cadena pesada que comprende la CDR1, CDR2 y CDR3, y las regiones de marco circundantes.

La “región variable de cadena ligera” o “V_L” significa la parte de la ligera que comprende la CDR4, CDR5 y CDR6, y las regiones de marco circundantes.

Una “CDR” significa las regiones hipervariables de las cadenas pesada y ligera, que determinan la especificidad de unión/complementariedad de un anticuerpo o de un fragmento de anticuerpo. El orden de las CDR en la presente solicitud es simplemente numérico.

Un “epitopo” significa en la presente una parte de un antígeno, que es reconocida por un anticuerpo o por un fragmento de anticuerpo. En particular, este término se refiere a partes del CD33, que pueden ser reconocidas por un anticuerpo.

En la presente se emplea “mAb” para referirse a los anticuerpos monoclonales.

Un anticuerpo puede tener una o más "funciones efectoras", que se refieren a las actividades biológicas atribuibles a la región Fc (una región Fc con la secuencia nativa o un variante de secuencia de aminoácidos de la región Fc) de un anticuerpo. Los ejemplos de funciones efectoras de anticuerpos incluyen la unión a CIq; la citotoxicidad dependiente del complemento (CDC); la unión al receptor de Fc; la citotoxicidad mediada por células dependiente de anticuerpos (ADCC); la fagocitosis; la infrarregulación de receptores de la superficie celular (por ejemplo, receptor de células B; BCR), etc.

Los fragmentos de anticuerpos "Fv monocatenarios" o "scFv" comprenden los dominios V_H y V_L de un anticuerpo, en los que dichos dominios están presentes en una única cadena polipeptídica. Los polipéptidos de Fv también comprenden generalmente

un conector polipeptídico entre los dominios V_H y V_L. que permite al scFv formar la estructura deseada para la unión al antígeno. Para un informe sobre scFv, véase Plückthun en *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg y Moore, *eds.*, Springer-Verlag, Nueva York, pp. 269-315 (1994).

Un agente de unión, tal como un anticuerpo, que "se dirige a," "que se une a" o que "se une de modo específico a" un antígeno de interés (es decir, un antígeno diana) es aquel capaz de unirse al antígeno con una afinidad suficiente de forma que el agente de unión es útil para dirigirse a una célula que expresa el antígeno. Generalmente, el agente de unión se une con una afinidad de al menos aproximadamente 1 x 10^7 M^{-1}, y se une al antígeno predeterminado con una afinidad que es al menos dos veces mayor que su afinidad para unirse a un antígeno no específico (por ejemplo, BSA, caseína) distinto del antígeno predeterminado o un antígeno estrechamente relacionado.

Un "derivado de anticuerpo", tal como se emplea en la presente, se refiere a un anticuerpo, tal como se definió anteriormente, que se modifica mediante una unión covalente a una molécula heteróloga tal como, por ejemplo, mediante la unión a un polipéptido heterólogo, o mediante glicosilación, desglicosilación, acetilación o fosforilación, u otra modificación que normalmente no está asociada con el anticuerpo. En algunas realizaciones, la molécula heteróloga no es un agente terapéutico. En algunas realizaciones, la molécula heteróloga no muestra un efecto citostático ni citotóxico en sí misma.

Una referencia más exhaustiva de todos los términos, expresiones y procedimientos utilizados en la presente es Sambrook at al., *Molecular Cloning*, Cold Spring Harbor Laboratory Press, 3ª edición (15 de enero, 2001).

Los agentes de unión a CD33 se unen de modo específico a un receptor, CD33, asociado a una población de células diana concreta. El CD33 es un miembro de la familia de sialoadhesión que se expresa sobre células del linaje hematopoyético, que incluyen precursores mieloides, monocitos, macrófagos, células dendríticas, y células cebadas. El CD33 también se expresa sobre células tumorales asociadas con enfermedades mieloproliferativas o proliferativas de células cebadas, incluyendo la leucemia mieloide aguda y los síndromes mieloplásicos, y sobre células precursoras leucémicas. Los anticuerpos que se dirigen a CD33 y sus usos se han descrito de modo general (véase, por ejemplo, Pierelli et al., 1993, *Br. J. Haematol.*, 84:24-30; Matutes et al., 1985, *Hemaiol.* Oncol., 3:179-186; Taussig et al., 2005, *Blood,* 106:4086-4092; Florian et al., 2006, *Leuk. & Lymph.*, 47:207-222).

En algunas realizaciones, el agente de unión a CD33 es un anticuerpo (por ejemplo, un anticuerpo monoclonal). Los anticuerpos monoclonales útiles pueden ser poblaciones homogéneas de anticuerpos contra un CD33 (por ejemplo, el dominio extracelular del CD33 humano). Un anticuerpo monoclonal (mAb) puede prepararse utilizando cualquier técnica conocida en la técnica. Estas incluyen, pero no se limitan a la técnica de hibridomas descrita por primera vez por Köhler y Milstein (1975, *Nature,* 256:495- 497), la técnica de hibridomas de células B humanas (Kozbor et al., 1983, *Immunology Today,* 4:72), y la técnica de EBV-hibridoma (Cole et al., 1985, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Estos anticuerpos pueden ser de cualquier clase de inmunoglobulinas, incluyendo IgG, IgM, IgE, IgA, e IgD, y de cualquier subclase de éstas. El hibridoma que produce un anticuerpo monoclonal puede cultivarse in vitro o in vivo.

Los anticuerpos monoclonales útiles incluyen, pero no se limitan a anticuerpos monoclonales humanos, anticuerpos monoclonales humanizados, anticuerpos monoclonales quiméricas, y los fragmentos de anticuerpo funcionalmente activos de cualquiera de éstos.

Los anticuerpos CD33 útiles incluyen los anticuerpos que pueden lograr un efecto terapéutico mediante diversos mecanismos conocidos en la técnica, tales como una citotoxicidad mediada por células dependiente de anticuerpos (ADCC), una fagocitosis celular dependiente de anticuerpos (ADCP) y/o una citotoxicidad dependiente del complemento (CDC). Por ejemplo, el anticuerpo puede mediar en la ADCC interaccionando con células efectoras inmunológicas, tales como células NK, monocitos, y macrófagos.

Los anticuerpos recombinantes, tales como anticuerpos monoclonales quiméricos y humanizados, comprenden porciones humanas y no humanas, que puede fabricarse utilizando técnicas de ADN recombinante convencionales (véase, por ejemplo, Cabilly et al., patente de EEUU nº 4.816.567; y Boss et al., patente de EEUU nº 4.816.397; incorporándose ambas como referencia en su totalidad). Los "anticuerpos humanizados" son moléculas de anticuerpos procedentes de una especie no humana que tiene una o más regiones determinantes de la complementariedad (CDR) procedentes de la especie no humana, y una región de marco procedente de una molécula de inmunoglobulina humana (véase, por ejemplo, Queen, patente de EEUU nº 5.585.089, que se incorpora en la presente como referencia en su totalidad). Estos anticuerpos monoclonales quiméricos y humanizados pueden producirse mediante técnicas de ADN recombinante conocidas en la técnica, por ejemplo

utilizando los métodos descritos en la publicación internacional nº WO 87/02671 ; la publicación de patente europea nº 0184187; la publicación de patente europea nº 0171496; la publicación de patente europea nº 0173494; la publicación internacional nº WO 86/01533; la patente de EEUU nº 4.816.567; la publicación de patente europea nº 012023; Berter et al., 1988, Science, 240:1041-1043; Liu et al., 1987, Proc. Natl. Acad. Sci. USA, 84:3439-3443; Liu et al., 1987, J. Immunol., 139:3521-3526; Sun el al., 1987, Proc. Natl. Acad. Sci. USA, 84:214-218; Nishimura et al., 1987, Cancer. Res., 47:999-1005; Wood et al., 1985, Nature, 314:446-449; Shaw et al., 1988, J. Natl. Cancer Inst., 80:1553-1559; Morrison, 1985, Science, 229:1202-1207; Oi et al., 1986, BioTechniques, 4:214; la patente de EEUU nº 5.225.539; Jones et al., 1986, Nature, 321:552-525; Verhoeyan et al., 1988, Science, 239:1534; y Beidler et al., 1988, J. Immunol., 141:4053-4060; incorporándose cada una de las cuales como referencia en su totalidad.

Los anticuerpos monoclonales humanos puede producirse mediante cualquiera de numerosas técnicas conocidas en la técnica (véase, por ejemplo, Teng el al., 1983, Proc. Natl. Acad. Sci. USA., 80:7308-7312; Kozbor et al., 1983, Immunology Today, 4:72-79; Olsson at al., 1982, Meth. Enzymol., 92:3-16; y las patentes de EEUU nº 5.939.598 y 5.770.429).

Los anticuerpos totalmente humanos pueden producirse utilizando ratones transgénicos que sean incapaces de expresar los genes de las cadenas pesada y ligera de inmunoglobulinas endógenas, pero que pueden expresar genes de la cadena pesada y ligera humanos. Los ratones transgénicos se inmunizan de la manera normal con un antígeno seleccionado, por ejemplo, todo o una porción de un polipéptido de CD33. Los anticuerpos monoclonales dirigidos contra el antígeno pueden obtenerse utilizando la tecnología de hibridomas convencional. Los transgenes de inmunoglobulina humanos que portan los ratones transgénicos se redisponen durante la diferenciación de las células B, y después sufren una conmutación de clase y una mutación somática. Por tanto, utilizando dicha técnica, es posible producir anticuerpos IgG, IgA, IgM e IgE terapéuticamente útiles. Para una visión general de esta tecnología para producir anticuerpos humanos véase, por ejemplo, Lonberg y Huszar (1995, Int. Rev. Immunol., 13:65-93). Para un análisis detallado de esta tecnología para producir anticuerpos humanos y anticuerpos monoclonales humanos, y protocolos para producir dichos anticuerpos, veánse, por ejemplo, las patentes de EEUU nº 5.625.126; 5.633.425; 5.569.825; 5.661.016; y 5.545.806. Otros anticuerpos humanos pueden obtenerse en el mercado, por ejemplo, en Medarex (Princeton, NJ), que se obtienen

inmunizando ratones.

Los anticuerpos totalmente humanos que reconocen un epitopo seleccionado también puede generarse utilizando una técnica denominada "selección guiada". En esta estrategia, un anticuerpo monoclonal no humano seleccionado, por ejemplo un anticuerpo de ratón, se emplea para guiar la selección de un anticuerpo totalmente humano que reconozca el mismo epitopo (véase, por ejemplo, Jespers et al., 1994, Biotechnology, 12:899-903). Los anticuerpos humanos también pueden producirse utilizando diversas técnicas conocidas en la técnica, que incluyen los bancos de presentación de fagos (véase, por ejemplo, Hoogenboom y Winter, 1991, J. Mol. Biol., 227:381; Marks et al., 1991, J. Mol. Biol., 222:581 ; Quan y Carter, 2002, The rise of monoclonal antibodies as therapeutics, en Anti-IgE and Allergic Disease, Jardieu y Fick Jr., eds., Marcel Dekker, Nueva York, NY, capítulo 20. pp. 427- 469).

Los fragmentos de anticuerpos útiles incluyen, pero no se limitan a fragmentos $F(ab')_2$, fragmentos Fab', fragmentos Fab, Fv, anticuerpos monocatenarios (SCA) (por ejemplo, según se describe en la patente de EEUU nº 4.946.778; Bird, 1988, Science, 242:423-42; Huston et al., 1988, Proc. Natl. Acad. Sci. USA, 85:5879-5883; y Ward et al., 1989, Nature, 334:544-54), scFv, scFv-Fc, FvdsFv, minicuerpos, diacuerpos, triacuerpos, tetracuerpos, SMIP (véase, por ejemplo, la solicitud de patente de EEUU publicada nº 2005-0238646) y cualquier otra molécula que comprenda una o más CDR y que tenga la misma especificidad que el anticuerpo.

En otras realizaciones, el anticuerpo es una proteína de fusión de un anticuerpo, o su fragmento funcionalmente activo, unida a otra proteína. Por ejemplo, un anticuerpo o un fragmento de anticuerpo puede condensarse a través de un enlace covalente (por ejemplo, un enlace peptídico) en el N-terminal o el C-terminal, a una secuencia de aminoácidos de otra proteína (o su porción, generalmente una porción de al menos 10, 20 ó 50 aminoácidos de la proteína) que no sea el anticuerpo o el fragmento del anticuerpo. En algunas realizaciones, el anticuerpo o su fragmento puede unirse de modo covalente a la otra proteína en el C-terminal del dominio variable o un dominio constante.

Los anticuerpos pueden modificarse, por ejemplo mediante la unión covalente de cualquier tipo de molécula con la condición de que dicha unión covalente permita al anticuerpo mantener su inmunoespecificidad de unión al antígeno. Por ejemplo, un derivado de un anticuerpo puede ser aquel que se ha modificado aún más, por ejemplo mediante glicosilación, desglicosilación, acetilación, pegilación, fosforilación, amidación, desrealización mediante grupos protectores/bloqueantes conocidos,

ruptura proteolítica, enlace a otra proteína, etc. Puede realizarse cualquiera de numerosas modificaciones químicas mediante técnicas conocidas que incluyen, pero no se limitan a ruptura química específica, acetilación, formilación, síntesis metabólica en presencia de tunicamicina o similares. Además, el derivado puede contener uno o más aminoácidos no naturales.

En realizaciones específicas, puede resultar deseable mejorar la afinidad de unión y/u otras propiedades biológicas del anticuerpo (véanse, por ejemplo, las publicaciones de patente de EEUU nº 2006-0003412 y 2006-0008882). Pueden prepararse variantes de secuencia de aminoácidos de los anticuerpos mediante la introducción de cambios de nucleótidos apropiados en el ácido nucleico del anticuerpo, o por síntesis peptídica. Estas modificaciones incluyen, por ejemplo, deleciones y/o inserciones y/o sustituciones de restos dentro de las secuencias de aminoácidos del anticuerpo. Se realiza cualquier combinación de deleciones, inserciones y/o sustituciones para conseguir la construcción final, con la condición de que la construcción final posea las características deseadas. Los cambios de aminoácidos también pueden alterar procesos postraduccionales del anticuerpo, tales como cambiando el número o la posición de los sitios de glicosilación.

Un método útil para la identificación de ciertos restos o regiones del anticuerpo que son localizaciones preferidas para la mutagénesis se denomina "mutagénesis de barrido de alanina," tal como se describe en Cunningham y Wells (Science, 244:1081-1085). En este caso, se identifica un resto o grupo de restos diana (por ejemplo, restos cargados, tales como Arg, Asp, His, Lys y Glu) y se reemplazan por un aminoácido neutro o cargado negativamente (generalmente alanina o polialanina) para afectar a la interacción de los aminoácidos con el antígeno. Después se refinan las localizaciones de los aminoácidos que muestran sensibilidad funcional a las sustituciones mediante la introducción de variantes adicionales u otras variantes en los sitios de sustitución o para los sitios de sustitución. De esta manera, mientras que el sitio para introducir una variación en la secuencia de aminoácidos está predeterminado, no necesita predeterminarse la naturaleza de la mutación per se. Por ejemplo, para analizar el comportamiento de una mutación en un sitio dado, se realiza una mutagénesis de barrido de alanina o una mutagénesis aleatoria en el codón o región diana y se seleccionan los variantes de los anticuerpo expresados con respecto a la actividad deseada.

Las inserciones en la secuencia de aminoácidos incluyen fusiones amino- y/o carboxilo-terminales que varían en longitud desde un resto a polipéptidos que

contienen cien o más restos, así como inserciones intrasecuencia de uno solo o de múltiples restos aminoacídicos. Los ejemplos de inserciones terminales incluyen un anticuerpo con un resto metionilo N-terminal, o el anticuerpo condensado con un polipéptido citotóxico.

Otro tipo de anticuerpo es un variante de sustitución de aminoácidos de un anticuerpo. Estos variantes tienen al menos un resto aminoácido en la molécula del anticuerpo reemplazado por un resto diferente. Los sitios de mayor interés para la mutagénesis de sustitución incluyen las regiones hipervariables, pero también se contemplan alteraciones en la región de marco.

Unas modificaciones sustanciales en las propiedades biológicas del anticuerpo pueden conseguirse mediante la selección de sustituciones que difieran significativamente en su efecto sobre el mantenimiento (a) de la estructura del esqueleto polipeptídico en el área de la sustitución, por ejemplo, como una lámina o conformación helicoidal, (b) de la carga o hidrofobicidad de la molécula en el sitio diana, o (c) del volumen de la cadena lateral. Los restos naturales se dividen en grupos basándose en propiedades comunes de las cadenas laterales:

(1) hidrófoba: norleucina, met, ala, val, leu, ile;

(2) hidrófila neutra: cys, ser, thr;

(3) ácida: asp, glu;

(4) básica: asn, gln, his, lys, arg;

(5) restos que influyen en la orientación de la cadena: gly, pro; y

(6) aromática: trp, tyr, phe.

Las sustituciones no conservativas incluirán cambiar un miembro de una de estas clases por otra clase.

Resulta deseable modificar el anticuerpo con respecto a la función efectora, por ejemplo para potenciar la citotoxicidad mediada por células dependiente de anticuerpos (ADCC), la fagocitosis celular dependiente de anticuerpos (ADCP) y/o la citotoxicidad dependiente del complemento (CDC) del anticuerpo. En particular, la actividad ADCC puede potenciarse introduciendo mutaciones de aminoácidos en la región constante del anticuerpo (Lazar et al., PNAS, 103, 11, 4005-4010, 2006). Esto puede lograrse introduciendo una o más sustituciones de aminoácidos en una región Fc del anticuerpo; véase, por ejemplo, la solicitud de patente de EEUU publicada nº 2006-0160996. Como alternativa, o además, puede introducirse uno o más restos cisteína en la región Fc, permitiendo con ello la formación de enlaces disulfuro intercatenarios en esta región. El anticuerpo homodimérico generado de esta manera

puede tener una mejor capacidad de internalización y/o una mayor CDC y ADCC (véase, por ejemplo, Caron et al., 1992, J. Exp. Med., 176:1191-1195; y Shopes, 1992, J. Immunol., 148:2918-2922.) Los anticuerpos homodiméricos con actividad antitumoral potenciada pueden también prepararse usando conectores cruzados heterobifuncionales, tal como se describe en Wolff et al., 1993, Cancer Research 53:2560-2565. Como alternativa, un anticuerpo puede modificarse para tener regiones Fc duales y, con ello, puede tener unas capacidades mejoradas de lisis del complemento y de ADCC (véase, por ejemplo, Stevenson et al., 1989, Anti-Cancer Drug Design, 3:219-230).

En la técnica se ha sugerido una diversidad de modificaciones de la región Fc, tanto en la bibliografía científica como en documentos de patentes, por ejemplo, en los documentos EP 0307434, WO 9304173, WO 9734631, WO 9744362, WO 9805787, WO 9943713, WO 9951642, WO 9958572, WO 02060919, WO 03074679, WO 2004016750, WO 2004029207, WO 2004063351, WO 2004074455, WO 2004035752, WO 2004099249, WO 2005077981, WO 2005092925, WO 2006019447, WO 2006031994, WO 2006047350, WO 2006053301, WO 2006088494 y WO 2007041635.

En realizaciones preferidas, los anticuerpos de la invención son variantes de Fc con sustituciones de aminoácidos en las posiciones 332 y/o 239 y/o 236. En realizaciones preferidas, los anticuerpos de la invención tienen mutaciones en el dominio Fc seleccionadas del grupo de:

i) una única sustitución en la posición 332, preferiblemente I332E;

ii) una combinación de sustituciones en las posiciones 239 y 332, preferiblemente S239D/I332E;

iii) una combinación de sustituciones en las posiciones 236 y 332, preferiblemente G236A/I332E;

iv) una combinación de sustituciones en las posiciones 236, 239 y 332, preferiblemente G236A/S239D/I332E;

En este contexto, resulta particularmente preferido introducir mutaciones en el dominio F_c en una o más posiciones seleccionadas de los aminoácidos en las posiciones 332 y/o 239 y/o 236, según el índice de numeración de EU de Kabat. Se prefieren en particular las sustituciones en las posiciones 239 y 332, en especial S239D/I332E.

Los variantes de Fc en los anticuerpos de la presente invención se definen según las modificaciones de los aminoácidos que los componen. Así, por ejemplo,

I332E es un variante de Fc con la sustitución I332E con relación al polipéptido de Fc de origen. De forma similar, S239D/I332E define un variante de Fc con las sustituciones S239D y I332E, y S239D/I332E/G236A define un variante de Fc con las sustituciones S239D, I332E, y G236A con relación al polipéptido de Fc de origen.

Para aumentar la semivida sérica del anticuerpo se puede incorporar un epitopo de unión al receptor de reciclaje en el anticuerpo (en especial un fragmento de anticuerpo), según se describe, por ejemplo, en la patente de EEUU nº 5.739.277. Tal como se emplea en la presente, la expresión "epítopo de unión al receptor de reciclaje" se refiere a un epítopo de la región Fc de una molécula de IgG (por ejemplo, IgG_1, IgG_2, IgG_3 o IgG_4) que es responsable de aumentar la semivida sérica in vivo de la molécula de IgG.

Los anticuerpos pueden estar glicosilados en posiciones conservadas en sus regiones constantes (véase, por ejemplo, Jefferis y Lund, 1997, Chem. Immunol., 65:111-128; Wright y Morrison, 1997, TibTECH, 15:26-32). Las cadenas laterales de oligosacáridos de las inmunoglobulinas pueden afectar a la función de la proteína (véase, por ejemplo, Boyd et al., 1996, Mol. Immunol., 32:1311 -1318; Wittwe y Howard, 1990, Biochem. 29:4175-4180), y a la interacción intramolecular entre porciones de la glicoproteína que puede afectar a la conformación y a la superficie tridimensional presentada de la glicoproteína (véase, por ejemplo, Jefferis y Lund, supra; Wyss y Wagner, 1996, Current Opin. Biotech., 7:409-416). Los oligosacáridos también puede actuar para dirigir una glicoproteína concreta a ciertas moléculas basándose en sus estructuras de reconocimiento específicas. Por ejemplo, se ha indicado que en una IgG galactosilada, el resto oligosacárido 'se voltea' hacia fuera del espacio inter-C_H2, y los restos N-acetilglucosamina terminales quedan disponibles para unirse a la proteína de unión a manosa (véase, por ejemplo, Malhotra et al., 1995, Nature Med., 1 :237-243). La eliminación mediante una glicopeptidasa de los oligosacáridos de CAMPATH-1H (un anticuerpo monoclonal IgG1 murino humanizado recombinante que reconoce el antígeno CDw52 de los linfocitos humanos) producida en células de ovario de hámster chino (CHO) produce una reducción completa de la lisis mediada por el complemento (CMCL o CDC) (Boyd et al., 1996, Mol. Immunol., 32:131, 1-1318), mientras que la eliminación selectiva de los restos ácido siálico utilizando neuraminidasa no produce pérdida de CMCL. También se ha indicado que la glicosilación de anticuerpos afecta a la ADCC. En particular, se ha indicado que las células CHO con expresión regulada por tetraciclina de β(1.4)-N-acetilglucosaminiltransferasa III (GnTIII), una glicosiltransferasa que cataliza la

formación de GlcNAc bisectado, tienen una mejor actividad ADCC (véase, por ejemplo, Umana et al., 1999, Nature Biotech., 17:176-180).

La glicosilación de los anticuerpos generalmente es una N-glicosilación o una O-glicosilación. La N-glicosilación se refiere a la unión del resto carbohidrato a la cadena lateral de un resto asparagina. Las secuencias tripeptídicas asparagina-X-serina y asparagina-X-treonina, en las que X es cualquier aminoácido excepto prolina, son las secuencias de reconocimiento para la unión enzimática del resto carbohidrato a la cadena lateral de la asparagina. De esta manera, la presencia de cualquiera de estas secuencias tripeptídicas en un polipéptido crea un sitio de glicosilación potencial. La O-glicosilación se refiere a la unión de uno de los azúcares N-acetilgalactosamina, galactosa o xilosa a un hidroxiaminoácido, de modo más habitual serina o treonina, aunque también puede usarse 5-hidroxiprolina o 5-hidroxilisina.

Los variantes de glicosilación de los anticuerpos son variantes en los que el patrón de glicosilación del anticuerpo está alterado. La alteración significa delecionar uno o más restos carbohidrato del anticuerpo, añadir uno o más restos carbohidrato al anticuerpo, cambiar la composición de la glicosilación (es decir, el patrón de glicosilación), el grado de la glicosilación, o similares.

La adición de sitios de glicosilación al anticuerpo se logra de modo conveniente alterando la secuencia de aminoácidos de manera que contenga una o más de las secuencias tripeptídicas descritas anteriormente (para los sitios de N-glicosilación). La alteración también puede realizarse mediante la adición o la sustitución de uno o más restos serina o treonina en la secuencia del anticuerpo original (para los sitios de O-glicosilación). De manera similar, la eliminación de sitios de glicosilación puede lograrse mediante la alteración de aminoácidos dentro de los sitios de glicosilación nativos del anticuerpo.

La secuencia de aminoácidos se altera habitualmente alterando la secuencia del ácido nucleico subyacente. Estos métodos incluyen, aunque no se limitan al aislamiento de una fuente natural (en el caso de variantes de secuencia de aminoácidos naturales) o la preparación mediante mutagénesis mediada por oligonucleótidos (o dirigida al sitio), una mutagénesis de PCR, o una mutagénesis de módulos de un variante preparado anteriormente o una versión no variante del anticuerpo.

La glicosilación (incluyendo el patrón de glicosilación) de los anticuerpos también puede alterarse sin alterar la secuencia de aminoácidos o la secuencia de nucleótidos subyacente. La glicosilación depende en gran medida de la célula

hospedante utilizada para expresar el anticuerpo. Puesto que el tipo de célula utilizado para la expresión de glicoproteínas recombinantes, por ejemplo anticuerpos, como producto terapéutico potencial en muy pocas ocasiones es la célula nativa, pueden esperarse variaciones significativas en el patrón de glicosilación de los anticuerpos (véase, por ejemplo, Hse el al., 1997, Biol. Chem., 272:9062-9070.) Además de la elección de las células hospedantes, otros factores que afectan a la glicosilación durante la producción recombinante de anticuerpos incluyen el modo de crecimiento, la formulación del medio, la densidad del cultivo, la oxigenación, el pH, los programas de purificación, y similares. Se han propuestos diversos métodos para alterar el patrón de glicosilación que se obtiene en un organismo hospedante concreto, que incluyen introducir o sobre-expresar ciertas enzimas implicadas en la producción de oligosacáridos (véanse, por ejemplo, las patentes de EEUU nº 5.047.335; 5.510.261; y 5.278.299). La glicosilación, o ciertos tipos de glicosilación, puede eliminarse de forma enzimática de la glicoproteína, por ejemplo utilizando una endoglicosidasa H (Endo H). Además, la célula hospedante recombinante puede modificarse genéticamente, por ejemplo, hacer que sea defectuosa para procesar ciertos tipos de polisacáridos. Estas técnicas y otras similares son muy conocidas en la técnica.

La estructura de glicosilación de los anticuerpos puede analizarse con facilidad mediante técnicas convencionales de análisis de carbohidratos, incluyendo una cromatografía de lectina, RMN, espectrometría de masas, HPLC, GPC, análisis de la composición de monosacáridos, digestión enzimática secuencial, y HPAEC-PAD, que emplea una cromatografía de intercambio aniónico a pH alto para separar oligosacáridos basándose en la carga. También se conocen métodos para liberar los oligosacáridos para objetivos analíticos e incluyen, sin limitación, un tratamiento enzimático (realizado de modo habitual empleando péptido-N-glicosidasa F/endo-β-galactosidasa), la eliminación utilizando un entorno alcalino severo para liberar principalmente estructuras O-enlazadas, y métodos químicos que emplean la hidrazina anhidra para liberar los oligosacáridos N- y O-enlazados.

Los anticuerpos también pueden tener modificaciones (por ejemplo, sustituciones, deleciones o adiciones) en restos aminoácidos que interaccionan con receptores Fc. En particular, los anticuerpos pueden tener modificaciones en los restos aminoácidos identificados por su implicación en la interacción entre el dominio anti-Fc y el receptor FcRn (véase, por ejemplo, la publicación internacional nº WO 97/34631).

En su aspecto más amplio, la presente invención se refiere a agentes de unión a CD33 que se unen al CD33 humano, y que se definen porque:

a) tienen una región variable de cadena pesada que comprende CDR1, CDR2 y CDR3, y una región variable de cadena ligera que comprende CDR4, CDR5 y CDR6, en los que CDR1 tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:1-14, CDR2 tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:15-28, CDR3 tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:29-42, CDR4 tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:43-56, CDR5 tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:57-70, CDR6 tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:71-84, o

b) reconocen un epitopo dentro de la secuencia de aminoácidos FFHPIPYYDKNSPVHGYW (SEQ ID NO:141) del CD33 humano.

La presente invención proporciona además agentes de unión a CD33, en los que la cinética de internalización de los agentes de unión a CD33 es tal que al menos 30% de la cantidad inicial del anticuerpo permanece sobre la superficie celular de células HL60 a las 4 horas después de la incubación.

Se ha descubierto que los agentes de unión a CD33 según la presente invención se unen a un epitopo diferente que el del lintuzumab (véase el ejemplo 4 en la presente). Ambos epitopos (SEQ ID NO:141 y SEQ ID NO:142) no se solapan. Se cree que los diferentes epitopos sobre el dominio extracelular del CD33, que son reconocidos por los agentes de unión a CD33 según la presente invención y por el lintuzumab, son la razón del distinto comportamiento de internalización y actuación de ADCC de los agentes de unión a CD33 según la presente invención y el lintuzumab (cf. los ejemplos 2 y 3 en la presente).

En una realización preferida, al menos 40% de la cantidad inicial de los agentes de unión a CD33 permanecen sobre la superficie celular a las 4 horas después de la incubación.

En una realización preferida, la región variable de cadena pesada comprende una secuencia de aminoácidos seleccionada de SEQ ID NO:85-98, y la región variable de cadena ligera comprende una secuencia de aminoácidos seleccionada de SEQ ID NO:99-112.

En una realización preferida, el agente de unión a CD33 tiene una cadena pesada que tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:113-126, y una cadena ligera que tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:127-140.

En una realización preferida, el agente de unión a CD33 tiene una afinidad por

el CD33 humano y por el CD33 del macaco cangrejero con una K_D igual o menor que 10 nM.

En una realización preferida en la presente, el agente de unión a CD33 está humanizado.

En una realización preferida en la presente, el agente de unión a CD33 es totalmente humano.

En una realización preferida, el agente de unión a CD33 comprende también una función efectora.

En una realización preferida, la función efectora está mediada por un dominio F_c.

En una realización preferida, el agente de unión a CD33 comprende una o más mutaciones en el dominio F_c que modulan la función del dominio F_c.

En una realización preferida, la modulación de la función del dominio F_c es un aumento de la ADCC en al menos 10%, preferiblemente 50% o 100%.

Los agentes de unión a CD33 particularmente preferidos según la presente invención se listan en la tabla 1:

nº	Conmº ID	SEQ ID CDR1	SEQ ID CDR2	SEQ ID CDR3	SEQ ID CDR4	SEQ ID CDR5	SEQ ID CDR6	SEQ ID V_H	SEQ ID V_L	SEQ ID cadena pesada	SEQ ID cadena ligera
1	280-03-08	1	15	29	43	57	71	85	99	113	127
2	280-21-09	2	16	30	44	58	72	86	100	114	128
3	280-29-12	3	17	31	45	59	73	87	101	115	129
4	280-31-01	4	18	32	46	60	74	88	102	116	130
5	280-31-01(mut)	5	19	33	47	61	75	89	103	117	131
6	280-34-02	6	20	34	48	62	76	90	104	118	132
7	280-50-01	7	21	35	49	63	77	91	105	119	133
8	280-50-01(mut)	8	22	36	50	64	78	92	106	120	134
9	280-61-07	9	23	37	51	65	79	93	107	121	135
10	283-03-03	10	24	38	52	66	80	94	108	122	136
11	283-05-01	11	25	39	53	67	81	95	109	123	137
12	283-07-03	12	26	40	54	68	82	96	110	124	138
13	283-11-03	13	27	41	55	69	83	97	111	125	139
14	283-14-01	14	28	42	56	70	84	98	112	126	140

Tabla 1

Tratamiento del cáncer

Varias publicaciones han descrito al CD33 como marcador de la superficie celular principalmente sobre células AML y CML que se expresa sobre las células malignas del 70% al 100% de los pacientes (Scheinberg et al., 1989; Hauswirt et al., 2007; Plesa et al., 2007; Webber et al., 2008). El CD33 se expresa sobre células blásticas mieloides malignas, que representan la mayoría de las células malignas en la sangre periférica y en la médula ósea de pacientes con leucemia, y sobre células precursores leucémicas, un número relativamente pequeño de células menos diferenciadas en la médula ósea que se caracterizan por su capacidad para la autorrenovación y el mantenimiento de la jerarquía clonal leucémica. La viabilidad clínica del transporte dirigido al CD33 utilizando anticuerpos se ha demostrado con Mylotarg® (gemtuzumab ozogamizina), un conjugado de anticuerpo-calicheamicina que ha sido aprobado para el tratamiento de pacientes con AML en recaída no elegible para otras opciones de tratamiento. Una estrategia alternativa dirigida al CD33 es el desarrollo del lintuzumab (SGN-33, HuM195), un anticuerpo monoclonal IgG1 humanizado que ha demostrado señales tempranas de eficacia en ensayos clínicos de fase I (Raza et al., 2009). Tomados conjuntamente, existe una abundancia de datos preclínicos y clínicos que subrayan la importancia y la viabilidad del transporte dirigido a CD33 para el tratamiento de la AML y otras malignidades positivas a CD33.

La leucemia mieloide aguda (AML) es una malignidad del linaje mieloide de los leucocitos. Esta neoplasia hematológica es una enfermedad de la sangre y de la médula ósea que si se deja sin tratar generalmente resulta fatal en semanas a meses. Hay una frecuencia estimada de 30000 casos de AML en EEUU y de 47000 en la Unión Europea (datos de frecuencia de 10 años confirmados por Mattson-Jack, 2010). La AML es la forma más frecuente de leucemia aguda en adultos (aproximadamente 90%), y comprende aproximadamente 33% de los nuevos casos de leucemia. La edad media de los pacientes diagnosticados con AML es de 67 años. La AML es responsable de aproximadamente 1,2% de las muertes por cáncer en EEUU.

La AML provoca síntomas no específicos, tales como pérdida de peso, fatiga, fiebre, y sudores nocturnos. La AML se diagnostica mediante ensayos sanguíneos, estudio de la médula ósea, y ensayos de laboratorio para determinar el subtipo de AML y para determinar las decisiones de tratamiento.

La terapia para la AML depende en gran medida de la edad y el estado de actuación del paciente. Los pacientes que pueden tolerar una quimioterapia de inducción intensiva (y la posterior consolidación y mantenimiento) se tratarán intensamente con una combinación de fármacos citotóxicos. Es probable que estos

pacientes logren una respuesta completa de aproximadamente 75%. En esta población de pacientes, el objetivo terapéutico es la curación. Aún así, se produce una recaída de la AML en aproximadamente la mitad de los pacientes en el año después de haber logrado una respuesta completa. Las proporciones de curación a largo plazo están en el radio del 30%.

Sin embargo, una edad avanzada en el momento del diagnóstico o la existencia de co-morbilidades no permite la administración de la terapia de inducción intensiva, lo cual conduce a un objetivo de tratamiento paliativo. Por tanto, las proporciones de remisión disminuyen significativamente en pacientes más ancianos con AML. La mediana de la supervivencia en pacientes más ancianos con AML es menor que 6 meses.

En un aspecto, los agentes de unión a CD33 son útiles para tratar el cáncer, tal como retrasando el avance de un cáncer y/o reduciendo la caquexia asociada al cáncer, o previniendo o retrasando la recurrencia de una malignidad hematológica (por ejemplo, leucemia) en un mamífero, preferiblemente un paciente humano. El agente de unión a CD33 puede administrarse por sí solo o coadministrarse con otro agente terapéutico. En algunas realizaciones, el agente de unión a CD33 se coadministra con un producto quimioterapéutico según las normas asistenciales. El agente de unión a CD33 puede administrarse en forma no conjugada (es decir, no conjugado con una citotoxina) o como un conjugado.

En esta subsección, un "paciente" es un ser humano u otro mamífero que está sometiéndose a un tratamiento o que se le ha diagnosticado cáncer.

En algunas realizaciones, los agentes de unión a CD33 son útiles para retrasar el avance de un cáncer y/o para reducir la caquexia asociada a cáncer en un paciente, mediante la administración al paciente que lo necesite una dosificación eficaz del agente de unión a CD33. Sin querer limitarse a un mecanismo concreto, el agente de unión a CD33 se une a células efectoras o accesorias de los linajes mieloides o monocíticos (por ejemplo, monocitos, macrófagos, células dendríticas, y neutrófilos), inhibiendo o reduciendo con ello la producción de diversas citoquinas, quimioquinas y factores del crecimiento desde las células efectoras o accesorias y/o las células tumorales. Estas citoquinas, quimioquinas y factores del crecimiento, que pueden estimular el crecimiento y la proliferación de células tumorales y/o contribuir a la caquexia del cáncer, incluyen, pero no se limitan a interleuquina-1β (IL-1β), factor de necrosis tumoral-α (TNF-α), interleuquina-6 (IL-6), interleuquina-8 (IL-8), interferón-γ (IFN-γ), factor del crecimiento endotelial vascular (VEGF), factor inhibidor de leucemia

(LIF), proteína quimioatrayente de monocitos-1 (MCP-1), RANTES, interleuquina-10 (IL-10), interleuquina-12 (IL-12), metaloproteinasa de matriz 2 (MMP2), IP-10 y/o proteína inflamatoria de macrófagos 1α (MIP1α). Los agentes de unión a CD33 también pueden reducir la migración de macrófagos al sitio de las células tumorales.

En algunas realizaciones, la administración de una dosificación eficaz de un agente de unión a CD33 a un paciente reduce los niveles de al menos una citoquina, quimioquina o factor del crecimiento, pudiendo dicha citoquina, quimioquina o factor del crecimiento estimular el crecimiento y la proliferación de las células tumorales, estimular la migración de células efectoras no malignas, tales como macrófagos asociados a tumores (TAMS), hacia la vecindad del sitio del tumor y/o contribuir a la caquexia del cáncer. En realizaciones específicas, la citoquina, la quimioquina o el factor del crecimiento es, por ejemplo, interleuquina-1β (IL-1β), factor de necrosis tumoral-α (TNF-α), interleuquina-6 (IL-6), interleuquina-8 (IL-8), interferón-γ (IFN-γ), factor del crecimiento endotelial vascular (VEGF), factor inhibidor de leucemia (LIF), proteína quimioatrayente de monocitos-1 (MCP-1), RANTES, interleuquina-10 (IL-10), interleuquina-12 (IL-12), metaloproteinasa de matriz 2 (MMP2), IP-10 y/o proteína inflamatoria de macrófagos 1α (MIP1α).

En otra realización, se proporciona un método para retrasar el avance de un cáncer mediante la administración a un paciente de un régimen eficaz de un agente de unión a CD33 que puede unirse de modo específico a CD33. Como resultado de la administración del agente de unión a CD33 se retrasa el avance del cáncer, tal como reduciendo el crecimiento o la proliferación de las células tumorales, disminuyendo la metástasis, reduciendo el nivel de al menos una citoquina, quimioquina o factor del crecimiento, reduciendo la células efectoras no malignas en la vecindad de las células tumorales, o similares.

En otra realización, se proporciona un método para retrasar la carga tumoral en un paciente mediante la administración al paciente de un régimen eficaz de un agente de unión a CD33 que puede unirse de modo específico a CD33. Como resultado de la administración del agente de unión a CD33, la carga tumoral en el paciente se detiene o se reduce, tal como reduciendo el tamaño o la masa del tumor, reduciendo el nivel de al menos una citoquina, quimioquina o factor del crecimiento, reduciendo las células efectoras no malignas en la vecindad de las células tumorales, inhibiendo la migración de macrófagos en la vecindad de las células tumorales, reduciendo el número de células efectoras no malignas (por ejemplo, TAMS o macrófagos) en el tumor, o similares.

En otra realización, se proporciona un método para reducir la carga tumoral o para retrasar el avance de un cáncer en un paciente mediante la administración al paciente de un régimen eficaz de un agente de unión a CD33 que puede unirse de modo específico a CD33. Como resultado de la administración del agente de unión a CD33, la carga tumoral en el paciente se detiene o se reduce, tal como reclutando células efectoras inmunológicas, como células NK o macrófagos o monocitos, que pueden destruir las células tumorales mediante mecanismos de mediación inmunológica.

La citotoxicidad celular dependiente de anticuerpos (ADCC) es un mecanismo mediado por células efectoras inmunológicas que puede contribuir a la actividad antitumoral de anticuerpos monoclonales (Weiner G.J., Monoclonal antibody mechanisms of action in cancer, Immunol Res., 2007, 39(1-3):271-278). La importancia de la ADCC para una eficacia antitumoral ha sido demostrada en modelos preclínicos, por ejemplo, en modelos tumorales de ratón (por ejemplo, Clynes R.A., Towers T.L., Presta L.G., Ravetch J.V., Inhibitory Fc receptors modulate in vivo cytoxicity against tumor targets, Nat. Med., abril de 2000, 6(4):443-446). Los datos de ensayos clínicos apoyan la importancia de la ADCC para una eficacia clínica de anticuerpos terapéuticos (por ejemplo, Weng W.K., Levy R., Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma, J. Clin. Oncol., 1 de noviembre de 2003, 21(21):3940-3947, Epub 15 de septiembre de 2003). Las interacciones de los anticuerpos monoclonales con los receptores de Fc sobre células inmunológicas contribuyen a la ADCC. El Fc de los anticuerpos puede modificarse para que muestre una afinidad potenciada por los receptores de Fc (por ejemplo, Presta L.G., Engineering of therapeutic antibodies to minimize immunogenicity and optimize function, Adv. Drug Deliv. Rev., 7 de agosto de 2006, 58(5-6):640-656, Epub 23 de mayo de 2006). Esta afinidad potenciada por los receptores de Fc produce una mayor actividad ADCC que puede conducir a una mayor eficacia antitumoral en pacientes.

En las diversas realizaciones descritas en esta sección, el agente de unión a CD33 puede utilizarse para tratar un cáncer positivo a CD33 (es decir, un cáncer que comprende células del cáncer que sobre-expresan el CD33 sobre su superficie celular, o que expresan el CD33 a niveles considerados aceptables para la terapia con anticuerpos CD33). El agente de unión a CD33 también puede utilizarse para tratar un cáncer que no sobre-expresa el CD33 sobre las células efectoras no malignas, con relación al tejido normal del mismo tipo. El cáncer puede ser, por ejemplo, una

malignidad no hematológica o una malignidad hematológica. En ejemplos específicos, la malignidad hematológica puede ser positiva a CD33, y puede ser, por ejemplo leucemia linfoide aguda, leucemia mieloide aguda, leucemia mielomonocítica crónica, una leucemia eritrocítica, leucemia megacarioblástica aguda, linfoma histiocítico, un sarcoma mieloide, un trastorno proliferativo de células cebadas, o el síndrome mielodisplásico (MDS). En algunas realizaciones, la malignidad hematológica es una malignidad positiva a CD33, tal como leucemia mieloide aguda o síndrome mielodisplásico (MDS).

En las diversas realizaciones descritas en esta sección, el agente de unión a CD33 puede ser un anticuerpo anti-CD33 no conjugado. Por ejemplo, el anticuerpo puede ser un anticuerpo totalmente humano, humanizado o quimérico, tal como un anticuerpo M 195 quimérico o humanizado. El anticuerpo también puede ser otro anticuerpo, tal como un anticuerpo que compite con el anticuerpo M 195 por la unión específica a CD33. El anticuerpo también puede unirse al mismo epitopo que el anticuerpo M 195 o a un epitopo diferente.

En otras realizaciones, el agente de unión a CD33 puede unirse (es decir, conjugarse) con una citotoxina. La citotoxina puede ser, por ejemplo, una toxina peptídica, tal como saporina, ricina, clorotoxina, exotoxina de *Pseudomonas*, endotoxina de *Pseudomonas* o toxina de la difteria. La citotoxina también puede ser una toxina química (es decir, con una base no peptídica), tal como calicheamicina, doxorrubicina, una camptotecina, daunorrubicina, u otros agentes de unión al ADN. La citotoxina también puede ser una auristatina, un maitansinoide, una dolastatina, u otros agentes bloqueantes de microtúbulos.

Un agente de unión a CD33 que es un anticuerpo anti-CD33 puede administrarse al paciente por vía intravenosa o subcutánea, a una dosis de 0,1 mg/kg o menor, a aproximadamente 25 mg/kg, preferiblemente de 1,0 mg/kg a aproximadamente 10 mg/kg. Un agente de unión a CD33 que es un fragmento de un anticuerpo anti-CD33 u otra proteína de unión a CD33 puede administrarse en una dosificación equivalente a una dosis de 0,1 mg/kg a aproximadamente 25 mg/kg, de 1,0 mg/kg a aproximadamente 10 mg/kg del anticuerpo intacto. El agente de unión a CD33 puede administrarse por vía intravenosa o subcutánea al paciente según un programa que sea, por ejemplo, diario, semanal, bisemanal, trisemanal (es decir, cada tres semanas) o mensual, o una combinación de éstos, al paciente. El agente de unión a CD33 puede administrarse durante un periodo de al menos un mes, al menos dos meses, al menos tres meses, al menos cuatro meses, al menos cinco meses, al

menos seis meses, o más, según sea necesario. En algunas realizaciones, a la fase de tratamiento (supra) con el agente de unión a CD33 le sigue una fase de mantenimiento, en la que se administran dosis del agente de unión a CD33 con menos frecuencia que durante la fase de tratamiento. Por ejemplo, pueden administrarse dosis de mantenimiento semanales, bisemanales, trisemanales o mensuales, durante un periodo de 1-6 meses. Las dosificaciones en la fase de mantenimiento pueden ser iguales que las dosificaciones en la fase de tratamiento.

Tratamiento de una malignidad hematológica en remisión

En otro aspecto, se proporcionan métodos para prevenir o retrasar la recurrencia de una malignidad hematológica (por ejemplo, leucemia) en un paciente, mediante la administración al paciente en remisión de la malignidad hematologica de una dosificación eficaz de un agente de unión a CD33, lo cual resulta en prevenir o retrasar la recurrencia de la malignidad hematológica subyacente. El agente de unión a CD33 se une de modo específico al CD33 sobre la superficie de la malignidad hematológica (es decir, la célula leucémica) y/o a células efectoras no malignas.

En esta descripción, un "paciente" generalmente es un ser humano que está sometiéndose a un tratamiento o que se le ha diagnosticado una malignidad hematológica. En algunas realizaciones, la malignidad hematológica es una malignidad hematológica positiva a CD33. Las malignidades hematológicas incluyen, pero no se limitan a leucemias (por ejemplo, leucemia linfoblástica aguda (ALL), leucemia mielógena aguda (AML), leucemia mielógena crónica (CML), leucemia mielomonocítica crónica, y leucemia de células pilosas. Los trastornos sanguíneos relacionados incluyen, pero no se limitan a síndrome mielodisplásico (MDS), mielofibrosis, enfermedad mieloproliferativa (por ejemplo, policitemia vera (PV, PCV o PRV), trombocitosis esencial (ET), y amiloides debidos a una enfermedad de la cadena ligera.

La expresión "malignidad hematológica positiva a CD33" se refiere a una malignidad hematológica caracterizada por la expresión del CD33 sobre la superficie de las células malignas. Las malignidades hematológicas positivas a CD33 incluyen, pero no se limitan a leucemia mieloide aguda (AML), leucemia mieloide crónica (CML), leucemia mielomonocítica crónica, leucemia de trombocitos, un síndrome mielodisplásico, un trastorno mieloproliferativo, anemia refractaria, un síndrome de preleucemia, una leucemia linfoide, o una leucemia indiferenciada.

En algunas realizaciones, los métodos incluyen la administración a un paciente en remisión de una malignidad hematológica positiva a CD33 de un régimen eficaz de un agente de unión a CD33, por lo cual la recurrencia de la malignidad hematológica se previene o se retrasa. En algunas realizaciones, el paciente carece de células detectables de la malignidad hematológica. Tal como se emplea en la presente, una "falta de células detectables" se determina mediante métodos de diagnóstico o pronóstico convencionales. Un paciente en remisión de la AML muestra generalmente una resolución de las características clínicas anómalas, la vuelta a los recuentos sanguíneos normales, y una hematopoyesis normal en la médula ósea con <5% de células blásticas, un recuento de neutrófilos de >1.000-1.500, un recuento de plaquetas de >100.000, y la desaparición del clon leucémico. Véase, por ejemplo, The Merck Manual, sec. 11, cap. 138 (17ª ed., 1997); Estey, 2001, Cancer, 92(5):1059-1073.

El agente de unión a CD33 puede ser, por ejemplo, un anticuerpo que se une de modo específico a CD33 y la malignidad hematológica puede ser leucemia mieloide aguda (AML), leucemia mieloide crónica (CML), leucemia mielomonocítica crónica, leucemia timoide, un síndrome mielodisplásico, un trastorno mieloproliferativo, anemia refractaria, una síndrome de preleucemia, una leucemia linfoide, o una leucemia indiferenciada.

En algunas realizaciones, el paciente en remisión de la malignidad hematológica no ha sido sometido a un transplante de médula ósea. En otras realizaciones, el paciente en remisión de la malignidad hematológica sí ha sido sometido a un transplante de médula ósea. El transplante de médula ósea puede ser autólogo o puede ser un transplante de médula ósea alogénico.

Los siguientes tipos de cáncer son especialmente adecuados para ser tratados con los anticuerpos según la presente invención: cánceres portados en la sangre que incluyen, pero no se limitan a leucemia linfoblástica aguda "ALL", leucemia de células B linfoblástica aguda, leucemia de células T linfoblástica aguda, leucemia mieloblástica aguda "AML", leucemia promielocitica aguda "APL", leucemia monoblástica aguda, leucemia eritroleucémica aguda, leucemia megacarioblástica aguda, leucemia mielomonocítica aguda, leucemia no linfocítica aguda, leucemia indiferenciada aguda, leucemia mielocítica crónica "CML", leucemia linfocítica crónica "CLL", leucemia de células pilosas, y mieloma múltiple.

Las leucemias agudas y crónicas que son adecuadas para ser tratadas mediante los agente de unión a CD33 incluyen: leucemias linfoblásticas, mielógenas,

linfocíticas, mielocíticas y leucemia trombocítica. Además el síndrome de mielodisplasia, el trastorno mieloproliferativ, la anemia refractaria, el síndrome de preleucemia, la leucemia linfoide o la leucemia indiferenciada pueden tratarse con los agentes de unión a CD33.

Combinaciones con otras sustancias activas

Dependiendo del trastorno a tratar, se pueden usar los agentes de unión a CD33 de la invención por sí mismos o en combinación con uno o más agentes terapéuticos adicionales, en particular seleccionados de agentes que dañan al ADN, que desmetilan el ADN o de unión a tubulina, o compuestos terapéuticamente activos que inhiben la angiogénesis, las vías de transducción de señales o los puntos de control mitóticos en células cancerosas o que tienen función inmunomoduladora (IMID).

El agente terapéutico adicional se puede administrar de modo simultáneo, opcionalmente como un componente de la misma preparación farmacéutica, o antes o después de la administración del agente de unión a CD33.

En ciertas realizaciones, el agente terapéutico adicional puede ser, sin limitación, uno o más inhibidores seleccionados del grupo de inhibidores de la familia EGFR, la familia VEGFR, VEGF, IGF-1R, receptores de insulina, auroraA, auroraB, PLK y PI3 quinasa, FGFR, PDGFR, Raf, KSP o PDK1.

Otros ejemplos de agentes terapéuticos adicionales son los inhibidores de CDK, Akt, Src, Bcr-Abl, cKit, cMet/HGF, Her2, Her3, c-Myc, Flt3, HSP90, antagonistas de erizo, inhibidores de JAK/STAT, Mek, mTor, NFkappaB, el proteasoma, Rho, un inhibidor de la señalización de Wnt o de la señalización de Notch, o un inhibidor de la vía de la ubiquitinación.

Otros ejemplos de agentes terapéuticos adicionales son los inhibidores de la ADN polimerasa, topoisomerasa II, inhibidores de múltiples tirosina quinasas, antagonistas de CXCR4, inhibidores de IL3RA, antagonistas de RAR, inhibidores de KIR, vacunas inmunoterapéuticas, inhibidores de TUB, inductores de Hsp70, inhibidores de la familia de IAP, inhibidores del ADN metiltransferasa, inhibidores de TNF, inhibidores de la tirosina quinasa receptores ErbB1, inhibidores de múltiples quinasas, inhibidores de JAK2, inhibidores de RR, inductores de la apoptosis, inhibidores de HGPRTasa, antagonistas del receptor de histamina H2 y agonistas del receptor CD25.

Ejemplos de inhibidores de aurora son, sin limitación, PHA-739358, AZD-1152, AT-9283, CYC-116, R-763, VX-667, MLN-8045, PF-3814735, SNS-314, VX-689, GSK-1070916, TTP-607, PHA-680626, MLN-8237, BI847325 y ENMD-2076.

Ejemplos de inhibidores de PLK son GSK-461364, BI2536 y BI6727.

Ejemplos de inhibidores de raf son BAY-73-4506 (también un inhibidor de VEGFR), PLX-4032, RAF-265 (también un inhibidor de VEGFR), sorafenib (también un inhibidor de VEGFR), XL-281, nevavar (también un inhibidor de VEGFR) y PLX4032.

Ejemplos de inhibidores de KSP son ispinesib, ARRY-520, AZD-4877, CK-1122697, GSK-246053A, GSK-923295, MK-0731, SB-743921, LY-2523355 y EMD-534085.

Ejemplos de inhibidores de src y/o bcr-abl son dasatinib, AZD-0530, bosutinib, XL-228 (también un inhibidor de IGF-1R), nilotinib (también un inhibidor de PDGFR y de cKit), imatinib (también un inhibidor de cKit), NS-187, KX2-391, AP-24534 (también un inhibidor de EGFR, FGFR, Tie2, Flt3), KM-80 y LS-104 (también un inhibidor de Flt3, Jak2).

Un ejemplo de un inhibidor de PDK1 es AR-12.

Un ejemplo de un inhibidor de Rho es BA-210.

Ejemplos de inhibidores de PI3 quinasa son PX-866, PX-867, BEZ-235 (también un inhibidor de mTor), XL-147, y XL-765 (también un inhibidor de mTor), BGT-226, CDC-0941.

Ejemplos de inhibidores de cMet o HGF son XL-184 (también un inhibidor de VEGFR, cKit, Flt3), PF-2341066, MK-2461, XL-880 (también un inhibidor de VEGFR), MGCD-265 (también un inhibidor de VEGFR, Ron, Tie2), SU-11274, PHA-665752, AMG-102, AV-299, ARQ-197, MetMAb, CGEN-241, BMS-777607, JNJ-38877605, PF-4217903, SGX-126, CEP-17940, AMG-458, INCB-028060, y E-7050.

Un ejemplo de un inhibidor de c-Myc es CX-3543.

Ejemplos de inhibidores de Flt3 son AC-220 (también un inhibidor de cKit y PDGFR), KW-2449, LS-104 (también un inhibidor de bcr-abl y Jak2), MC-2002, SB-1317, lestaurtinib (también un inhibidor de VEGFR, PDGFR, PKC), TG-101348 (también un inhibidor de JAK2), XL-999 (también un inhibidor de cKit, FGFR, PDGFR y VEGFR), sunitinib (también un inhibidor de PDGFR, VEGFR y cKit), y tandutinib (también un inhibidor de PDGFR, y cKit).

Ejemplos de inhibidores de HSP90 son tanespimicina, alvespimicina, IPI-504, STA-9090, MEDI-561, AUY-922, CNF-2024 y SNX-5422.

Ejemplos de inhibidores de JAK/STAT son CYT-997 (que también interacciona

con la tubulina), TG-101348 (también un inhibidor de Flt3), y XL-019.

Ejemplos de inhibidores de Mek son ARRY-142886, AS-703026, PD-325901, AZD-8330, ARRY-704, RDEA-119 y XL-518.

Ejemplos de inhibidores de mTor son temsirolimus, deforolimus (que también actúa como inhibidor de VEGF), everolimus (también un inhibidor de VEGF), XL-765 (también un inhibidor de la PI3 quinasa), y BEZ-235 (también un inhibidor de la PI3 quinasa).

Ejemplos de inhibidores de Akt son perifosina, GSK-690693, RX-0201, y triciribina.

Ejemplos de inhibidores de cKit son masitinib, OSI-930 (también actúa como un inhibidor de VEGFR), AC-220 (también un inhibidor de Flt3 y PDGFR), tandutinib (también un inhibidor de Flt3 y PDGFR), axitinib (también un inhibidor de VEGFR y PDGFR), sunitinib (también un inhibidor de Flt3, PDGFR, VEGFR) y XL-820 (también actúa como un inhibidor de VEGFR- y PDGFR), imatinib (también un inhibidor de bcr-abl), nilotinib (también un inhibidor de bcr-abl y PDGFR).

Ejemplos de antagonistas de erizo son IPI-609, CUR-61414, GDC-0449, IPI-926 y XL-139.

Ejemplos de inhibidores de CDK son seliciclib, AT-7519, P-276, ZK-CDK (que también inhibe a VEGFR2 y PDGFR), PD-332991, R-547, SNS-032, PHA-848125 y SCH-727965.

Ejemplos de inhibidores de proteasomas son bortezomib, carfilzomib, y NPI-0052 (también un inhibidor de NFkappaB).

Ejemplos de inhibidores de proteasomas/inhibidores de la vía de NFkappaB son bortezomib, carfilzomib, NPI-0052, CEP-18770, MLN-2238, PR-047, PR-957, AVE-8680 y SPC-839.

Un ejemplo de un inhibidor de la vía de ubiquitinación es HBX-41108.

Ejemplos de agentes desmetilantes son 5-azacitidina y decitabina.

Ejemplos de agentes antiangiogénicos son los inhibidores de FGFR, PDGFR y VEGF(R), y las talidomidas, tales como agentes seleccionados, sin limitación, de bevacizumab, motesanib, CDP-791, SU-14813, telatinib, KRN-951, ZK-CDK (también un inhibidor de CDK), ABT-869, BMS-690514, RAF-265, IMC-KDR, IMC-18F1, IMiD, talidomida, CC-4047, lenalidomida, ENMD-0995, IMC-D11, Ki-23057, brivanib, cediranib, 1B3, CP-868596, IMC-3G3, R-1530 (también un inhibidor de Flt3), sunitinib (también un inhibidor de cKit y Flt3), axitinib (también un inhibidor de cKit), lestaurtinib (también un inhibidor de Flt3 y PKC), vatalanib, tandutinib (también un inhibidor de Flt3

y cKit), pazopanib, PF-337210, aflibercept, E-7080, CHIR-258, tosilato de sorafenib (también un inhibidor de Raf), vandetanib, CP-547632, OSI-930, AEE-788 (también un inhibidor de EGFR y Her2), BAY-57-9352 (también un inhibidor de Raf), BAY-73-4506 (también un inhibidor de Raf), XL-880 (también un inhibidor de cMet), XL-647 (también un inhibidor de EGFR y EphB4), XL-820 (también un inhibidor de cKit), nilotinib (también un inhibidor de cKit y brc-abl), CYT-116, PTC-299, BMS-584622, CEP-11981, dovitinib, CY-2401401, ENMD-2976 y BIBF1120.

El agente terapéutico adicional se puede seleccionar también de inhibidores de EGFR, y puede ser un inhibidor de EGFR de molécula pequeña o un anticuerpo anti-EGFR. Ejemplos de anticuerpos anti-EGFR, sin limitación, son cetuximab, panitumumab, nimotuzumab, zalutumumab; ejemplos de inhibidores de EGFR de molécula pequeña son gefitinib, erlotinib ,vandetanib (también un inhibidor de VEGFR) y afatinib (también un inhibidor de Her2). Otro ejemplo de un agente modulador de EGFR es la toxina de fusión EGF.

Otros inhibidores de EGFR y/o Her2 útiles para su combinación con un agente de unión a CD33 de la invención son lapatinib, trastuzumab, pertuzumab, XL-647, neratinib, BMS-599626 ARRY-334543, AV-412, mAB-806, BMS-690514, JNJ-26483327, AEE-788 (también un inhibidor de VEGFR), AZD-8931, ARRY-380 ARRY-333786, IMC-11F8, zemab, TAK-285, AZD-4769, y afatinib (inhibidor dual de Her2 y EGFR).

Los inhibidores de la ADN polimerasa útiles para su combinación con un agente de unión a CD33 de la invención son Ara-C/citarabina, clolar/clofarabina.

Un inhibidor de la ADN metiltransferasa útil para su combinación con un agente de unión a CD33 de la invención es vidaza/azacitidina.

Un inductor de la apoptosis útil para su combinación con un agente de unión a CD33 de la invención es trisenox/trióxido de arsénico.

Los inhibidores de la topoisomerasa II útiles para su combinación con un agente de unión a CD33 de la invención son idarrubicina, daunorrubicina y mitoxantrona.

Un antagonista de RAR útil para su combinación con un agente de unión a CD33 de la invención es vesanoide/tretinoína.

Un inhibidor de la HGPRTasa útil para su combinación con un agente de unión a CD33 de la invención es mercapto/mercaptopurina.

Un antagonista del receptor de histamina H2 útil para su combinación con un agente de unión a CD33 de la invención es cepleno/diclorhidrato de histamina.

Un agonista del receptor CD25 útil para su combinación con un agente de unión a CD33 de la invención es IL-2.

El fármaco adicional también puede seleccionarse de agentes que se dirigen a las vías de IGF-1R y del receptor de insulina. Tales agentes incluyen anticuerpos que se unen a IGF-1R (por ejemplo CP-751871, AMG-479, IMC-A12, MK-0646, AVE-1642, R-1507, BIIB-022, SCH-717454, rhu Mab IGFR y nuevas entidades químicas que tienen como diana el dominio quinasa del IGF1-R (por ejemplo OSI-906 o BMS-554417, XL-228, BMS-754807).

Otros agentes pueden combinarse de modo ventajoso en una terapia con el agente de unión a CD33 de la invención son moléculas que se dirigen a CD20, incluyendo anticuerpos específicos de CD20, tales como rituximab, LY-2469298, ocrelizumab, MEDI-552, IMMU-106, GA-101 (= R7159), XmAb-0367, ofatumumab, anticuerpos CD20 radiomarcados, tales como tositumumab y ibritumomab tiuxetano u otras proteínas dirigidas a CD20, tales como SMIP Tru015, PRO-131921, FBT-A05, veltuzumab, y R-7159.

Los agentes de unión a CD33 pueden combinarse con inhibidores de otros antígenos de superficie expresados sobre leucocitos, en particular anticuerpos o moléculas de tipo anticuerpo, por ejemplo anti-CD2 (siplizumab), anti-CD4 (zanolimumab), anti-CD19 (MT-103, MDX-1342, SAR-3419, XmAb-5574), anti-CD22 (epratuzumab), anti-CD23 (lumiliximab), anti-CD30 (iratumumab), anti-CD32B (MGA-321), anti-CD38 (HuMax-CD38), anti-CD40 (SGN40), anti-CD52 (alemtuzumab), y anti-CD80 (galiximab).

Otros agentes que pueden combinarse con los agentes de unión a CD33 son inmunotoxinas, tales como BL-22 (una inmunotoxina anti-CD22), inotuzumab ozogamicina (un conjugado de anticuerpo anti-CD23-calicheamicina), RFT5.dgA (una cadena de la toxina A de ricina anti-CD25), SGN-35 (un conjugado de anti-CD30-auristatina E), y gemtuzumab ozogamicina (un conjugado de anti-CD33-calicheamicina), MDX-1411 (conjugado anti-CD70), o anticuerpos radiomarcados, tales como ^{90}Y-epratuzumab (radioinmunoconjugado anti-CD22).

Además, los agentes de unión a CD33 pueden combinarse con inmunomoduladores, agentes, por ejemplo anticuerpos, que induzcan la apoptosis o modifiquen las vías de transducción de señales, tales como los moduladores del receptor TRAIL mapatumumab (un agonista del receptor TRAIL-1), lexatumumab (un agonista del receptor TRAIL-2), tigatuzumab, apomab, AMG-951 y AMG-655; un anticuerpo anti-HLA-DR (tal como 1D09C3), un anti-CD74, un inhibidor del ligando del

factor de diferenciación de osteoclastos (tal como denosumab), un antagonista de BAFF (tal como AMG-623a) o un agonista de un receptor de tipo Toll (por ejemplo, TLR-4 o TLR-9).

Otros fármacos que pueden utilizarse en combinación con los agentes de unión a CD33 de la presente invención se seleccionan, pero no se limitan a hormonas, análogos hormonales y compuestos antihormonales (por ejemplo, tamoxifeno, toremifeno, raloxifeno, fulvestrant, acetato de megestrol, flutamida, nilutamida, bicalutamida, acetato de ciproterona, finasterida, acetato de buserelina, fludrocortinsona, fluoximesterona, medroxiprogesterona, caproato de hidroxiprogesterona, dietilestilbestrol, propionato de testosterona, fluoximesterona/equivalentes, octreotida, arzoxifeno, pasireotida, vapreotida, adrenocorticoesteroides/antagonists, prednisona, dexametasona, ainoglutetimida), inhibidores de aromatasas (por ejemplo, anastrozol, letrozol, liarozol, exemestano, atamestano, formestano), agonistas y antagonistas de LHRH (por ejemplo, acetato de goserelina, leuprolida, abarelix, cetrorelix, deslorelina, histrelina, triptorelina), antimetabolitos (por ejemplo, antifolatos, tales como metotrexato, trimetrexato, pemetrexed, análogos de pirimidina, tales como 5-fluorouracilo, fluorodesoxiuridina, capecitabina, decitabina, nelarabina, 5-azacitidina, y gemcitabina, análogos de purina y de adenosina, tales como mercaptopurina, tioguanina, azatioprina, cladribina y pentostatina, citarabina, fludarabina, clofarabina); antibióticos antitumorales (por ejemplo, antraciclinas, tales como doxorrubicina, daunorrubicina, epirrubicina y idarrubicina, mitomicina-C, bleomicina, dactinomicina, plicamicina, esplicamicina, actimomicina D, mitoxantrona, mitoxantronidarrubicina, pixantrona, estreptozocina, afidicolina); derivados del platino (por ejemplo, cisplatino, oxaliplatino, carboplatino, lobaplatino, satraplatino); agentes alquilantes (por ejemplo, estramustina, semustina, mecloretamina, melfalano, clorambucilo, busulfano, dacarbazina, ciclofosfamida, ifosfamida, hidroxiurea, temozolomida, nitrosoureas, tales como carmustina y lomustina, tiotepa); agentes antimitóticos (por ejemplo, vinca-alcaloides, tal como vinblastina, vindesina, vinorelbina, vinflunina y vincristina; y taxanos, tales como paclitaxel, docetaxel y sus formulaciones, larotaxel; simotaxel, y epotilonas, tales como ixabepilona, patupilona, ZK-EPO); inhibidores de la topoisomerasa (por ejemplo, epipodofilotoxinas, tales como etopósido y etopofós, tenipósido, amsacrina, topotecano, irinotecano, banoxantrona, camptotecina) y diversos productos quimioterapéuticos, tales como derivados del ácido retinoico, amifostina, anagrelida, interferón-alfa, interferón-beta, interferón-gamma, interleuquina-2, procarbazina, N-

metilhidrazina, mitotano, y porfímero, bexaroteno, celecoxib, etilenmina/metilmelamina, trietilenmelamina, trietilentiofosforamida, hexametilmelamina, y las enzimas L-asparaginasa, L-arginasa y metronidazol, misonidazol, desmetilmisonidazol, pimonidazol, etanidazol, nimorazol, RSU 1069, EO9, RB 6145, SR4233, nicotinamida, 5-bromodesoxiuridina, 5-yododesoxiuridina, bromodesoxicitidina, eritrohidroxinoniladenina, antracendiona, GRN-163L (un antagonista de molde de telomerasa competitivo), SDX-101 (un agonista de PPAR), talabostato (un inhibidor de DPP), forodesina (un inhibidor de PNP), atacicepto (un receptor soluble que se dirige a los miembros de la familia del TNF BLyS y APRIL), agentes neutralizantes de TNF-alfa (Enbrel, Humira, Remicade), XL-844 (un inhibidor de CHK1/2), VNP-40101M (un agente ADN-alquilante), SPC-2996 (un inhibidor de bcl2 antisentido), obatoclax (un inhibidor de bcl2), enzastaurina (un beta-modulador de PKC), vorinistato (un inhibidor de HDAC), romidepsina (un inhibidor de HDAC), AT-101 (un inhibidor de Bcl-2/Bcl-xL), plitidepsina (un depsipéptido de múltiples acciones), SL-11047 (un modulador del metabolismo de poliaminas).

Los agentes de unión a CD33 de la invención también pueden utilizarse en combinación con otras terapias, que incluyen cirugía, transplante de células precursoras, radioterapia, terapia endocrina, modificadores de la respuesta biológica, hipertemia y crioterapia, y agentes para atenuar cualquier efecto adverso (por ejemplo, antieméticos), G-CSF, GM-CSF, fotosensibilizantes, tales como derivados de hematoporfirina, fotofrina, derivados de benzoporfirina, Npe6, etioporpirina de estaño, feoborida-a, bacterioclorofila-a, naftalocianinas, ftalocianinas, ftalocianinas de cinc.

Composiciones farmacéuticas y métodos de administración

Los agentes de unión a CD33 puede estar en cualquier forma que permita administrar la composición a un paciente. Por ejemplo, la composición puede estar en forma de un sólido o de un líquido. El modo preferido de aplicación es parenteral, por infusión o inyección (intravenosa, intramuscular, subcutánea, intraperitoneal, intradérmica), pero también pueden ser aplicables otros modos de aplicación, tales como por inhalación, transdérmico, intranasal, bucal, oral e intratumoral. La administración parenteral incluye inyecciones subcutáneas, inyección intravenosa, intramuscular, intraestemal, o técnicas de infusión. En un aspecto, las composiciones se administran por vía parenteral. En otro aspecto, los compuestos se administran por vía intravenosa.

Las composiciones farmacéuticas pueden formularse para permitir que un compuesto esté biodisponible tras la administración de la composición a un paciente. Las composiciones pueden tomar la forma de una o más unidades de dosificación, en las que, por ejemplo, un recipiente de un compuesto en forma de aerosol puede albergar una pluralidad de unidades de dosificación.

Los materiales utilizados para preparar las composiciones farmacéuticas pueden ser no tóxicos en las cantidades empleadas. Será evidente para los expertos en la técnica que la dosificación óptima del ingrediente o ingredientes activos en la composición farmacéutica dependerá de una diversidad de factores. Los factores pertinentes incluyen, sin limitación, el tipo de paciente (por ejemplo, un ser humano), la forma concreta del compuesto, el modo de administración, y la composición empleada.

El vehículo o portador farmacéuticamente aceptable puede estar en partículas, de forma que las composición están, por ejemplo, en forma de polvo. El vehículo o vehículos pueden ser líquidos, y las composiciones pueden ser, por ejemplo, un líquido inyectable. La composición puede estar en forma de un líquido, por ejemplo, para la inyección parenteral. En una composición para la administración mediante inyección, también puede incluirse uno o más tensioactivos, conservantes, agentes humectantes, agentes dispersantes, agentes suspensores, tampones, estabilizantes y agentes isotónicos.

Las composiciones líquidas, tanto si son disoluciones, suspensiones u otra forma parecida, también pueden incluir uno o más de los siguientes: diluyentes estériles, tales como agua para inyección, disolución salina, preferiblemente disolución salina fisiológica, disolución de Ringer, cloruro de sodio isotónico, aceites no volátiles, tales como mono- o diglicéridos sintéticos que pueden actuar como disolvente o medio de suspensión, polietilenglicoles, glicerina, ciclodextrina, propilenglicol u otros disolventes; estabilizantes, tales como aminoácidos; tensioactivos, tales como polisorbatos; agentes antibacterianos, tales como alcohol bencílico o metilparabeno; antioxidantes, tales como ácido ascórbico o bisulfito de sodio; agentes quelantes, tales como ácido etilendiaminatetraacético; tampones, tales como acetatos, citratos o fosfatos; y agentes para el ajuste de la tonicidad, tales como cloruro de sodio o dextrosa. Una composición parenteral puede estar dentro de una ampolla, una jeringa desechable o un vial de múltiples dosis fabricado de vidrio, plástico u otro material. Un ejemplo de adyuvante es la disolución salina fisiológica. Una composición inyectable es preferiblemente estéril.

Los agentes de unión a CD33 también pueden secarse (liofilizarse, secarse por

pulverización, secarse por pulverización-congelación, secarse con gases supercríticos o casi supercríticos, secarse al vacío, secarse al aire), precipitarse o cristalizarse, o atraparse en microcápsulas que se preparan, por ejemplo, mediante técnicas de coacervación o mediante polimerización interfacial utilizando, por ejemplo, hidroximetilcelulosa o gelatina y poli(metacrilato de metilo), respectivamente, en sistemas de transporte de fármacos coloidales (por ejemplo, liposomas, microesferas de albúmina, nanopartículas y nanocápsulas), en macroemulsiones, o precipitarse o inmovilizarse sobre vehículos o superficies, por ejemplo mediante la tecnnología de pcmc ("protein coated microcrystals", microcristales revestidos con proteína). Estas técnicas se describen en Remington, The Science and Practice of Pharmacy, 21ª edición, Hendrickson R. ed.

La cantidad de la composición que es eficaz para el tratamiento de un trastorno o una afección concretos dependerá de la naturaleza del trastorno o de la afección, y puede determinarse mediante técnicas clínicas convencionales. Además, opcionalmente pueden emplearse ensayos in vitro o in vivo para ayudar a identificar los intervalos de dosificación óptimos. La dosis precisa que se empleará en las composiciones dependerá también de la vía de administración, y de la gravedad de la enfermedad o del trastorno, y deberá decidirse según el criterio del médico y de las circunstancias de cada paciente.

Las composiciones comprenden una cantidad eficaz de un fármaco o fármacos, o agente o agentes, de tal forma que se obtenga una dosificación adecuada. Generalmente, esta cantidad es al menos aproximadamente 0,01% de un fármaco o agente en peso de la composición. Cuando se prevé una administración oral, esta cantidad puede variar en un intervalo de aproximadamente 0,1% a aproximadamente 80% en peso de la composición. En un aspecto, las composiciones orales pueden comprender de aproximadamente 4% a aproximadamente 50% del compuesto en peso de la composición. En otro aspecto, las presentes composiciones se preparan de modo que una unidad de dosificación parenteral contenga de aproximadamente 0,01% a aproximadamente 2% en peso del compuesto.

Para la administración intravenosa, la composición puede comprender de aproximadamente 1 a aproximadamente 50 mg de un fármaco o agente por kg de peso corporal del paciente. En un aspecto, la composición puede incluir de aproximadamente 1, 1,5 ó 2,5 a aproximadamente 50 mg de un fármaco o agente por kg de peso corporal del paciente. En otro aspecto, la cantidad administrada estará en el intervalo de aproximadamente 1, 1,5 ó 2,5 a aproximadamente 25 mg/kg de peso

corporal de un fármaco o agente.

En algunas realizaciones, la dosificación administrada a un paciente es menor que 0,1 mg/kg a aproximadamente 50 mg/kg del peso corporal del paciente (para la conversión a mg/mm^2, puede utilizarse un BSA de 1,8 m^2 y un peso corporal de 80 kg).

Tal como se analiza en la presente, el agente de unión a CD33 puede administrarse por vía intravenosa o subcutánea al paciente según un programa que sea, por ejemplo, diario, semanal, bisemanal, trisemanal o mensual al paciente. Por ejemplo, un agente de unión a CD33 puede administrarse de modo semanal, durante un periodo de 2 a 10 semanas, generalmente 3-6 semanas. En algunas realizaciones, el régimen de dosificación del agente de unión a CD33 mantiene una concentración en suero sanguíneo de anticuerpo de al menos 5 µg/ml o al menos 10 µg/ml durante el ciclo de dosificación. El agente de unión a CD33 puede administrarse, por ejemplo, en 1-8 o más ciclos. En algunas realizaciones, un agente de unión a CD33 se administra de modo crónico a un sujeto.

Como ejemplo, la invención incluye un método para tratar un cáncer, tal como leucemia mieloide, mediante la administración de 0,1 mg/kg a 50 mg/kg, por ejemplo aproximadamente 1,5-8 ó 2,5-8 mg/kg, de un anticuerpo anti-CD33 según la invención de modo semanal. Este tratamiento habitualmente puede continuar durante aproximadamente 1-3 meses, generalmente durante aproximadamente dos meses. En una realización, el programa de dosificación se mantiene hasta que se advierte una reducción en los blastos. Por ejemplo, la dosificación puede continuar hasta aproximadamente 6 meses. A este tratamiento le puede seguir un programa de dosificación menos frecuente, que implica, por ejemplo, dosis bisemanales (o dos veces al mes). Este programa de dosificación puede mantenerse durante 1, 2, 3, 4, 5, 6 meses o más para mantener una reducción en los blastos y/o una remisión.

En algunas realizaciones, un agente profiláctico puede administrarse con un agente de unión a CD33 para minimizar las reacciones de infusión. Los agentes profilácticos adecuados incluyen, por ejemplo, metilprednisolona, difenildramina, acetaminofeno u otro agente adecuado. El agente profiláctico puede administrarse antes o aproximadamente al mismo tiempo que el agente de unión a CD33.

El fármaco o fármacos, o el agente o los agentes, o las composiciones pueden administrarse mediante cualquier vía conveniente, por ejemplo, mediante infusión o inyección en embolada, mediante absorción a través de revestimientos epiteliales o mucocutáneos (por ejemplo, mucosa oral , mucosa rectal e intestinal, etc.). La administración puede ser sistémica o local. Se conocen diversos sistemas de

transporte, por ejemplo, encapsulación en liposomas, micropartículas, microcápsulas, cápsulas, etc., y pueden utilizarse para administrar un compuesto. En ciertas realizaciones, se administra más de un fármaco o agente o composición a un paciente.

Puede resultar deseable administrar uno o más fármacos o agentes o composiciones de modo local al área que necesita tratamiento, según se apropiado para el fármaco o el agente. Esto puede lograrse, por ejemplo, y no como limitación, mediante la infusión local durante una cirugía; la aplicación tópica, por ejemplo junto con una venda para heridas después de una cirugía; mediante inyección: mediante un catéter; mediante un supositorio; o mediante un implante, estando fabricado el implante de un material poroso, no poroso, o gelatinoso, incluyendo membranas, tales como membranas sialásticas, o fibras. En una realización, la administración puede realizarse mediante una inyección directa en el sitio (o el sitio anterior) de un cáncer, un tumor, o un tejido neoplásico o preneoplásico.

El fármaco o fármacos, o el agente o agentes, o las composiciones pueden administrarse en un sistema de liberación controlada, tal como una bomba o diversos materiales poliméricos. En otra realización, un sistema de liberación controlada puede colocarse cerca de la diana del fármaco o fármacos, o del agente o agentes, o de las composiciones, con lo cual se requiere sólo una fracción de la dosis sistémica (véase, por ejemplo, Goodson, en Medical Applications of Controlled Release, vol. 2, pp. 115-138 (1984)). Pueden utilizarse otros sistemas de liberación controlada que se analizan en el informe de Langer (1990, Science, 249:1527-1533).

Los fármacos o los agentes se formulan según procedimientos habituales en forma de una composición farmacéutica adaptada a la administración intravenosa a animales, en particular seres humanos, según sea apropiado para el fármaco o el agente. De forma típica, los vehículos o portadores para la administración intravenosa son disoluciones tampón acuosas isotónicas estériles. Cuando sea necesario, las composiciones también pueden incluir un agente solubilizante. Las composiciones para la administración intravenosa pueden comprender opcionalmente un anestésico local, tal como lignocaína, para mitigar el dolor en el sitio de la inyección. En general, los ingredientes se suministran por separado o mezclados entre sí en la forma de dosificación unitaria, por ejemplo, como un polvo liofilizado seco o concentrado exento de agua en un recipiente sellado herméticamente, tal como una ampolla o un sobre que indica la cantidad de agente activo. Cuando el fármaco o el agente se va a administrar mediante infusión, se puede dispensar, por ejemplo, con una botella de infusión que contenga disolución salina o agua de calidad farmacéutica estéril. Cuando

el fármaco o el agente se va a administrar mediante inyección, puede proporcionarse una ampolla de agua estéril para inyección o disolución salina, de forma que los ingredientes puedan mezclarse antes de la administración.

Las composiciones de agentes terapéuticos también pueden administrarse según formas de dosificación aceptadas, en forma de comprimidos, pastillas, suspensiones acuosas u oleosas, gránulos, polvos, emulsiones, cápsulas, jarabes, o elixires, por ejemplo. Las composiciones para la administración oral pueden contener uno o más agente opcionales, por ejemplo, agentes edulcorantes, tales como fructosa, aspartamo o sacarina; agentes aromatizantes, tales como menta, aceite de gualteria, o cereza; agentes colorantes; y agentes conservantes, para proporcionar una preparación farmacéuticamente agradable al paladar. Además, cuando están en forma de comprimidos o píldoras, las composiciones puede revestirse para retrasar la disgregación y la absorción en el tracto gastrointestinal, proporcionando con ello una acción sostenida a lo largo de un periodo extendido de tiempo. Las membranas selectivamente permeables que rodean a un compuesto conductor osmóticamente activo también son adecuadas para fármacos o agentes para la administración oral. En estas plataformas, el fluido del entorno que rodea a la cápsula es embebido por el compuesto conductor, que se hincha para desplazar al agente o a la composición de agente a través de un orificio. Estas plataformas de transporte pueden proporcionar un perfil de transporte de orden fundamentalmente cero, en oposición a los perfiles con picos de las formulaciones de liberación inmediata. También puede utilizarse un material de retraso en el tiempo, tal como monoestearato de glicerol o estearato de glicerol.

La composición puede incluir diversos materiales que modifican la forma física de una unidad de dosificación sólida o líquida. Por ejemplo, la composición puede incluir materiales que formen una envuelta de revestimiento alrededor de los ingredientes activos. Los materiales que forman la envuelta de revestimiento generalmente son inertes, y pueden seleccionarse, por ejemplo, de azúcar, goma laca, y otros agentes de revestimiento entérico. Como alternativa, los ingredientes activos pueden encapsularse en una cápsula de gelatina.

Las composiciones pueden administrarse a un paciente que las necesite con una frecuencia, o a lo largo de un periodo de tiempo, que es determinado por el médico encargado. Las composiciones pueden administrarse a lo largo de un periodo de 1 día, 2 días, 3 días, 5 días, 7 días, 10 días, 14 días, 21 días, 28 días, un mes, dos meses, o periodos de tiempo más largos. Se entiende que las composiciones pueden

administrarse durante cualquier periodo de tiempo entre 1 día y dos meses o más.

Producción de anticuerpos

Pueden producirse anticuerpos utilizando cualquier método útil para la síntesis de anticuerpos, en particular, tal como mediante expresión recombinante o síntesis química.

La expresión recombinante de anticuerpos, o sus fragmentos o derivados, generalmente implica la construcción de un ácido nucleico que codifique el anticuerpo. Si la secuencia de nucleótidos del anticuerpo es conocida, un ácido nucleico que codifique el anticuerpo o un polipéptido de éste puede ensamblarse a partir de oligonucleótidos sintetizados de modo químico (por ejemplo, según se describe en Kutmeier at al., 1994, BioTechniques, 17:242), lo cual implica la síntesis de oligonucleótidos solapantes que contengan porciones de la secuencia que codifica el anticuerpo, la hibridación y el acoplamiento de estos oligonucleótidos, y después la amplificación de los oligonucleótidos acoplados, por ejemplo mediante PCR.

Como alternativa, una molécula de ácido nucleico que codifica un anticuerpo o un polipéptido de éste puede generarse a partir de una fuente adecuada. Si no está disponible un clon que contenga el ácido nucleico que codifica el anticuerpo concreto, pero la secuencia del anticuerpo es conocida, puede obtenerse un ácido nucleico que codifique el anticuerpo a partir de una fuente adecuada (por ejemplo, un banco de ADNc de anticuerpos, o un banco de ADNc generado a partir de cualquier tejido o célula que exprese la inmunoglobulina), por ejemplo mediante amplificación con PCR empleando cebadores sintéticos hibridables con los extremos 3' y 5' de la secuencia, o mediante clonación utilizando una sonda oligonucleotídica específica para la secuencia génica concreta.

Si un anticuerpo que reconoce de modo específico un antígeno concreto no está disponible en el mercado (o si no está disponible una fuente para un banco de ADNc para clonar un ácido nucleico que codifique dicha inmunoglobulina) pueden generarse anticuerpos específicos para un antígeno concreto mediante cualquier método conocido en la técnica, por ejemplo, mediante la inmunización de un paciente, o un modelo animal adecuado, tal como un conejo o un ratón, para generar anticuerpos policlonales o, más preferiblemente, mediante la generación de anticuerpos monoclonales, por ejemplo como se describe en Kohler y Milstein (1975, Nature, 256:495-497) o como se describe en Kozbor et al. (1983, Immunology Today,

4:72), o en Cole et al. (1985, en Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Como alternativa, puede obtenerse un clon que codifique al menos la porción Fab del anticuerpo mediante la selección de bancos de expresión de Fab (por ejemplo, como se describe en Huse et al., 1989, Science, 246, 1275-1281) para clones de fragmentos Fab que se unen al antígeno específico, o mediante la selección de bancos de anticuerpos (véase, por ejemplo, Clackson et al., 1991, Nature, 352:624; Hane et al., 1997, Proc. Natl. Acad. Sci. USA, 94:4937).

Cuando se ha obtenido una secuencia de ácido nucleico que codifique al menos el dominio variable del anticuerpo, puede introducirse en un vector que contenga la secuencia de nucleótidos que codifica las regiones constantes del anticuerpo (véase, por ejemplo, la publicación internacional nº WO 86/05807; el documento WO 89/01036; y la patente de EEUU nº 5.122.464). Están disponibles vectores que contienen la cadena ligera o pesada completa que permiten la expresión de una molécula de anticuerpo completa. Después, el ácido nucleico que codifica el anticuerpo puede utilizarse para introducir la sustitución o sustituciones, o la deleción o deleciones, necesarias para sustituir (o delecionar) el resto o los restos cisteína de la región variable que participan en un enlace disulfuro intracaternario, por un resto aminoácido que no contenga un grupo sulfhidrilo. Estas modificaciones pueden realizarse mediante cualquier método conocido en la técnica para la introducción de mutaciones o deleciones específicas en una secuencia de nucleótidos, por ejemplo, pero sin limitarse a la mutagénesis química y la mutagénesis dirigida al sitio in vitro (véase, por ejemplo, Hutchinson et al., 1978, J. Biol. Chem., 253:6551).

Además, se han desarrollado técnicas para la producción de "anticuerpos quiméricos" (véase, por ejemplo, Morrison et al., 1984, Proc. Natl. Acad. Sci. USA, 81:851-855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-454). Un anticuerpo quimérico es una molécula en la que diferentes porciones se derivan de diferentes especies animales, tales como aquellas que tienen una región variable derivada de un anticuerpo monoclonal murino y una región constante de una inmunoglobulina humana, por ejemplo, anticuerpos humanizados.

Cuando se ha obtenido una secuencia de ácido nucleico que codifica un anticuerpo, el vector para la producción del anticuerpo puede producirse mediante la tecnología del ADN recombinante utilizando técnicas conocidas en la técnica. Pueden utilizarse los métodos conocidos por los expertos en la técnica para construir vectores de expresión que contengan las secuencias codificadoras del anticuerpo y las señales de control de la transcripción y de la traducción adecuadas. Estos métodos incluyen,

por ejemplo, técnicas de ADN recombinante in vitro, técnicas sintéticas, y recombinación genética in vivo. Véanse, por ejemplo, las técnicas descritas en Sambrook et al. (1990, Molecular Cloning, A Laboratory Manual, 2ª Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; y Sambrook et al., 2001, Molecular Cloning, A Laboratory Manual, 3ª ed., Cold Spring Harbor Publish., Cold Spring Harbor, N.Y.), y Ausubel et al. (eds., 1993-2006, Current Protocols in Molecular Biology, John Wiley & Sons, NY).

Un vector de expresión que comprende la secuencia de nucleótidos de un anticuerpo, o la secuencia de nucleótidos de un anticuerpo, puede transferirse a una célula hospedante mediante técnicas convencionales (por ejemplo, electroporación, transacción liposómica, precipitación con fosfato de calcio o transducción), y las células resultantes entonces se cultivan mediante técnicas convencionales para producir el anticuerpo. En realizaciones específicas, la expresión del anticuerpo se regula mediante un promotor constitutivo, inducible o específico de tejido.

Las células hospedantes utilizadas para expresar el anticuerpo recombinante pueden ser células bacterianas, tales como *Escherichia coli*, o preferiblemente células eucariotas, en especial para la expresión de moléculas de inmunoglobulina recombinantes. En particular, las células de mamífero, tales como las células de ovario de hámster chino (CHO), junto con un vector que contenga el elemento promotor génico temprano intermedio principal del citomegalovirus humano, son un sistema de expresión eficaz para las inmunoglobulinas (véase, por ejemplo, Foecking et al., 1986, Gene, 45:101; Cockett el al., 1990, BioTechnology, 8:2). La línea de células CHO puede ser, por ejemplo, DG44 o CHO-S. En otro ejemplo, un anticuerpo puede expresarse utilizando el sistema CHEF (véase, por ejemplo, la patente de EEUU nº 5.888.809).

Puede utilizarse una diversidad de otros sistemas de vectores de expresión-hospedante para expresar anticuerpos. Estos sistemas de expresión-hospedante representan vehículos mediante los cuales las secuencias codificadoras del anticuerpo pueden producirse y posteriormente purificarse, pero también representan células que, cuando se transforman o se transfectan con las secuencias de nucleótidos codificadoras apropiadas, expresan una molécula de anticuerpo de inmunoglobulina in situ. Estas incluyen, pero no se limitan a microorganismos, tales como bacterias (por ejemplo, *E. coli* y *B. subtilis*) transformadas con vectores de expresión de ADN de bacteriófago recombinante, de ADN plasmídico o de ADN cosmídico que contienen secuencias codificadoras de inmunoglobulinas; levaduras (por ejemplo,

Saccharomyces pichia) transformadas con vectores de expresión de levaduras recombinantes que contiene secuencias codificadoras del anticuerpo; sistemas de células de insecto infectadas con vectores de expresión de virus recombinantes (por ejemplo, baculovirus) que contienen las secuencias codificadoras de inmunoglobulinas; sistemas de células vegetales infectadas con vectores de expresión de virus recombinantes (por ejemplo, virus del mosaico de la coliflor (CaMV) y virus del mosaico del tabaco (TMV)), o transformadas con vectores de expresión de plásmidos recombinantes (por ejemplo, el plásmido Ti) que contiene secuencias codificadoras de anticuerpos; o sistemas de células de mamífero (por ejemplo, células COS, CHO, CHO-S, BH, 293, 293T o 3T3) que albergan construcciones de expresión recombinantes que contienen promotores derivados del genoma de células de mamífero (por ejemplo, el promotor de metalotioneína) o de virus de mamífero (por ejemplo, el promotor tardío de adenovirus; el promotor del virus vacuna 7.5K).

En sistemas bacterianos, una serie de vectores de expresión puede seleccionarse de modo ventajoso dependiendo del uso previsto para el anticuerpo que se está expresando. Por ejemplo, cuando una gran cantidad de dicha proteína se quiere producir, pueden resultar deseables vectores que dirijan la expresión de niveles altos de productos de proteínas de fusión que puedan purificarse con facilidad. Estos vectores incluyen, pero no se limitan al vector de expresión de *E. coli* pUR278 (Ruther et al., 1983, EMBO J., 2:1791-94), en el que la secuencia codificadora del anticuerpo puede acoplarse de modo individual al vector dentro de marco con la región codificadora de lac Z, de modo que se produce una proteína de fusión; vectores pIN (Inouye y Inouye, 1985, Nucleic Acids Res., 13:3101-3109; Van Heeke y Schuster, 1989, J. Biol. Chem., 24:5503-5509); y similares. Los vectores pGEX también pueden utilizarse para expresar polipéptidos extraños en forma de proteínas de fusión con glutatión S-transferasa (GST). En general, dichas proteínas de fusión son solubles y pueden purificarse con facilidad a partir de células lisadas mediante adsorción y unión a una matriz de esferas de glutatión-agarosa, seguido de una elución en presencia de glutatión libre. Los vectores pGEX se diseñan para que incluyan sitios de ruptura de proteasas de factor Xa o trombina, de modo que el producto génico diana clonado pueda liberarse del resto GST.

En un sistema de insectos, puede utilizarse el virus de la polihedrosis nuclear de *Autographa californica* (AcNPV) o el virus análogo de *Drosophila melanogaster* como vector para expresar genes extraños. El virus se desarrolla en células de *Spodopiera frugipenta*. La secuencia que codifica el anticuerpo puede clonarse de

modo individual en regiones no esenciales (por ejemplo, el gen de polihedrina) del virus y colocarse bajo el control de un promotor de AcNPV (por ejemplo, el promotor de polihedrina).

En células hospedantes de mamífero puede utilizarse una serie de sistemas de expresión basados en virus. En los casos en que se utiliza un adenovirus como vector de expresión, la secuencia codificadora del anticuerpo de interés puede acoplarse a un complejo de control de la transcripción/traducción de adenovirus, por ejemplo, el promotor tardío y la secuencia conductora tripartita. Este gen quimérico entonces puede insertarse en el genoma del adenovirus mediante recombinación in vitro o in vivo. La inserción de una región no esencial del genoma vírico (por ejemplo, región E1 o E3) produce un virus recombinante que es viable y es capaz de expresar la molécula de inmunoglobulina en hospedantes infectados (véase, por ejemplo, Logan y Shenk, 1984, Proc. Natl. Acad. Sci. USA, 81 :355-359). También pueden ser necesarias señales de inicio específicas para la traducción eficaz de las secuencias codificadoras del anticuerpo insertadas. Estas señales incluyen el codón de inicio ATG y las secuencia adyacentes. Además, el codón de inicio se relaciona operablemente con el marco de lectura de la secuencia codificadora deseada para asegurar la traducción del inserto entero. Estas señales de control de la traducción y codones de inicio exógenos pueden tener una diversidad de orígenes, tanto naturales como sintéticos. La eficacia de la expresión puede potenciarse mediante la inclusión de elementos potenciadores de la transcripción apropiados, terminadores de la transcripción apropiados, etc. (véase, por ejemplo, Bittner et al., 1987, Methods in Enzymol., 153:51-544).

Además puede elegirse una cepa de célula hospedante para que module la expresión de las secuencias insertadas, o para que modifique y procese el producto génico de la manera específica deseada. Estas modificaciones (por ejemplo, glicosilación) y procesamiento (por ejemplo, ruptura y escisión) de los productos de proteína pueden ser importantes para la función de la proteína. Las diferentes células hospedantes tienen mecanismos característicos y específicos para el procesamiento y la modificación postraduccional de proteínas y productos génicos. Pueden elegirse las líneas celulares o sistemas de hospedantes apropiados para asegurar la correcta modificación y procesamiento de la proteína extraña expresada. Para este objetivo, pueden utilizarse células hospedantes eucariotas que posean la maquinaria celular para el procesamiento adecuado del transcrito primario, la glicosilación y la fosforilación del producto génico. Estas células hospedantes de mamífero incluyen, pero no se limitan a CHO (por ejemplo, DG44 o CHO-S), VERY, BH, HeIa, COS,

MDCK, 293, 293T, 3T3, WI38, BT483, Hs578T, HTB2, BT20 y T47D, CRL7030 y Hs578Bst.

Para la producción de alto rendimiento a largo plazo de proteínas recombinantes se prefiere la expresión estable. Por ejemplo, pueden modificarse líneas celulares que expresen de forma estable un anticuerpo. En lugar de utilizar vectores de expresión que contengan orígenes de la replicación víricos, las células hospedantes pueden transformarse con ADN controlado por los elementos de control de la expresión apropiados (por ejemplo, secuencias promotoras, potenciadoras, terminadores de la transcripción, sitios de poliadenilación, etc.), y un marcador seleccionable. Tras la introducción del ADN extraño, las células modificadas se cultivan durante 1-2 días en un medio enriquecido y después se cambian a un medio selectivo. El marcador seleccionable en el plásmido recombinante confiere resistencia a la selección y permite a las células integrar el plásmido de modo estable en sus cromosomas y crecer para formar focos que, a su vez, pueden clonarse y expandirse en líneas celulares. Este método puede emplearse de modo ventajoso para modificar líneas celulares que expresan el anticuerpo. Estas líneas celulares modificadas pueden ser particularmente útiles para la selección y la evaluación de antígenos tumorales que interaccionan directa o indirectamente con el anticuerpo.

Puede utilizarse una serie de sistemas de selección. Por ejemplo, pueden emplearse los genes de la timidina quinasa del virus del herpes simplex (véase, por ejemplo, Wigler et al., 1977, Cell, 11:223), hipoxantina-guanina fosforribosiltransferasa (véase, por ejemplo, Szybalska y Szybalski, 1992, Proc. Natl Acad. Sci. USA, 48:202), y adenina fosforribosiltransferasa (véase, por ejemplo, Lowy et al. , 1980, Cell, 22:817) en células tk-, hgprt- o aprt-, respectivamente. Además puede utilizarse la resistencia a antimetabolitos como base para la selección de los siguientes genes: DHFR, que confiere resistencia al metotrexato (véase, por ejemplo, Wigler et al., 1980, Proc. Natl. Acad Sci. USA, 77:3567-3570; O'Hare et al., 1981, Proc. Natl. Acad. Sci. USA, 78:1527-1531); gpt, que confiere resistencia al ácido micofenólico (véase, por ejemplo, Mulligan y Berg, 1981, Proc. Natl. Acad. Sci. USA, 78:2072-2076); neo, que confiere resistencia al aminoglicósido G-418 (véase, por ejemplo, Clinical Pharmacy, 12:488-505; Wu y Wu, 1991, Biotherapy, 3:87-95; Tolstoshev, 1993, Arm. Rev. Pharmacol. Toxicol., 32:573-596; Mulligan, 1993, Science, 260:926-932; Morgan y Anderson, 1993, Ann. Rev. Biochem., 62:191-217; y May, 1993, TIB TECH, 11(5):155- 215); e hygro, que confiere resistencia a la higromicina (véase, por ejemplo, Santerre et al. , 1984, Gene, 30:147-150). Los métodos conocidos habitualmente en la técnica de la

tecnología del ADN recombinante que pueden utilizarse se describen en Ausubel et al. (eds.), 1993-2006, Current Protocols in Molecular Biology, John Wiley & Sons, NY; Kriegler, 1990, Gene Transfer and Expression. A laboratory Manual, Stockton Press, NY; y en los capítulos 12 y 13, Dracopoli et al. (eds.), 1994, Current Protocols in Human Genetics, John Wiley & Sons, NY; y Colberre-Garapin et al., 1981 , J. Mol. Biol., 150:1-14).

Los niveles de expresión de un anticuerpo pueden aumentar mediante la amplificación del vector (para un informe, véase Bebbington y Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, vol. 3 (Academic Press, Nueva York, 1987)). Cuando un marcador en el sistema de vector que expresa un anticuerpo es amplificable, un aumento en el nivel de inhibidor presente en el cultivo de células hospedantes aumentará el número de copias del gen marcador. Puesto que la región amplificada está asociada con la secuencia de nucleótidos del anticuerpo, la producción del anticuerpo también aumentará (véase, por ejemplo, Crouse et al., 1983, Mol. Cell. Biol., 3:257-266).

La célula hospedante puede cotransfectarse con dos vectores de expresión, codificando el primer vector un polipéptido derivado de la cadena pesada, y codificando el segundo vector un polipéptido derivado de la cadena ligera. Los dos vectores pueden contener el mismo o diferentes marcadores seleccionables que permitan la expresión igual de los polipéptidos de cadena pesada y ligera. Como alternativa, puede utilizarse un único vector para codificar ambos polipéptidos de cadena pesada y ligera. En estas situaciones, la cadena ligera generalmente se coloca antes que la cadena pesada para evitar un exceso de cadena pesada libre tóxica (véase, por ejemplo, Proudfoot, 1986, Nature, 322:562-565; Kohler, 1980, Proc. Natl. Acad. Sci. USA, 77:2197-2199). Las secuencias codificadoras de las cadenas pesada y ligera pueden comprender ADNc o ADN genómico.

Cuando el anticuerpo ha sido expresado de modo recombinante puede purificarse utilizando cualquier método adecuado para la purificación de un anticuerpo, por ejemplo mediante cromatografía (por ejemplo, de intercambio iónico, de afinidad, en particular de afinidad por un antígeno específico después de la proteína A, y cromatografía en columna de exclusión molecular), centrifugación, solubilidad diferencial, o mediante cualquier otra técnica convencional para la purificación de proteínas.

Una referencia exhaustiva para todas la etapas utilizadas para preparar los

anticuerpos monoclonales según la presente invención es Yokoyama et al., “Production of Monoclonal Antibodies”, Current Protocols in Immunology, unidad 2.5, 2006.

Ejemplos

Ejemplo 1, Afinidad por CD33

Los agentes de unión a CD33 tienen una K_D de afinidad por CD33 humano y de macaco cangrejero de 10 nM o menor en la línea celular HL60 y HEK293-cynomolgus CD33, respectivamente.

Catorce agentes de unión a CD33 (los anticuerpos monoclonales totalmente humanos listados en la tabla 1 como nº 1-14, respectivamente) al CD33 humano y de macao cangrejero se determinaron mediante análisis de Scatchard con FACS según se describe en Brockhoff et al. (Cytometry, 1994, 1 de septiembre,17(1):75-83) sobre células que expresan CD33 (línea celular HL60 derivada de AML, línea celular recombinante HEK293-cynomolgus CD33). Brevemente, se prepararon diluciones de un agente de unión a CD33 en una placa de 96 pocillos, comenzando con 100-400 nM en el primer pocillo (80 µl), seguido de 11 etapas de dilución (1:2, 40 + 40 µl). Se añadieron 50 µl de agente de unión a CD33 a los tubos de FACS, y se añadieron 150 µl de células (0,8 x 10^6/ ml = 1,2 x 10^5 células/tubo) a cada tubo de FACS. Las células se mezclaron con suavidad y se incubaron durante 1 h sobre hielo. Después se añadieron 50 µl de anticuerpo secundario conjugado con FITC (concentración de 15 µg/ml; mAb de ratón anti-IgG humana), se mezcló y se incubó durante 30 min en hielo. Después se añadieron 4 ml de PBS, pH 7,2, que contenía ácido al 0,02%, las células se sedimentaron y se resuspendieron en 300 µl de PBS, pH 7,2, y se sometieron a un análisis de FACS utilizando un BD FACS Canto. Todas las etapas experimentales se realizaron sobre hielo húmedo, y todas las diluciones del agente de unión a CD33 se realizaron en PBS/BSA al 0,5% + ácido al 0,02%. La calibración del FACS se realizó utilizando esferas Quantum FITC MESF (Premix) (Bangs Laboratories). Todas las muestras se midieron utilizando los mismos parámetros de FACS. La proporción de IgG unida frente a IgG libre se calculó a partir de los valores de MFI a diferentes concentraciones de agentes de unión a CD33 y se muestran como una transferencia Scatchard. Se trazó una línea de regresión a través de los puntos de datos resultantes, y la pendiente de esta línea se corresponde con el valor negativo de la constante de

asociación. Los resultados se listan en la tabla 2.

nº	Clon nº ID	CD33 humano K_D (nM)	CD33 de Cynomolgus K_D (nM)
1	280-03-08	0,3	0,03
2	280-21-09	0,4	0,3
3	280-29-12	0,5	0,6
4	280-31-01	0,4	0,3
5	280-31-01(mut)	1	0,5
6	280-34-02	0,3	0,5
7	280-50-01	0,3	0,5
8	280-50-01(mut)	0,4	1,7
9	280-61-07	0,3	0,4
10	283-03-03	0,3	1,9
11	283-05-01	0,2	1,1
12	283-07-03	0,3	2,1
13	283-11-03	0,3	0,6
14	283-14-01	3,2	2,3

Tabla 2

Ejemplo 2: Cinética de internalización

La internalización del anticuerpo se refiere a la reducción de la cantidad de complejos de anticuerpo/antígeno sobre la superficie celular de la célula diana después de la incubación con anticuerpo. Los ensayos de internalización se realizaron con la línea celular HL60 que expresa el CD33. Las células se incubaron con una cantidad fija de un agente de unión a CD33 (10 µg/ml de los anticuerpos monoclonales totalmente humanos listados en la tabla 1 como nº 1-14) durante periodos definidos de tiempo (0 h, 1 h, 4 h, 24 h) a 37 °C para permitir la internalización del complejo de anticuerpo/antígeno. En los momentos del tiempo indicados se añadió ácido a la mezcla de incubación para evitar posteriores internalizaciones. Después se añadió una cantidad fija de agente de unión a CD33 para saturar todos los sitios de antígeno de CD33 sobre la superficie celular. La cantidad total de agente de unión a CD33 unida a la superficie celular se determinó mediante un análisis de FACS utilizando un

anticuerpo secundario anti-IgG humana conjugado con FITC. El momento del tiempo de 0 h se usó para determinar el nivel inicial de complejo de antígeno/anticuerpo de sobre la superficie, y se definió como 100%. Los resultados se listan en la tabla 3 y se ilustran en las figuras 1-3.

nº	Clon nº ID	Complejo de antígeno/anticuerpo de CD33 que permanecesobre la superficie celular después de 4 h de incubación con anticuerpo (%)
1	280-03-08	49
2	280-21-09	41
3	280-29-12	49
4	280-31-01	44
5	280-31-01(mut)	52
6	280-34-02	44
7	280-50-01	53
8	280-50-01(mut)	55
9	280-61-07	47
10	283-03-03	45
11	283-05-01	51
12	283-07-03	48
13	283-11-03	46
14	283-14-01	47

Tabla 3

Se incluye el lintuzumab como anticuerpo de referencia. Los complejos de lintuzumab/CD33 se internalizan con rapidez tras la unión del lintuzumab, lo cual está de acuerdo con los datos publicados. Después de un periodo de incubación de 4 h sólo quedó aproximadamente 20% de la cantidad inicial de los complejos de CD33/lintuzumab sobre la superficie celular. Se pudo demostrar que, de manera inesperada, la internalización de los 14 agentes de unión a CD33 según la presente invención fue frenada comparado con el lintuzumab.

Ejemplo 3: Actividad ADCC

La velocidad de internalización frenada se traduce en una mayor actividad ADCC *in vitro*. Para evaluar el efecto de la internalización frenada sobre la actividad ADCC de los agentes de unión a CD33 (los anticuerpos monoclonales totalmente humanos listados en la tabla 1 como nº 1-14), se incubaron células diana (HL60) con un agente de unión a CD33 durante 0, 1, 4 y 24 h. Posteriormente se realizó un ensayo de ADCC con PBMC estimuladas con IL-2 como células efectoras, y células HL60 revestidas con anticuerpos como células diana. Para todos los experimentos, se empleó una concentración de mAb de 30 µg/ml. El co-cultivo de las células efectoras con las células diana en presencia del agente de unión a CD33 se realizó por cuadruplicado o por triplicado en placas de microvaloración de fondo redondo de 96 pocillos en un volumen final de 200 µl de medio de ensayo por pocillo que consiste en suero humano al 10% y BSA al 1% en RPMI en una proporción 1:1. En primer lugar se cultivaron las células efectoras (células PBMC recién aisladas en 100 µl de suero humano al 10% en RPMI por pocillo), seguido de las células diana y una disolución del agente de unión a CD33 (diluida en 50 µl de BSA al 1% en RPMI). Como control, se cultivaron células efectoras en medio de ensayo solo (control de células efectoras), y las células diana se cultivaron en medio de ensayo solo (lisis espontánea) o en medio de ensayo suplementado con Triton X-100 al 1% (lisis máxima). El co-cultivo se incubó a 37 ºC en un incubador de CO_2 húmedo durante 3 horas. Al final de la incubación, las células se retiraron del medio de cultivo mediante centrifugación (200 x g, es decir, 1000 rpm; 10 min) a temperatura ambiente. Los sobrenadantes exentos de células (100 µl/pocillo) se trasladaron a los correspondientes pocillos de una placa de fondo plano de 96 pocillos. Para determinar la actividad LDH en estos sobrenadantes se añadieron 100 µl de mezcla de reacción (250 µl de catalizador recién mezclado con 11,25 ml de disolución de tinte) a cada pocillo y se incubaron durante 30 min a temperatura ambiente en la oscuridad. Entonces se mide la absorbancia como se describe a continuación.

Se empleó un kit de detección de citotoxicidad (LDH; Roche 11 644 793 001) para medir la actividad ADCC. La detección de la citotoxicidad se basa en la medición de la actividad de la enzima LDH liberada de las células con la membrana plasmática dañada. La LDH liberada hacia los sobrenadantes del cultivo reduce la sal tetrazolio del kit para formar formazano. El máximo de absorción del tinte de formazano se mide a 490 nm frente a una longitud de onda de referencia de 650 nm en un lector de placas de ELISA. Para determinar el porcentaje de citotoxicidad mediada por células, se calculó la absorbancia media de los cultivos realizados por cuadruplicado o por

triplicado, y se restó el fondo. Estos valores corregidos se sustituyeron en la siguiente ecuación para calcular la ADCC (%):

(mezcla de células efectoras/diana-control de células efectoras-liberación espontánea) dividido entre (liberación máxima-liberación espontánea)

La actividad ADCC en el momento 0 h (no se preincuba el anticuerpo con las células diana) se define como 100% de actividad ADCC. La actividad ADCC para diversos momentos del tiempo de preincubación del anticuerpo se calculó con relación al momento 0 h y se muestra como citotoxicidad relativa (%).

La internalización frenada de los agentes de unión a CD33, comparado con el lintuzumab, produjo una mayor actividad ADCC comparado con el lintuzumab. En conclusión, una internalización frenada conduce al aumento de la actividad ADCC. La internalización de los agentes de unión a CD33 descritos se correlaciona inversamente con la actividad ADCC de los agentes de unión a CD33, que es indicativo de las ventajas con respecto a la actividad clínica de dichos agentes de unión a CD33. Los resultados se ilustran en la figura 4 para la cinética de internalización en este experimento, y en la figura 5 para la actividad ADCC.

Ejemplo 4: Cartografiado de epitopos

El epitopo de unión de los agentes de unión a CD33 descritos en la presente, con relación al epitopo del lintuzumab, se determinó mediante una espectrometría de masas de intercambio de hidrógeno (HXMS).

Este método determina la susceptibilidad de los hidrógenos del esqueleto de amida de la proteína de CD33 para intercambiarse con D_2O. Los experimentos se realizaron con la proteína de CD33 recombinante sola, y con la proteína de CD33 con agente de unión a CD33/lintuzumab añadidos (denominado en lo sucesivo en este ejemplo “anticuerpo/anticuerpos”). Así se identifican las regiones de la proteína de CD33 que muestran una protección significativa frente al intercambio debido a la unión del anticuerpo. La resolución del método se determina mediante los péptidos producidos mediante la digestión con pepsina, por ejemplo, la secuencia de aminoácidos resultante puede ser más grande que el epitopo real del anticuerpo. Estos péptidos derivados de CD33 se identificaron mediante otros experimientos de control con muestras no intercambiadas que emplean las tecnologías de masas y de

HPLC MS/MS precisas convencionales.

Para la muestra de proteína + anticuerpo, la proteína de CD33 y el anticuerpo se incubaron durante 15 min a temperatura ambiente. La proporción molar final de anticuerpo/CD33 fue de 2:1. Utilizando un sistema de robot LEAP (la placa de intercambio se mantiene a 25 °C, la placa de muestra/extinción se mantiene a 4 °C), se añadieron 8 ul de muestra a 80 µl de tampón de intercambio (NaH_2PO_4 10 mM en D_2O, pH = 7, o NaH_2PO_4 10 mM en H_2O, pH = 7), se mezcló y se dejó que intercambiase durante diversos tiempos (15, 60, 120, 240, y 600 segundos) a 25 °C. Entonces se trasladaron 80 ul de esta disolución a 80 µl de tampón de extinción (urea 1 M, TCEP-HCl 0,1 M) a 4 °C y se mezcló. Entonces se trasladaron 90 µl de esta disolución a 10 µl de pepsina (4 mg/ml) a 4 °C y se mezcló. Después de 2 minutos se inyectaron 60 µl de esta disolución sobre un cartucho de trampa Michrom C18. El cartucho se lavó con H_2O + TFA al 0,1% durante 2 minutos a 100 µl/min. Entonces se puso en marcha una válvula y el cartucho eluyó sobre una columna Phenomenex Jupiter C5, 1,0 x 50 mm, 5 µm, 300 A. La fase móvil A fue agua/acetonitrilo/TFA (99/0,95/0,05) y la fase móvil B fue acetonitrilo/agua/TFA (95/4,95/0,05). El caudal fue de 100 µl/min. El gradiente fue: 0 minutos (B al 0%), 6 minutos (B al 40%), 7 minutos (B al 40%), 8 minutos (B al 90%), 10 minutos (B al 90%), 11 minutos (B al 0%). El sistema LEAP preenfría la fase móvil hasta 4 °C y también mantiene la columna de trampa y la columna analitica a 4 °C. Para los experimentos de MS (empleados para cuantificar el intercambio con el tampón de D_2O), se empleó un método de un único barrido de 300-2000 durante 14 minutos a una resolución de 60.000. Para los experimentos de MS/MS (empleados para identificar péptidos con el tampón de intercambio de H_2O), se empleó un método con 7 barridos durante 14 minutos. El primer barrido fue un barrido de gama completa de 300-2000 con una resolución de 30.000. Los posteriores barridos fueron barridos CID de los 6 iones más intensos del barrido nº 1. El ancho de aislamiento fue de 1,5 amu, la energía de colisión fue de 35 V, y el tiempo de activación fue de 30 ms. Los péptidos de pepsina se identificaron utilizando los datos de fragmentación y el programa Proteome Discoverer (Thermo). Los péptidos identificados se analizaron utilizando un programa interno que calcula la masa media para los péptidos intercambiados.

Todos los agentes de unión a CD33 protegían el mismo fragmento de péptido con la secuencia de aminoácidos FFHPIPYYDKNSPVHGYW (SEQ ID NO:141) (tabla 4). La secuencia de CD33 protegida por los agentes de unión a CD33 descritos en la presente es diferente y no se solapa con la secuencia peptídica del CD33 protegido

por la unión del lintuzumab (MDPNFWLQVQE, SEQ ID NO:142). En la formación de modelos de silicio, que emplea la estructura cristalina de SIGLEC-5, un miembro de la familia SIGLEC homólogo a CD33, se revelaron los epitopos de unión de todos los anticuerpos en el dominio proximal de la proteína, en los que el epitopo de unión del lintuzumab es diferente de los de los agentes de unión a CD33 descritos en la presente. En conclusión, los agentes de unión a CD33 descritos en esta solicitud de patente se unen a un epitopo diferente que el lintuzumab.

nº	Clon nº ID	Epitopo de CD33
1	280-03-08	FFHPIPYYDKNSPVHGYW
2	280-21-09	FFHPIPYYDKNSPVHGYW
3	280-29-12	FFHPIPYYDKNSPVHGYW
4	280-31-01	FFHPIPYYDKNSPVHGYW
5	280-31-01(mut)	FFHPIPYYDKNSPVHGYW
6	280-34-02	FFHPIPYYDKNSPVHGYW
7	280-50-01	FFHPIPYYDKNSPVHGYW
8	280-50-01(mut)	FFHPIPYYDKNSPVHGYW
9	280-61-07	FFHPIPYYDKNSPVHGYW
10	283-03-03	FFHPIPYYDKNSPVHGYW
11	283-05-01	FFHPIPYYDKNSPVHGYW
12	283-07-03	FFHPIPYYDKNSPVHGYW
13	283-11-03	FFHPIPYYDKNSPVHGYW
14	283-14-01	FFHPIPYYDKNSPVHGYW
15	lintuzumab	MDPNFWLQVQE

Tabla 4

REIVINDICACIONES

1.- Un agente de unión a CD33 que se une al CD33 humano, definiéndose el agente de unión a CD33 porque:

a) tiene una región variable de cadena pesada que comprende CDR1, CDR2 y CDR3, y una región variable de cadena ligera que comprende CDR4, CDR5 y CDR6, en el que CDR1 tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:1-14, CDR2 tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:15-28, CDR3 tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:29-42, CDR4 tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:43-56, CDR5 tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:57-70, CDR6 tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:71-84, o

b) reconoce un epitopo dentro de la secuencia de aminoácidos FFHPIPYYDKNSPVHGYW (SEQ ID NO:141) del CD33 humano.

2.- Un agente de unión a CD33 según la reivindicación 1, en el que el epitopo se determina mediante una espectroscopía de masas de intercambio de hidrógeno.

3.- Un agente de unión a CD33 según una cualquiera de las reivindicaciones anteriores, en el que la cinética de internalización del agente de unión a CD33 es tal que al menos 30% de la cantidad inicial del agente de unión a CD33 permanece sobre la superficie celular de células HL60 a las 4 horas después de la incubación.

4.- Un agente de unión a CD33 según una cualquiera de las reivindicaciones anteriores, en el que la cinética de internalización del agente de unión a CD33 es tal que al menos 40% de la cantidad inicial del agente de unión a CD33 permanece sobre la superficie celular a las 4 horas después de la incubación.

5.- Un agente de unión a CD33 según una cualquiera de las reivindicaciones anteriores, en el que la región variable de cadena pesada comprende una secuencia de aminoácidos seleccionada de SEQ ID NO:85-98, y la región variable de cadena ligera comprende una secuencia de aminoácidos seleccionada de SEQ ID NO:99-112.

6.- Un agente de unión a CD33 según una cualquiera de las reivindicaciones

anteriores, en el que el agente de unión a CD33 tiene una cadena pesada que tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:113-126, y una cadena ligera que tiene una secuencia de aminoácidos seleccionada de SEQ ID NO:127-140.

7.- Un agente de unión a CD33 según una cualquiera de las reivindicaciones anteriores, en el que el agente de unión a CD33 tiene afinidad por el CD33 humano y por el CD33 de macaco cangrejero, con una K_D igual o menor que 10 nM.

8.- Un agente de unión a CD33 según una cualquiera de las reivindicaciones anteriores, en el que el agente de unión a CD33 está humanizado.

9.- Un agente de unión a CD33 según una cualquiera de las reivindicaciones anteriores, en el que el agente de unión a CD33 es totalmente humano.

10.- Un agente de unión a CD33 según una cualquiera de las reivindicaciones anteriores, en el que el agente de unión a CD33 comprende además una función efectora.

11.- Un agente de unión a CD33 según la reivindicación 10, en el que la función efectora es mediada por un dominio F_c.

12.- Un agente de unión a CD33 según la reivindicación 10, en el que el agente de unión a CD33 comprende una o más mutaciones en el dominio F_c que modulan la función del dominio F_c.

13.- Un agente de unión a CD33 según la reivindicación 12, en el que la modulación de la función del dominio Fc es un aumento de la ADCC en al menos 10%, preferiblemente 50%, y más preferiblemente 100%.

14.- Un agente de unión a CD33 según cualquiera de las reivindicaciones 12-13, en el que las mutaciones en el dominio F_c se localizan en una o más posiciones seleccionadas de los aminoácidos en las posiciones 332 y/o 239 y/o 236 según el índice de numeración de EU de Kabat.

15.- Un agente de unión a CD33 según cualquiera de las reivindicaciones 12-

14, en el que las mutaciones en el dominio F_c son una combinación de sustituciones en las posiciones 239 y 332, preferiblemente S239D/I332E.

16.- Un agente de unión a CD33 seleccionado de un anticuerpo que comprende una CDR1 de SEQ ID NO:1, una CDR2 de SEQ ID NO:15, una CDR3 de SEQ ID NO:29, una CDR4 de SEQ ID NO:43, una CDR5 de SEQ ID NO:57, y una CDR6 de SEQ ID NO:71, un anticuerpo que comprende una CDR1 de SEQ ID NO:2, una CDR2 de SEQ ID NO:16, una CDR3 de SEQ ID NO:30, una CDR4 de SEQ ID NO:44, una CDR5 de SEQ ID NO:58, y una CDR6 de SEQ ID NO:72, un anticuerpo que comprende una CDR1 de SEQ ID NO:3, una CDR2 de SEQ ID NO:17, una CDR3 de SEQ ID NO:31, una CDR4 de SEQ ID NO:45, una CDR5 de SEQ ID NO:59, y una CDR6 de SEQ ID NO:73, un anticuerpo que comprende una CDR1 de SEQ ID NO:4, una CDR2 de SEQ ID NO:18, una CDR3 de SEQ ID NO:32, una CDR4 de SEQ ID NO:46, una CDR5 de SEQ ID NO:60, y una CDR6 de SEQ ID NO:74, un anticuerpo que comprende una CDR1 de SEQ ID NO:5, una CDR2 de SEQ ID NO:19, una CDR3 de SEQ ID NO:33, una CDR4 de SEQ ID NO:47, una CDR5 de SEQ ID NO:61, y una CDR6 de SEQ ID NO:75, un anticuerpo que comprende una CDR1 de SEQ ID NO:6, una CDR2 de SEQ ID NO:20, una CDR3 de SEQ ID NO:34, una CDR4 de SEQ ID NO:48, una CDR5 de SEQ ID NO:62, y una CDR6 de SEQ ID NO:76, un anticuerpo que comprende una CDR1 de SEQ ID NO:7, una CDR2 de SEQ ID NO:21, una CDR3 de SEQ ID NO:35, una CDR4 de SEQ ID NO:49, una CDR5 de SEQ ID NO:63, y una CDR6 de SEQ ID NO:77, un anticuerpo que comprende una CDR1 de SEQ ID NO:8, una CDR2 de SEQ ID NO:22, una CDR3 de SEQ ID NO:36, una CDR4 de SEQ ID NO:50, una CDR5 de SEQ ID NO:64, y una CDR6 de SEQ ID NO:78, un anticuerpo que comprende una CDR1 de SEQ ID NO:9, una CDR2 de SEQ ID NO:23, una CDR3 de SEQ ID NO:37, una CDR4 de SEQ ID NO:51, una CDR5 de SEQ ID NO:65, y una CDR6 de SEQ ID NO:79, un anticuerpo que comprende una CDR1 de SEQ ID NO:10, una CDR2 de SEQ ID NO:24, una CDR3 de SEQ ID NO:38, una CDR4 de SEQ ID NO:52, una CDR5 de SEQ ID NO:66, y una CDR6 de SEQ ID NO:80, un anticuerpo que comprende una CDR1 de SEQ ID NO:11, una CDR2 de SEQ ID NO:25, una CDR3 de SEQ ID NO:39, una CDR4 de SEQ ID NO:53, una CDR5 de SEQ ID NO:67, y una CDR6 de SEQ ID NO:81, un anticuerpo que comprende una CDR1 de SEQ ID NO:12, una CDR2 de SEQ ID NO:26, una CDR3 de SEQ ID NO:40, una CDR4 de SEQ ID NO:54, una CDR5 de SEQ ID NO:68, y una CDR6 de SEQ ID NO:82, un anticuerpo que comprende una CDR1 de SEQ ID NO:13,

una CDR2 de SEQ ID NO:27, una CDR3 de SEQ ID NO:41, una CDR4 de SEQ ID NO:55, una CDR5 de SEQ ID NO:69, y una CDR6 de SEQ ID NO:83, un anticuerpo que comprende una CDR1 de SEQ ID NO:14, una CDR2 de SEQ ID NO:28, una CDR3 de SEQ ID NO:42, una CDR4 de SEQ ID NO:56, una CDR5 de SEQ ID NO:70, y una CDR6 de SEQ ID NO:84.

17.- Un agente de unión a CD33 seleccionado de un anticuerpo que comprende una región variable de cadena pesada de SEQ ID NO:85 y una región variable de cadena ligera de SEQ ID NO:99, un anticuerpo que comprende una región variable de cadena pesada de SEQ ID NO:86 y una región variable de cadena ligera de SEQ ID NO:100, un anticuerpo que comprende una región variable de cadena pesada de SEQ ID NO:87 y una región variable de cadena ligera de SEQ ID NO:101, un anticuerpo que comprende una región variable de cadena pesada de SEQ ID NO:88 y una región variable de cadena ligera de SEQ ID NO:102, un anticuerpo que comprende una región variable de cadena pesada de SEQ ID NO:89 y una región variable de cadena ligera de SEQ ID NO:103, un anticuerpo que comprende una región variable de cadena pesada de SEQ ID NO:90 y una región variable de cadena ligera de SEQ ID NO:104, un anticuerpo que comprende una región variable de cadena pesada de SEQ ID NO:91 y una región variable de cadena ligera de SEQ ID NO:105, un anticuerpo que comprende una región variable de cadena pesada de SEQ ID NO:92 y una región variable de cadena ligera de SEQ ID NO:106, un anticuerpo que comprende una región variable de cadena pesada de SEQ ID NO:93 y una región variable de cadena ligera de SEQ ID NO:107, un anticuerpo que comprende una región variable de cadena pesada de SEQ ID NO:94 y una región variable de cadena ligera de SEQ ID NO:108, un anticuerpo que comprende una región variable de cadena pesada de SEQ ID NO:95 y una región variable de cadena ligera de SEQ ID NO:109, un anticuerpo que comprende una región variable de cadena pesada de SEQ ID NO:96 y una región variable de cadena ligera de SEQ ID NO:110, un anticuerpo que comprende una región variable de cadena pesada de SEQ ID NO:97 y una región variable de cadena ligera de SEQ ID NO:111, un anticuerpo que comprende una región variable de cadena pesada de SEQ ID NO:98 y una región variable de cadena ligera de SEQ ID NO:113.

18.- Un agente de unión a CD33 seleccionado de un anticuerpo que comprende una cadena pesada de SEQ ID NO:113 y una cadena ligera de SEQ ID

NO:127, un anticuerpo que comprende una cadena pesada de SEQ ID NO:114 y una cadena ligera de SEQ ID NO:128, un anticuerpo que comprende una cadena pesada de SEQ ID NO:115 y una cadena ligera de SEQ ID NO:129, un anticuerpo que comprende una cadena pesada de SEQ ID NO:116 y una cadena ligera de SEQ ID NO:130, un anticuerpo que comprende una cadena pesada de SEQ ID NO:117 y una cadena ligera de SEQ ID NO:131, un anticuerpo que comprende una cadena pesada de SEQ ID NO:118 y una cadena ligera de SEQ ID NO:132, un anticuerpo que comprende una cadena pesada de SEQ ID NO:119 y una cadena ligera de SEQ ID NO:133, un anticuerpo que comprende una cadena pesada de SEQ ID NO:120 y una cadena ligera de SEQ ID NO:134, un anticuerpo que comprende una cadena pesada de SEQ ID NO:121 y una cadena ligera de SEQ ID NO:135, un anticuerpo que comprende una cadena pesada de SEQ ID NO:122 y una cadena ligera de SEQ ID NO:136, un anticuerpo que comprende una cadena pesada de SEQ ID NO:123 y una cadena ligera de SEQ ID NO:137, un anticuerpo que comprende una cadena pesada de SEQ ID NO:124 y una cadena ligera de SEQ ID NO:138, un anticuerpo que comprende una cadena pesada de SEQ ID NO:125 y una cadena ligera de SEQ ID NO:139, un anticuerpo que comprende una cadena pesada de SEQ ID NO:126 y una cadena ligera de SEQ ID NO:140.

19.- Una molécula de ADN que comprende una región que codifica la región variable de cadena pesada de un agente de unión a CD33 según cualquiera de las reivindicaciones anteriores.

20.- Una molécula de ADN que comprende una región que codifica la región variable de cadena ligera de un agente de unión a CD33 según cualquiera de las reivindicaciones 1-18.

21.- Un vector de expresión que contiene una molécula de ADN de la reivindicación 19 ó 20.

22.- Una célula hospedante que porta uno o más vectores de la reivindicación 21.

23.- Un método para producir un agente de unión a CD33 de una cualquiera de las reivindicaciones 1-18, que comprende transfectar una célula hospedante con uno o

más vectores de la reivindicación 21, cultivar la célula hospedante, y recuperar y purificar la molécula de anticuerpo.

24.- Una composición farmacéutica que comprende, como ingrediente activo, uno o más agentes de unión a CD33 según una cualquiera de las reivindicaciones 1 a 18, y al menos un vehículo fisiológicamente aceptable.

25.- La composición farmacéutica de la reivindicación 24, que comprende además uno o más agentes terapéuticos adicionales.

26.- La composición farmacéutica de una cualquiera de las reivindicaciones 24-25, para disminuir el número de células que expresan el CD33 sobre su superficie.

27.- La composición farmacéutica de una cualquiera de las reivindicaciones 24-25, para el tratamiento de malignidades de células mieloides y del síndrome mielodisplásico (MDS).

28.- La composición farmacéutica de la reivindicación 27, en la que dicha malignidad mieloide se selecciona de leucemia mieloide aguda y leucemia mieloide crónica.

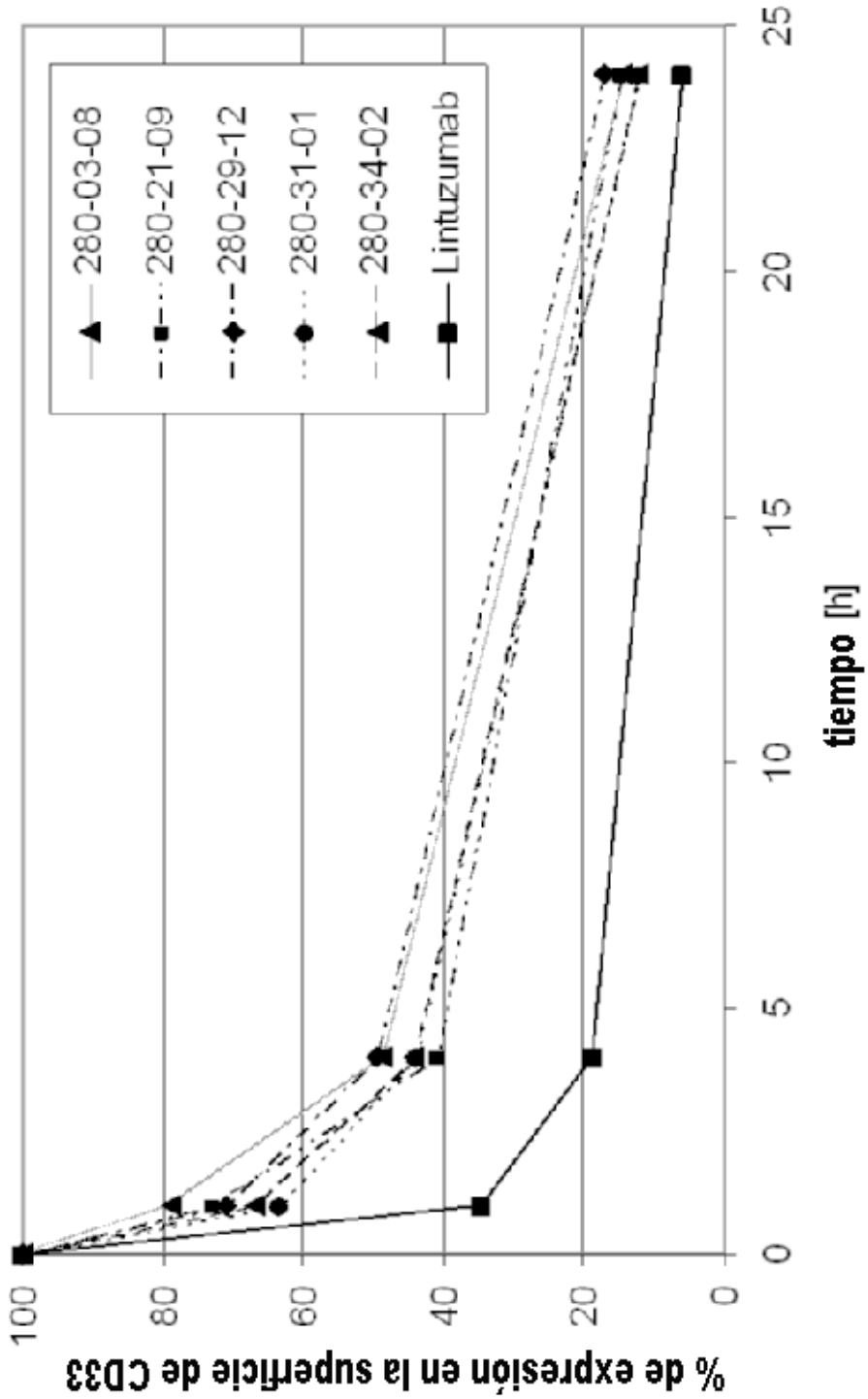
29.- La composición farmacéutica de una cualquiera de las reivindicaciones 24-25, para el tratamiento de enfermedades autoinmunológicas e inflamatorias que implican a las células mieloides en su patología.

30.- El método para disminuir el número de células mieloides que expresan CD33 en un paciente, que comprende administrar a dicho paciente uno o más agentes de unión a CD33 según una cualquiera de las reivindicaciones 1-18 o una composición farmacéutica según cualquiera de las reivindicaciones 24-25.

RESUMEN

La presente invención se refiere a inmunoterapias que están basadas en la disminución del número de células mieloides. En particular, la presente invención se refiere a agentes de unión a CD33 para su uso en dichas terapias, por ejemplo para el tratamiento de malignidades de células mieloides y el síndrome mielodisplásico (MDS).

Figura 1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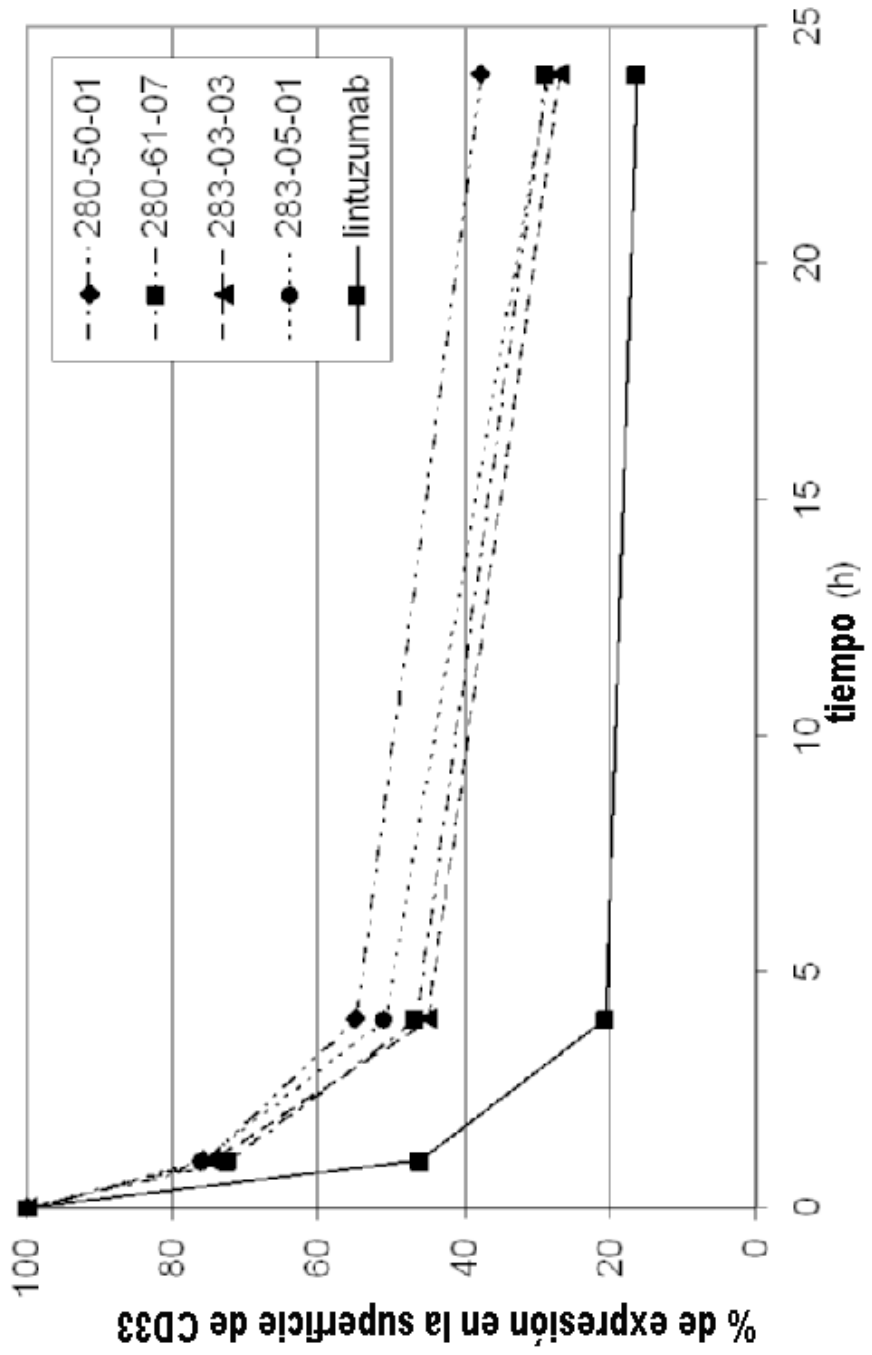
Lintuzumab

Figura 2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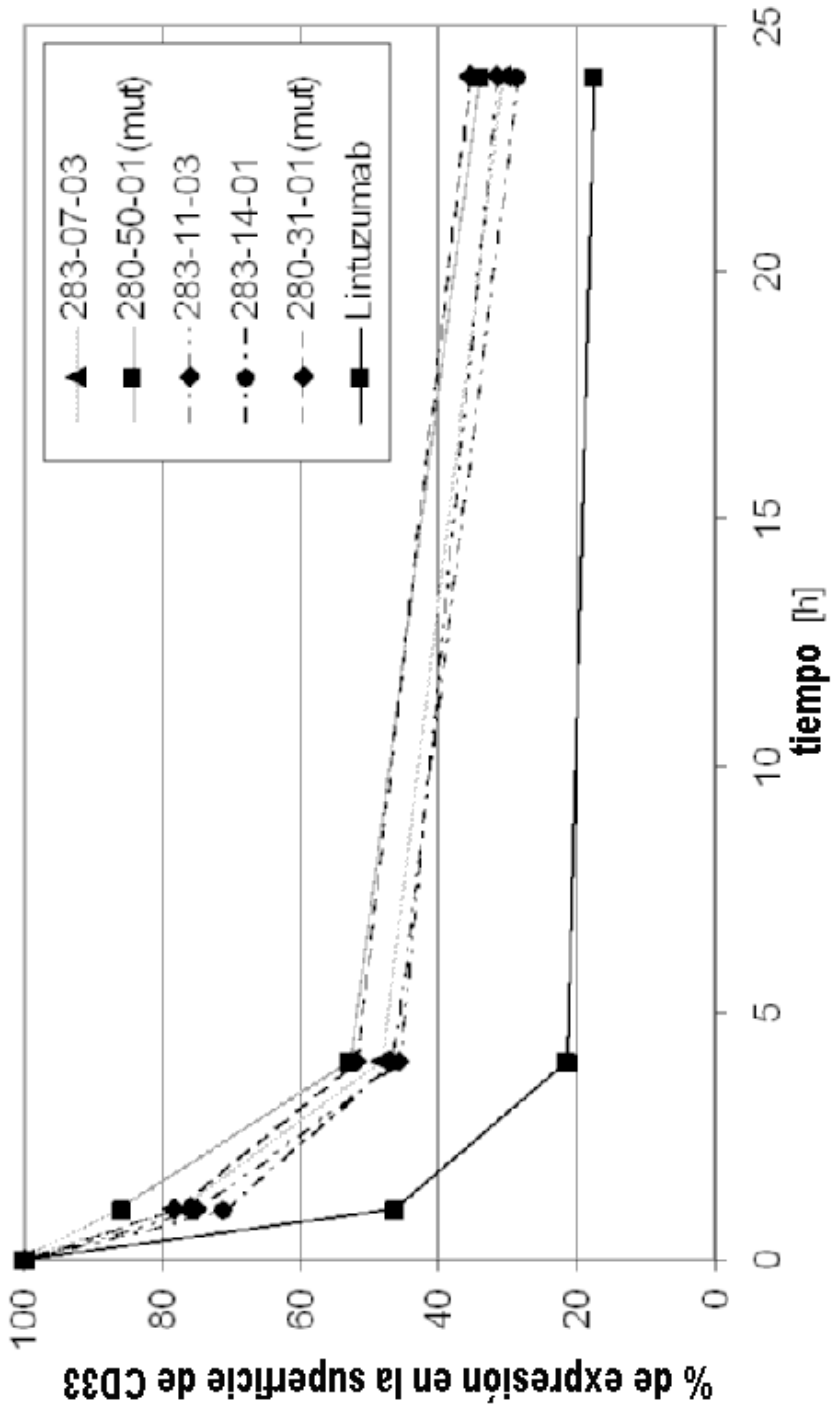
lintuzumab

Figura 3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15
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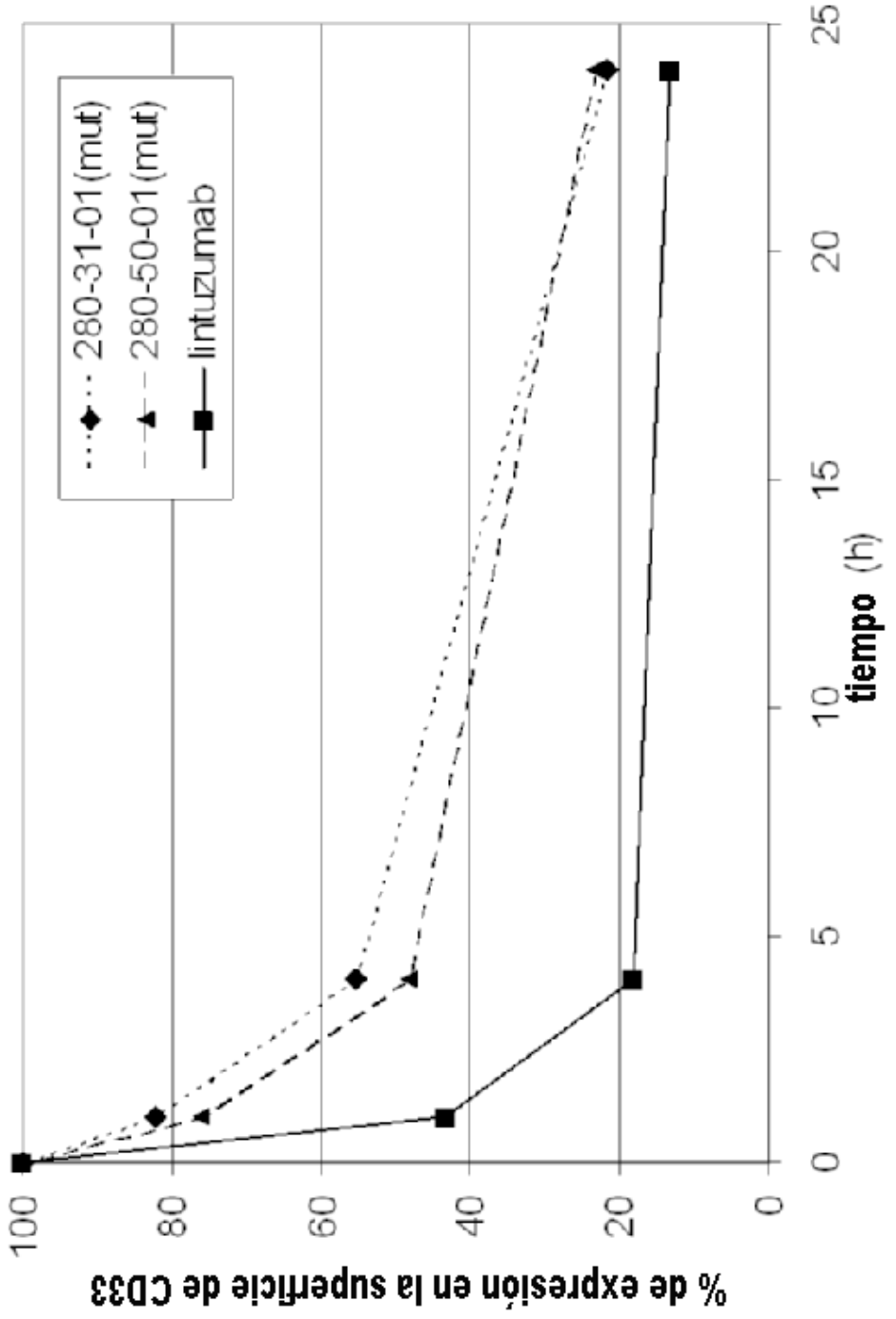
25
tiempo [h]

Figura 4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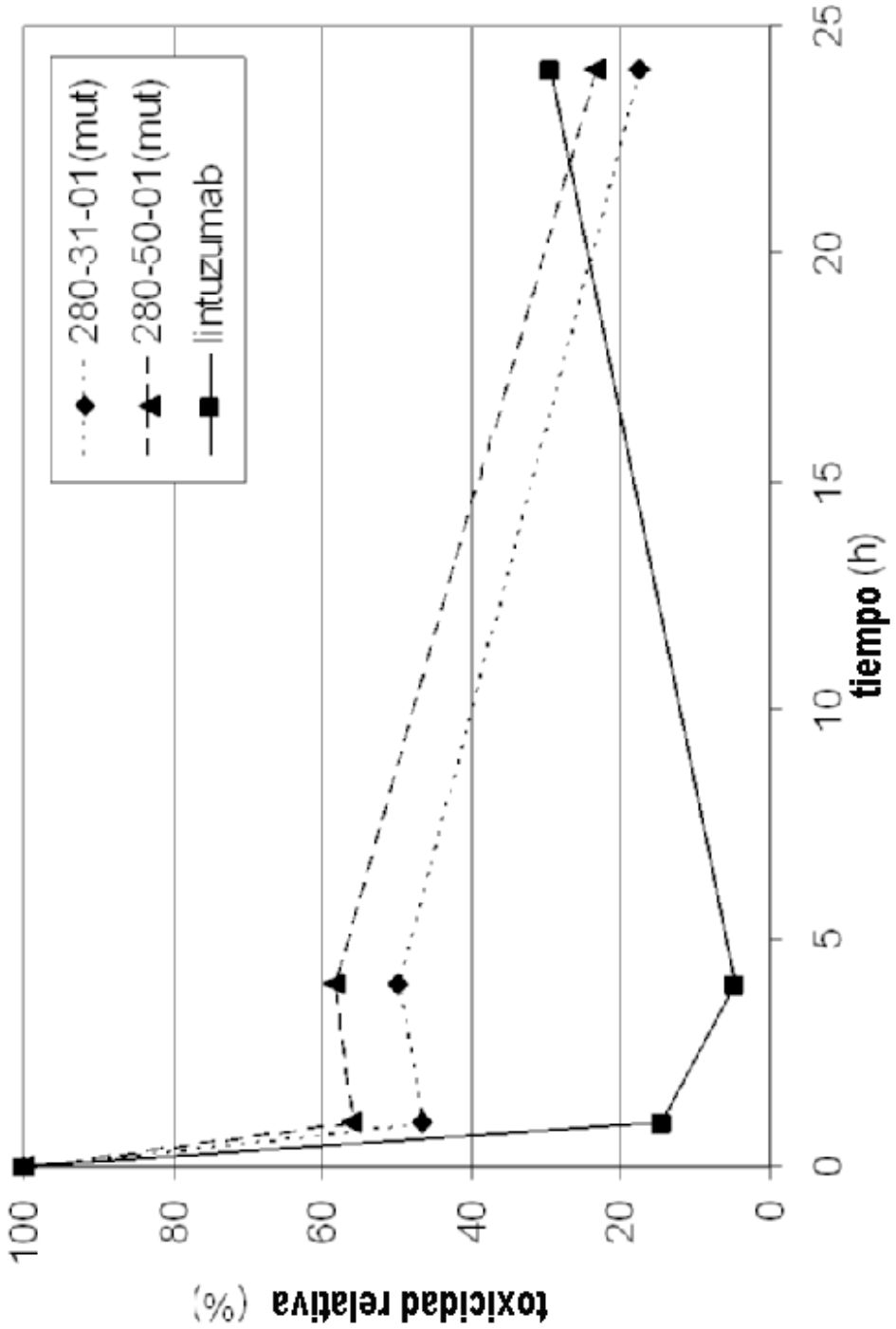
lintuzumab

Figura 5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toxicidad relativa (%)
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60
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15
20
25
tiempo (h)

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Gln Gln Phe Asn Ser Ser Ile Thr
1 5

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Asp Ala Ser Ser Leu Glu Ser
1 5

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Asp Ala Ser Ser Leu Glu Ser
1 5

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Asp Ala Ser Ser Leu Glu Ser
1 5

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1 5

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Asp Ala Ser Ser Leu Glu Ser
1 5

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Asp Ala Ser Arg Leu Glu Ser
1 5

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Ala Ala Ser Ser Leu Glu Ser
1 5

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Ala Ala Ser Ser Leu Glu Ser
1 5

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Ala Ala Ser Ser Leu Glu Ser
1 5

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<400> 66

Asp Ala Ser Ser Leu Glu Ser
1 5

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Asp Ala Ser Ser Leu Glu Ser
1 5

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<400> 68

Asp Ala Ser Ser Leu Glu Ser
1 5

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<400> 69

Asp Ala Ser Ser Leu Glu Ser
1 5

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<400> 70

Asp Ala Ser Ser Leu Glu Ser
1 5

<210> 71
<211> 11
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<400> 71

Arg Ala Ser Gln Gly Ile Ser Ser Val Leu Ala
1 5 10

<210> 72
<211> 11
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<220>
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<400> 72

Arg Ala Ser Gln Gly Ile Ser Ser Ala Leu Ala
1 5 10

<210> 73
<211> 11
<212> PRT
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<220>
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<400> 73

Arg Ala Ser Gln Gly Ile Ser Ser Ala Leu Ala
1 5 10

<210> 74
<211> 11
<212> PRT
<213> Artificial

<220>
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<400> 74

Arg Ala Ser Gln Gly Ile Ser Ser Val Leu Ala
1 5 10

<210> 75
<211> 11
<212> PRT
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<400> 75

Arg Ala Ser Gln Gly Ile Ser Ser Val Leu Ala
1 5 10

<210> 76
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<400> 76

Arg Ala Ser Gln Gly Ile Ser Ser Ala Leu Ala
1 5 10

<210> 77

<211> 11
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<220>
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<400> 77

Arg Ala Ser Gln Gly Ile Ser Ser Val Leu Ala
1 5 10

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<211> 11
<212> PRT
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<220>
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<400> 78

Arg Ala Ser Gln Gly Ile Ser Ser Val Leu Ala
1 5 10

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<211> 11
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<400> 79

Arg Ala Ser Gln Gly Ile Ser Ser Val Leu Ala
1 5 10

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<400> 80

Arg Ala Ser Gln Gly Ile Ser Ser Ala Leu Ala
1 5 10

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<211> 11
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<400> 81

Arg Ala Ser Gln Gly Ile Ser Ser Ala Leu Ala
1 5 10

<210> 82
<211> 11
<212> PRT
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<400> 82

Arg Ala Ser Gln Gly Ile Ser Ser Ala Leu Ala
1 5 10

<210> 83
<211> 11
<212> PRT
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<220>
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<400> 83

Arg Ala Ser Gln Gly Ile Ser Ser Ala Leu Ala
1 5 10

<210> 84
<211> 11
<212> PRT
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<220>
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<400> 84

Arg Ala Ser Gln Gly Ile Ser Ser Ala Leu Ala
1 5 10

<210> 85
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<212> PRT
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<220>
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<400> 85

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Asp Tyr

20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Val Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Arg Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

Val Thr Val Ser Ser
115

<210> 86
<211> 117
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 86

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Asp Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Ile Asn Ile Ala Ser Tyr Ala Gln Asn Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Asn Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

Val Thr Val Ser Ser
115

<210> 87
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<212> PRT
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<220>
<223> VH

<400> 87

Gln Val Gln Leu Val Gln Ser Gly Thr Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Val Ser Gly Asp Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Ile Gly Ile Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg His Trp Leu Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

Val Thr Val Ser Ser
115

<210> 88
<211> 117
<212> PRT
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<400> 88

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Asp Tyr

20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Val Ala Asp Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Arg Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

Val Thr Val Ser Ser
115

<210> 89
<211> 117
<212> PRT
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<220>
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<400> 89

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Asp Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Val Ala Asp Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Arg Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

Val Thr Val Ser Ser
        115

<210> 90
<211> 117
<212> PRT
<213> Artificial

<220>
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<400> 90

Gln Val Glu Leu Val Gln Ser Gly Ala Glu Val Gln Lys Pro Gly Ser
1   5   10   15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Asp Thr Phe Ser Ser Tyr
            20   25   30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45

Gly Arg Ile Ile Pro Ile Val Gly Ile Val Asn Tyr Ala Glu Lys Phe
    50   55   60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Met Ser Thr Ala Tyr
65   70   75   80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
            100   105   110

Val Thr Val Ser Ser
        115

<210> 91
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<212> PRT
<213> Artificial

<220>
<223> VH

<400> 91

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1   5   10   15

Ser Val Lys Val Ala Cys Lys Ala Ser Gly Asp Thr Phe Ser Ser Tyr

20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Val Ile Pro Ile Ile Gly Ile Ala Ser Tyr Ala Gln Asn Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Arg Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Ala Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

Val Thr Val Ser Ser
115

<210> 92
<211> 117
<212> PRT
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<220>
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<400> 92

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ala Cys Lys Ala Ser Gly Asp Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Val Ile Pro Ile Ile Gly Ile Ala Ser Tyr Ala Gln Asn Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Arg Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Ala Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

Val Thr Val Ser Ser
        115

<210> 93
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<212> PRT
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<400> 93

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1   5   10   15

Ser Val Lys Val Ala Cys Lys Ala Ser Gly Asp Thr Phe Ser Ser Tyr
            20   25   30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45

Gly Arg Val Ile Pro Ile Ile Gly Ile Ala Ser Tyr Ala Gln Asn Phe
    50   55   60

Glu Gly Arg Val Thr Ile Thr Ala Asp Arg Ser Thr Ser Thr Ala Tyr
65   70   75   80

Met Ala Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
            100   105   110

Val Thr Val Ser Ser
        115

<210> 94
<211> 119
<212> PRT
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<400> 94

Gln Val Glu Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1   5   10   15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr

20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Asp Met Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Tyr Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Gly Val Asp Trp Tyr Phe Asp Leu Trp Gly Arg Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 95
<211> 119
<212> PRT
<213> Artificial

<220>
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<400> 95

Gln Val Glu Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Glu Asp Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Ile Gly Ile Val Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Tyr Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Gly Val Asp Trp Tyr Phe Asp Leu Trp Gly Arg Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
        115

<210> 96
<211> 119
<212> PRT
<213> Artificial

<220>
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<400> 96

Gln Val Glu Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1   5   10   15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20   25   30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45

Gly Arg Ile Ile Pro Ile Leu Gly Met Ala Asn Tyr Ala Gln Lys Phe
    50   55   60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Tyr Thr Ala Tyr
65   70   75   80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Arg Glu Gly Gly Val Asp Trp Tyr Phe Asp Leu Trp Gly Arg Gly
            100   105   110

Thr Leu Val Thr Val Ser Ser
        115

<210> 97
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<212> PRT
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<400> 97

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1   5   10   15

Ser Met Lys Val Ser Cys Lys Ala Ser Glu Asp Thr Phe Ser Ser Tyr

20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Ile Gly Ile Val Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Cys Thr Ala Tyr
65 70 75 80

Met Glu Leu Thr Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Gly Val Asp Trp Tyr Phe Asp Leu Trp Gly Arg Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 98
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<212> PRT
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<220>
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<400> 98

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Tyr Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Gly Val Asp Trp Tyr Phe Asp Leu Trp Gly Arg Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
        115

<210> 99
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<212> PRT
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<220>
<223> VL

<400> 99

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
            20   25   30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35   40   45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50   55   60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65   70   75   80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
                85   90   95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
            100   105

<210> 100
<211> 107
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 100

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
            20   25   30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile

35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 101
<211> 107
<212> PRT
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<220>
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<400> 101

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Ser Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 102
<211> 107
<212> PRT
<213> Artificial

<220>

<223> VL

<400> 102

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 103
<211> 107
<212> PRT
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<220>
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<400> 103

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asp Ser Ser Ile Thr

85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 104
<211> 107
<212> PRT
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<220>
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<400> 104

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Arg Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Thr Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Tyr Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 105
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<212> PRT
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<220>
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<400> 105

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 106
<211> 107
<212> PRT
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<400> 106

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asp Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 107
<211> 107
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<400> 107

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 108
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<212> PRT
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<400> 108

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Phe Met Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 109
<211> 107
<212> PRT
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<400> 109

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Phe Met Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 110
<211> 107
<212> PRT
<213> Artificial

<220>
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<400> 110

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

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Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Phe Met Ile
        35                  40                  45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
            100                 105
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<210> 111
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<400> 111

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Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Phe Met Ile
        35                  40                  45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
            100                 105
```

<210> 112
<211> 107
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 112

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Phe Met Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 113
<211> 447
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 113

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Asp Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Val Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Arg Thr Ala Tyr
65 70 75 80

```
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
            100                 105                 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
        115                 120                 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
    130                 135                 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335
```

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
340 345 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 114
<211> 447
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 114

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Asp Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Ile Asn Ile Ala Ser Tyr Ala Gln Asn Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Asn Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

```
Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
            100                 105                 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
        115                 120                 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
    130                 135                 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350
```

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 115
<211> 447
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 115

Gln Val Gln Leu Val Gln Ser Gly Thr Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Val Ser Gly Asp Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Ile Gly Ile Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg His Trp Leu Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

```
Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
        115                 120                 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
    130                 135                 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365
```

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 116
<211> 447
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 116

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Asp Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Val Ala Asp Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Arg Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125

```
Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
    130                 135                 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380
```

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 117
<211> 447
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 117

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Asp Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Val Ala Asp Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Arg Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130 135 140

```
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400
```

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 118
<211> 447
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 118

Gln Val Glu Leu Val Gln Ser Gly Ala Glu Val Gln Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Asp Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Val Gly Ile Val Asn Tyr Ala Glu Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Met Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145 150 155 160

```
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415
```

```
Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445
```

<210> 119
<211> 447
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 119

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Val Lys Val Ala Cys Lys Ala Ser Gly Asp Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Arg Val Ile Pro Ile Ile Gly Ile Ala Ser Tyr Ala Gln Asn Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Arg Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Ala Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
            100                 105                 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
        115                 120                 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
    130                 135                 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175
```

```
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430
```

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 120
<211> 447
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 120

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ala Cys Lys Ala Ser Gly Asp Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Val Ile Pro Ile Ile Gly Ile Ala Ser Tyr Ala Gln Asn Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Arg Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Ala Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180 185 190

```
Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445
```

<210> 121
<211> 447
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 121

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Val Lys Val Ala Cys Lys Ala Ser Gly Asp Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Arg Val Ile Pro Ile Ile Gly Ile Ala Ser Tyr Ala Gln Asn Phe
    50                  55                  60

Glu Gly Arg Val Thr Ile Thr Ala Asp Arg Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Ala Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
            100                 105                 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
        115                 120                 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
    130                 135                 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205
```

```
Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445
```

<210> 122
<211> 449

<212> PRT
<213> Artificial

<220>
<223> VH

<400> 122

```
Gln Val Glu Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Arg Ile Ile Pro Ile Leu Asp Met Ala Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Tyr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Gly Gly Val Asp Trp Tyr Phe Asp Leu Trp Gly Arg Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        115                 120                 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    130                 135                 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        195                 200                 205

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220
```

```
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225                 230                 235                 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    290                 295                 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                 390                 395                 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445

Lys
```

<210> 123
<211> 449

<212> PRT
<213> Artificial

<220>
<223> VH

<400> 123

```
Gln Val Glu Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Glu Asp Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Arg Ile Ile Pro Ile Ile Gly Ile Val Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Tyr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Gly Gly Val Asp Trp Tyr Phe Asp Leu Trp Gly Arg Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        115                 120                 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    130                 135                 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        195                 200                 205

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220
```

```
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225                 230                 235                 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    290                 295                 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                 390                 395                 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445

Lys
```

<210> 124
<211> 449

<212> PRT
<213> Artificial

<220>
<223> VH

<400> 124

```
Gln Val Glu Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Arg Ile Ile Pro Ile Leu Gly Met Ala Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Tyr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Gly Gly Val Asp Trp Tyr Phe Asp Leu Trp Gly Arg Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        115                 120                 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    130                 135                 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        195                 200                 205

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220
```

```
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225                 230                 235                 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    290                 295                 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                 390                 395                 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445

Lys
```

<210> 125
<211> 449

<212> PRT
<213> Artificial

<220>
<223> VH

<400> 125

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Met Lys Val Ser Cys Lys Ala Ser Glu Asp Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Arg Ile Ile Pro Ile Ile Gly Ile Val Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Cys Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Thr Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Gly Gly Val Asp Trp Tyr Phe Asp Leu Trp Gly Arg Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        115                 120                 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    130                 135                 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        195                 200                 205

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220
```

```
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225                 230                 235                 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    290                 295                 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                 390                 395                 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445

Lys
```

<210> 126
<211> 449

<212> PRT
<213> Artificial

<220>
<223> VH

<400> 126

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Arg Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Tyr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Gly Gly Val Asp Trp Tyr Phe Asp Leu Trp Gly Arg Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        115                 120                 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    130                 135                 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        195                 200                 205

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220
```

```
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225                 230                 235                 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    290                 295                 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                 390                 395                 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445

Lys
```

<210> 127
<211> 213

<212> PRT
<213> Artificial

<220>
<223> VL

<400> 127

```
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
            100                 105                 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
        115                 120                 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
    130                 135                 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145                 150                 155                 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
                165                 170                 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
            180                 185                 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
        195                 200                 205

Asn Arg Gly Glu Cys
    210
```

<210> 128
<211> 213
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 128

```
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
            20                  25                  30


Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45


Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
                85                  90                  95


Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
            100                 105                 110


Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
        115                 120                 125


Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
    130                 135                 140


Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145                 150                 155                 160


Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
                165                 170                 175


Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
            180                 185                 190


Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
        195                 200                 205
```

Asn Arg Gly Glu Cys
210

<210> 129
<211> 213
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 129

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Ser Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe

195 200 205

Asn Arg Gly Glu Cys
210

<210> 130
<211> 213
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 130

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
210

<210> 131
<211> 213
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 131

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asp Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
210

<210> 132
<211> 213
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 132

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Arg Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Thr Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Tyr Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
210

<210> 133
<211> 213
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 133

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu

145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
210

<210> 134
<211> 213
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 134

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asp Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
210

<210> 135
<211> 213
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 135

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

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Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
    130                 135                 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145                 150                 155                 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
                165                 170                 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
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Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
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Asn Arg Gly Glu Cys
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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Phe Met Ile
        35                  40                  45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
            100                 105                 110
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Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Phe Met Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro

100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Phe Met Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
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Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Phe Met Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

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Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
            100                 105                 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
        115                 120                 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
    130                 135                 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145                 150                 155                 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
                165                 170                 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
            180                 185                 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
        195                 200                 205

Asn Arg Gly Glu Cys
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Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Phe Met Ile
        35                  40                  45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
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Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
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Tyr Trp

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Met Asp Pro Asn Phe Trp Leu Gln Val Gln Glu
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Asn Trp Ala Asp Ala Phe Asp Ile
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Gly

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Arg Ile Ile Pro Ile Ile Asn Ile Ala Ser Tyr Ala Gln Asn Phe Gln
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Gly

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Arg Ile Ile Pro Ile Ile Gly Ile Ala Asn Tyr Ala Gln Lys Phe Gln
1 5 10 15

Gly

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Gly

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Arg Ile Ile Pro Ile Leu Gly Val Ala Asp Tyr Ala Gln Lys Phe Gln
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Gly

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Gly

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Gly

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Gly

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Gly

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Gly

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Gly

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Gly

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Gly

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Ser Tyr Ala Ile Ser
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Gln Gln Phe Asn Ser Ser Ile Thr
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Asp Ala Ser Ser Leu Glu Ser
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Asp Ala Ser Ser Leu Glu Ser
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Asp Ala Ser Ser Leu Glu Ser
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Arg Ala Ser Gln Gly Ile Ser Ser Ala Leu Ala
1 5 10

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Arg Ala Ser Gln Gly Ile Ser Ser Val Leu Ala
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Arg Ala Ser Gln Gly Ile Ser Ser Val Leu Ala
1 5 10

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Arg Ala Ser Gln Gly Ile Ser Ser Ala Leu Ala
1 5 10

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Arg Ala Ser Gln Gly Ile Ser Ser Val Leu Ala
1 5 10

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Arg Ala Ser Gln Gly Ile Ser Ser Val Leu Ala
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Arg Ala Ser Gln Gly Ile Ser Ser Val Leu Ala
1 5 10

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Arg Ala Ser Gln Gly Ile Ser Ser Ala Leu Ala
1 5 10

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Arg Ala Ser Gln Gly Ile Ser Ser Ala Leu Ala
1 5 10

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Arg Ala Ser Gln Gly Ile Ser Ser Ala Leu Ala
1 5 10

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Arg Ala Ser Gln Gly Ile Ser Ser Ala Leu Ala
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Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Asp Tyr

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Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Val Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Arg Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

Val Thr Val Ser Ser
115

<210> 86
<211> 117
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 86

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Asp Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Ile Asn Ile Ala Ser Tyr Ala Gln Asn Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Asn Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

Val Thr Val Ser Ser
        115

<210> 87
<211> 117
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 87

Gln Val Gln Leu Val Gln Ser Gly Thr Glu Val Lys Lys Pro Gly Ser
1   5   10   15

Ser Val Lys Val Ser Cys Lys Val Ser Gly Asp Thr Phe Ser Ser Tyr
            20   25   30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45

Gly Arg Ile Ile Pro Ile Ile Gly Ile Ala Asn Tyr Ala Gln Lys Phe
    50   55   60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65   70   75   80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Arg His Trp Leu Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
            100   105   110

Val Thr Val Ser Ser
        115

<210> 88
<211> 117
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 88

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1   5   10   15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Asp Tyr

20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Val Ala Asp Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Arg Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

Val Thr Val Ser Ser
115

<210> 89
<211> 117
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 89

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Asp Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Val Ala Asp Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Arg Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

Val Thr Val Ser Ser
        115

<210> 90
<211> 117
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 90

Gln Val Glu Leu Val Gln Ser Gly Ala Glu Val Gln Lys Pro Gly Ser
1   5   10   15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Asp Thr Phe Ser Ser Tyr
            20   25   30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45

Gly Arg Ile Ile Pro Ile Val Gly Ile Val Asn Tyr Ala Glu Lys Phe
    50   55   60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Met Ser Thr Ala Tyr
65   70   75   80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
            100   105   110

Val Thr Val Ser Ser
        115

<210> 91
<211> 117
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 91

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1   5   10   15

Ser Val Lys Val Ala Cys Lys Ala Ser Gly Asp Thr Phe Ser Ser Tyr

20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Val Ile Pro Ile Ile Gly Ile Ala Ser Tyr Ala Gln Asn Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Arg Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Ala Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

Val Thr Val Ser Ser
115

<210> 92
<211> 117
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 92

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ala Cys Lys Ala Ser Gly Asp Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Val Ile Pro Ile Ile Gly Ile Ala Ser Tyr Ala Gln Asn Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Arg Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Ala Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

Val Thr Val Ser Ser
        115

<210> 93
<211> 117
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 93

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1   5   10   15

Ser Val Lys Val Ala Cys Lys Ala Ser Gly Asp Thr Phe Ser Ser Tyr
            20   25   30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45

Gly Arg Val Ile Pro Ile Ile Gly Ile Ala Ser Tyr Ala Gln Asn Phe
    50   55   60

Glu Gly Arg Val Thr Ile Thr Ala Asp Arg Ser Thr Ser Thr Ala Tyr
65   70   75   80

Met Ala Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
            100   105   110

Val Thr Val Ser Ser
        115

<210> 94
<211> 119
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 94

Gln Val Glu Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1   5   10   15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr

20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Asp Met Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Tyr Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Gly Val Asp Trp Tyr Phe Asp Leu Trp Gly Arg Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 95
<211> 119
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 95

Gln Val Glu Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Glu Asp Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Ile Gly Ile Val Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Tyr Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Gly Val Asp Trp Tyr Phe Asp Leu Trp Gly Arg Gly
100 105 110

```
Thr Leu Val Thr Val Ser Ser
        115


<210>  96
<211>  119
<212>  PRT
<213>  Artificial

<220>
<223>  VH

<400>  96

Gln Val Glu Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15


Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20                  25                  30


Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45


Gly Arg Ile Ile Pro Ile Leu Gly Met Ala Asn Tyr Ala Gln Lys Phe
    50                  55                  60


Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Tyr Thr Ala Tyr
65                  70                  75                  80


Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Glu Gly Gly Val Asp Trp Tyr Phe Asp Leu Trp Gly Arg Gly
            100                 105                 110


Thr Leu Val Thr Val Ser Ser
        115


<210>  97
<211>  119
<212>  PRT
<213>  Artificial

<220>
<223>  VH

<400>  97

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15


Ser Met Lys Val Ser Cys Lys Ala Ser Glu Asp Thr Phe Ser Ser Tyr
```

20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Ile Gly Ile Val Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Cys Thr Ala Tyr
65 70 75 80

Met Glu Leu Thr Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Gly Val Asp Trp Tyr Phe Asp Leu Trp Gly Arg Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 98
<211> 119
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 98

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Tyr Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Gly Val Asp Trp Tyr Phe Asp Leu Trp Gly Arg Gly
100 105 110

```
Thr Leu Val Thr Val Ser Ser
        115


<210>  99
<211>  107
<212>  PRT
<213>  Artificial

<220>
<223>  VL

<400>  99

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
            20                  25                  30


Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45


Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
                85                  90                  95


Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
            100                 105


<210>  100
<211>  107
<212>  PRT
<213>  Artificial

<220>
<223>  VL

<400>  100

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
            20                  25                  30


Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
```

35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 101
<211> 107
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 101

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Ser Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 102
<211> 107
<212> PRT
<213> Artificial

<220>

<223> VL

<400> 102

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 103
<211> 107
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 103

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asp Ser Ser Ile Thr

85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 104
<211> 107
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 104

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Arg Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Thr Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Tyr Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 105
<211> 107
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 105

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
20 25 30

```
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
            100                 105
```

<210> 106
<211> 107
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 106

```
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asp Ser Ser Ile Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
            100                 105
```

<210> 107
<211> 107
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 107

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 108
<211> 107
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 108

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Phe Met Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 109
<211> 107
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 109

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Phe Met Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 110
<211> 107
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 110

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Phe Met Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 111
<211> 107
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 111

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Phe Met Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 112
<211> 107
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 112

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Phe Met Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 113
<211> 447
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 113

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Asp Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Val Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Arg Thr Ala Tyr
65 70 75 80

```
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
            100                 105                 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
        115                 120                 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
    130                 135                 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335
```

```
Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445
```

<210> 114
<211> 447
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 114

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Asp Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Arg Ile Ile Pro Ile Ile Asn Ile Ala Ser Tyr Ala Gln Asn Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Asn Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
```

```
Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
            100                 105                 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
        115                 120                 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
    130                 135                 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350
```

```
Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445


<210>  115
<211>  447
<212>  PRT
<213>  Artificial

<220>
<223>  VH

<400>  115

Gln Val Gln Leu Val Gln Ser Gly Thr Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Val Ser Gly Asp Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Arg Ile Ile Pro Ile Ile Gly Ile Ala Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg His Trp Leu Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
            100                 105                 110
```

```
Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
        115                 120                 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
    130                 135                 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365
```

```
Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445


<210>  116
<211>  447
<212>  PRT
<213>  Artificial

<220>
<223>  VH

<400>  116

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Asp Tyr
            20                  25                  30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Arg Ile Ile Pro Ile Leu Gly Val Ala Asp Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Arg Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
            100                 105                 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
        115                 120                 125
```

```
Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
    130                 135                 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380
```

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 117
<211> 447
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 117

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Asp Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Val Ala Asp Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Arg Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130 135 140

```
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400
```

```
Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415


Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430


His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445


<210>  118
<211>  447
<212>  PRT
<213>  Artificial

<220>
<223>  VH

<400>  118

Gln Val Glu Leu Val Gln Ser Gly Ala Glu Val Gln Lys Pro Gly Ser
1               5                   10                  15


Ser Val Lys Val Ser Cys Lys Ala Ser Gly Asp Thr Phe Ser Ser Tyr
            20                  25                  30


Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45


Gly Arg Ile Ile Pro Ile Val Gly Ile Val Asn Tyr Ala Glu Lys Phe
    50                  55                  60


Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Met Ser Thr Ala Tyr
65                  70                  75                  80


Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
            100                 105                 110


Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
        115                 120                 125


Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
    130                 135                 140


Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160
```

```
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415
```

```
Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445
```

<210> 119
<211> 447
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 119

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Val Lys Val Ala Cys Lys Ala Ser Gly Asp Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Arg Val Ile Pro Ile Ile Gly Ile Ala Ser Tyr Ala Gln Asn Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Arg Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Ala Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
            100                 105                 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
        115                 120                 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
    130                 135                 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175
```

```
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430
```

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 120
<211> 447
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 120

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ala Cys Lys Ala Ser Gly Asp Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Val Ile Pro Ile Ile Gly Ile Ala Ser Tyr Ala Gln Asn Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Arg Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Ala Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180 185 190

```
Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445
```

<210> 121
<211> 447
<212> PRT
<213> Artificial

<220>
<223> VH

<400> 121

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ala Cys Lys Ala Ser Gly Asp Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Val Ile Pro Ile Ile Gly Ile Ala Ser Tyr Ala Gln Asn Phe
50 55 60

Glu Gly Arg Val Thr Ile Thr Ala Asp Arg Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Ala Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Trp Ala Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180 185 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
195 200 205

```
Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445
```

<210> 122
<211> 449

<212> PRT
<213> Artificial

<220>
<223> VH

<400> 122

```
Gln Val Glu Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Arg Ile Ile Pro Ile Leu Asp Met Ala Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Tyr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Gly Gly Val Asp Trp Tyr Phe Asp Leu Trp Gly Arg Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        115                 120                 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    130                 135                 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        195                 200                 205

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220
```

```
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225                 230                 235                 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    290                 295                 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                 390                 395                 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445

Lys
```

<210> 123
<211> 449

<212> PRT
<213> Artificial

<220>
<223> VH

<400> 123

```
Gln Val Glu Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Glu Asp Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Arg Ile Ile Pro Ile Ile Gly Ile Val Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Tyr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Gly Gly Val Asp Trp Tyr Phe Asp Leu Trp Gly Arg Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        115                 120                 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    130                 135                 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        195                 200                 205

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220
```

```
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225                 230                 235                 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    290                 295                 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                 390                 395                 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445

Lys
```

<210> 124
<211> 449

<212> PRT
<213> Artificial

<220>
<223> VH

<400> 124

```
Gln Val Glu Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Arg Ile Ile Pro Ile Leu Gly Met Ala Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Tyr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Gly Gly Val Asp Trp Tyr Phe Asp Leu Trp Gly Arg Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        115                 120                 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    130                 135                 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        195                 200                 205

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220
```

```
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225                 230                 235                 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    290                 295                 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                 390                 395                 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445

Lys
```

<210> 125
<211> 449

<212> PRT
<213> Artificial

<220>
<223> VH

<400> 125

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Met Lys Val Ser Cys Lys Ala Ser Glu Asp Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Arg Ile Ile Pro Ile Ile Gly Ile Val Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Cys Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Thr Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Gly Gly Val Asp Trp Tyr Phe Asp Leu Trp Gly Arg Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        115                 120                 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    130                 135                 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        195                 200                 205

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220
```

```
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225                 230                 235                 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    290                 295                 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                 390                 395                 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445

Lys
```

<210> 126
<211> 449

<212> PRT
<213> Artificial

<220>
<223> VH

<400> 126

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Arg Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Tyr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Gly Gly Val Asp Trp Tyr Phe Asp Leu Trp Gly Arg Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        115                 120                 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    130                 135                 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        195                 200                 205

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220
```

```
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225                 230                 235                 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    290                 295                 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                 390                 395                 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445

Lys
```

<210> 127
<211> 213

<212> PRT
<213> Artificial

<220>
<223> VL

<400> 127

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
210

<210> 128
<211> 213
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 128

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
210

<210> 129
<211> 213
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 129

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Ser Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe

195 200 205

Asn Arg Gly Glu Cys
210

<210> 130
<211> 213
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 130

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
210

<210> 131
<211> 213
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 131

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asp Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

```
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
            180                 185                 190


Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
        195                 200                 205


Asn Arg Gly Glu Cys
    210


<210>  132
<211>  213
<212>  PRT
<213>  Artificial

<220>
<223>  VL

<400>  132

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
            20                  25                  30


Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45


Tyr Asp Ala Ser Arg Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Phe Thr Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Tyr Thr
                85                  90                  95


Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
            100                 105                 110


Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
        115                 120                 125


Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
    130                 135                 140


Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145                 150                 155                 160
```

```
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
                165                 170                 175


Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
            180                 185                 190


Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
        195                 200                 205


Asn Arg Gly Glu Cys
    210


<210>  133
<211>  213
<212>  PRT
<213>  Artificial

<220>
<223>  VL

<400>  133

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
            20                  25                  30


Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45


Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
                85                  90                  95


Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
            100                 105                 110


Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
        115                 120                 125


Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
    130                 135                 140


Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
```

145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
210

<210> 134
<211> 213
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 134

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asp Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
210

<210> 135
<211> 213
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 135

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

```
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
    130                 135                 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145                 150                 155                 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
                165                 170                 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
            180                 185                 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
        195                 200                 205

Asn Arg Gly Glu Cys
    210


<210>  136
<211>  213
<212>  PRT
<213>  Artificial

<220>
<223>  VL

<400>  136

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Phe Met Ile
        35                  40                  45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
            100                 105                 110
```

```
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
        115                 120                 125


Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
    130                 135                 140


Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145                 150                 155                 160


Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
                165                 170                 175


Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
            180                 185                 190


Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
        195                 200                 205


Asn Arg Gly Glu Cys
    210


<210>  137
<211>  213
<212>  PRT
<213>  Artificial

<220>
<223>  VL

<400>  137

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
            20                  25                  30


Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Phe Met Ile
        35                  40                  45


Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
                85                  90                  95


Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
```

100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
210

<210> 138
<211> 213
<212> PRT
<213> Artificial

<220>
<223> VL

<400> 138

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Phe Met Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

```
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
            100                 105                 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
        115                 120                 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
    130                 135                 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145                 150                 155                 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
                165                 170                 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
            180                 185                 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
        195                 200                 205

Asn Arg Gly Glu Cys
    210


<210>  139
<211>  213
<212>  PRT
<213>  Artificial

<220>
<223>  VL

<400>  139

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Phe Met Ile
        35                  40                  45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
```

```
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
            100                 105                 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
        115                 120                 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
    130                 135                 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145                 150                 155                 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
                165                 170                 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
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            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Phe Met Ile
        35                  40                  45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
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Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Ser Ile Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

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<400> 142

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1 5 10

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 12-0321-WO1	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/EP2011/067339	International filing date *(day/month/year)* 04.10.2011	Priority date *(day/month/year)* 04.10.2010
International Patent Classification (IPC) or national classification and IPC INV. C07K16/28		
Applicant BOEHRINGER INGELHEIM INTERNATIONAL GMBH		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 1-20 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and/or sheets containing rectifications authorized by this Authority, unless those sheets were superseded or cancelled, and any accompanying letters (see Rules 46.5, 66.8, 70.16, 91.2, and Section 607 of the Administrative Instructions).
      ☐ sheets containing rectifications, where the decision was made by this Authority not to take them into account because they were not authorized by or notified to this Authority at the time when this Authority began to draw up this report, and any accompanying letters (Rules 66.4bis, 70.2(e), 70.16 and 91.2).
      ☐ superseded sheets and any accompanying letters, where this Authority either considers that the superseding sheets contain an amendment that goes beyond the disclosure in the international application as filed, or the superseding sheets were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in item 4 of Box No. I and the Supplemental Box (see Rule 70.16(b)).
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see paragraph 3*bis* of Annex C of the Administrative Instructions).

4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03.05.2012	15.01.2013
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399 - 4465	Authorized officer Sirim, Pinar Telephone No. +49 89 2399-7732

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a) and (b))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-57 as originally filed

Sequence listings, SEQ ID NO

1-142 as originally filed

Claims, Numbers

1-30 filed with telefax on 03-05-2012

Drawings, Sheets

1/5-5/5 as originally filed

☒ a sequence listing - see Supplemental Box Relating to Sequence Listing.

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 1-30
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since either they are considered to go beyond the disclosure as filed, or they were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in the Supplemental Box (Rules 70.2(c) and (c-bis)):
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:

5. ☐ This report has been established:
   - ☐ taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91 (Rules 66.1(d-bis) and 70.2(e)).
   - ☐ without taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91(Rules 66.4bis and 70.2(e)).

6. ☐ Supplementary international search report(s) from Authority(ies) has/have been received and taken into account in establishing this report (Rule 45bis.8(b) and (c)).

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-30
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-30
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-30
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search and/or examination

   ☐ to this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the sequence listing, which forms part of the basis of the report, may be marked "superseded."*

1 Documents

The following documents have been taken into consideration:

D1 CARON PHILIP C ET AL: "Biological and immunological features of humanized M195 (anti-CD33) monoclonal antibodies", CANCER RESEARCH, vol. 52, no. 24, 1992, pages 6761-6767, XP009142412, ISSN: 0008-5472

D2 CARON P C ET AL: "MURINE AND HUMANIZED CONSTRUCTS OF MONOCLONAL ANTIBODY M195 (ANTI-CD33) FOR THE THERAPY OF ACUTE MYELOGENOUS LEUKEMIA", CANCER, AMERICAN CANCER SOCIETY, PHILADELPHIA, PA, US, vol. 73, no. 3, SUPPL, 1 January 1994 (1994-01-01), pages 1049-1056, XP009036638, ISSN: 0008-543X

D3 CARON P C ET AL: "ENGINEERED HUMANIZED DIMERIC FORMS OF IGG ARE MORE EFFECTIVE ANTIBODIES", THE JOURNAL OF EXPERIMENTAL MEDICINE, ROCKEFELLER UNIVERSITY PRESS, US, vol. 176, no. 4, 1 January 1992 (1992-01-01), pages 1191-1195, XP000914982, ISSN: 0022-1007, DOI: DOI:10.1084/JEM.176.4.1191

2 Subject-matter of the application

The present application relates to immunotherapies that are based on myeloid cell depletion. In particular, the present invention relates to anti-CD33 antibodies for use in such therapies, e.g. in the treatment of myeloid cell malignancies and myelodysplastic syndrome (MDS).

3 General Remarks

3.1 The amendments filed by the Applicant in response to the Written Opinion of the International Search Authority (WOISA), appear to meet the requirements of Art. 19(2) PCT and/or Art. 34(2)(b) PCT.

3.2 The lack of unity objection raised in the WOISA does no longer maintained for the following reasons:

3.2.1 Present claim 1 is directed to a CD33 binding agent which binds to an epitope within the amino acid sequence defined by SEQ ID NO.141 of human CD33 (i.e. alternative b) of original claim 1). Thus, the binding agents share the common property of binding to human CD33 and recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (SeqID No: 141) of human CD33.

CD33 binding agents are already known in the art (see any of the documents D1 to D3). These documents deal with Lintuzumab, a humanized CD33 antibody based on the Hu195 or SGN-33 antibody. Table 4 on page 57 of present application discloses that Lintuzumab binds to a different epitope than the binding agents of claim 1, namely to the epitope MDPNFWLQVQE (SEQ ID NO. 142) of CD33.

D1 discloses that by 4 hours 25% of the anti-CD33 antibody HuG M195 was internalized (page 6763, right hand column; Figure 3(B)),

D2 discloses that after incubation of HL60 cells by 4 hours 30% of the total cell-associated antibody was internalized into the cell (see D2, page 1051, right-column, last paragraph to page 1053, right-column, figure 1).

D3 discloses that by 5 hours, 30-40% of the CD33 binding antibody HuG1-M195 was internalized into HL60 cells with the remainder on the cell-surface (see D3, page 1192, left hand column, second paragraph; page 1193, left hand column, second paragraph - page 1194, left hand column, first paragraph; Figure 2).

Thus, the prior art teaches that about 25-40% of a precursor antibody of Lintuzumab has been internalized after at least 4 hours incubation of HL60 cells. However, each of these documents applies different internalization assays with varying conditions as regards t the amounts of antibody or test cells or both. This makes it difficult to compare the results obtained with each of these assays. In contrast thereto, the present application discloses a side-by-side comparison between the claimed antibodies and Lintuzumab which binds to the same epitope as any of antibodies of D1 to D3 and therefore has been taken as a representative for said prior art antibodies. The results of said

comparison show that the claimed antibodies are such that at least 40% of the initial amount of the CD33 binding agents remain on the cell surface at at time of 4 hours after incubation. However, when Lintuzumab was tested after the same incubation period only about 20% of the initial amount of CD33/ Lintuzumab complexes was left on the cell surface (see application example 2, pages 53/54, and figures 1-4).

Therefore, it can be accepted that antibodies binding to the specific CD33 epitope as recited in present claim 1 have an improved internalisation kinetics when compared (directly) to antibodies binding to the same epitope as Lintuzumab. Binding to the specific epitope is associated with the unexpected effect of decelerated internalization of CD33 receptor/antibody complexes, and therefore constitutes a special technical feature according to Rule 13.1 PCT. The requirements of unity of invention are met as the binding agents of claim 1 are linked by a single inventive concept (Rule 13.1 PCT).

Re Item V. Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

4

5 **Inventive step**

In view of the arguments submitted together with the Applicant's reply to the WOISA, an inventive step for the presently claimed subject-matter can be acknowledged for the following reasons:

Claim 1 relates to a CD33 binding agent binding to a specific epitope defined by SEQ ID NO.141 of human CD33.

The application shows that after 4 hours of incubation with antibodies which bind to this epitope of CD33 at least 40% of the initial amount of said anti-CD33 antibodies remain on the cell surface.

As already discussed above (see item 3.2), anti-CD33 antibodies binding to human CD33 with these internalization kinetics are known in the art (see any of D1 to D3). All of these antibodies bind to the same epitope as Lintuzumab.

This epitope differs from those of claim 1. The application discloses a direct comparison with Lintuzumab under the same assay conditions. These assays showed that antibodies binding to the epitope of claim 1 have decelerated internalization kinetics compared to Lintuzumab taken as representative of the known closest prior art anti-CD33 antibodies.

Thus, taking any of D1 to D3 as the closest state of the art, the technical problem to be solved may be seen as "the provision of further anti-CD33 antibodies".

As a solution to this problem, the present application provides binding agents binding to the epitope as specified in present claim 1.

The agent according to claim 1 differs from the prior art agents by its binding to a different CD33 epitope. The application has shown that binding to this epitope is associated with the unexpected technical effect of retarded internalization of the antibody when directly compared to an antibody which binds to the different CD33 epitope of D1 to D3. Retarded antibody internalization ensures prolonged availability of the antibody on the cell surface which then can longer exert cell toxicity by itself or mediate such effects by attracting effector cells.

Therefore, an inventive step can be acknowledged for the subject-matter of claim 1 and claims 2 to 30 all of which are based on binding agents binding to an epitope within the specific amino acid sequence of CD33 as recited in claim 1.

6 Further Remarks

6.1 The term "binding agents" as used in present claim 1 is unclear and not sufficiently disclosed by the application. The effect of retarded internalization has only been shown for antibodies binding to the epitope defined by SEQ ID NO. 141. Therefore, this finding should be properly reflected in the claims.

6.2 Claim 30 relates to a method of treatment of the human body by therapy. The patentability of such claims can be dependent upon the formulation of the claims. The EPO, for example, does not recognise as patentable claims to the use of a compound in medical treatment, but may allow claims to a product, in particular substances or compositions for use in a first or further medical treatment.

Telefax

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Patents RCV

European Patent Office Munich

0049-89/ 2399 4465

Number of Pages: 33

May 03, 2012

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Sitz: Wien
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Gesellschaft mit beschränkter
Haftung

International Application PCT/EP2011/067339
Title: "CD33 binding agents"
Applicant: BOEHRINGER INGELHEIM INTERNATIONAL GMBH
Our Ref.: P12-0321-WO1

RESPONSE

to the Written Opinion of the International Searching Authority ("WOISA") dated 4 October 2011

Concomitantly with this response including amended claims pursuant to Article 34 PCT ("new claims"), a Chapter II PCT Demand is filed and the corresponding fee is paid. International Preliminary Examination should thus be carried out on the enclosed new claims and by kindly considering our arguments and explanations which follow herein below.

I. REQUESTS

1. Based on the amended set of claims (copy enclosed), International Preliminary Examination according to Article 34 PCT is requested.

2. It is further kindly requested that a positive International Preliminary Report on Patentability be issued given the amendments made to the original claims and the arguments and explanations provided herein below which take into account the concerns that the ISA had with the original claims.

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3. In the event that the arguments presented below might require further discussion, the undersigned offers and kindly requests that the Examiner contacts the undersigned via phone. The undersigned is prepared to discuss the case with the Examiner.

II. CLAIM AMENDMENTS

Applicant herewith files an amended set of claims which should be the basis for International Preliminary Examination. The following amendments, which should be apparent from the enclosed copy showing the changes highlighted (see the enclosed marked-up copy), have been effected:

1. New Claim 1 is based on original claim 1 which was amended by deleting alternative (a). In addition, for the sake of clarity, the claimed binding agent is now defined to bind an epitope, rather than to *recognize* an epitope. This amendment finds support on page 5, lines 22-28 and page 7 and 8 of the application as originally filed.

2. New claim 2 has been added and depends from new claim 1. New claim 2 is entirely based on alternative (a) of original claim 1.

3. New claim 3 corresponds to original claim 2.

4. Original claim 3 was deleted.

5. The remainder of the original claims remains unchanged.

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III. COMMENTS ON THE OBJECTIONS SET OUT IN THE WOISA

1. Remarks on the gist of the claimed invention

1.1 Applicant has found a new epitope on the CD33 molecule which, upon binding of an antibody, results in a decelerated internalization of CD33 and thus in a prolonged availability of the antibody on the cell surface such that it can mediate cell dependent cytotoxicity for a longer time than prior art antibodies.. CD33 is a so-called tumor marker molecule and is thus an attractive target for binding molecules such as antibodies. In fact, CD33 is a myeloid lineage-specific antigen and is therefore particularly expressed on hematopoietic tumors and therefore a target for treating in particular acute myeloid leukemia (AML), since it is does not appear in abundance on "normal" cells. Thus far, a couple of companies such as Seattle Genetics and Wyeth/Pfizer were involved in the development of an antibody against CD33. A promising candidate was a humanized anti-CD33 antibody, called Lintuzumab (*note* that documents **D1** to **D3** (all naming Caron as first author) as cited in the ISR concern the "precursor" antibody (Hu195 or SGN-33) of Lintuzumab). However, trials for the treatment of AML were abandoned in 2010 when a phase IIb trial failed to show increased survival.

A similar drug, called Gemtuzumab ozogamicin – an antibody conjugated with a chemotherapeutic agent – which was approved by FDA was withdrawn from the market in 2010 after further trials showed only little benefit to patients and an increased rate of venoocclusive disease and liver toxicity which could even lead to death.

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1.2 CD33 is a molecule known to be internalized by cells rather quickly. For antibodies that are designed to deliver a chemotherapeutic agent this property is rather desirable, while for antibodies that should opsonize cells in order to exert an antibody dependent killing mode such as antibody dependent cell cytotoxicity (ADCC) or antibody dependent phagocytosis (ADPC) it would be more desirable that the antibody bound to CD33 would not be internalized too fast, since internalization and mediating cell dependent cytotoxicity are inversely correlated.
However, both Lintuzumab and Gemtuzumab ozogamicin are internalized rather fast; see Figure 6 on page 3202 in van der Velden et al. in Blood (2001); 97:3197-3204:

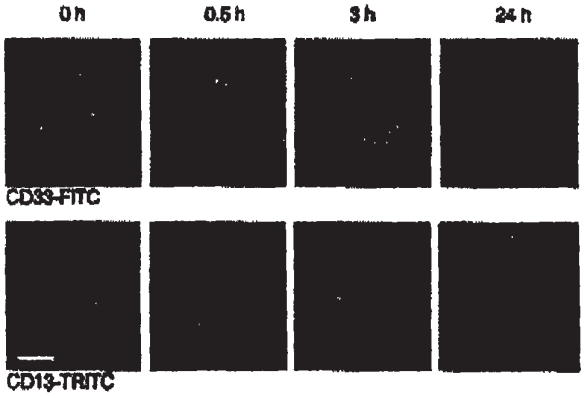

Figure 6. Dual color laser microscopic images showing the internalization of the CD33 antibody P67.6. The CD33+ myeloid cell line MEG-01 was incubated at 37°C with P67.6-FITC for different periods as indicated, followed by labeling with CD13 and GoM-TRITC. The upper panel shows the P67.6-FITC staining (green channel), and the bottom panel shows the red channel of exactly the same image displaying the membrane staining with CD13/GoM-TRITC (bar = 10 μm).

Indeed, after 3hrs Gemtuzumab ozogamicin is almost fully internalized by CD33-positive tumor cells. For Gemtuzumab

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ozogamicin this makes perfectly sense, since it is designed to deliver its cytotoxcic load (chalichcamicin) to the cytoplasm.

For Lintuzumab it would be preferable if the antibody would not be internalized too fast, but rather slowly, because it is designed to either exert ADCC or ADCP; see page 483, paragraph bridging left and right column of Sutherland et al in MAbs (2009) 1: 5; 481-490:

> In the presence of primary human NK cells, lintuzumab mediated the tumor cell killing of MDR- KG-1 cells and the MDR+ HEL9217 and TF1-a cell lines in a dose-dependent manner (Fig. 3A). Low, but clearly detectable, ADCC activity could also be detected against the AML blasts from an AML patient with refractory disease (Fig. 3A). In contrast, the hIgG1κ control antibody demonstrated minimal activity. Decreased levels of specific lysis observed with primary cells as compared to the cell lines may result from reduced chromium-51 uptake by the AML patient cells and a lower cell viability contributing to a higher background lysis.
>
> Removal of antibody-coated tumor cells by FcγR-mediated phagocytosis (ADCP) has been hypothesized to be a key mechanism for target cell depletion mediated by therapeutic mAbs in vivo.[34,35] In the current study, the ability of lintuzumab to mediate ADCP against three different AML cell lines with macrophages generated from GM-CSF-treated normal human PBMCs as effector cells, was investigated. These mac-

For both killing modes (ADCC and ADCP), the antibody should remain bound on the cell surface of a tumor cell, and should not be internalized quickly. However, a direct comparison between Lintuzumab and the antibodies of the present invention clearly revealed that, while at least 40% of the initial amount of a claimed CD33 antibody remains left on the cell surface of HL60 cells at a time of 4 hrs after incubation, Lintuzumab was internalized to an extent of about 80% after 4 hrs incubation, thereby only 20% of the initial amount of Lintuzumab remained left on the surface of HL60 cells; see Example 2 on pages 53 and 54 as well as Figures 1-4.

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1.3 In sharp contrast to the ultra-fast internalization of Gemtuzumab ozogamicin and the somewhat "slower", but even fast internalization of Lintuzumab, the antibodies of the present invention are internalized much slower and are thus improved, since they can opsonize tumor cells for a prolonged period of time and thus mediate cytotoxicity; see pages 53 and 54 as well as page 55, lines 18-24 of the present application for a direct comparison between Lintuzumab and the antibodies of the present invention.

This improvement is achieved by the binding of the antibodies to an epitope different from that bound by Gemtuzumab ozogamicin and Lintuzumab, respectively. It could not at all have been expected that the binding of an anti-CD33 antibody to the epitope to which the antibodies of the present invention bind, would decrease the internalization of such an antibody.
Astonishingly, even though the antibodies of the present invention differ to a certain degree in their CDRs, they all bind to the same epitope; see Table 4 on page 57, which summarizes the epitope mapping data of the antibodies that are now subject of new claim 2.

1.4 All in all, the antibodies of the present invention are both novel and inventive over prior art antibodies due to their binding to a novel epitope within CD33, whereby binding to said epitope effects a decreased internalization of CD33 which, as explained above, is highly desirable. Accordingly, antibodies of the present invention can be expected to show the improved effect that, for example, Lintuzumab could not achieve in the late phase clinical trial, i.e. an improvement in mediating cell-dependent cytotoxicity and thus an improvement in the treatment of cancer such as AML.

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1.5 In sum, the binding of the claimed antibodies to a novel epitope within CD33 and the effect caused by their binding (decreased internalization of CD33) is the novel and inventive feature that unifies these antibodies.

2. Unity (Rule 13 PCT)

2.1 The ISA objected that the present application would lack unity. The applicant traverses this assessment. Based on the amended claims, this objection should be overcome.

2.2 According to Rule 13 PCT the international application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept. Where a group of inventions is claimed in one and the same international application, the requirement of unity of invention referred to in Rule 13.1 PCT shall be fulfilled only when there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. The expression "special technical features" shall mean those technical features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art.
New claim 1 relates to a CD33 binding agent binding to human CD33, the CD33 binding agent being defined by binding to an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (SeqID No: 141) of human CD33.
All further claims are directly or indirectly dependent on claim 1, thus the unity argumentation relating to claim 1 relates to the entire claim set.

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As said, new claim 1 defines the CD33 binding agent by way of its binding capacity to the specific epitope, FFIIPIPYYDKNSPVHGYW. This specifically claimed antibody-epitope binding is a special technical feature according to Rule 13.1 PCT. Indeed, this particular epitope does not belong to prior art knowledge and the effect (decelerated internalization of CD33) linked with the binding of a claimed binding molecule to said epitope is surprising.
In particular, the relevant prior art antibodies such as those disclosed in all prior art documents dealing with Lintuzumab (humanized CD33 antibody based on the HuM195 antibody), i.e., **D1**, **D2** and **D3** bind to a different epitope and do not cause a deceleration of CD33 internalization; see also Table 4 on page 57, where Lintuzumab's epitope – which is different from the epitope referred to in new claim 1 – is shown.

As explained above in section III.1, the claimed antibodies, though they show differences in their CDRs do nevertheless all surprisingly bind to the same epitope. This fact is reflected in new claim 1. Hence, although the antibodies of new claim 2 differ in their CDRs, there is a technical relationship (unifying feature/technical relationship) among the alleged 13 inventions: their binding to the novel and inventive epitope as referred to in new claim 1.
This special technical feature, namely the capacity of binding to the novel epitope, is accompanied with an important technical improvement of the claimed CD33 binding agent. In detail, the binding agent according to new claim 1 which is able to bind to the novel epitope, provides improved internalization kinetics compared to correspondent binding agents known from the prior art.
Internalization of antibody means the reduction of the amount of antibody/ antigen complexes on the cell surface of the target cell

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after incubation with antibody. The more the internalization of an antibody is retarded the higher is its potential medical efficacy. Unexpectedly the inventors found (see e.g. example 2 of the present application) that internalization of all new found CD33 binding agents which bind to the novel epitope was significally decelerated compared to lintuzumab which represents the closest prior art antibody.

The single general inventive concept is thus, the provision of a new and improved group of CD33 binding agents which share one special technical feature in common: binding to a novel epitope within CD33 which has the unexpected technical effect of decelerated internalization, i.e., at least 40% of the initial amount of the CD33 binding agents remain on the cell surface at a time of 4 hrs after incubation; see Example 2 and Figures 1-4.

As the invention underlying the entire claimed subject matter is linked by such a single general inventive concept, the claimed subject matter complies with the unity requirements according to the PCT.

3. **Novelty (Art. 33(2) PCT)**

Novelty was acknowledged in the WOISA and in view of the explanations provided above and the data shown in present application, we submit that there can be no doubt whatsoever about novelty of the (original and) new claims.

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4. Inventive step (Art. 33(3) PCT)

4.1 The present invention relates to CD33 binding agents which can be used in cancer therapy, e.g. in the treatment of myeloid cell malignancies such as AML and myelodysplastic syndrome (MDS). In the prior art it has been shown that CD33 antigen is frequently expressed on tumor cells that originate from the myelomonocytic lineage, such as myeloid leukemia cells. Unfortunately, clinical development of lintuzumab was discontinued after results from a phase II trial, either as monotherapy or in combination with chemotherapy, since it did not yield the expected improvement in efficacy. Gemtuzumab ozogamicin, a CD33-binding antibody conjugated to a chemotherapeutic, was withdrawn from the market because of undesired side effects that were not outweighed by a beneficial therapeutic effect. Therefore, there has been a clear and unmet need for the development of improved CD33-targeting treatment modalities.

4.2 The objective technical problem over the closest prior art **D1** (or alternatively **D2** or **D3**), is the provision of a CD33 binding agent that shows prolonged availability to remain on the cell surface in order to mediate cell dependent cytotoxicity for a longer period of time, since CD33 is rather quickly internalized, thereby taking the antibody out of action.
The solution for this problem is realized for the first time by the binding agents according to the present application that are characterized by their capacity to bind to the new specific epitope.
The solution is plausible since the present application demonstrates in a side-by-side comparison the internalization kinetics of the claimed antibodies and Lintuzumab, the closest prior art antibody.

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4.3 This finding was neither foreseeable nor expectable as none of the prior art documents contains a hint as to how an antibody should be constructed in order to achieve a prolonged availability to remain on the cell surface. Even worse, all prior art documents that address internalisation kinetics of CD33 binding agents lead rather away from the claimed subject matter of the present application. All the disclosed antibodies according to **D1-D3** bind to a different epitope, namely to MDPNFWLQVQE (see e.g. Table 4 on page 57 of the present application). Binding to this epitope results in an internalisation kinetic profile that is significantly worse in comparison to the profile of the claimed antibodies. Specifically, the claimed antibodies are such that at least 40% of the initial amount of the CD33 binding agents remain on the cell surface at a time of 4 hrs after incubation, while the closest prior art antibody Lintuzumab fails to reach such a level after 4 hrs incubation; see Example 2 and Figures 1-4. Such an improved internalisation kinetics profile however could neither be expected nor could its reason have been expected to lie in the binding to the special epitope within CD33 as referred to in new claim 1.

The fact that **D1**, **D2** or **D3** assert that about 25-40% of a precursor antibody of Lintuzumab, Lintuzumab itself or a dimerized Lintuzumab has been internalized, seems to implicate[1] that the remaining antibody is cell surface bound, is more or less irrelevant, since each of the documents applies a different internalization assay. Indeed, either different amounts of antibody or test cells or both is/are varied (see **D1** on page 6762, left column "Modulation of cell surface antigen"; **D2** on page 1051, right column "Internalization

[1] Note that none of D1, D2 or D3 explicitly mentions which amount of antibody remains left on the cell surface. They only mention which amount is internalized, but are silent about the fate of the antibody that is allegedly not internalized.

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studies"; **D3** on page 1192, left column "Internalization studies").
This makes each of the internalization assays of **D1** to **D3** unreliable which is, all the more, reflected by the fact that even though the authors of **D1** to **D3** are more or less the same, they obtain each time different results. Would they have applied the same conditions as was done by the present inventors, they would have observed what the present inventors have found: an antibody which binds the epitope of Lintuzumab does not show the decelerated internalization kinetics as do the claimed antibodies.
Be that as it may, the side-by-side comparison between the claimed antibodies and Lintuzumab (being representative for prior art antibodies that bind to the same epitope as Lintuzumab) shows that the claimed antibodies are superior and thus advantageous!

The claimed subject matter of the present application is thus inventive over the prior art, since it could neither have been expected that an epitope binding other than to the known prior art epitopes, specifically other than to MDPNFWLQVQE (**D1**-**D3**) would provide for the technical effect observed by the present inventors, i.e., a delayed/retarded internalization which ensures prolonged availability of the antibody on the cell surface in order to exert by itself ADCC or mediate a cell toxicity effect on the target cell by attracting T-cells, NK cells and phagocytes.

Thus, the subject matter underlying the new claims of the present application was not obvious in view of the prior art, but involves an inventive step.

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Respectfully submitted,

Dr. Conrad Eckhardt
European Patent Attorney
GA 31039

Enclosures:
New set of claims (marked-up and clean copy)
Chapter II PCT Demand
Fee Calculation Sheet
Form 1037

New Claims

1. A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by binding to an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (SeqID No: 141) of human CD33.

2. A CD 33 binding agent according to claim 1, characterized in that having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from SeqID No:1-14, CDR2 has an amino acid sequence selected from SeqID No:15-28, CDR3 has an amino acid sequence selected from SeqID No:29-42, CDR4 has an amino acid sequence selected from SeqID No:43-56, CDR5 has an amino acid sequence selected from SeqID No:57-70, CDR6 has an amino acid sequence selected from SeqID No:71-84

3. A CD33 binding agent according to claim 1 or 2, wherein the epitope is determined by Hydrogen Exchange Mass Spectroscopy.

4. A CD33 binding agent according to to any of the preceding claims, wherein the internalization kinetics of the CD33 binding agents are such that at least 40% of the initial amount of the CD33 binding agents remain on the cell surface at a time of 4 hours after incubation.

5. A CD33 binding agent according to any of the preceding claims, wherein the heavy chain variable region comprises an amino acid sequence selected from SeqID No:85-98 and the light chain variable region comprises an amino acid sequence selected from SeqID No:99-112.

6. A CD33 binding agent according to any of the preceding claims, wherein the CD33 binding agent has a heavy chain having an amino acid sequence selected from SeqID No:113-126 and a light chain having an amino acid sequence selected from SeqID No:127-140.

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7. A CD33 binding agent according to any of the preceding claims, wherein the CD33 binding agent has an affinity to both human CD33 and cynomolgus CD33 with a K_D of equal to or less than 10 nM.

8. A CD33 binding agent according to any of the preceding claims, wherein the CD33 binding agent is humanized.

9. A CD33 binding agent according to any of the preceding claims, wherein the CD33 binding agent is fully human.

10. A CD33 binding agent according to any of the preceding claims, wherein the CD33 binding agent further comprises an effector function.

11. A CD33 binding agent according to claim 10, wherein the effector function is mediated by an F_c domain.

12. A CD33 binding agent according to claim 10, wherein the CD33 binding agent comprises one or more mutations in the F_c domain that modulate the function of the F_c domain.

13. A CD33 binding agent according to claim 12, wherein the modulation of the function of the Fc domain is an increase of ADCC by at least 10%, preferably 50% and more preferably 100%.

14. A CD33 binding agent according to any of claim 12-13, wherein the mutations in the F_c domain are located at one or more positions selected from amino acids at positions 332 and/or 239 and/or 236 according to the Kabat EU numbering index.

15. A CD33 binding agent according to any of claim 12-14, wherein the mutations in the F_c domain are a combination of substitutions at positions 239 and 332, preferably S239D/I332E.

16. A CD33 binding agent selected from
an antibody comprising a CDR1 of SeqID No: 1, a CDR2 of SeqID No: 15, a CDR3

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of SeqID No: 29, a CDR4 of SeqID No: 43, a CDR5 of SeqID No: 57 and a CDR6 of SeqID No: 71,

an antibody comprising a CDR1 of SeqID No: 2, a CDR2 of SeqID No: 16, a CDR3 of SeqID No: 30, a CDR4 of SeqID No: 44, a CDR5 of SeqID No: 58 and a CDR6 of SeqID No: 72,

an antibody comprising a CDR1 of SeqID No: 3, a CDR2 of SeqID No: 17, a CDR3 of SeqID No: 31, a CDR4 of SeqID No: 45, a CDR5 of SeqID No: 59 and a CDR6 of SeqID No: 73,

an antibody comprising a CDR1 of SeqID No: 4, a CDR2 of SeqID No: 18, a CDR3 of SeqID No: 32, a CDR4 of SeqID No: 46, a CDR5 of SeqID No: 60 and a CDR6 of SeqID No: 74,

an antibody comprising a CDR1 of SeqID No: 5, a CDR2 of SeqID No: 19, a CDR3 of SeqID No: 33, a CDR4 of SeqID No: 47, a CDR5 of SeqID No: 61 and a CDR6 of SeqID No: 75,

an antibody comprising a CDR1 of SeqID No: 6, a CDR2 of SeqID No: 20, a CDR3 of SeqID No: 34, a CDR4 of SeqID No: 48, a CDR5 of SeqID No: 62 and a CDR6 of SeqID No: 76,

an antibody comprising a CDR1 of SeqID No: 7, a CDR2 of SeqID No: 21, a CDR3 of SeqID No: 35, a CDR4 of SeqID No: 49, a CDR5 of SeqID No: 63 and a CDR6 of SeqID No: 77,

an antibody comprising a CDR1 of SeqID No: 8, a CDR2 of SeqID No: 22, a CDR3 of SeqID No: 36, a CDR4 of SeqID No: 50, a CDR5 of SeqID No: 64 and a CDR6 of SeqID No: 78,

an antibody comprising a CDR1 of SeqID No: 9, a CDR2 of SeqID No: 23, a CDR3 of SeqID No: 37, a CDR4 of SeqID No: 51, a CDR5 of SeqID No: 65 and a CDR6 of SeqID No: 79,

an antibody comprising a CDR1 of SeqID No: 10, a CDR2 of SeqID No: 24, a CDR3 of SeqID No: 38, a CDR4 of SeqID No: 52, a CDR5 of SeqID No: 66 and a CDR6 of SeqID No: 80,

an antibody comprising a CDR1 of SeqID No: 11, a CDR2 of SeqID No: 25, a CDR3 of SeqID No: 39, a CDR4 of SeqID No: 53, a CDR5 of SeqID No: 67 and a CDR6 of SeqID No: 81,

an antibody comprising a CDR1 of SeqID No: 12, a CDR2 of SeqID No: 26, a CDR3 of SeqID No: 40, a CDR4 of SeqID No: 54, a CDR5 of SeqID No: 68 and a CDR6 of

SeqID No: 82,
an antibody comprising a CDR1 of SeqID No: 13, a CDR2 of SeqID No: 27, a CDR3 of SeqID No: 41, a CDR4 of SeqID No: 55, a CDR5 of SeqID No: 69 and a CDR6 of SeqID No: 83,
an antibody comprising a CDR1 of SeqID No: 14, a CDR2 of SeqID No: 28, a CDR3 of SeqID No: 42, a CDR4 of SeqID No: 56, a CDR5 of SeqID No: 70 and a CDR6 of SeqID No: 84.

17. A CD33 binding agent selected from
an antibody comprising a heavy chain variable region of SeqID No: 85 and a light chain variable region of SeqID No: 99,
an antibody comprising a heavy chain variable region of SeqID No: 86 and a light chain variable region of SeqID No: 100,
an antibody comprising a heavy chain variable region of SeqID No: 87 and a light chain variable region of SeqID No: 101,
an antibody comprising a heavy chain variable region of SeqID No: 88 and a light chain variable region of SeqID No: 102,
an antibody comprising a heavy chain variable region of SeqID No: 89 and a light chain variable region of SeqID No: 103,
an antibody comprising a heavy chain variable region of SeqID No: 90 and a light chain variable region of SeqID No: 104,
an antibody comprising a heavy chain variable region of SeqID No: 91 and a light chain variable region of SeqID No: 105,
an antibody comprising a heavy chain variable region of SeqID No: 92 and a light chain variable region of SeqID No: 106,
an antibody comprising a heavy chain variable region of SeqID No: 93 and a light chain variable region of SeqID No: 107,
an antibody comprising a heavy chain variable region of SeqID No: 94 and a light chain variable region of SeqID No: 108,
an antibody comprising a heavy chain variable region of SeqID No: 95 and a light chain variable region of SeqID No: 109,
an antibody comprising a heavy chain variable region of SeqID No: 96 and a light chain variable region of SeqID No: 110,
an antibody comprising a heavy chain variable region of SeqID No: 97 and a light

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chain variable region of SeqID No: 111,
an antibody comprising a heavy chain variable region of SeqID No: 98 and a light chain variable region of SeqID No: 113,

18. A CD33 binding agent selected from
an antibody comprising a heavy chain of SeqID No: 113 and a light chain of SeqID No: 127,
an antibody comprising a heavy chain of SeqID No: 114 and a light chain of SeqID No: 128,
an antibody comprising a heavy chain of SeqID No: 115 and a light chain of SeqID No: 129,
an antibody comprising a heavy chain of SeqID No: 116 and a light chain of SeqID No: 130,
an antibody comprising a heavy chain of SeqID No: 117 and a light chain of SeqID No: 131,
an antibody comprising a heavy chain of SeqID No: 118 and a light chain of SeqID No: 132,
an antibody comprising a heavy chain of SeqID No: 119 and a light chain of SeqID No: 133,
an antibody comprising a heavy chain of SeqID No: 120 and a light chain of SeqID No: 134,
an antibody comprising a heavy chain of SeqID No: 121 and a light chain of SeqID No: 135,
an antibody comprising a heavy chain of SeqID No: 122 and a light chain of SeqID No: 136,
an antibody comprising a heavy chain of SeqID No: 123 and a light chain of SeqID No: 137,
an antibody comprising a heavy chain of SeqID No: 124 and a light chain of SeqID No: 138,
an antibody comprising a heavy chain of SeqID No: 125 and a light chain of SeqID No: 139,
an antibody comprising a heavy chain of SeqID No: 126 and a light chain of SeqID No: 140.

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19. A DNA molecule comprising a region encoding the heavy chain variable region of a CD33 binding agent according to any of the preceding claims.

20. A DNA molecule comprising a region encoding the light chain variable region of a CD33 binding agent according to any of claims 1-18.

21. An expression vector containing a DNA molecule of claim 19 or 20.

22. A host cell carrying one or more vectors of claim 21.

23. A method for producing a CD33 binding agent of any one of claims 1-18, comprising transfecting a host cell with one or more vectors of claim 21, culturing the host cell and recovering and purifying the antibody molecule.

24. A pharmaceutical composition comprising, as the active ingredient, one or more CD33 binding agents according to any one of claims 1 to 18, and at least a physiologically acceptable carrier.

25. The pharmaceutical composition of claim 24, further comprising one or more additional therapeutic agents.

26. The pharmaceutical composition of any one of claims 24-25 for depleting cells that express CD33 on their surface.

27. The pharmaceutical composition of any one of claims 24-25 for the treatment of myeloid cell malignancies and myelodysplastic syndrome (MDS).

28. The pharmaceutical composition of claim 27, wherein said myeloid malignancy is selected from acute myeloid leukemia and chronic myeloid leukemia.

29. The pharmaceutical composition of any one of claims 24-25 for the treatment of autoimmune and inflammatory diseases that involve myeloid cells in their pathology.

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30. A method of depleting CD33 expressing myeloid cells in a patient, comprising administering to said patient one or more CD33 binding agents according to any one of claims 1-18 or a pharmaceutical composition according to any of claims 24-25.

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PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2011/067339	04.10.2011	04.10.2010

International Patent Classification (IPC) or both national classification and IPC
INV. C07K16/28 A61K39/395

Applicant
BOEHRINGER INGELHEIM INTERNATIONAL GMBH

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office

D-80298 Munich
Tel. +49 89 2399 - 0
Fax: +49 89 2399 - 4465

Date of completion of this opinion

see form PCT/ISA/210

Authorized Officer

Sirim, Pinar

Telephone No. +49 89 2399-7732

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☒ in electronic form

   b. (time)

      ☒ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. IV Lack of unity of invention

1. ☐ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has, within the applicable time limit:

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☐ not paid additional fees

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos. 1-30

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-30
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-30
Industrial applicability (IA)	Yes:	Claims	1-30
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item IV. Lack of unity of invention

As already indicated on the ISR this Authority has considered that the application lacks unity as required by Rule 13 PCT and that there are 13 inventions covered by the claims. The following inventions have been identified:

Invention 1: claims: 1-30 (partially) = A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:1, CDR2 has an amino acid sequence selected from Seq ID No:15, CDR3 has an amino acid sequence selected from Seq ID No:29, CDR4 has an amino acid sequence selected from Seq ID No: 43, CDR5 has an amino acid sequence selected from Seq ID No:57, CDR6 has an amino acid sequence selected from Seq ID No:71, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

Invention 2: claims: 1-30 (partially) = A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:2, CDR2 has an amino acid sequence selected from Seq ID No:16, CDR3 has an amino acid sequence selected from Seq ID No:30, CDR4 has an amino acid sequence selected from Seq ID No:44, CDR5 has an amino acid sequence selected from Seq ID No:58, CDR6 has an amino acid sequence selected from Seq ID No:72, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

Invention 3: claims: 1-30 (partially) = A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:3, CDR2 has an amino acid sequence selected from Seq ID No:17, CDR3 has an amino acid sequence selected from Seq ID No:31, CDR4 has an amino acid sequence selected from Seq ID

No:45, CDR5 has an amino acid sequence selected from Seq ID No:59, CDR6 has an amino acid sequence selected from Seq ID No:73, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

Invention 4: claims: 1-30 (partially) = A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:4, CDR2 has an amino acid sequence selected from Seq ID No:18, CDR3 has an amino acid sequence selected from Seq ID No:32, CDR4 has an amino acid sequence selected from Seq ID No:46, CDR5 has an amino acid sequence selected from Seq ID No:60, CDR6 has an amino acid sequence selected from Seq ID No:74, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

Invention 5: claims: 1-30 (partially) = A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:5, CDR2 has an amino acid sequence selected from Seq ID No:19, CDR3 has an amino acid sequence selected from Seq ID No:33, CDR4 has an amino acid sequence selected from Seq ID No:47, CDR5 has an amino acid sequence selected from Seq ID No:61, CDR6 has an amino acid sequence selected from Seq ID No:75, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

Invention 6: claims: 1-30 (partially) = A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:6, CDR2 has an amino acid sequence selected from Seq ID No:20, CDR3 has an amino acid sequence selected from Seq ID No:34, CDR4 has an amino acid sequence selected from Seq ID No:48, CDR5 has an amino acid sequence selected from Seq ID No:62,

CDR6 has an amino acid sequence selected from Seq ID No:76, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (SeqID No: 141) of human CD33

Invention 7: claims: 1-30 (partially) = A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:7, CDR2 has an amino acid sequence selected from Seq ID No:21, CDR3 has an amino acid sequence selected from Seq ID No:35, CDR4 has an amino acid sequence selected from Seq ID No:49, CDR5 has an amino acid sequence selected from Seq ID No:63, CDR6 has an amino acid sequence selected from Seq ID No:77, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

Invention 8: claims: 1-30 (partially) = A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:8, CDR2 has an amino acid sequence selected from Seq ID No:22, CDR3 has an amino acid sequence selected from Seq ID No:36, CDR4 has an amino acid sequence selected from Seq ID No:50, CDR5 has an amino acid sequence selected from Seq ID No:64, CDR6 has an amino acid sequence selected from Seq ID No:78, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

Invention 9: claims: 1-30 (partially) = A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:9, CDR2 has an amino acid sequence selected from Seq ID No:23, CDR3 has an amino acid sequence selected from Seq ID No:37, CDR4 has an amino acid sequence selected from Seq ID No:51, CDR5 has an amino acid sequence selected from Seq ID No:65,

CDR6 has an amino acid sequence selected from Seq ID No:79, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

Invention 10: claims: 1-30 (partially) = A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:10, CDR2 has an amino acid sequence selected from Seq ID No:24, CDR3 has an amino acid sequence selected from Seq ID No:38, CDR4 has an amino acid sequence selected from Seq ID No:52, CDR5 has an amino acid sequence selected from Seq ID No:66, CDR6 has an amino acid sequence selected from Seq ID No:80, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

Invention 11: claims: 1-30 (partially) = A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:11 or 13, CDR2 has an amino acid sequence selected from Seq ID No:25 or 27, CDR3 has an amino acid sequence selected from Seq ID No:39 or 41, CDR4 has an amino acid sequence selected from Seq ID No:53 or 55, CDR5 has an amino acid sequence selected from Seq ID No:67 or 69, CDR6 has an amino acid sequence selected from Seq ID No:81 or 83, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

Invention 12 = claims: 1-30 (partially) = A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:12, CDR2 has an amino acid sequence selected from Seq ID No:26, CDR3 has an amino acid sequence selected from Seq ID No:40, CDR4 has an amino acid sequence selected from Seq ID No:54, CDR5 has an amino acid sequence selected from Seq ID

No:68, CDR6 has an amino acid sequence selected from Seq ID No:82, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

Invention 13: claims: 1-30 (partially) = A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:14, CDR2 has an amino acid sequence selected from Seq ID No:28, CDR3 has an amino acid sequence selected from Seq ID No:42, CDR4 has an amino acid sequence selected from Seq ID No:56, CDR5 has an amino acid sequence selected from Seq ID No:70, CDR6 has an amino acid sequence selected from Seq ID No:84, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

An international application must relate to one invention only or to a group of inventions so linked as to form a single general inventive concept (Rule 13.1 PCT). Unity of invention is fulfilled only when there is a technical relationship among the inventions involving one or more of the same or corresponding special technical features.

Special technical features are such features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art.

The binding agents share the common property of binding to human CD33 and recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (SeqID No: 141) of human CD33. However, neither the mere presence of 6 CDRs can be regarded as the shared significant structural element because it is the specific sequence of all 6 CDRs in combination that is essential to the common property shared by the individual binding agents nor the binding to a common epitope since this binding is not associated with a previously unknown technical effect as outlined in the following:

CD33 binding agents having 6 CDRs and the same property are already known in the art. Caron et al., CANCER RESEARCH, vol. 52, no. 24, 1992, pages 6761-6767 discloses (page 6761, right hand column, last paragraph; Figure 3(B)) a humanized anti-CD33 mAb having 6 CDRs. Here the internalization kinetics of the antibody are such that 75% of the total cell-associated antibody remained on the cell surface of HL60 cells by 4 hours. Caron et al., CANCER, vol. 73, no. 3, SUPPL, 1 January 1994 (1994-01-01), pages 1049-1056 discloses (page 1050, left hand column, second paragraph - page 1053, right hand column, second paragraph; Figure 1) that after 4 hours 30% of the CD33 binding antibody HuG1 (has 6 CDRs) had entered the HL60 cells whereas the rest (i.e. 70%) remained on the cell surface. Caron et al., THE JOURNAL OF EXPERIMENTAL MEDICINE, vol. 176, no. 4, 1 January 1992 (1992-01-01), pages 1191-1195 discloses (page 1191, right hand column, second paragraph; page 1192, left hand column, second paragraph; page 1193, left hand column, second paragraph - page 1194, left hand column, first paragraph; Figure 2) that by 5 hours, 40% of the CD33 binding antibody HuG1 (has 6 CDRs) remained on the cell surface of HL60 cells.

Thus, although these antibodies may bind to a different epitope than those specified in present claims, they have the same internalisation kinetics as defined in the dependent claims. These prior art documents disclose that after 4 hours incubation of HL60 cells also at least of the 30% of the initial amount of the anti-CD33 antibody remains on the cell surface.

In view of the prior art the problem underlying the application can be defined as the provision of further CD33 binding agents binding to human CD33 having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6 binding and recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33. Claim 1 provides 13 different groups of solutions to this problem. In view of the structural differences between the claimed binding agents and in the absence of any technical feature that would provide a unifying link between the claimed binding agents and distinguish these binding agents from the binding agents disclosed in the prior art, each and every binding agent provided by the

present application has to be considered a different solution to this problem. Claim 1 as originally filed thus provides 13 different solutions to this problem resulting in 13 inventions.

For the sake of efficiency, all inventions have been searched without asking for additional fees. Therefore, this opinion has been established in respect of all 13 inventions. However, when entering national examination phase, the Applicant will be required to restrict the application to one of the 13 inventions searched.

Re Item V.Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 **Documents**

The following documents have been taken into consideration:

D1 CARON PHILIP C ET AL: "Biological and immunological features of humanized M195 (anti-CD33) monoclonal antibodies", CANCER RESEARCH, vol. 52, no. 24, 1992, pages 6761-6767, XP009142412, ISSN: 0008-5472

D2 CARON P C ET AL: "MURINE AND HUMANIZED CONSTRUCTS OF MONOCLONAL ANTIBODY M195 (ANTI-CD33) FOR THE THERAPY OF ACUTE MYELOGENOUS LEUKEMIA", CANCER, AMERICAN CANCER SOCIETY, PHILADELPHIA, PA, US, vol. 73, no. 3, SUPPL, 1 January 1994 (1994-01-01), pages 1049-1056, XP009036638, ISSN: 0008-543X

D3 CARON P C ET AL: "ENGINEERED HUMANIZED DIMERIC FORMS OF IGG ARE MORE EFFECTIVE ANTIBODIES", THE JOURNAL OF EXPERIMENTAL MEDICINE, ROCKEFELLER UNIVERSITY PRESS, US, vol. 176, no. 4, 1 January 1992 (1992-01-01), pages 1191-1195, XP000914982, ISSN: 0022-1007, DOI: DOI:10.1084/JEM.176.4.1191

D4 KUEGLER MARKUS ET AL: "A recombinant trispecific single-chain Fv derivative directed against CD123 and CD33 mediates effective elimination of acute myeloid leukaemia cells by dual targeting", BRITISH JOURNAL OF HAEMATOLOGY, WILEY-

BLACKWELL PUBLISHING LTD, GB, vol. 150, no. 5, 1 September 2010 (2010-09-01), pages 574-586, XP009142424, ISSN: 0007-1048, DOI: DOI:10.1111/J.1365-2141.2010.08300.X [retrieved on 2010-07-16]

D5 SINGER HEIKO ET AL: "Effective Elimination of Acute Myeloid Leukemic Cells by Recombinant Bispecific Antibody Derivatives Directed Against CD33 and CD16", JOURNAL OF IMMUNOTHERAPY, LIPPINCOTT WILLIAMS & WILKINS, HAGERSTOWN, MD, US, vol. 33, no. 6, 1 July 2010 (2010-07-01), pages 599-608, XP009142457, ISSN: 1524-9557, DOI: DOI:10.1097/CJI.0B013E3181DDA225

D6 LINENBERGER MICHAEL L ET AL: "Antibody-directed therapies for hematological malignancies", TRENDS IN MOLECULAR MEDICINE, ELSEVIER CURRENT TRENDS, GB, vol. 8, no. 2, 1 February 2002 (2002-02-01), pages 69-76, XP009142419, ISSN: 1471-4914

D7 WO 2008/058021 A2 (SEATTLE GENETICS INC [US]; DRACHMAN JONATHAN G [US]; SUTHERLAND MAY KU) 15 May 2008 (2008-05-15)

D8 KORVER W ET AL: "Monoclonal antibodies against IREM-1: potential for targeted therapy of AML",LEUKEMIA, MACMILLAN PRESS LTD, US, vol. 23, no. 9, 1 September 2009 (2009-09-01), pages 1587-1597, XP009142413, ISSN: 0887-6924

D9 LAZAR GREG A ET AL: "Engineered antibody Fc variants with enhanced effector function", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES (PNAS), NATIONAL ACADEMY OF SCIENCE, US, vol. 103, no. 11, 14 March 2006 (2006-03-14), pages 4005-4010, XP002590010, ISSN: 0027-8424, DOI: DOI:10.1073/PNAS.0508123103

D10 CARTER P J: "POTENT ANTIBODY THERAPEUTICS BY DESIGN", NATURE REVIEWS. IMMUNOLOGY, NATURE PUBLISHING GROUP, GB, vol. 6, 7 April 2006 (2006-04-07), pages 343-357, XP007901440, ISSN: 1474-1733, DOI: DOI: 10.1038/NRI1837

D11 MAN SUNG CO: "CHIMERIC AND HUMANIZED ANTIBODIES WITH SPECIFICITY FOR THE CD33 ANTIGEN1", JOURNAL OF IMMUNOLOGY, AMERICAN ASSOCIATION OF IMMUNOLOGISTS, US, vol. 148, no. 4, 15 February 1992 (1992-02-15), pages 1149-1154, XP000368716, ISSN: 0022-1767

2 Subject-matter of the application

The present application relates to immunotherapies that are based on myeloid cell depletion. In particular, the present invention relates to anti-CD33 antibody molecules for use in such therapies, e.g. in the treatment of myeloid cell malignancies and myelodysplastic syndrome (MDS).

3 Inventive step

The present application does not meet the requirements of Art. 33(3) PCT because none of the 13 different antibodies recited in claims 1-30 involves an inventive step. The reasons are as follows:

Claim 1 relates to a CD33 binding agent binding to human CD33, the CD33 binding agent being defined by its 6 CDRs, claim 3 further specifies that the internalization kinetics of the CD33 binding agent is such that at least 30% of the initial amount of the CD33 binding agents remain on the cell surface of HL60 cells at a time of 4 hours after incubation.

CD33 binding agents binding to human CD33 with these internalization kinetics are known in the art, see D1 (page 6761, right hand column, last paragraph; Figure 3(B)), D2 (page 1050, left hand column, second paragraph - page 1053, right hand column, second paragraph; Figure 1) and D3 (page 1192, left hand column, second paragraph; page 1193, left hand column, second paragraph - page 1194, left hand column, first paragraph; Figure 2). The agent according to claim 1 differs from the prior art agents by its CDRs. The problem underlying the application can be defined as the provision of further CD33 binding agents binding to human CD33, wherein the internalization kinetics of the CD33 binding agent are such that at least 30% of the initial amount of the CD33 binding agent remain on the cell surface of HL60 cells at a time of 4 hours after incubation. The solution according to

claim 1 taken alone or combined with the feature of claim 3 is considered devoid of any inventive merit in the absence of any surprising or unexpected effects linked to the particular CDR sequences.

None of dependent claims 2-18 adds any technical feature that would warrant acknowledgement of an inventive step either.

Claims 19-30 relate to embodiments that are familiar to the skilled person. Consequently, they would only considered inventive if based upon a novel and inventive binding agent. For present claims 19-30 this is not the case. Hence they lack an inventive step.

4 **Further Remarks**

Claim 30 relates to a method of treatment of the human body by therapy. The patentability of such claims can be dependent upon the formulation of the claims. The EPO, for example, does not recognise as patentable claims to the use of a compound in medical treatment, but may allow claims to a product, in particular substances or compositions for use in a first or further medical treatment.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
12-0321-pct		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP2011/067339	04/10/2011	04/10/2010
Applicant		
BOEHRINGER INGELHEIM INTERNATIONAL GMBH		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 13 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language,** the international search was carried out on the basis of:
      [X] the international application in the language in which it was filed
      [ ] a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
   b. [ ] This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. [X] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. [ ] **Certain claims were found unsearchable** (See Box No. II)

3. [X] **Unity of invention is lacking** (see Box No III)

4. With regard to the **title,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the **drawings,**
   a. the figure of the **drawings** to be published with the abstract is Figure No. _____
      [ ] as suggested by the applicant
      [ ] as selected by this Authority, because the applicant failed to suggest a figure
      [ ] as selected by this Authority, because this figure better characterizes the invention
   b. [X] none of the figures is to be published with the abstract

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2011/067339

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   - [ ] on paper
   - [X] in electronic form

   b. (time)

   - [X] in the international application as filed
   - [ ] together with the international application in electronic form
   - [ ] subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2011/067339

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/067339

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61K39/395
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CARON PHILIP C ET AL: "Biological and immunological features of humanized M195 (anti-CD33) monoclonal antibodies", CANCER RESEARCH, vol. 52, no. 24, 1992, pages 6761-6767, XP009142412, ISSN: 0008-5472 the whole document ----- -/--	1-30

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 January 2012	30/01/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Sirim, Pinar

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CARON P C ET AL: "MURINE AND HUMANIZED CONSTRUCTS OF MONOCLONAL ANTIBODY M195 (ANTI-CD33) FOR THE THERAPY OF ACUTE MYELOGENOUS LEUKEMIA", CANCER, AMERICAN CANCER SOCIETY, PHILADELPHIA, PA, US, vol. 73, no. 3, SUPPL, 1 January 1994 (1994-01-01), pages 1049-1056, XP009036638, ISSN: 0008-543X the whole document	1-30
X	CARON P C ET AL: "ENGINEERED HUMANIZED DIMERIC FORMS OF IGG ARE MORE EFFECTIVE ANTIBODIES", THE JOURNAL OF EXPERIMENTAL MEDICINE, ROCKEFELLER UNIVERSITY PRESS, US, vol. 176, no. 4, 1 January 1992 (1992-01-01), pages 1191-1195, XP000914982, ISSN: 0022-1007, DOI: DOI:10.1084/JEM.176.4.1191 the whole document	1-30
A	KUEGLER MARKUS ET AL: "A recombinant trispecific single-chain Fv derivative directed against CD123 and CD33 mediates effective elimination of acute myeloid leukaemia cells by dual targeting", BRITISH JOURNAL OF HAEMATOLOGY, WILEY-BLACKWELL PUBLISHING LTD, GB, vol. 150, no. 5, 1 September 2010 (2010-09-01), pages 574-586, XP009142424, ISSN: 0007-1048, DOI: DOI:10.1111/J.1365-2141.2010.08300.X [retrieved on 2010-07-16] the whole document	1-30
A	SINGER HEIKO ET AL: "Effective Elimination of Acute Myeloid Leukemic Cells by Recombinant Bispecific Antibody Derivatives Directed Against CD33 and CD16", JOURNAL OF IMMUNOTHERAPY, LIPPINCOTT WILLIAMS & WILKINS, HAGERSTOWN, MD, US, vol. 33, no. 6, 1 July 2010 (2010-07-01), pages 599-608, XP009142457, ISSN: 1524-9557, DOI: DOI:10.1097/CJI.0B013E3181DDA225 the whole document	1-30

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LINENBERGER MICHAEL L ET AL: "Antibody-directed therapies for hematological malignancies", TRENDS IN MOLECULAR MEDICINE, ELSEVIER CURRENT TRENDS, GB, vol. 8, no. 2, 1 February 2002 (2002-02-01), pages 69-76, XP009142419, ISSN: 1471-4914 the whole document	1-30
A	WO 2008/058021 A2 (SEATTLE GENETICS INC [US]; DRACHMAN JONATHAN G [US]; SUTHERLAND MAY KU) 15 May 2008 (2008-05-15) the whole document	1-30
A	KORVER W ET AL: "Monoclonal antibodies against IREM-1: potential for targeted therapy of AML", LEUKEMIA, MACMILLAN PRESS LTD, US, vol. 23, no. 9, 1 September 2009 (2009-09-01), pages 1587-1597, XP009142413, ISSN: 0887-6924 the whole document	1-30
A	LAZAR GREG A ET AL: "Engineered antibody Fc variants with enhanced effector function", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES (PNAS), NATIONAL ACADEMY OF SCIENCE, US, vol. 103, no. 11, 14 March 2006 (2006-03-14), pages 4005-4010, XP002590010, ISSN: 0027-8424, DOI: DOI:10.1073/PNAS.0508123103 the whole document	1-30
A	CARTER P J: "POTENT ANTIBODY THERAPEUTICS BY DESIGN", NATURE REVIEWS. IMMUNOLOGY, NATURE PUBLISHING GROUP, GB, vol. 6, 7 April 2006 (2006-04-07), pages 343-357, XP007901440, ISSN: 1474-1733, DOI: DOI:10.1038/NRI1837 the whole document	1-30

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International application No
PCT/EP2011/067339

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MAN SUNG CO: "CHIMERIC AND HUMANIZED ANTIBODIES WITH SPECIFICITY FOR THE CD33 ANTIGEN1", JOURNAL OF IMMUNOLOGY, AMERICAN ASSOCIATION OF IMMUNOLOGISTS, US, vol. 148, no. 4, 15 February 1992 (1992-02-15), pages 1149-1154, XP000368716, ISSN: 0022-1767 the whole document -----	1-30

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2011/067339

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008058021 A2	15-05-2008	AU 2007317333 A1	15-05-2008
		CA 2667808 A1	15-05-2008
		EP 2084527 A2	05-08-2009
		JP 2010509234 A	25-03-2010
		US 2008267960 A1	30-10-2008
		US 2010215653 A1	26-08-2010
		US 2011038856 A1	17-02-2011
		WO 2008058021 A2	15-05-2008

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:1, CDR2 has an amino acid sequence selected from SeqID No:15, CDR3 has an amino acid sequence selected from SeqID No:29, CDR4 has an amino acid sequence selected from SeqID No: 43, CDR5 has an amino acid sequence selected from SeqID No:57, CDR6 has an amino acid sequence selected from SeqID No:71, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

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2. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:2, CDR2 has an amino acid sequence selected from Seq ID No:16, CDR3 has an amino acid sequence selected from Seq ID No:30, CDR4 has an amino acid sequence selected from Seq ID No:44, CDR5 has an amino acid sequence selected from Seq ID No:58, CDR6 has an amino acid sequence selected from Seq ID No:72, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

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3. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:3, CDR2 has an amino acid sequence selected from Seq ID No:17, CDR3 has an amino acid sequence selected from Seq ID No:31, CDR4 has an amino acid sequence selected from Seq ID No:45, CDR5 has an amino acid sequence selected from Seq ID No:59, CDR6 has an amino acid sequence selected from Seq ID No:73, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

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4. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:4, CDR2 has an amino acid sequence selected from Seq ID No:18, CDR3 has an amino acid sequence selected from Seq ID No:32, CDR4 has an amino acid sequence selected from Seq ID No:46, CDR5 has an amino acid sequence selected from Seq ID No:60, CDR6 has an amino acid sequence selected from Seq ID No:74, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

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5. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:5, CDR2 has an amino acid sequence selected from Seq ID No:19, CDR3 has an amino acid sequence selected from Seq ID No:33, CDR4 has an amino acid sequence selected from Seq ID No:47, CDR5 has an amino acid sequence selected from Seq ID No:61, CDR6 has an amino acid sequence selected from Seq ID No:75, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

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6. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:6, CDR2 has an amino acid sequence selected from Seq ID No:20, CDR3 has an amino acid sequence selected from Seq ID No:34, CDR4 has an amino acid sequence selected from Seq ID No:48, CDR5 has an amino acid sequence selected from Seq ID No:62, CDR6 has an amino acid sequence selected from Seq ID No:76, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

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7. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1,

CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:7, CDR2 has an amino acid sequence selected from Seq ID No:21, CDR3 has an amino acid sequence selected from Seq ID No:35, CDR4 has an amino acid sequence selected from Seq ID No:49, CDR5 has an amino acid sequence selected from Seq ID No:63, CDR6 has an amino acid sequence selected from Seq ID No:77,or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

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8. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:8, CDR2 has an amino acid sequence selected from Seq ID No:22, CDR3 has an amino acid sequence selected from Seq ID No:36, CDR4 has an amino acid sequence selected from Seq ID No:50, CDR5 has an amino acid sequence selected from Seq ID No:64, CDR6 has an amino acid sequence selected from Seq ID No:78, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

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9. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:9, CDR2 has an amino acid sequence selected from Seq ID No:23, CDR3 has an amino acid sequence selected from Seq ID No:37, CDR4 has an amino acid sequence selected from Seq ID No:51, CDR5 has an amino acid sequence selected from Seq ID No:65, CDR6 has an amino acid sequence selected from Seq ID No:79, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

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10. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:10, CDR2 has an amino acid sequence selected from Seq ID No:24, CDR3 has an amino acid sequence

selected from Seq ID No:38, CDR4 has an amino acid sequence selected from Seq ID No:52, CDR5 has an amino acid sequence selected from Seq ID No:66, CDR6 has an amino acid sequence selected from Seq ID No:80, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

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11. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:11 or 13, CDR2 has an amino acid sequence selected from Seq ID No:25 or 27, CDR3 has an amino acid sequence selected from Seq ID No:39 or 41, CDR4 has an amino acid sequence selected from Seq ID No:53 or 55, CDR5 has an amino acid sequence selected from Seq ID No:67 or 69, CDR6 has an amino acid sequence selected from Seq ID No:81 or 83, or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

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12. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:12, CDR2 has an amino acid sequence selected from Seq ID No:26, CDR3 has an amino acid sequence selected from Seq ID No:40, CDR4 has an amino acid sequence selected from Seq ID No:54, CDR5 has an amino acid sequence selected from Seq ID No:68, CDR6 has an amino acid sequence selected from Seq ID No:82,or b) recognizing an epitope within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33

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13. claims: 1-30(partially)

A CD33 binding agent binding to human CD33, the CD33 binding agent being defined by
a) having a heavy chain variable region comprising CDR1, CDR2 and CDR3, and a light chain variable region comprising CDR4, CDR5 and CDR6, wherein CDR1 has an amino acid sequence selected from Seq ID No:14, CDR2 has an amino acid sequence selected from Seq ID No:28, CDR3 has an amino acid sequence selected from Seq ID No:42, CDR4 has an amino acid sequence selected from Seq ID No:56, CDR5 has an amino acid sequence selected from Seq ID No:70, CDR6 has an amino acid sequence selected from Seq ID No:84, or b) recognizing an epitope

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

within the amino acid sequence FFHPIPYYDKNSPVHGYW (Seq ID No: 141) of human CD33