OLARTERAISBECK
OLARTE RAISBECK MOURE & CASTRO LTDA.

SEÑORES
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES
E. S. D.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 07-010436- -00003-0000
Fecha: 2008-10-24 16:48:28 Dep. 2020 NUECREACIONI
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 538 PETICION Folios: 2

Ref.: Expediente No. 07-010.436
Solicitud de Patente de Invención titulada: "ANTICUERPOS ANTAGONISTAS DE IL-17"
Solicitante: NOVARTIS A.G.
Nuestra Ref.: P2007/000001

SOLICITUD DE EXAMEN DE PATENTABILIDAD SEGÚN EL ARTÍCULO 44 DE LA DECISION 486, GACETA No. 595

CARLOS R. OLARTE, mayor de edad y vecino de Bogotá, D.C., identificado tal como aparece al pie de mi firma, en mi condición de apoderado de la sociedad de la referencia, respetuosamente me permito solicitar que se examine si la invención reúne los requisitos de patentabilidad previstos en la misma, dando cumplimiento a lo establecido en el articulo 44 de la Decisión 486 de la comisión de la Comunidad Andina. Adjunto recibo por el monto de $420.000 pesos para cubrir la tasa correspondiente, según la Resolución 44372 de 26 de diciembre 2007.

Atentamente,

CARLOS R. OLARTE
C.C. 79.782.747 de Bogotá
T.P. 74.295

Anexo: Recibo por $420.000 pesos

235

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 08 - 84,232
FECHA : OCTUBRE 24 DE 2008

***** C O N S I G N A C I O N *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
OLARTE RAISBEK	CONSIGNACION	BANCO DE BOGOTA	062754387	81240622	23/10/2008	4,502,000.00
OLARTE RAISBEK	CONSIGNACION	BANCO DE BOGOTA	062754387	81240633	23/10/2008	8,307,000.00

***** C O N C E P T O *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	85 EXAMEN DE PATENTABILIDAD DE INVEN	420,000.00
		TOTAL : $	420,000.00

SON: CUATROCIENTOS VEINTE MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No._____

Industria y Comercio
SUPERINTENDENCIA

DIRECCIÓN DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No. 07-10436

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCIÓN**

FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No **595** DE

FECHA **29 DE AGOSTO DE 2008** EL TÉRMINO HÁBIL SEÑALADO POR LA

LEY EXPIRA EL **26 DE NOVIEMBRE DE 2008**

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI _____ NO ____X____

D1 257

Industria y Comercio

SUPERINTENDENCIA

Delegatura de propiedad Industrial

División de Nuevas Creaciones

PCT

SOLICITUD

PATENTE DE INVENCIÓN

21. **EXPEDIENTE No.** 06-13073

54. **TÍTULO** ANTICUERPOS HUMANOS MODIFICADOS ANTI-IGF-IR

51. **CLASIFICACIÓN INTERNACIONAL** C07K 16/00, A61P 35/00, C07K 16/28, A61K 39/395

71. **SOLICITANTE** PFIZER PRODUCTS INC.

DOMICILIO Eastern Point Road, Groton, Connecticut 06340, E.U.A.

74. **APODERADO** CARLOS R. OLARTE

22. **BOGOTÁ, D. C.,** Febrero 9 de 2006

(FORMA P-10)

OLARTERAISBECK

Industria y Comercio
SUPERINTENDENCIA

Radicación : [illegible] Folios: 205
Fecha (CCD): [illegible]
Presentó: [illegible]
Dependencia: [illegible]

PETITORIO - FORMULARIO ÚNICO DE SOLICITUD
PATENTE PCT
ENTRADA EN FASE NACIONAL 13-03-2006

SOLICITUD DE:

(1) [X] Patente de Invención. [ ] Patente de Modelo de Utilidad.

[ ] Capítulo I. [X] Capítulo II.

(2) **SOLICITANTE (71)**	**Nombre:** PFIZER PRODUCTS INC **Dirección:** Eastern Point Road, Groton, Connecticut 06340 E.U.A. **Nacionalidad o Domicilio:** ESTADOUNIDENSE **Lugar de Constitución:** Delaware, Estados Unidos de América. **Teléfono:** _____ **Fax:** _____ **E-mail:** _____	**IDENTIFICACIÓN** C.C. [ ] NIT [ ] C.E. [ ] Otro [ ] Cual _____ Número _____
REPRESENTANTE APODERADO	**Nombre:** CARLOS R. OLARTE **Dirección:** World Trade Center Torre A, Calle 100 No. 8A-37 Piso 10 **Teléfono:** 601-7700 **Fax:** 601-7799 **E-mail:** office@olarteraisbeck.com	**IDENTIFICACIÓN** C.C. [X] NIT [ ] C.E. [ ] Otro [ ] Cual _____ Número 79782747Bta TP 74.295
INVENTOR (ES) (72)	**Nombre:** (1) BRUCE DAVID COHEN, (2) VAHE BEDIAN **Dirección:** (1 y 2) Eastern Point Road, Groton, Connecticut 06340, Estados Unidos de América. **Nacionalidad o Domicilio:** ESTADOUNIDENSE	

(3) **PCT (86)**

Solicitud Internacional No. PCT/IB2004/002555 Fecha Agosto 3, 2004

Publicación Internacional No. WO 2005/016967 Fecha Febrero 24, 2005

(4) **Título (54)** ANTICUERPOS HUMANOS MODIFICADOS ANTI-IGF-1R

Clasificación Internacional (51) C07K 16/00, A61P 35/00, C07K 16/28, A61K 39/395

Prioridad SI [X] NO [ ]

(33) País de Origen	(32) Fecha	(31) Número de Solicitud
US	Agosto 13, 2003	60/495.200

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proteínas que se unen a IGF-I, antineoplásicos, agentes quimioterapéuticos, antitumorales, oligonucleótidos complementarios frente a IGF-IR o IGF-I, análogos peptídicos que bloquean la activación de IGF-IR, IGF-IR soluble, y/o uno o más agentes químicos que inhiban la producción o la actividad de IGF-I, que se conocen en la técnica, por ejemplo, la octreotida. Para una composición farmacéutica que comprende un anticuerpo activador, el anticuerpo anti-IGF-IR se puede formular con un factor que aumenta la proliferación celular o previene la apoptosis. Tales factores incluyen factores de crecimiento tales como el IGF-I, y/o análogos de IGF-I que activan al IGF-IR. Tales terapias combinadas pueden requerir dosis menores del anticuerpo anti-IGF-IR así como de agentes coadministrados, por lo que se evitan posibles toxicidades o complicaciones asociadas con las diversas monoterapias. En una realización, el anticuerpo y uno o más agentes terapéuticos adicionales.

Las composiciones farmacéuticas de la invención pueden incluir una «cantidad terapéuticamente eficaz» o una «cantidad profilácticamente eficaz» de un anticuerpo o de una fracción de un anticuerpo de la invención. Una «cantidad terapéuticamente eficaz» se refiere a una cantidad eficaz, a unas dosificaciones y durante periodos de tiempo necesarios, para conseguir el efecto terapéutico deseado. Una cantidad terapéuticamente eficaz del anticuerpo o una fracción del anticuerpo puede oscilar según factores tales como la patología, la edad, el sexo y el peso de la persona, y la capacidad del anticuerpo o de una fracción de un anticuerpo para inducir una respuesta deseada en la persona. Una cantidad terapéuticamente eficaz es también aquella en la que los efectos terapéuticamente beneficiosos superan en valor a cualquier efecto tóxico o perjudicial del anticuerpo o de la fracción del

puede incluir agentes de diagnóstico o terapéuticos, además del anticuerpo o la composición farmacéutica. Un equipo de reactivos puede incluir también las instrucciones para su uso en un procedimiento diagnóstico o terapéutico. En una realización preferida, el equipo de reactivos incluye el anticuerpo o una composición farmacéutica de éste y un agente de diagnóstico que se pueda usar en un procedimiento descrito a continuación. En otra realización preferida, el equipo de reactivos incluye el anticuerpo o una composición farmacéutica de éste y uno o más agentes terapéuticos, tales como un antineoplásico adicional, un antitumoral o un agente quimioterapéutico, que se pueda utilizar en un procedimiento descrito a continuación.

Esta invención se refiere también a composiciones farmacéuticas para inhibir el crecimiento celular anómalo en un mamífero que comprende una cantidad de un compuesto de la invención en combinación con una cantidad de un agente quimioterapéutico, en el que las cantidades del compuesto, la sal, el solvato o el profármaco y del agente quimioterapéutico son eficaces juntas en la inhibición del crecimiento celular anómalo. Muchos agentes quimioterapéuticos se conocen actualmente en la técnica. En una realización, los agentes quimioterapéuticos se seleccionan del grupo constituido por inhibidores de la mitosis, compuestos alquilantes, antimetabolitos, antibióticos intercalantes, inhibidores de factores de crecimiento, inhibidores del ciclo celular, enzimas, inhibidores de la topoisomerasa, compuestos antisupervivencia, modificadores de la respuesta biológica, anti-hormonas, por ejemplo anti-andrógenos y antiangiogénicos.

Los antiangiogénicos, tales como los inhibidores de la MMP-2

bencenosulfonilamino]-8-oxa-biciclo[3.2.1]octano-3-carboxílico; y hidroxiamida del ácido (R) 3-[4-(4-fluoro-fenoxi)-bencenosulfonilamino]-tetrahidro-furan-3-carboxílico; y las sales y los solvatos farmacéuticamente aceptables de dichos compuestos.

Un compuesto de la invención se puede utilizar también con inhibidores de la transducción de señales, tales como agentes que pueden inhibir las respuestas del EGF-R (receptor del factor de crecimiento epidérmico), tales como anticuerpos anti-EGF-R, anticuerpos anti-EGF y moléculas inhibidoras del EGF-R; inhibidores del VEGF (factor de crecimiento del endotelio vascular), tales como receptores del VEGF y moléculas que puedan inhibir el VEGF; y los inhibidores de receptor erbB2, tales como las moléculas orgánicas o los anticuerpos que se unen al receptor erbB2, por ejemplo, HERCEPTIN™ (Genentech, Inc.). Los inhibidores de EGF-R se describen por ejemplo en los documentos WO 95/19970 (publicada el 27 de julio de 1995), WO 98/14451 (publicada el 9 de abril de 1998), WO 98/02434 (publicada el 22 de enero de 1998), y Patente de los Estados Unidos 5.747.498 (presentada el 5 de mayo de 1998), y dichas sustancias se pueden utilizar en la presente invención como se describe en la presente memoria. Los agentes que inhiben el EGFR incluyen, pero no se limitan a los anticuerpos monoclonales C225 y anti-EGFR 22Mab (ImClone Systems Incorporated), ABX-EGF (Abgenix/Cell Genesys), EMD-7200 (Merck KgaA), EMD-5590 (Merck KgaA), MDX-447/H-477 (Medarex Inc. and Merck KgaA), y los compuestos ZD-1834, ZD-1838 y ZD-1839 (AstraZeneca), PKI-166 (Novartis), PKI-166/CGP-75166 (Novartis), PTK 787 (Novartis), CP 701 (Cephalon), leflunomida (Pharmacia/Sugen), CI-1033 (Warner Lambert Parke Davis), CI-1033/PD 183.805 (Warner Lambert Parke

de enero de 1999, incorporadas ambas íntegramente en la presente memoria como referencia. Los compuestos y las sustancias inhibidores del receptor erbB2 descritos en las solicitudes PCT, patentes de los Estados Unidos, y solicitudes provisionales de los Estados Unidos mencionadas anteriormente, así como otros compuestos y sustancias que inhiben el receptor erbB2, se pueden utilizar con el compuesto de la presente invención según la presente memoria.

El anticuerpo de la invención se puede usar también con un anticuerpo anti-CTLA-4, tales como los descritos en la patente de los Estados Unidos 6.682.736, que incluye un anticuerpo con la secuencia del anticuerpo 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1 ó 12.9.1.1. El anticuerpo se puede utilizar también con anticuerpos anti-CD40, tales como los descritos en el documento WO 03040170 publicado el 15 de mayo de 2003, que incluye uno con la secuencia del anticuerpo 3.1. 1,3. 1.1H-A78T, 3.1. 1H-A78T-V88A-V97A, 7.1.2, 10.8.3, 15.1.1, 21.4.1, 21.2.1, 22.1.1, 22.1.1H-C109A, 23.5.1, 23.25.1, 23.28.1, 23.28.1H-D16E, 23.29.1 ó 24.2.

Los anticuerpos también se pueden combinar con sustancias anti-integrina, tales como anticuerpos anti-integrina.

Algunos ejemplos de agentes específicos con los que se puede combinar el anticuerpo incluyen los siguientes:

Agentes alquilantes: N-óxido de una mostaza nitrogenada, ciclofosfamida, ifosfamida, melfalan, busulfanmitobronitol, carboquona, tiotepa, ranimustina, nimustina y temozolomida;

antimetabolitos: metotrexato, 6-mercaptopurina, ribósido, mercaptopurina, 5-fluorouracilo, tegafur, doxifluridina, carmofur, citarabina, citarabina, ocfosfato,

enocitabina, S-1, gemcitabina, fludarabina y capecitabina;

antibióticos: actinomicina D, doxorubicina, daunorubicina, neocarzinostatina, bleomicina, peplomicina, mitomicina C, aclarubicina, pirarubicina, epirubicina, zinostatina estimalámero y idarubicina;

antitumorales vegetales: vincristina, vinblastina, vindeshina, etopósido, sobuzoxano, docetaxel, paclitaxel y vinorelbina;

los compuestos coordinados con platino: cisplatino, carboplatino, nedaplatino y oxaliplatino;

derivados de la camptotecina: irinotecán, topotecán y camptotecina;

el inhibidor de la tirosina quinasa gefitinib;

agentes anti-CD20: rituximab, tositumomab y ibritumomab tiuxetán;

interferones: interferón alfa, interferón α-2a, interferón α-2b, interferón β, interferón γ-1a and interferón γ-n1;

modificadores de la respuesta biológica: krestina, lentinan, sizofiran, picibanil y ubenimex; y

otros antitumorales: mitoxantrona, l-asparaginasa, procarbazina, dacarbazina, hidroxicarbamida, pentostatina y tretinoína.

Además, el anticuerpo de la invención se puede combinar con los antineoplásicos exemestano, edotecarina (J-107088) y SU11248.

El anticuerpo anti-IGF-IR se puede usar para detectar IGF-IR en una muestra biológica *in vitro* o *in vivo*. El anticuerpo anti-IGF-IR se puede usar en un inmunoensayo convencional, incluyendo, sin limitación, un ELISA, un RIA, citometría de flujo, inmunohistoquímica de tejido, inmunotransferencia o inmunoprecipitación. El anticuerpo anti-IGF-IR de la invención se puede utilizar para detectar IGF-IR de humanos. En otra realización, el anticuerpo anti-IGF-

EJEMPLO I: Generación del hibridoma que produce el anticuerpo anti-IGF-IR

El anticuerpo de la invención se preparó, se seleccionó y se analizó como sigue:

Se inmunizaron ratones XENOMICE™ de ocho a diez semanas de edad por vía intraperitoneal o en sus almohadillas de las patas traseras tanto con el dominio extracelular del IGF-IR humano (10 µg/dosis/ratón), o con células 3T3-IGF-IR ó 300.19-IGF-IR, que son dos líneas celulares transfectadas que expresan IGF-IR humano en sus membranas plasmáticas (10 x 10^6 células/ dosis/ratón). Esta dosis se repitió de cinco a siete veces durante un periodo de tres a ocho semanas. Cuatro días antes de la fusión, los ratones recibieron una última inyección del dominio extracelular del IGF-IR humano en PBS. Los linfocitos de los ganglios linfáticos y del bazo procedentes de los ratones inmunizados se fusionaron con la línea celular del mieloma no secretor P3-X63-Ag8.653 y se sometieron a la selección en medio HAT como se ha descrito antes (Galfre and Milstein, *Meteds Enzymol.* 73:3-46, 1981). Se seleccionó un hibridoma, 2.12.1, que producía anticuerpos monoclonales específicos para IGF-IR para su posterior estudio y se depositó en la American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, el 12 de diciembre de 2000 con el siguiente número de depósito:

Hibridoma	Depósito nº
2.12.1	PTA-2792

Este hibridoma, y otros que producen anticuerpos específicos para IGF-IR,

se describen el documento WO 02/05359, publicado el 11 de julio de 2002. El texto de esta publicación, incluyendo todas las secuencias descritas, se incorpora por la presente como referencia.

Dos mutaciones marco conservadas de la cadena pesada y tres mutaciones marco conservadas de la cadena ligera del anticuerpo 2.12.1 se corrigieron hacia la línea germinal para producir el anticuerpo 2.12.1fx.

Todos los cambios para generar las mutaciones 2.12.1fx se realizaron utilizando el equipo de reactivos para mutagénesis dirigida QuikChange (Stratagene™) preparando un plásmido con un sitio diana para la mutación por desnaturalización del plásmido e hibridación con los cebadores oligonucleótido que contienen la mutación deseada. La actividad no desplazante de hebra de la ADN polimerasa *Pfu Turbo* se empleó para ampliar e incorporar los cebadores mutágenos dando lugar a hebras circulares melladas. La digestión del ADN molde parental metilado y no mutado con *Dpn*I siguió con la transformación con el ADN bicatenario circular y mellado en células supercompetentes XL1Blue. Tras la transformación, las células supercompetentes XL1Blue repararon la mella en el plásmido mutado. Los plásmidos que contenían las mutaciones se seleccionaron y se verificó la secuencia.

La figura 1 muestra la secuencia de ADN que codifica la cadena pesada del anticuerpo 2.12.1fx, incluyendo la secuencia que codifica la secuencia señal utilizada para expresar el anticuerpo maduro (ID SEC Nº 1). La figura 2 muestra la secuencia de ADN que codifica la cadena ligera del anticuerpo 2.12.1fx, incluyendo la secuencia que codifica la secuencia señal utilizada para expresar el anticuerpo maduro (ID SEC Nº 2). La figura 3 muestra una

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Habiendo descrito la invención como antecede, se declara como propiedad lo contenido en las siguientes

REIVINDICACIONES

1. Un anticuerpo monoclonal humano o una porción de unión al antígeno de éste que comprende al menos un resto de aminoácido mutado en una posición seleccionada que se ha reemplazado por el correspondiente resto de aminoácido de la línea germinal.

2. El anticuerpo humano o una porción de unión al antígeno de éste de acuerdo a la reivindicación 1 en el que dicho resto reemplazado es una mutación somática contenida en una región marco conservada de una región variable de dicho anticuerpo.

3. El anticuerpo humano o una porción de unión al antígeno de éste de acuerdo a la reivindicación 2 en el que dicho anticuerpo humano o una porción de unión al antígeno de éste se une específicamente al receptor del factor de crecimiento insulinoide I humano (IGF-IR).

4. El anticuerpo humano o una porción de unión al antígeno de éste de acuerdo a la reivindicación 3 en el que la región variable de la cadena ligera comprende los aminoácidos 23 a 130 de la secuencia de aminoácidos ID SEC Nº 5.

5. El anticuerpo humano de acuerdo a la reivindicación 4, en el que la cadena ligera comprende los aminoácidos 23 a 236 de ID SEC Nº 5.

6. El anticuerpo humano o una porción de unión al antígeno de éste de acuerdo a la reivindicación 4 en el que la región variable de la cadena pesada comprende los aminoácidos 20 a 144 de ID SEC Nº 3.

7. El anticuerpo humano de acuerdo a la reivindicación 6 en el que la cadena pesada comprende los aminoácidos 20 a 470 de ID SEC Nº 3 y la

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cadena ligera comprende los aminoácidos 23 a 236 de ID SEC Nº 5.

8. El anticuerpo humano de acuerdo a la reivindicación 6 en el que la cadena pesada de dicho anticuerpo está constituida por los aminoácidos 20 a 470 de ID SEC Nº 3 y la cadena ligera está constituida por los aminóácidos 23 a 236 de ID SEC Nº 5.

9. Una composición farmacéutica para el tratamiento del cáncer que comprende el anticuerpo o una porción de unión al antígeno de éste de acuerdo con una cualquiera de las reivindicaciones 1-8 y un vehículo farmacéuticamente aceptable.

10. La composición farmacéutica de acuerdo con la reivindicación 9 que comprende además un antineoplásico, un agente quimioterapéutico o un antitumoral.

11. Un procedimiento de tratamiento del cáncer en un humano con el anticuerpo humano o una porción de unión al antígeno de éste de acuerdo con una cualquiera de las reivindicaciones 1-8 que comprende la etapa de administración a un humano de una cantidad del anticuerpo que sea eficaz para tratar dicho cáncer.

12. Un polinucleótido aislado que comprende una secuencia de un ácido nucleico que codifica una cadena pesada o una porción de unión al antígeno de ésta o una cadena ligera o una porción de unión al antígeno de ésta de un anticuerpo de acuerdo con una cualquiera de las reivindicaciones 1-8.

13. Un vector que comprende la molécula de ácido nucleico aislada de la reivindicación 12.

14. Una célula huéspad que comprende el vector de la reivindicación 13.

15. Un procedimiento de producción de un anticuerpo monoclonal

US 20040086503A1

(19) United States

(12) Patent Application Publication

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Cohen et al.

(54) [redacted]

(76) Inventors: Bruce D. Cohen, East Lyme, CT (US); Jean Beebe, Salem, CT (US); Penelope E. Miller, Mystic, CT (US); James D. Moyer, East Lyme, CT (US); Jose R. Corvalan, Foster City, CA (US); Michael Gallo, North Vancouver (CA)

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(21) Appl. No.: 10/038,591

(22) Filed: Jan. 4, 2002

Related U.S. Application Data

(60) Provisional application No. 60/259,927, filed on Jan. 5, 2001.

Publication Classification

(51) Int. Cl.[7] A61K 39/395; C07K 16/28

(52) U.S. Cl. 424/143.1; 530/388.22

(57) ABSTRACT

The present invention relates to antibodies and antigen-binding portions thereof that specifically bind to insulin like growth factor I receptor (IGF-IR), which is preferably human IGF-IR. The invention also relates to human anti-IGF-IR antibodies, including chimeric, bispecific, derivatized, single chain antibodies or portions of fusion proteins. The invention also relates to isolated heavy and light chain immunoglobulin molecules derived from anti-IGF-IR antibodies and nucleic acid molecules encoding such molecules. The present invention also relates to methods of making anti-IGF-IR antibodies, pharmaceutical compositions comprising these antibodies and methods of using the antibodies and compositions thereof for diagnosis and treatment. The invention also provides gene therapy methods using nucleic acid molecules encoding the heavy and/or light immunoglobulin molecules that comprise the human anti-IGF-IR antibodies. The invention also relates to gene therapy methods and transgenic animals comprising nucleic acid molecules of the present invention.

ANTIBODIES TO INSULIN-LIKE GROWTH [redacted]

[0001] This application claims the benefit of U.S. Provisional Application No. 60/259,927, filed Jan. 5, 2001.

BACKGROUND OF THE INVENTION

[0002] Insulin-like growth factor (IGF-I) is a 7.5-kD polypeptide that circulates in plasma in high concentrations and is detectable in most tissues. IGF-I stimulates cell differentiation and cell proliferation, and is required by most mammalian cell types for sustained proliferation. These cell types include, among others, human diploid fibroblasts, epithelial cells, smooth muscle cells, T lymphocytes, neural cells, myeloid cells, chondrocytes, osteoblasts and bone marrow stem cells. For a review of the wide variety of cell types for which IGF-I/IGF-I receptor interaction mediates cell proliferation, see Goldring et al., Eukar. Gene Express., 1:31-326 (1991).

[0003] The first step in the transduction pathway leading to IGF-I-stimulated cellular proliferation or differentiation is binding of IGF-I or IGF-II (or insulin at supraphysiological concentrations) to the IGF-I receptor. The IGF-I receptor is composed of two types of subunits: an alpha subunit (a 130-135 kD protein that is entirely extracellular and functions in ligand binding) and a beta subunit (a 95-kD transmembrane protein, with transmembrane and cytoplasmic domains). The IGF-IR belongs to the family of tyrosine kinase growth factor receptors (Ullrich et al., Cell 61: 203-212, 1990), and is structurally similar to the insulin receptor (Ullrich et al., EMBO J. 5: 2503-2512, 1986). The IGF-IR is initially synthesized as a single chain proreceptor polypeptide which is processed by glycosylation, proteolytic cleavage, and covalent bonding to assemble into a mature 460-kD heterotetramer comprising two alpha-subunits and two beta-subunits. The beta subunit(s) possesses ligand-activated tyrosine kinase activity. This activity is implicated in the signaling pathways mediating ligand action which involve autophosphorylation of the beta-subunit and phosphorylation of IGF-IR substrates.

[0004] In vivo, serum levels of IGF-I are dependent upon the presence of pituitary growth hormone (GH). Although the liver is a major site of GH-dependent IGF-I synthesis, recent work indicates that the majority of normal tissues also produce IGF-I. A variety of neoplastic tissues may also produce IGF-I. Thus IGF-I may act as a regulator of normal and abnormal cellular proliferation via autocrine or paracrine, as well as endocrine mechanisms. IGF-I and IGF-II bind to IGF binding proteins (IGFBPs) in vivo. The availability of free IGF for interaction with the IGF-IR is modulated by the IGFBPs. For a review of IGFBPs and IGF-I, see Grimberg et al., J. Cell. Physiol. 183: 1-9, 2000.

[0005] There is considerable evidence for a role for IGF-I and/or IGF-IR in the maintenance of tumor cells in vitro and in vivo. IGF-IR levels are elevated in tumors of lung (Kaiser et al., J. Cancer Res. Clin. Oncol. 119: 665-668, 1993; Moody et al., Life Sciences 52: 1161-1173, 1993; Macauley et al., Cancer Res., 50: 2511-2517, 1990), breast (Pollak et al., Cancer Lett. 38: 223-230, 1987; Foekens et al., Cancer Res. 49: 7002-7009, 1989; Cullen et al., Cancer Res. 49: 7002-7009, 1990; Arteaga et al., J. Clin. Invest. 84: 1418-1423, 1989), prostate and colon (Remaole-Bennet et al., J. Clin. Endocrinol. Metab. 75: 609-616, 1992; Guo et al., Gastroenterol. 102: 1101-1108, 1992). Deregulated expression of IGF-I in prostate epithelium leads to neoplasia in transgenic mice (DiGiovanni et al., Proc. Natl. Acad. Sci. USA 97: 3455-60, 2000). In addition, IGF-I appears to be an autocrine stimulator of human gliomas (Sandberg-Nordqvist et al., Cancer Res. 53: 2478-2478, 1993), while IGF-I stimulated the growth of fibrosarcomas that overexpressed IGF-IR (Butler et al., Cancer Res. 58: 3021-27, 1998). Further, individuals with "high normal" levels of IGF-I have an increased risk of common cancers compared to individuals with IGF-I levels in the "low normal" range (Rosen et al., Trends Endocrinol. Metab. 10: 136-41, 1999). Many of these tumor cell types respond to IGF-I with a proliferative signal in culture (Nakanishi et al., J. Clin. Invest. 82: 354-359, 1988; Freed et al., J. Mol. Endocrinol. 3: 509-514, 1989), and autocrine or paracrine loops for proliferation in vivo have been postulated (LeRoith et al., Endocrine Revs. 16: 143-163, 1995; Yee et al., Mol. Endocrinol. 3: 509-514, 1989). For a review of the role IGF-I/IGF-I receptor interaction plays in the growth of a variety of human tumors, see Macaulay, Br. J. Cancer, 65: 311-320, 1992.

[0006] Increased IGF-I levels are also correlated with several noncancerous pathological states, including acromegaly and gigantism (Barkan, Cleveland Clin. J. Med. 65: 343, 347-349, 1998), while abnormal IGF-I/IGF-I receptor function has been implicated in psoriasis (Wraight et al., Nat. Biotech. 18: 521-526, 2000), atherosclerosis and smooth muscle restenosis of blood vessels following angioplasty (Bayes-Genis et al., Circ. Res. 86: 125-130, 2000). Increased IGF-I levels also can be a problem in diabetes or in complications thereof, such as microvascular proliferation (Smith et al., Nat. Med. 5: 1390-1395, 1999). Decreased IGF-I levels, which occur, inter alia, in cases when GH serum levels are decreased or when there is an insensitivity or resistance to GH, is associated with such disorders as small stature (Laron, Paediatr. Drugs 1: 155-159, 1999), neuropathy, decrease in muscle mass and osteoporosis (Rosen et al., Trends Endocrinol. Metab. 10: 136-141, 1999).

[0007] Using antisense expression vectors or antisense oligonucleotides to the IGF-IR RNA, it has been shown that interference with IGF-IR leads to inhibition of IGF-I-mediated or IGF-II-mediated cell growth (see, e.g., Wraight et al., Nat. Biotech. 18: 521-526, 2000). The antisense strategy was successful in inhibiting cellular proliferation in several normal cell types and in human tumor cell lines. Growth can also be inhibited using peptide analogues of IGF-I (Pietrzkowski et al., Cell Growth & Diff. 3: 199-205, 1992; and Pietrzkowski et al., Mol. Cell. Biol., 12: 3883-3889, 1992), or a vector expressing an antisense RNA to the IGF-I RNA (Trojan et al., Science 259: 94-97, 1992). In addition, antibodies to IGF-IR (Arteaga et al., Breast Canc. Res. Treatm., 22: 101-106, 1992; and Kalebic et al., Cancer Res. 54: 5531-5534, 1994), and dominant negative mutants of IGF-IR (Prager et al., Proc. Natl. Acad. Sci. U.S.A. 91: 2181-2185, 1994; Li et al., J. Biol. Chem., 269: 32558-32564, 1994 and Jiang et al., Oncogene 28: 6071-77, 1999), can reverse the transformed phenotype, inhibit tumorigenesis, and induce loss of the metastatic phenotype.

[0008] IGF-I is also important in the regulation of apoptosis. Apoptosis, which is programmed cell death, is involved in a wide variety of developmental processes, including immune and nervous system maturation. In addi-

[illegible] apoptosis also has been [illegible] (Williams, Cell [illegible]; [illegible] 1993). [illegible]

[0009] [illegible] with [illegible] cells (Rodriguez-Tarduchy et al. [illegible] 1990), and from serum withdrawal in Rat-1/mycER cells (Harrington E., et al., EMBO J. 13: 3286-3295 (1994)). The anti-apoptotic function of IGF-I is important in the post-commitment stage of the cell cycle and also in cells blocked in cell cycle progression by etoposide or thymidine. The demonstration that c-myc driven fibroblasts are dependent on IGF-I for their survival suggests that there is an important role for the IGF-IR in the maintenance of tumor cells by specifically inhibiting apoptosis, a role distinct from the proliferative effects of IGF-I or IGF-IR. This would be similar to a role thought to be played by other anti-apoptotic genes such as bcl-2 in promoting tumor survival (McDonnell et al., Cell 57: 79-88, 1989; Hockenberry et al., Nature 348: 334-336, 1990).

[0010] The protective effects of IGF-I on apoptosis are dependent upon having IGF-IR present on cells to interact with IGF-I (Resnicoff et al., Cancer Res. 55: 3739-3741, 1995). Support for an anti-apoptotic function of IGF-IR in the maintenance of tumor cells was also provided by a study using antisense oligonucleotides to the IGF-IR that identified a quantitative relationship between IGF-IR levels, the extent of apoptosis and the tumorigenic potential of a rat syngeneic tumor (Resnicoff et al., Cancer Res. 55: 3739-3741, 1995). An overexpressed IGF-IR has been found to protect tumor cells in vitro from etoposide-induced apoptosis (Sell et al., Cancer Res. 55: 303-306, 1995) and, even more dramatically, that a decrease of IGF-IR levels below wild type levels caused massive apoptosis of tumor cells in vivo (Resnicoff et al., Cancer Res. [illegible] 2463-246[illegible] 1995).

[0011] Potential strategies for inducing apoptosis or for inhibiting cell proliferation associated with increased IGF-I, increased IGF-II and/or increased IGF-IR receptor levels include suppressing IGF-I levels or IGF-II levels or preventing the binding of IGF-I to the IGF-IR. For example, the long acting somatostatin analogue octreotide has been employed to reduce IGF-I synthesis and/or secretion. Soluble IGF-IR [illegible] apoptosis in tumor cells in vivo [illegible] Anticancer Res. [illegible] Antisense oligonucleotides, peptide analogues of IGF-I, and antibodies to IGF-IR have been used to decrease IGF-I [illegible] expression [illegible]. However [illegible] In addition, [illegible] IGF-IR [illegible]

[0012] A [illegible]

[illegible]

IGF-I and/or IGF-II [illegible] would be desirable to generate antibodies to IGF-IR [illegible] be used to either [illegible] human IGF-IR. Although anti-IGF-IR antibodies [illegible] in certain patients [illegible] these antibodies has [illegible] to be suitable for inhibiting IGF-I activity for diagnostic or clinical procedures. See, e.g., Thompson et al., Pediat. Res. 32: 455-459 (1988); Tappy et al., Diabetes 37: 1708-1714 (1988); Wada et al., [illegible] (1993); Drakkas et al., Nether. [illegible] Thus, it would be desirable to obtain high-affinity human anti-IGF-IR antibodies that could be used to treat diseases in humans.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIGS. 1A-1C show alignments of the nucleotide sequences of the light chain variable regions from six human anti-IGF-IR antibodies to each other and to germline sequences. FIG. 1A shows the alignment of the nucleotide sequences of the variable region of the light chain (VL) of antibodies 2.12.1 (SEQ ID NO: 1), 2.13.2 (SEQ ID NO: 5), 2.14.3 (SEQ ID NO: 9) and 4.9.2 (SEQ ID NO: 13) to each other and to the germline Vκ A30 sequence (SEQ ID NO: 39). FIG. 1B shows the alignment of the nucleotide sequence of VL of antibody 4.17.3 (SEQ ID NO: 17) to the germline Vκ O12 sequence (SEQ ID NO: 41). FIG. 1C shows the alignment of the nucleotide sequence of VL of antibody 6.1.1 (SEQ ID NO: 21) to the germline Vκ A27 sequence (SEQ ID NO: 37). The alignments also show the CDR regions of the VL from each antibody. The consensus sequences for FIGS. 1A-1C are shown in SEQ ID NOS: 53-55, respectively.

[0014] FIGS. 2A-2D show alignments of the nucleotide sequences of the heavy chain variable regions from six human anti-IGF-IR antibodies to each other and to germline sequences. FIG. 2A shows the alignment of the nucleotide sequence of the VH of antibody 2.12.1 (SEQ ID NO: 3) to the germline VH DP-35 sequence (SEQ ID NO: 29). FIG. 2B shows the alignment of the nucleotide sequence of the VH of antibody 2.14.3 (SEQ ID NO: 11) to the germline VIV-4/4.35 sequence (SEQ ID NO: 43). FIGS. 2C-1 and 2C-2 show the alignments of the nucleotide sequences of the VH of antibodies 2.13.2 (SEQ ID NO: 7), 4.9.2 (SEQ ID NO: 15) and 6.1.1 (SEQ ID NO: 23) to each other and to the germline VH DP-47 sequence (SEQ ID NO: 31). FIG. 2D shows the alignment of the nucleotide sequence of the VH of antibody 4.17.3 (SEQ ID NO: 19) to the germline VH DP-71 sequence (SEQ ID NO: 35). The alignment also shows the CDR regions of the antibodies. The consensus sequences for FIGS. 2A-2D are shown in SEQ ID NOS: 56-59, respectively.

[0015] FIG. 3 shows that anti-IGF-IR antibodies [illegible] IGF-IR [illegible]

[0016] FIG. 4 shows that anti-IGF-IR antibody [illegible] IGF-I-induced receptor tyrosine phosphorylation [illegible]

[0017] FIG. 5 shows [illegible] anti-IGF-IR antibodies [illegible] IGF-IR [illegible]

[0018] FIG. 6 [illegible]

[0019] FIG. 7 shows [illegible] anti-IGF-IR antibody 2.13.2 inhibits 3T3/IGF-IR tumor growth in vivo alone (left panel) or in combination with [illegible] (right panel).

[0020] FIG. 8 shows [illegible] anti-IGF-IR antibody 2.13.2 serum levels [illegible] IGF-IR downregulation in 3T3/IGF-IR tumors.

[0021] FIG. 9 shows that multiple doses of anti-IGF-IR antibody 2.13.2 inhibit 3T3/IGF-IR tumor growth in vivo alone or in combination with adriamycin.

[0022] FIG. 10 shows that anti-IGF-IR antibody 2.13.2 inhibits large tumor growth in vivo in combination with adriamycin.

[0023] FIG. 11 shows that anti-IGF-IR antibody 2.13.2 inhibits Colo 205 tumor growth in vivo alone or in combination with 5-deoxyuridine (5-FU).

[0024] FIG. 12 shows that multiple doses of anti-IGF-IR antibody 2.13.2 inhibit Colo 205 tumor growth in vivo alone or in combination with 5-FU.

[0025] FIG. 13 shows that multiple doses of anti-IGF-IR antibody 2.13.2 inhibit MCF-7 tumor growth in vivo alone or in combination with taxol.

[0026] FIG. 14 shows that anti-IGF-IR antibody 2.13.2 inhibits MCF-7 tumor growth in vivo alone (left panel) or in combination with adriamycin (right panel).

[0027] FIG. 15 shows that multiple doses of anti-IGF-IR antibody 2.13.2 inhibit MCF-7 tumor growth in vivo alone or in combination with tamoxifen.

[0028] FIG. 16 shows that multiple doses of anti-IGF-IR antibody 2.13.2 inhibit A431 tumor growth in vivo alone or in combination with the epidermal growth factor receptor (EGF-R) tyrosine kinase inhibitor CP-358,774.

[0029] FIG. 17 shows a pharmacokinetic evaluation of a single intravenous injection of anti-IGF-IR antibody 2.13.2 in Cynomolgus monkeys.

[0030] FIG. 18 shows that the combination of anti-IGF-IR antibody 2.13.2 and adriamycin increases the downregulation of IGF-IR on 3T3/IGF-IR tumors in vivo.

[0031] FIG. 19A shows the number of mutations in different regions of the heavy and light chains of 2.13.2 and 2.12.1 compared to the germline sequences.

[0032] FIGS. 19A-D show alignments of the amino acid sequences from the heavy and light chains of antibodies 2.13.2 and 2.12.1 with the germline sequences from which they are derived. FIG. 19B shows an alignment of the amino acid sequence of the heavy chain of antibody 2.13.2 (SEQ ID NO: [illegible]) with that of the germline sequence [illegible] FIG. 19C [illegible] antibody 2.13.2 (SEQ ID NO: [illegible]) with that of the germline sequence A30 (SEQ ID NO: [illegible]). FIG. 19D [illegible] FIG. 19E [illegible]

[illegible]

(left) mutations are highlighted with a [illegible] above the amino acid residue and CDR mutations are highlighted with [illegible] the amino acid residue.

SUMMARY OF THE INVENTION

[0033] The present invention provides an isolated antibody or antigen-binding portion thereof that binds IGF-IR, preferably one that binds to primate and human IGF-IR, and [illegible] a human antibody. The invention provides an anti-IGF-IR antibody that inhibits the binding of IGF-I or IGF-II to IGF-IR and also provides an anti-IGF-IR antibody that activates IGF-IR.

[0034] The invention provides a pharmaceutical composition comprising the antibody and a pharmaceutically acceptable carrier. The pharmaceutical composition may further comprise another component, such as an anti-tumor agent or an imaging reagent.

[0035] Diagnostic and therapeutic methods are also provided by the invention. Diagnostic methods include a method for diagnosing the presence or location of an IGF-IR-expressing tissue using an anti-IGF-IR antibody. A therapeutic method comprises administering the antibody to a subject in need thereof, preferably in conjunction with administration of another therapeutic agent.

[0036] The invention provides an isolated cell line, such as a hybridoma, that produces an anti-IGF-IR antibody.

[0037] The invention also provides nucleic acid molecules encoding the heavy and/or light chain or antigen-binding portions thereof of an anti-IGF-IR antibody.

[0038] The invention provides vectors and host cells comprising the nucleic acid molecules, as well as methods of recombinantly producing the polypeptides encoded by the nucleic acid molecules.

[0039] Non-human transgenic animals that express the heavy and/or light chain or antigen-binding portions thereof of an anti-IGF-IR antibody are also provided. The invention also provides a method for treating a subject in need thereof with an effective amount of a nucleic acid molecule encoding the heavy and/or light chain or antigen-binding portions thereof of an anti-IGF-IR antibody.

DETAILED DESCRIPTION OF THE INVENTION

[0040] Definitions and General Techniques

[0041] Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry [illegible]

[illegible]

reference to a nucleic acid molecule having a particular sequence should be understood to encompass its complementary strand, with its complementary sequence.

[0079] In the molecular biology art, researchers use the terms "percent sequence identity", "percent sequence similarity" and "percent sequence homology" interchangeably. In this application, these terms shall have the same meaning with respect to nucleic acid sequences only.

[0080] The term "substantial similarity" or "substantial sequence similarity," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 85%, preferably at least about 90%, and more preferably at least about 95%, 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed above.

[0081] As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 75% or 80% sequence identity, preferably at least 90% or 95% sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. See, e.g., Pearson, Methods Mol. Biol. 24: 307-31 (1994), herein incorporated by reference. Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; and 6) sulfur-containing side chains are cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine.

[0082] Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet et al., Science 256: 1443-45 (1992), herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0083] Sequence similarity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG contains programs such as "Gap" and "Bestfit" which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, e.g., GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters. a program in GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (1990); Pearson (2000). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially blastp or tblastn, using default parameters. See, e.g., Altschul et al., J. Mol. Biol. 215: 403-410 (1990); Altschul et al., Nucleic Acids Res. 25:3389-402 (1997); herein incorporated by reference.

[0084] The length of polypeptide sequences compared for homology will generally be at least about 16 amino acid residues, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues, and preferably more than about 35 residues. When searching a database containing sequences from a large number of different organisms, it is preferable to compare amino acid sequences.

[0085] As used herein, the terms "label" or "labeled" refers to incorporation of another molecule in the antibody. In one embodiment, the label is a detectable marker, e.g., incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). In another embodiment, the label or marker can be therapeutic, e.g., a drug conjugate or toxin. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I), fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase), chemiluminescent markers, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags), magnetic agents, such as gadolinium chelates, toxins such as pertussis toxin, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

[0086] ~~The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials. The term "therapeutically effective amount" as used herein refers to the amount of an agent or a combination of agents that is sufficient to inhibit, or prevent, or ameliorate the pathology and/or symptomology or reverse the progression of pathology. The therapeutically effective amount will vary depending on the agent, the disease and its severity and the age, weight, etc., of the patient to be treated. The term "prophylactically effective amount" as used herein refers to the amount of an agent or a combination of agents that is sufficient to prevent the development of pathology or symptomology in a patient. The prophylactically effective amount will vary depending on the agent, the disease and its severity and the age, weight, etc., of the patient to be treated. The term "pharmaceutical agent or drug" as used herein refers to a chemical compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient. Other chemistry terms herein are used according to conventional usage in the art, as exemplified by The McGraw-Hill Dictionary of Chemical Terms (Parker, S., Ed., McGraw-Hill, San Francisco (1985)), incorporated herein by reference).~~

[0087] ~~The term "antineoplastic agent" is used herein to refer to agents that have the functional property of inhibiting a development or progression of a neoplasm in a human, particularly a malignant (cancerous) lesion, such as a carcinoma, sarcoma, lymphoma, or leukemia. Inhibition of metastasis is frequently a property of antineoplastic agents.~~

[0088] The term patient includes human and veterinary subjects.

[0089] ~~Human Anti-IGF-1R Antibodies and Characterization Thereof~~

[0090] Human antibodies avoid certain of the problems associated with antibodies that possess mouse or rat variable and/or constant regions. The presence of such mouse or rat derived sequences can lead to the rapid clearance of the antibodies or can lead to the generation of an immune response against the antibody by a patient. Therefore, in one embodiment, the invention provides humanized anti-IGF-IR antibodies. In a preferred embodiment, the invention provides fully human anti-IGF-IR antibodies by introducing human immunoglobulin genes into a rodent so that the rodent produces fully human antibodies. More preferred are fully human anti-human IGF-IR antibodies. Fully human anti-IGF-IR antibodies are expected to minimize the immunogenic and allergic responses intrinsic to mouse or mouse-derivatized monoclonal antibodies (Mabs) and thus to increase the efficacy and safety of the administered antibodies. The use of fully human antibodies can be expected to provide a substantial advantage in the treatment of chronic and recurring human diseases, such as inflammation and cancer, which may require repeated antibody administrations. In another embodiment, the invention provides an anti-IGF-IR antibody that does not bind complement.

[0091] ~~In a preferred embodiment, the anti-IGF-IR antibody is 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In another preferred embodiment, the anti-IGF-IR antibody comprises a light chain comprising an amino acid sequence selected from SEQ ID NO: 2, 6, 10, 14, 18 or 22, or one or more CDRs from these amino acid sequences. In another preferred embodiment, the anti-IGF-IR antibody comprises a heavy chain comprising an amino acid sequence selected from SEQ ID NO: 4, 8, 12, 16, 20 or 24 or one or more CDRs from these amino acid sequences.~~

[0092] Class and Subclass of Anti-IGF-IR Antibodies

[0093] The antibody may be an IgG, an IgM, an IgE, an IgA or an IgD molecule. In a preferred embodiment, the antibody is an IgG and is an IgG1, IgG2, IgG3 or IgG4 subtype. In a more preferred embodiment, the anti-IGF-IR antibody is subclass IgG2. In another preferred embodiment, the anti-IGF-IR antibody is the same class and subclass as antibody 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1, which is IgG2.

[0094] The class and subclass of anti-IGF-IR antibodies may be determined by any method known in the art. In general, the class and subclass of an antibody may be determined using antibodies that are specific for a particular class and subclass of antibody. Such antibodies are available commercially. The class and subclass can be determined by ELISA, Western Blot as well as other techniques. Alternatively, the class and subclass may be determined by sequencing all or a portion of the constant domains of the heavy and/or light chains of the antibodies, comparing their amino acid sequences to the known amino acid sequences of various class and subclasses of immunoglobulins, and determining the class and subclass of the antibodies.

[0095] Species and Molecule Selectivity

[0096] In another aspect of the invention, the anti-IGF-IR antibody demonstrates both species and molecule selectivity. In one embodiment, the anti-IGF-IR antibody binds to human, cynomologous or rhesus IGF-IR. In a preferred embodiment, the anti-IGF-IR antibody does not bind to mouse, rat, guinea pig, dog or rabbit IGF-IR. In another preferred embodiment, the anti-IGF-IR antibody does not bind to a New World monkey species such as a marmoset. Following the teachings of the specification, one may determine the species selectivity for the anti-IGF-IR antibody using methods well known in the art. For instance, one may determine species selectivity using Western blot, FACS, ELISA or RIA. In a preferred embodiment, one may determine the species selectivity using Western blot.

[0097] In another embodiment, the anti-IGF-IR antibody has a selectivity for IGF-IR that is at least 50 times greater than its selectivity for insulin receptor. In a preferred embodiment, the selectivity of the anti-IGF-IR antibody is more than 100 times greater than its selectivity for insulin receptor. In an even more preferred embodiment, the anti-IGF-IR antibody does not exhibit any appreciable specific binding to any other protein other than IGF-IR. One may determine the selectivity of the anti-IGF-IR antibody for IGF-IR using methods well known in the art following the teachings of the specification. For instance, one may determine the selectivity using Western blot, FACS, ELISA or RIA. In a preferred embodiment, one may determine the molecular selectivity using Western blot.

[0098] ~~Binding Affinity of Anti-IGF-IR to IGF-IR~~

[0099] ~~In another aspect of the invention, the anti-IGF-IR antibodies bind to IGF-IR with high affinity. In one embodiment, the anti-IGF-IR antibody binds to IGF-IR with a K_d of 1×10^{-8} M or less. In a more preferred embodiment, the antibody binds to IGF-IR with a K_d of 1×10^{-9} M or less. In an even more preferred embodiment, the antibody binds to IGF-IR with a K_d of 5×10^{-10} M or less. In another preferred embodiment, the antibody binds to IGF-IR with a K_d of 1×10^{-10} M or less. In another preferred embodiment, the antibody binds to IGF-IR with substantially the same K_d as an antibody selected from 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In another preferred embodiment, the antibody binds to IGF-IR with substantially the same K_d as an antibody that comprises one or more CDRs from an antibody selected from 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In still another preferred embodiment, the antibody binds to IGF-IR with substantially the same K_d as an antibody that comprises one of the amino acid sequences selected from SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 or 24. In another preferred embodiment, the antibody binds to IGF-IR with substantially the same K_d as an antibody that comprises one or more CDRs from an anti-

[illegible] comprises one of the amino acid sequences selected from SEQ ID NOS: [illegible], 14, 16, 18, 20 [illegible]

[0100] In [illegible] the invention, the anti-IGF-IR antibodies [illegible] dissociation constant. In one embodiment, the anti-IGF-IR antibody has a K_d of [illegible] or lower. In [illegible] preferred embodiment, the K_d is substantially the same [illegible] 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In another preferred embodiment, the antibody binds to IGF-IR with substantially the same K_d as an antibody that comprises one or more CDRs from an antibody selected from 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In still another preferred embodiment, the antibody binds to IGF-IR with substantially the same K_d as an antibody that comprises one of the amino acid sequences selected from SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 or 24. In another preferred embodiment, the antibody binds to IGF-IR with substantially the same K_d as an antibody that comprises one or more CDRs from an antibody that comprises one of the amino acid sequences selected from SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 [illegible]

[0101] The binding affinity and dissociation rate of an anti-IGF-IR antibody to IGF-IR may be determined by any method known in the art. In one embodiment, the binding affinity can be measured by competitive ELISAs, RIAs or surface plasmon resonance, such as BIAcore. The dissociation rate can also be measured by surface plasmon resonance. In a more preferred embodiment, the binding affinity and dissociation rate is measured by surface plasmon resonance. In an even more preferred embodiment, the binding affinity and dissociation rate is measured using a BIAcore. An example of determining binding affinity and dissociation rate is described below in Example II.

[0102] Half-Life of Anti-IGF-IR Antibodies

[0103] According to another object of the invention, the anti-IGF-IR antibody has a half-life of at least one day in vitro or in vivo. In a preferred embodiment, the antibody or portion thereof has a half-life of at least three days. In a more preferred embodiment, the antibody or portion thereof has a half-life of four days or longer. In another embodiment, the antibody or portion thereof has a half-life of eight days or longer. In another embodiment, the antibody or antigen-binding portion thereof is derivatized or modified such that it has a longer half-life, as discussed below. In another preferred embodiment, the antibody may contain [illegible] mutations [illegible] half-life, such as described WO 00/09560, published [illegible] 2000.

[0104] The antibody half-life may be measured by any means known [illegible] in the art. For [illegible] the antibody [illegible] measured by Western blot, [illegible] ELISA or RIA [illegible] period [illegible] The antibody half-life [illegible]

[0105] [illegible]

[0106] [illegible]

[illegible] anti-IGF-IR antibody that cross-competes with a human anti-IGF-IR antibody. In a preferred embodiment, the human anti-IGF-IR [illegible] 2.12.1, 2.13.2, 2.14.3, 3.1.1, [illegible] In another preferred embodiment, the human anti-IGF-IR comprises one or more CDRs from an antibody selected from: 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In still another preferred embodiment, the human anti-IGF-IR comprises one of the amino acid sequences selected from SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 or 24. In another preferred embodiment, the human anti-IGF-IR comprises one or more CDRs from an antibody that comprises one of the amino acid sequences selected from SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 or 24. In a highly preferred embodiment, the anti-IGF-IR antibody is another human antibody.

[0107] One may determine whether an anti-IGF-IR antibody binds to the same antigen using a variety of methods known in the art. For instance, one may determine whether a test anti-IGF-IR antibody binds to the same antigen by using an anti-IGF-IR antibody to capture an antigen that is known to bind to the anti-IGF-IR antibody, such as IGF-IR, eluting the antigen from the antibody, and then determining whether the test antibody will bind to the eluted antigen. One may determine whether an antibody binds to the same epitope as an anti-IGF-IR antibody by binding the anti-IGF-IR antibody to IGF-IR under saturating conditions, and then measuring the ability of the test antibody to bind to IGF-IR. If the test antibody is able to bind to the IGF-IR at the same time as the anti-IGF-IR antibody, then the test antibody binds to a different epitope as the anti-IGF-IR antibody. However, if the test antibody is not able to bind to the IGF-IR at the same time, then the test antibody binds to the same epitope as the human anti-IGF-IR antibody. This experiment may be performed using ELISA, RIA or surface plasmon resonance. In a preferred embodiment, the experiment is performed using surface plasmon resonance. In a more preferred embodiment, BIAcore is used. One may also determine whether an anti-IGF-IR antibody cross-competes with an anti-IGF-IR antibody. In a preferred embodiment, one may determine whether an anti-IGF-IR antibody cross-competes with another by using the same method that is used to measure whether the anti-IGF-IR antibody is able to bind to the same epitope as another anti-IGF-IR antibody.

[0108] Light and Heavy Chain Usage

[0109] The invention also provides an anti-IGF-IR antibody that comprises variable sequences encoded by a human κ gene. In a preferred embodiment, the variable sequences are encoded by either the Vκ A27, A30 or O12 gene family. In a preferred embodiment, the variable sequences are encoded by a human Vκ A30 gene family. In a more preferred [illegible] of the light chain comprises [illegible] amino acid [illegible] from the germline Vκ A27, A30 [illegible] substitutions [illegible]

[0110] [illegible] SEQ ID NOS [illegible] IGF-IR [illegible]

sequences of the variable regions of the light chains of the six anti-IGF-IR antibodies [illegible] to the germline sequences from which they are derived. Following the teachings of this specification, one of ordinary skill in the art could determine the encoded amino acid sequence of the six anti-IGF-IR light chains and the germline light chains and determine the differences between the germline sequences and the antibody sequences.

[0111] In a preferred embodiment, the VL of the anti-IGF-IR antibody contains the same amino acid substitutions, relative to the germline amino acid sequence, as any one or more of the VL of antibodies [illegible], 3.1.1, 4.9.2, 4.17.3 or 6.1.1. For example, the VL of the anti-IGF-IR antibody may contain one or more amino acid substitutions that are the same as those present in antibody [illegible], another amino acid substitution that is the same as that present in antibody 2.14.3, and another amino acid substitution that is the same as antibody 4.9.2. In this manner, one can mix and match different features of antibody binding in order to alter, e.g., the affinity of the antibody for IGF-IR or its dissociation rate from the antigen. In another embodiment, the amino acid substitutions are made in the same position as those found in any one or more of the VL of antibodies [illegible], 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1, but conservative amino acid substitutions are made rather than using the same amino acid. For example, if the amino acid substitution compared to the germline in one of the antibodies 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1 is glutamate, one may conservatively substitute aspartate. Similarly, if the amino acid substitution is serine, one may conservatively substitute threonine.

[0112] In another preferred embodiment, the light chain comprises an amino acid sequence that is the same as the amino acid sequence of the VL of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In another highly preferred embodiment, the light chain comprises amino acid sequences that are the same as the CDR regions of the light chain of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In another preferred embodiment, the light chain comprises an amino acid sequence from at least one CDR region of the light chain of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In another preferred embodiment, the light chain comprises amino acid sequences from CDRs from different light chains. In a more preferred embodiment, the CDRs from different light chains are obtained from [illegible], 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In another preferred embodiment, the light chain comprises an amino acid sequence selected from SEQ ID NOS: [illegible] In another embodiment, the light chain comprises an amino acid sequence encoded by a nucleic acid sequence selected from SEQ ID NOS: [illegible], 13, 17 or 21, or a nucleic acid sequence that encodes an amino acid sequence [illegible] Preferably, the amino acid substitutions are conservative amino acid substitutions. [illegible]

[0113] [illegible]

V[illegible] DP-47 [illegible] In another preferred embodiment, the heavy chain [illegible] germline V_H DP-35, DP-47, DP-70, DP-71 or VIV-4/4.35, or [illegible] changes, and even more preferably [illegible] acid changes.

[0114] SEQ ID NOS: 4, 8, 12, 16, 20 and 24 provide the amino acid sequences of the variable regions of six anti-IGF-IR heavy chains. SEQ ID NOS: 30, 32, 34, 36 and 44 provide the amino acid sequences and SEQ ID NOS: 29, 31, 33, 35 and 43 provide the nucleotide sequences of the germline heavy chains DP-35, DP-47, DP-70, DP-71 and VIV-4, respectively. FIGS. 2A-2D show the alignments of the amino acid sequences of the variable region of the six anti-IGF-IR antibodies to their corresponding germline sequences. Following the teachings of this specification, one of ordinary skill in the art could determine the encoded amino acid sequence of the six anti-IGF-IR heavy chains and the germline heavy chains and determine the differences between the germline sequences and the antibody sequences.

[0115] In a preferred embodiment, the VH of the anti-IGF-IR antibody contains the same amino acid substitutions, relative to the germline amino acid sequence, as any one or more of the VH of antibodies 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. Similar to what was discussed above, the VH of the anti-IGF-IR antibody may contain one or more amino acid substitutions that are the same as those present in antibody 2.13.2, another amino acid substitution that is the same as that present in antibody 2.14.3, and another amino acid substitution that is the same as antibody 4.9.2. In this manner, one can mix and match different features of antibody binding in order to alter, e.g., the affinity of the antibody for IGF-IR or its dissociation rate from the antigen. In another embodiment, the amino acid substitutions are made in the same position as those found in any one or more of the VH of antibodies [illegible], 4.17.3, 4.9.2 or 6.1.1, but conservative amino acid substitutions are made rather than using the same amino acid.

[0116] In another preferred embodiment, the heavy chain comprises an amino acid sequence that is the same as the amino acid sequence of the VH of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In another highly preferred embodiment, the heavy chain comprises amino acid sequences that are the same as the CDR regions of the heavy chain of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In another preferred embodiment, the heavy chain comprises an amino acid sequence from at least one CDR region of the heavy chain of [illegible] or 6.1.1. In another preferred embodiment, the heavy chain comprises amino acid sequences from CDRs from different heavy chains [illegible] CDRs [illegible] 2.14.3, 3.1.1, 4.9.2, [illegible] In another preferred embodiment, the heavy chain comprises an amino acid sequence selected from SEQ ID NOS: [illegible] amino acid sequence encoded by a nucleic acid sequence [illegible] SEQ ID NOS: 3, [illegible]

chains of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In another embodiment, the nucleic acid molecule comprises a nucleic acid sequence that encodes the amino acid sequence of one of SEQ ID NOS: 2, 6, 10, 14, 18 or 22 or comprises a nucleic acid sequence of one of SEQ ID NOS: 1, 5, 9, 13, 17 or 21. In another preferred embodiment, the nucleic acid molecule comprises a nucleic acid sequence that encodes the amino acid sequence of one or more of the CDRs of any one of SEQ ID NOS: 2, 6, 10, 14, 18 or 22 or comprises a nucleic acid sequence of one or more of the CDRs of any one of SEQ ID NOS: 1, 5, 9, 13, 17 or 21. In a more preferred embodiment, the nucleic acid molecule comprises a nucleic acid sequence that encodes the amino acid sequence of all of the CDRs of any one of SEQ ID NOS: 2, 6, 10, 14, 18 or 22 or comprises a nucleic acid sequence of all the CDRs of any one of SEQ ID NOS: 1, 5, 9, 13, 17 or 21.

[0151] The invention also provides a nucleic acid molecules that encodes an amino acid sequence of a VL that has an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a VL described above, particularly to a VL that comprises an amino acid sequence of one of SEQ ID NOS: 2, 6, 10, 14, 18 or 22. The invention also provides a nucleic acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence of one of SEQ ID NOS: 1, 5, 9, 13, 17 or 21. In another embodiment, the invention provides a nucleic acid molecule encoding a VL that hybridizes under highly stringent conditions to a nucleic acid molecule encoding a VL as described above, particularly a nucleic acid molecule that comprises a nucleic acid sequence encoding an amino acid sequence of SEQ ID NOS: 2, 6, 10, 14, 18 or 22. The invention also provides a nucleic acid sequence encoding an VL that hybridizes under highly stringent conditions to a nucleic acid molecule comprising a nucleic acid sequence of one of SEQ ID NOS: 1, 5, 9, 13, 17 or 21.

[0152] ~~The invention also provides a nucleic acid molecule encoding the variable region of the heavy chain (VH) is derived from the DP-35, DP-47, DP-71 or VH 4-34 35 VH gene, preferably the DP-35 VH gene. In another preferred embodiment, the nucleic acid molecule encoding the VH comprises the joining region derived from JH6 or JH5, most preferably JH6. In another preferred embodiment, the JH segment is derived from 3-3, 6-19 or 4-17. In an even more preferred embodiment, the nucleic acid molecule encoding the VH contains no more than ten amino acid changes from the germline DP-47 gene, preferably no more than six amino acid changes, and even more preferably no more than three amino acid changes.~~ In a highly preferred embodiment, the nucleic acid molecule encoding the VH contains at least one amino acid change compared to the germline sequence, wherein the amino acid change is identical to the amino acid change from the germline sequence from the heavy chain of one of the antibodies 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In an even more preferred embodiment, the VH contains at least three amino acid changes compared to the germline sequences wherein the changes are identical to those changes from the germline sequence from the VH of one of the antibodies 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1.

[0153] In one embodiment, the nucleic acid molecule comprises a nucleic acid sequence that encodes the amino acid sequence of the VH of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In another embodiment, the nucleic acid molecule comprises a nucleic acid sequence that encodes the amino acid sequence of one or more of the CDRs of the heavy chain of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In a preferred embodiment, the nucleic acid molecule comprises a nucleic acid sequence that encodes the amino acid sequences of all of the CDRs of the heavy chain of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In another preferred embodiment, the nucleic acid molecule comprises a nucleic acid sequence that encodes the amino acid sequence of one of SEQ ID NOS: 4, 8, 12, 16, 20 or 24 or that comprises a nucleic acid sequence of one of SEQ ID NOS: 3, 7, 11, 15, 19 or 23. In another preferred embodiment, the nucleic acid molecule comprises a nucleic acid sequence that encodes the amino acid sequence of one or more of the CDRs of any one of SEQ ID NOS: 4, 8, 12, 16, 20 or 24 or comprises a nucleic acid sequence of one or more of the CDRs of any one of SEQ ID NOS: 3, 7, 11, 15, 19 or 23. In a preferred embodiment, the nucleic acid molecule comprises a nucleic acid sequence that encodes the amino acid sequences of all of the CDRs of any one of SEQ ID NOS: 4, 8, 12, 16, 20 or 24 or comprises a nucleic acid sequence of all of the CDRs of any one of SEQ ID NOS: 3, 7, 11, 15, 19 or 23.

[0154] In another embodiment, the nucleic acid molecule encodes an amino acid sequence of a VH that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to one of the amino acid sequences encoding a VH as described immediately above, particularly to a VH that comprises an amino acid sequence of one of SEQ ID NOS: 4, 8, 12, 16, 20 or 24. The invention also provides a nucleic acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence of one of SEQ ID NOS: 3, 7, 11, 15, 19 or 23. In another embodiment, the nucleic acid molecule encoding a VH is one that hybridizes under highly stringent conditions to a nucleic acid sequence encoding a VH as described above, particularly to a VH that comprises an amino acid sequence of one of SEQ ID NOS: 4, 8, 12, 16, 20 or 24. The invention also provides a nucleic acid sequence encoding a VH that hybridizes under highly stringent conditions to a nucleic acid molecule comprising a nucleic acid sequence of one of SEQ ID NOS: 3, 7, 11, 15, 19or23.

[0155] The nucleic acid molecule encoding either or both of the entire heavy and light chains of an anti-IGF-IR antibody or the variable regions thereof may be obtained from any source that produces an anti-IGF-IR antibody. Methods of isolating mRNA encoding an antibody are well-known in the art. See, e.g., Sambrook et al. The mRNA may be used to produce cDNA for use in the polymerase chain reaction (PCR) or cDNA cloning of antibody genes. In one embodiment of the invention, the nucleic acid molecules may be obtained from a hybridoma that expresses an anti-IGF-IR antibody, as described above, preferably a hybridoma that has as one of its fusion partners a transgenic animal cell that expresses human immunoglobulin genes, such as a XENOMOUSE™, non-human mouse transgenic animal or a non human, non-mouse transgenic animal. In another embodiment, the hybridoma is derived from a non-human, non-transgenic animal, which may be used e.g. for humanized antibodies.

label may be used therapeutically as a toxin for cancerous cells or tumors. Examples of labels for polypeptides include, but are not limited to, the following radioisotopes or radionuclides: ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I.

[0202] An anti-IGF-IR antibody may also be derivatized with a chemical group such as polyethylene glycol (PEG), a methyl or ethyl group, or a carbohydrate group. These groups may be useful to improve the biological characteristics of the antibody, e.g., to increase serum half life or to increase tissue binding.

[0203] ~~Pharmaceutical Compositions and Kits.~~

[0204] ~~The invention also relates to a pharmaceutical composition for the treatment of a hyperproliferative disorder in a mammal which comprises a therapeutically effective amount of a compound of the invention and a pharmaceutically acceptable carrier. In one embodiment, said pharmaceutical composition is for the treatment of cancer such as~~ brain, lung, squamous cell, bladder, gastric, pancreatic, ~~breast~~, head, neck, renal, kidney, ~~ovarian, prostate~~, colorectal, esophageal, gynecological or thyroid cancer. In another embodiment, said pharmaceutical composition relates to non-cancerous hyperproliferative disorders such as, without limitation, restenosis after angioplasty and psoriasis. In another embodiment, the invention relates to pharmaceutical compositions for the treatment of a mammal that requires activation of IGF-IR, wherein the pharmaceutical composition comprises a therapeutically effective amount of an activating antibody of the invention and a pharmaceutically acceptable carrier. Pharmaceutical compositions comprising activating antibodies may be used to treat animals that lack sufficient IGF-I or IGF-II, or may be used to treat osteoporosis, frailty or disorders in which the mammal secretes too little active growth hormone or is unable to respond to growth hormone.

[0205] ~~The anti-IGF-IR antibodies of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises an antibody of the invention and a pharmaceutically acceptable carrier.~~ As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable substances such as wetting or minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody or antibody portion.

[0206] ~~The compositions of this invention may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories.~~ The preferred form depends on the intended mode of administration and the capeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. The preferred mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular or subcutaneous injection.

[0207] Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the anti-IGF-IR antibody in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[0208] The antibodies of the present invention can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is intraperitoneal, subcutaneous, intramuscular, intravenous or infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In one embodiment, the antibodies of the present invention can be administered as a single dose or may be administered as multiple doses.

[0209] In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978. In certain embodiments, the anti-IGF-IR of the invention may be orally administered, for example with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral

administration [illegible] may be necessary to [illegible] the compound with [illegible] to prevent [illegible] its inactivation.

[0210] Supplementary active compounds can also be incorporated into the compositions. In certain embodiments, an anti-IGF-IR of the invention is coformulated with and/or coadministered with one or more additional therapeutic agents, such as a chemotherapeutic agent, an antineoplastic agent or an anti-tumor agent. For example, an anti-IGF-IR antibody may be coformulated and/or coadministered with one or more additional therapeutic agents. These agents include, without limitation, antibodies that bind other targets (e.g., antibodies that bind one or more growth factors or cytokines, their cell surface receptors or IGF-I), IGF-I binding proteins, antineoplastic agents, chemotherapeutic agents, anti-tumor agents, antisense oligonucleotides against IGF-IR or IGF-I, peptide analogues that block IGF-IR activation, soluble IGF-IR, and/or one or more chemical agents that inhibit IGF-I production or activity, which are known in the art, e.g., octreotide. For a pharmaceutical composition comprising an activating antibody, the anti-IGF-IR antibody may be formulated with a factor that increases cell proliferation or prevents apoptosis. Such factors include growth factors such as IGF-I, and/or analogues of IGF-I that activate IGF-IR. Such combination therapies may require lower dosages of the anti-IGF-IR antibody as well as the co-administered agents, thus avoiding possible toxicities or complications associated with the various monotherapies. In one embodiment, the antibody and one or more additional therapeutic agent.

[0211] The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody or antibody portion of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody or antibody portion may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[0212] Dosage regimens may be adjusted to provide the optimum desired response [illegible]. [illegible] dosage unit form [illegible]

[illegible]

[illegible] dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals. A [illegible] formulation is 5 mg/ml anti-IGF-IR antibody in a buffer of 20 mM sodium citrate, pH [illegible], [illegible] mM NaCl and 0.2 mg/ml polysorbate 80.

[0213] An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody or antibody portion of the invention is [illegible]-100 mg/kg, more preferably 0.5-50 mg/kg, more preferably 1-20 mg/kg, and even more preferably 1-10 mg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. In one embodiment, the therapeutically or prophylactically effective amount of an antibody or antigen-binding portion thereof is administered along with one or more additional therapeutic agents.

[0214] In another aspect, the invention relates to administration of an anti-IGF-IR antibody for the treatment of cancer in a dose of less than 300 mg per month.

[0215] Another aspect of the present invention provides kits comprising the anti-IGF-IR antibodies and the pharmaceutical compositions comprising these antibodies. A kit may include, in addition to the antibody or pharmaceutical composition, diagnostic or therapeutic agents. A kit may also include instructions for use in a diagnostic or therapeutic method. In a preferred embodiment, the kit includes the antibody or a pharmaceutical composition thereof and a diagnostic agent that can be used in a method described below. In another preferred embodiment, the kit includes the antibody or a pharmaceutical composition thereof and one or more therapeutic agents, such as an additional antineoplastic agent, anti-tumor agent or chemotherapeutic agent, that can be used in a method described below.

[0216] This invention also relates to pharmaceutical compositions for inhibiting abnormal cell growth in a mammal which comprise an amount of a compound of the invention in combination with an amount of a chemotherapeutic agent, wherein the amounts of the compound, salt, solvate, or prodrug, and of the chemotherapeutic agent are together effective in inhibiting abnormal cell growth. Many chemotherapeutic agents are presently known in the art. In one embodiment, the chemotherapeutic agent is selected from the group consisting of mitotic inhibitors, alkylating agents, anti-metabolites, intercalating antibiotics, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, [illegible] biological response modifiers, anti-hormones, e.g. anti-androgens, and anti-angiogenesis agents.

[0217] Anti-angiogenesis agents, such as MMP-2 (matrix-metalloproteinase 2) inhibitors, MMP-9 (matrix-metalloproteinase 9) inhibitors, and COX-II (cyclooxygenase II) inhibitors, can be used in conjunction with a compound of the invention. Examples of useful COX-II inhibitors include CELEBREX™ (celecoxib), [illegible]. Examples of useful matrix metalloproteinase inhibitors are described in WO 96/33172 (published Oct. 24, 1996), WO 96/27583 (published Mar. 7, 1996), European Patent Application No. 97304971.1 (filed Jul. 8, 1997), European Patent Application No. 99308617.2 (filed Oct. 29, 1999), WO 98/07697 (published Feb. 26, 1998), WO 98/03516 (published Jan. 29, 1998), WO 98/34918 (published Aug. 13, 1998), WO 98/34915 (published Aug. 13, 1998), WO 98/33768 (published Aug. 6, 1998), WO 98/30566 (published Jul. 16, 1998), European Patent Publication 606,046 (published Jul. 13, 1994), European Patent Publication 931,788 (published Jul. 28, 1999), WO 90/05719 (published May 31, 1990), WO 99/52910 (published Oct. 21, 1999), WO 99/52889 (published Oct. 21, 1999), WO 99/29667 (published Jun. 17, 1999), PCT International Application No. PCT/IB98/01113 (filed Jul. 21, 1998), European Patent Application No. 99302232.1 (filed Mar. 25, 1999), Great Britain patent application number 9912961.1 (filed Jun. 3, 1999), U.S. Provisional Application No. 60/148,464 (filed Aug. 12, 1999), U.S. Pat. No. 5,863,949 (issued Jan. 26, 1999), U.S. Pat. No. 5,861,510 (issued Jan. 19, 1999), and European Patent Publication 780,386 (published Jun. 25, 1997), all of which are incorporated herein in their entireties by reference. Preferred MMP inhibitors are those that do not demonstrate arthralgia. More preferred, are those that selectively inhibit MMP-2 and/or MMP-9 relative to the other matrix-metalloproteinases (i.e. MMP-1, MMP-3, MMP-4, MMP-5, MMP-6, MMP-7, MMP-8, MMP-10, MMP-11, MMP-12, and MMP-13). Some specific examples of MMP inhibitors useful in the present invention are AG-3340, RO 32-3555, RS 13-0830, and the compounds recited in the following list: 3-[[4-(4-fluoro-phenoxy)-benzenesulfonyl]-(1-hydroxycarbamoyl-cyclopentyl)-amino]-propionic acid; 3-exo-3-[4-(4-fluoro-phenoxy)-benzenesulfonylamino]-8-oxa-bicyclo[3.2.1]octane-3-carboxylic acid hydroxyamide; (2R, 3R) 1-[4-(2-chloro-4-fluoro-benzyloxy)-benzenesulfonyl]-3-hydroxy-3-methyl-piperidine-2-carboxylic acid hydroxyamide; 4-[4-(4-fluoro-phenoxy)-benzenesulfonylamino]-tetrahydro-pyran-4-carboxylic acid hydroxyamide; 3-[[4-(4-fluoro-phenoxy)-benzenesulfonyl]-(1-hydroxycarbamoyl-cyclobutyl)-amino]-propionic acid; 4-[4-(4-chloro-phenoxy)-benzenesulfonylamino]-tetrahydro-pyran-4-carboxylic acid hydroxyamide; [illegible]

[illegible]

[0218] A compound of the invention can also be used with signal transduction inhibitors, such as agents that can inhibit EGF-R (epidermal growth factor receptor) responses, such as EGF-R antibodies, EGF antibodies, and molecules that are EGF-R inhibitors; VEGF (vascular endothelial growth factor) inhibitors, such as VEGF receptors and molecules that can inhibit VEGF; and erbB2 receptor inhibitors, such as organic molecules or antibodies that bind to the erbB2 receptor, for example, HERCEPTIN™ (Genentech, Inc.). EGFR inhibitors are described in, for example in WO 95/19970 (published Jul. 27, 1995), WO 98/14451 (published Apr. 9, 1998), WO 98/02434 (published Jan. 22, 1998), and U.S. Pat. No. 5,747,498 (issued May 5, 1998), and such substances can be used in the present invention as described herein. EGFR-inhibiting agents include, but are not limited to, the monoclonal antibodies C225 and anti-EGFR 22Mab (ImClone Systems Incorporated), ABX-EGF (Abgenix/Cell Genesys), EMD-7200 (Merck KgaA), EMD-5590 (Merck KgaA), MDX-447/H-477 (Medarex Inc. and Merck KgaA), and the compounds ZD-1834, ZD-1838 and ZD-1839 (AstraZeneca), PKI-166 (Novartis), PKI-166/CGP-75166 (Novartis), PTK 787 (Novartis), CP 701 (Cephalon), leflunomide (Pharmacia/Sugen), CI-1033 (Warner Lambert Parke Davis), CI-1033/PD 183,805 (Warner Lambert Parke Davis), CL-387,785 (Wyeth-Ayerst), BBR-1611 (Boehringer Mannheim GmbH/Roche), Naamidine A (Bristol Myers Squibb), RC-3940-II (Pharmacia), BIBX-1382 (Boehringer Ingelheim), OLX-103 (Merck & Co.), VRCTC-310 (Ventech Research), EGF fusion toxin (Seragen Inc.), DAB-389 (Seragen/Lilgand), ZM-252808 (Imperial Cancer Research Fund), RG-50864 (INSERM), LFM-A12 (Parker Hughes Cancer Center), WHI-P97 (Parker Hughes Cancer Center), GW-282974 (Glaxo), KT-8391 (Kyowa Hakko) and EGF-R Vaccine (York Medical/Centro de Immunologia Molecular (CIM)). These and other EGF-R-inhibiting agents can be used in the present invention.

[0219] VEGF inhibitors, for example SU-5416 and SU-6668 (Sugen Inc.), SH-268 (Schering), and NX-1838 (NeXstar) can also be combined with the compound of the present invention. VEGF inhibitors are described in, for example in WO 99/24440 (published May 20, 1999), PCT International Application PCT/IB99/00797 (filed May 3, 1999), in WO 95/21613 (published Aug. 17, 1995), WO 99/61422 (published Dec. 2, 1999), U.S. Pat. No. 5,834,504 (issued Nov. 10, 1998), WO 98/50356 (published Nov. 12, 1998), U.S. Pat. No. 5,883,113 (issued Mar. [illegible]), U.S. Pat. No. 5,886,020 (issued Mar. 23, 1999), U.S. Pat. No. 5,792,783 (issued Aug. 11, 1998), WO 99/10349 (published Mar. 4, 1999), WO 97/32856 (published Sep. [illegible]), WO [illegible] (published [illegible]), WO 98/[illegible] (published [illegible]), WO 98/[illegible] (published [illegible]), WO [illegible] (published [illegible]), WO 98/[illegible] (published [illegible] 1998), all of which are [illegible]

[illegible]

[0220] [illegible]

can furthermore be combined with the compound of the invention, for example those indicated in WO 98/02434 (published Jan. 22, 1998), WO 99/35146 (published Jul. 15, 1999), WO 99/35132 (published Jul. 15, 1999), WO 98/02437 (published Jan. 22, 1998), WO 97/13760 (published Apr. 17, 1997), WO 95/19970 (published Jul. 27, 1995), U.S. Pat. No. 5,587,458 (issued Dec. 24, 1996), and U.S. Pat. No. 5,877,305 (issued Mar. 2, 1999), which are all hereby incorporated herein in their entireties by reference. ErbB2 receptor inhibitors useful in the present invention are also described in U.S. Provisional Application No. 60/117,341, filed Jan. 27, 1999, and in U.S. Provisional Application No. 60/117,346, filed Jan. 27, 1999, both of which are incorporated in their entireties herein by reference. The erbB2 receptor inhibitor compounds and substance described in the aforementioned PCT applications, U.S. patents, and U.S. provisional applications, as well as other compounds and substances that inhibit the erbB2 receptor, can be used with the compound of the present invention in accordance with the present invention.

[0221] ~~Anti-survival agents include anti-IGF-IR antibod-~~ ~~-for and anti-integrin agents, such as anti-integrin antibodies.~~

[0222] Diagnostic Methods of Use

[0223] The anti-IGF-IR antibodies may be used to detect IGF-IR in a biological sample in vitro or in vivo. The anti-IGF-IR antibodies may be used in a conventional immunoassay, including, without limitation, an ELISA, an RIA, FACS, tissue immunohistochemistry, Western blot or immunoprecipitation. The anti-IGF-IR antibodies of the invention may be used to detect IGF-IR from humans. In another embodiment, the anti-IGF-IR antibodies may be used to detect IGF-IR from Old World primates such as cynomologous and rhesus monkeys, chimpanzees and apes.

[0224] The invention provides a method for detecting anti-IGF-IR in a biological sample comprising contacting a biological sample with an anti-IGF-IR antibody of the invention and detecting the bound antibody bound to anti-IGF-IR, to detect the IGF-IR in the biological sample. In one embodiment, the anti-IGF-IR antibody is directly labeled with a detectable label. In another embodiment, the anti-IGF-IR antibody (the first antibody) is unlabeled and a second antibody or other molecule that can bind the anti-IGF-IR antibody is labeled. As is well known to one of skill in the art, a second antibody is chosen that is able to specifically bind the specific species and class of the first antibody. For example, if the anti-IGF-IR antibody is a human IgG, then the secondary antibody may be an anti-human-IgG. Other molecules that can bind to antibodies include, without limitation, Protein A and Protein G, both of which are available commercially, e.g., from Pierce Chemical Co.

[0225] Suitable labels for the antibody or secondary have been disclosed supra, and include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, magnetic agents and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; an example of a magnetic agent includes gadolinium; and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

[0226] In an alternative embodiment, IGF-IR can be assayed in a biological sample by a competition immunoassay utilizing IGF-IR standards labeled with a detectable substance and an unlabeled anti-IGF-IR antibody. In this assay, the biological sample, the labeled IGF-IR standards and the anti-IGF-IR antibody are combined and the amount of labeled IGF-IR standard bound to the unlabeled antibody is determined. The amount of IGF-IR in the biological sample is inversely proportional to the amount of labeled IGF-IR standard bound to the anti-IGF-IR antibody.

[0227] One may use the immunoassays disclosed above for a number of purposes. In one embodiment, the anti-IGF-IR antibodies may be used to detect IGF-IR in cells in cell culture. In a preferred embodiment, the anti-IGF-IR antibodies may be used to determine the level of tyrosine phosphorylation, tyrosine autophosphorylation of IGF-IR, and/or the amount of IGF-IR on the cell surface after treatment of the cells with various compounds. This method can be used to test compounds that may be used to activate or inhibit IGF-IR. In this method, one sample of cells is treated with a test compound for a period of time while another sample is left untreated. If tyrosine autophosphorylation is to be measured, the cells are lysed and tyrosine phosphorylation of the IGF-IR is measured using an immunoassay described above or as described in Example III, which uses an ELISA. If the total level of IGF-IR is to be measured, the cells are lysed and the total IGF-IR level is measured using one of the immunoassays described above.

[0228] A preferred immunoassay for determining IGF-IR tyrosine phosphorylation or for measuring total IGF-IR levels is an ELISA or Western blot. If only the cell surface level of IGF-IR is to be measured, the cells are not lysed, and the cell surface levels of IGF-IR are measured using one of the immunoassays described above. A preferred immunoassay for determining cell surface levels of IGF-IR includes the steps of labeling the cell surface proteins with a detectable label, such as biotin or ^{125}I, immunoprecipitating the IGF-IR with an anti-IGF-IR antibody and then detecting the labeled IGF-IR. Another preferred immunoassay for determining the localization of IGF-IR, e.g., cell surface levels, is by using immunohistochemistry. Methods such as ELISA, RIA, Western blot, immunohistochemistry, cell surface labeling of integral membrane proteins and immunoprecipitation are well known in the art. See, e.g., Harlow and Lane, supra. In addition, the immunoassays may be scaled up for high throughput screening in order to test a large number of compounds for either activation or inhibition of IGF-IR.

[0229] The anti-IGF-IR antibodies of the invention may also be used to determine the levels of IGF-IR in a tissue or in cells derived from the tissue. In a preferred embodiment, the tissue is a diseased tissue. In a more preferred embodiment, the tissue is a tumor or a biopsy thereof. In a preferred embodiment of the method, a tissue or a biopsy thereof is excised from a patient. The tissue or biopsy is then used in an immunoassay to determine, e.g., IGF-IR levels, cell surface levels of IGF-IR, levels of tyrosine phosphorylation of IGF-IR or localization of IGF-IR by the methods discussed above. The method can be used to determine if a tumor expresses IGF-IR at a high level.

rectal cancer, cancer of the anal region, stomach cancer, colon cancer, breast cancer, gynecologic tumors (e.g., uterine sarcomas, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina or carcinoma of the vulva), Hodgkin's disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system (e.g., cancer of the thyroid, parathyroid or adrenal glands), sarcomas of soft tissues, cancer of the urethra, cancer of the penis, prostate cancer, chronic or acute leukemia, solid tumors of childhood, lymphocytic lymphomas, cancer of the bladder, cancer of the kidney or ureter (e.g., renal cell carcinoma, carcinoma of the renal pelvis), or neoplasms of the central nervous system (e.g., primary CNS lymphoma, spinal axis tumors, brain stem gliomas or pituitary adenomas).

[0238] The antibody may be administered once, but more preferably is administered multiple times. The antibody may be administered from three times daily to once every six months. The administering may be on a schedule such as three times daily, twice daily, once daily, once every two days, once every three days, once weekly, once every two weeks, once every month, once every two months, once every three months and once every six months. The antibody may be administered via an oral, mucosal, buccal, intranasal, inhalable, intravenous, subcutaneous, intramuscular, parenteral, intratumor or topical route. The antibody may be administered at a site distant from the site of the tumor. The antibody may also be administered continuously via a minipump. The antibody may be administered once, at least twice or for at least the period of time until the condition is treated, palliated or cured. The antibody generally will be administered for as long as the tumor is present provided that the antibody causes the tumor or cancer to stop growing or to decrease in weight or volume. The antibody will generally be administered as part of a pharmaceutical composition as described supra. The dosage of antibody will generally be in the range of 0.1-100 mg/kg, more preferably 0.5-50 mg/kg, more preferably 1-20 mg/kg, and even more preferably 1-10 mg/kg. The serum concentration of the antibody may be measured by any method known in the art. See, e.g., Example XVII below. The antibody may also be administered prophylactically in order to prevent a cancer or tumor from occurring. This may be especially useful in patients that have a "high normal" level of IGF-I because these patients have been shown to have a higher risk of developing common cancers. See Rosen et al., supra.

[0239] ~~In another aspect, the anti-IGF-IR antibody may be co-administered with other therapeutic agents, such as anti-neoplastic drugs or molecules, to a patient who has a hyperproliferative disorder, such as cancer or a tumor.~~ In one aspect, the invention relates to a method for the treatment of the hyperproliferative disorder in a mammal ~~comprising administering to said mammal a therapeutically effective amount of a compound of the invention in combination with an anti-tumor agent selected from the group consisting of, but not limited to, mitotic inhibitors, alkylating agents, anti-metabolites, intercalating agents, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, biological response modifiers, anti-hormones, kinase inhibitors, matrix metalloproteinase inhibitors, genetic therapeutics and anti-androgens.~~ In a more preferred embodiment, the antibody may be co-administered with a ~~chemotherapeutic agent, such as anti-neoplastic drugs or molecules.~~ In another preferred embodiment, the antibody or combination therapy is administered along with radiotherapy, chemotherapy, photodynamic therapy, surgery or other immunotherapy. In yet another preferred embodiment, the antibody will be administered with another antibody. For example, the anti-IGF-IR antibody may be administered with an antibody or other agent that is known to inhibit tumor or cancer cell proliferation, e.g., an antibody or agent that inhibits erbB2 receptor, EGF-R, CD20 or VEGF.

[0240] ~~The co-administration of the antibody with an additional therapeutic agent (combination therapy) encompasses co-administering a pharmaceutical composition comprising the anti-IGF-IR antibody and the additional therapeutic agent and administering two of more separate pharmaceutical compositions, one comprising the anti-IGF-IR antibody and the other(s) comprising the additional therapeutic agent(s).~~ Further, although co-administration or combination therapy generally means that the antibody and additional therapeutic agents are administered at the same time as one another, it also encompasses instances in which the antibody and additional therapeutic agents are administered at different times. For instance, the antibody may be administered once every three days, while the additional therapeutic agent is administered once daily. Alternatively, the antibody may be administered prior to or subsequent to treatment of the disorder with the additional therapeutic agent. Similarly, administration of the anti-IGF-IR antibody may be administered prior to or subsequent to other therapy, such as radiotherapy, chemotherapy, photodynamic therapy, surgery or other immunotherapy

[0241] ~~The antibody and one or more additional therapeutic agents (the combination therapy) may be administered once, twice or at least the period of time until the condition is treated, palliated or cured.~~ Preferably, the combination therapy is administered multiple times. The combination therapy may be administered from three times daily to once every six months. The administering may be on a schedule such as three times daily, twice daily, once daily, once every two days, once every three days, once weekly, once every two weeks, once every month, once every two months, once every three months and once every six months, or may be administered continuously via a minipump. The combination therapy may be administered via an oral, mucosal, buccal, intranasal, inhalable, intravenous, subcutaneous, intramuscular, parenteral, intratumor or topical route. The combination therapy may be administered at a site distant from the site of the tumor. The combination therapy generally will be administered for as long as the tumor is present provided that the antibody causes the tumor or cancer to stop growing or to decrease in weight or volume.

[0242] In a still further embodiment, the anti-IGF-IR antibody is labeled with a radiolabel, an immunotoxin or a toxin, or is a fusion protein comprising a toxic peptide. The anti-IGF-IR antibody or anti-IGF-IR antibody fusion protein directs the radiolabel, immunotoxin, toxin or toxic peptide to the IGF-IR-expressing tumor or cancer cell. In a preferred embodiment, the radiolabel, immunotoxin, toxin or toxic peptide is internalized after the anti-IGF-IR antibody binds to the IGF-IR on the surface of the tumor or cancer cell.

[0243] In another aspect, the anti-IGF-IR antibody may be used therapeutically to induce apoptosis of specific cells in a patient in need thereof. In many cases, the cells targeted for apoptosis are cancerous or tumor cells. Thus, in a preferred

—continued

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We claim

[illegible]

2. The antibody or portion thereof according to claim 1, wherein the IGF-IR is human.

3. The antibody or antigen-binding portion thereof according to any one of claims 1 or 2, wherein the antibody or portion thereof is selected from the group consisting of:

a) binds to the same epitope of IGF-IR as an antibody selected from the group consisting of [illegible];

b) binds to the same epitope of IGF-IR as an antibody comprising [illegible];

c) inhibits the binding of IGF-I to IGF-IR;

d) binds to IGF-IR with a K_d of [illegible];

e) binds to IGF-IR with a K_d of [illegible];

f) has an off rate for IGF-IR [illegible];

g) inhibits tumor growth in vivo;

h) causes IGF-IR disappearance from the cell surface when incubated with a cell expressing IGF-IR;

i) inhibits IGF-IR-induced tyrosine phosphorylation;

j) binds to IGF-IR with a K_d of 5×10^{-9} M or less; and

k) has an off rate for IGF-IR [illegible].

4. The antibody or antigen-binding portion thereof according to claim 3, wherein the antibody or portion thereof has all of said properties.

5. The antibody or antigen-binding portion thereof according to any one of claims 1 or 2, wherein the antibody or portion thereof is selected from the group consisting of:

a) an antibody selected from the group consisting of [illegible];

b) an antibody comprising [illegible];

c) inhibits tumor growth in vivo;

d) causes IGF-IR disappearance from the cell surface when incubated with a cell expressing IGF-IR;

e) inhibits IGF-IR-induced tyrosine phosphorylation;

f) binds to IGF-IR with a K_d of 5×10^{-9} M or less; and

g) has an off rate for IGF-IR [illegible].

6. The antibody or antigen-binding portion thereof according to claim 5, wherein the antibody or portion thereof comprises all of said properties.

7. The antibody or antigen-binding portion thereof according to any one of claims 1-6, wherein said antibody or portion thereof inhibits binding between IGF-IR and IGF-I or IGF-II with an IC_{50} of less than 500 nM.

8. The antibody or antigen-binding portion thereof according to any one of claims 1-7, wherein said antibody or antigen-binding portion thereof comprises a variable region of a κ light chain, wherein the sequence of said variable region of said κ light chain comprises no more than ten amino acid changes from the amino acid sequence encoded by a germline Vκ A30, A27 or O12 gene.

9. The antibody or antigen-binding portion thereof according to claim 8, wherein the variable region of the κ light chain comprises an amino acid sequence selected from the group consisting of the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 18 and SEQ ID NO: 22, or an amino acid sequence having 1-10 amino acid insertions, deletions or substitutions therefrom.

10. The antibody or antigen-binding portion thereof according to any one of claims 1-7, wherein said antibody or antigen-binding portion thereof comprises a variable region of a heavy chain, wherein the sequence of said variable region comprises no more than eight amino acid changes from the amino acid sequence encoded by a germline V_H DP47, DP35, DP73 or VIV-3 gene.

11. The antibody or antigen-binding portion thereof according to claim 10, wherein the variable region of the heavy chain comprises an amino acid sequence selected from the group consisting of the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO: 16, SEQ ID NO: 20 and SEQ ID NO: 24, or an amino acid sequence having 1-10 amino acid insertions, deletions or substitutions therefrom.

12. The antibody or antigen-binding portion thereof according to any one of claims 1-11, that is:

a) a monoclonal antibody (or fragment thereof) of the IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD or IgE class or subclass, or a hybrid thereof;

b) an Fab fragment, an F(ab')$_2$ fragment, an Fv fragment, a single chain antibody, a humanized antibody, a chimeric antibody or a bispecific antibody.

13. The antibody or antigen-binding portion thereof according to claim 12, wherein the antibody or portion thereof is a monoclonal antibody or portion thereof comprising a human heavy chain constant region and a human light chain constant region, wherein the human heavy chain constant region is selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD and IgE constant regions, and wherein the human light chain constant region is selected from the group consisting of Cκ and Cλ constant regions.

a) binds to the same epitope of IGF-IR as an antibody selected from the group consisting of 2.12.1, 2.14.3, 3.1.1, 49.2 and 4.17.3;

b) binds to the same antigenic determinant as an antibody selected from the group consisting of 2.12.1, 2.14.3, 3.1.1, 49.2 and 4.17.3;

c) binds to IGF-IR with substantially the same K_d as an antibody selected from the group consisting of 2.12.1, 2.14.3, 3.1.1, 49.2 and 4.17.3; and

d) binds to IGF-IR with substantially the same K_{off} as an antibody selected from the group consisting of 2.12.1, 2.14.3, 3.1.1, 49.2 and 4.17.3.

6. The antibody or antigen-binding portion thereof according to claim 5, wherein the antibody or portion thereof comprises all of said properties.

7. The antibody or antigen-binding portion thereof according to any one of claims 1-6, wherein said antibody or portion thereof inhibits binding between IGF-IR and IGF-I or IGF-II with an IC_{50} of less than 500 nM.

8. The antibody or antigen-binding portion thereof according to any one of claims 1-7, wherein said antibody or antigen-binding portion thereof comprises a variable region of a κ light chain, wherein the sequence of said variable region of said κ light chain comprises no more than ten amino acid changes from the amino acid sequence encoded by a germline Vκ A30, A27 or O12 gene.

9. The antibody or antigen-binding portion thereof according to claim 8, wherein the variable region of the κ light chain comprises an amino acid sequence selected from the group consisting of the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 18 and SEQ ID NO: 22, or an amino acid sequence having 1-10 amino acid insertions, deletions or substitutions therefrom.

10. The antibody or antigen-binding portion thereof according to any one of claims 1-7, wherein said antibody or antigen-binding portion thereof comprises a variable region of a heavy chain, wherein the sequence of said variable region comprises no more than eight amino acid changes from the amino acid sequence encoded by a germline V_H DP47, DP35, DP73 or VIV-3 gene.

11. The antibody or antigen-binding portion thereof according to claim 10, wherein the variable region of the heavy chain comprises an amino acid sequence selected from the group consisting of the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO: 16, SEQ ID NO: 20 and SEQ ID NO: 24, or an amino acid sequence having 1-10 amino acid insertions, deletions or substitutions therefrom.

12. The antibody or antigen-binding portion thereof according to any one of claims 1-11, that is:

a) a monoclonal antibody (or fragment thereof) of the IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD or IgE class or subclass, or a hybrid thereof;

b) an Fab fragment, an F(ab')$_2$ fragment, an Fv fragment, a single chain antibody, a humanized antibody, a chimeric antibody or a bispecific antibody.

13. The antibody or antigen-binding portion thereof according to claim 12, wherein the antibody or portion thereof is a monoclonal antibody or portion thereof comprising a human heavy chain constant region and a human light chain constant region, wherein the human heavy chain constant region is selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD and IgE constant regions, and wherein the human light chain constant region is selected from the group consisting of Cκ and Cλ constant regions.

14. The antibody or antigen-binding portion thereof according to claim 13, wherein the antibody or portion thereof comprises all of the amino acid sequences of the CDR regions a variable region, wherein said variable region is selected from the group consisting of selected from the group consisting of:

a) a variable region of the light chain of an antibody selected from the group consisting of 2.12.1, 2.14.3, 2.14.3, 3.1.1, 49.2 and 4.17.3;

b) a variable region of a light chain comprising an amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 18 and SEQ ID NO: 22, or an amino acid sequence having 1-30 amino acid insertions, deletions or substitutions therefrom;

c) a variable region of the heavy chain of an antibody selected from the group consisting of 2.12.1, 2.14.3, 2.14.3, 3.1.1, 49.2 and 4.17.3;

d) a variable region of a heavy chain comprising an amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO: 16, SEQ ID NO: 20 and SEQ ID NO: 24, or an amino acid sequence having 1-30 amino acid insertions, deletions or substitutions; and

e) the variable regions of the light chain and the heavy chain of an antibody selected from the group consisting of 2.12.1, 2.14.3, 2.14.3, 3.1.1, 49.2 and 4.17.3.

15. The antibody according to claim 14, wherein said antibody is selected from the group consisting of 2.12.1, 2.14.3, 2.14.3, 3.1.1, 49.2 and 4.17.3.

16. The antibody according to claim 14, wherein said antibody comprises a heavy chain and a light chain, and wherein the amino acid sequences of the light chain and heavy chain are selected from the group consisting of:

a) the amino acid sequence of the [illegible] and the amino acid sequence of the light chain of [illegible];

b) the amino acid sequence of the [illegible] and the amino acid sequence of the light chain of [illegible]; and

c) the amino acid sequence of SEQ ID NO: [illegible] and the amino acid sequence of SEQ ID NO: [illegible].

c) the amino acid sequence of SEQ ID NO: 49 and the amino acid sequence of SEQ ID NO: 51

17. The antibody according to claim 1, wherein said antibody has an amino acid sequence comprising the amino acid sequences of the CDRs of antibodies 2.12.1 or 2.13.2, or CDRs of that antibody having no more than 5 conservative amino acid changes.

~~18. A pharmaceutical composition comprising the antibody or portion thereof according to any one of claims 1-17; and a pharmaceutically acceptable carrier.~~

~~19. The pharmaceutical composition according to claim 18, further comprising an antineoplastic, chemotherapeutic, or anti-tumor agent.~~

20. A process for making an antibody that binds to IGF-IR, comprising the steps of:

a) immunizing a non-human mammal with an immunogen comprising IGF-IR, wherein the mammal is capable of expressing human antibodies in B cells of the animal;

b) isolating B cells from the mammal;

c) screening said B cells, or cell lines derived therefrom, to identify a cell line that produces antibodies that bind to IGF-IR;

d) culturing the cell line that expresses antibodies that bind to IGF-IR; and

e) isolating antibodies that bind to IGF-IR from the cell line.

21. An isolated cell line that produces the antibody according to any one of claims 1-17.

22. The cell line according to claim 20 that produces an antibody selected from the group consisting of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2 and 4.17.3, or wherein the antibody has the same amino acid sequences thereof

23. A method of diagnosing the presence or location of an IGF-IR-expressing tumor in a subject in need thereof, comprising the steps of

a) injecting the antibody according to any one of claims 1-17 into the subject,

b) determining the expression of IGF-IR in the subject by localizing where the antibody has bound,

c) comparing the expression in part (b) with that of a normal reference subject or standard, and

d) diagnosing the presence or location of the tumor.

24. A method of treating cancer in a human with an antibody or antigen-binding portion thereof that specifically binds to IGF-IR, comprising the step of administering to the human an amount of the antibody effective to treat said cancer

25. A method of treating a patient in need thereof with the antibody or antigen-binding portion thereof according to any one of claims 1-17, comprising the step of administering to the patient an effective amount of the antibody.

~~26. The method according to either of claims 24 or 25, further comprising the step of administering an anti-neoplastic, anti-tumor, anti-angiogenic or chemotherapeutic agent.~~

27. An isolated nucleic acid molecule that comprises a nucleic acid sequence that encodes a heavy chain or antigen-binding portion thereof or a light chain or antigen-binding portion thereof of an antibody according to any one of claims 1-17.

28. The isolated nucleic acid molecule according to claim 27, wherein the nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of:

a) a nucleic acid sequence encoding at least one CDR region from the heavy chain of the antibody selected from the group consisting of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2 and 4.17.3;

b) a nucleic acid sequence encoding the three CDR regions from the heavy chain of the antibody selected from the group consisting of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2 and 4.17.3;

c) a nucleic acid sequence encoding the amino acid sequence of the heavy chain or the antigen-binding portion thereof of the antibody selected from the group consisting of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2 and 4.17.3;

d) a nucleic acid sequence encoding at least one CDR sequence from the light chain of the antibody selected from the group consisting of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2 and 4.17.3;

e) a nucleic acid sequence encoding the three CDR sequences from the heavy chain of the antibody selected from the group consisting of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2 and 4.17.3;

f) a nucleic acid sequence encoding the amino acid sequence of the light chain or the antigen-binding portion thereof of the antibody selected from the group consisting of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2 and 4.17.3;

g) a nucleic acid sequence encoding the amino acid sequence of selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22; and

h) a nucleic acid sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 and 23;

wherein said nucleic acid molecule optionally comprises a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 28 or SEQ ID NO: 26.

29. A vector comprising the nucleic acid molecule according to either of claims 27 or 28, wherein the vector optionally comprises an expression control sequence operably linked to the nucleic acid molecule.

30. A host cell comprising the vector according to claim 29 or the nucleic acid molecule according to either of claims 27 or 28

31. A method of making an anti-IGF-IR antibody or antigen-binding portion thereof, comprising culturing the host cell according to claim 30 or the cell line according to claim 21 under suitable conditions and recovering said antibody or antigen-binding portion.

32. A non-human transgenic animal comprising the nucleic acid according to either of claims 27 or 28, wherein the non-human transgenic animal expresses said nucleic acid.

33. A method of treating a subject in need thereof with an antibody or antigen-binding portion thereof that specifically binds to IGF-IR, comprising the steps of

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Phase 2 trial of carboplatin, paclitaxel, and irinotecan in ovarian, fallopian tube, and primary peritoneal cancers

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Abstract

Objective. Our goal in this nonrandomized phase 2 trial was to evaluate the toxicity and obtain preliminary data on the potential efficacy of a novel three-drug combination regimen (carboplatin–paclitaxel–irinotecan) when employed as initial therapy of advanced ovarian cancer or as second-line treatment in the setting of a prolonged (≥12 months) treatment-free interval.

Methods. Patients with a histologically confirmed diagnosis of advanced ovarian cancer, primary adenocarcinoma of the peritoneum, or fallopian tube cancer were enrolled in the study. Patients received carboplatin (AUC 5), paclitaxel (150 mg/m^2 over 3 h), and irinotecan (100 mg/m^2 over 90 min). The three-drug combination was initially administered on an every 21-day schedule, but due to toxicity was subsequently changed to a 28-day program.

Results. A total of 30 patients were enrolled into this phase 2 trial. Twenty three patients were chemotherapy naive while 7 had received prior chemotherapy. Seventeen patients completed all six cycles of treatment. Eight patients (27%) were removed from the study after a median of three cycles due to toxicities. Seventeen patients (57%) experienced grade 4 neutropenia, with three individuals requiring hospitalization for neutropenic fever and dehydration. Grades 3 and 4 thrombocytopenia were experienced by three patients each. The principal nonhematologic toxicities were diarrhea (grade 3, three patients) and fatigue. The overall objective clinical response rate was 83%.

Conclusions. The combination of carboplatin–paclitaxel–irinotecan can be administered to women with advanced ovarian cancer with significant, but overall acceptable toxicity. Modification of the regimen from a 3-week to a 4-week schedule permits a greater percentage of patients to complete the program without experiencing excessive toxicity. The overall objective response rate observed in this trial is comparable to other combination regimens employed in this setting. Defining a place for this three-drug program in the standard management of ovarian cancer will require the conduct of an appropriately designed randomized trial.

Keywords: Ovarian cancer; Carboplatin; Paclitaxel; Irinotecan

The majority of women with advanced ovarian cancer achieve an objective response to treatment consisting of cytoreductive surgery followed by chemotherapy [1–4]. The standard initial combination chemotherapy for advanced ovarian cancer includes a platinum agent (cisplatin or carboplatin) and a taxane (paclitaxel or docetaxel) [1–4]. Nevertheless, over 50% of patients relapse and treatment with second-line agents becomes necessary.

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Irinotecan (Camptosar®, CPT-11) is a semisynthetic derivative of camptothecin, with demonstrated activity in many tumor types [5]. The drug possesses greater aqueous solubility, in vitro and in vivo activity, and is associated with less severe and more predictable toxicity than camptothecin [6].

Irinotecan has recently been demonstrated to be an active agent in both cervix cancer and platinum-resistant ovarian cancer [7–9]. Further, several phase 1–2 clinical trials have shown that the agent can be safely administered when combined with carboplatin [10], or with both carboplatin and paclitaxel in a three-drug treatment program [11].

Thus, it is natural to question the potential clinical utility of this three-drug program as a management strategy for women with advanced ovarian cancer. As a result, we initiated a phase 2 trial to examine the toxicity and obtain a preliminary assessment of efficacy of a carboplatin, paclitaxel, and irinotecan regimen in this clinical setting, and report here the results of this study.

Methods and materials

Patient eligibility

To be eligible for entry into this nonrandomized phase 2 trial, patients had to meet all of the following criteria: (a) histologically documented advanced ovarian cancer, primary adenocarcinoma of the peritoneum, or fallopian tube cancer; (b) no prior chemotherapy for the malignancy, or if previously treated, no therapy for ≥12 months; (c) measurable (by physical or radiographic [CT scan] examination) or evaluable disease (serum CA-125 antigen level >50 units/ml at the time of initiation of chemotherapy considered acceptable as evaluable disease); (d) GOG performance status 0–2; (e) age ≥18; (f) laboratory parameters: WBC >3000/mm^2, neutrophils >1500/mm^2, platelets >100,000/mm^2, serum creatinine <1.8 mg%, serum bilirubin ≤1.5 mg%; (g) life expectancy >12 weeks; (h) patients had to sign an informed consent document (approved by the institutional IRB) stating that they understood the investigational nature of the proposed treatment program.

Exclusion criteria: (a) patients who have previously received irinotecan or topotecan; (b) patients with any active or uncontrolled infection, including known HIV infection; (c) patients with psychiatric disorders that interfered with consent or follow up; (d) patients with a history of myocardial infarction within the previous 6 months or congestive heart failure requiring therapy; (e) patients with clinically apparent central nervous system metastases or carcinomatous meningitis; (f) patients who have received prior radiation therapy; (g) patients with known Gilbert's Syndrome, as these patients may experience excessive irinotecan-induced toxicity.

Treatment regimen

Although patients were grouped according to prior treatment (previously untreated or no chemotherapy within the previous 12 months), the treatment plan for both groups was identical. All treatments were initially delivered in the outpatient setting on an every 21-day schedule (however, due to delayed blood count recovery, the treatment regimen was modified to an every 28-day program after entry of the first 12 patients onto this protocol). Drug administration of paclitaxel and CPT-11 was based on actual calculated body surface area.

Carboplatin AUC 5 infused over 30 min
Paclitaxel 150 mg/m^2 infused over 3 h
Irinotecan 100 mg/m^2 infused over 90 min

The three-drug combination was given on Day 1 of each course, with retreatment delayed 1 week if recovery from the toxicity of the prior cycle was not complete. Patients received standard antiemetics and medications to prevent paclitaxel-associated hypersensitivity reactions (diphenhydramine, famotidine, dexamethasone) and irinotecan-associated diarrhea (loperamide). Patients requiring palliative radiation therapy were removed from treatment on this protocol. Patients with complete response (CR) were treated for a maximum of six courses; patients with partial response (PR) and stable disease (SD) were permitted to continue treatment until disease progression.

Routine prophylactic use of a colony-stimulating factor (G-CSF or GM-CSF) was not employed in this trial. Therapeutic use in patients who experienced neutropenic complications such as tissue infection was permitted at the investigators discretion. With subsequent treatments patients had their chemotherapy doses reduced, rather than maintained with prophylactic colony-stimulating factor support. Patients experiencing grade 4 neutropenia in the absence of fever were to be administered a broad-spectrum oral antibiotic (e.g., ciprofloxacin).

Statistical considerations

It was planned to treat a total of 25 (minimum) to 40 (maximum) patients on this phase 2 trial. As patients were receiving two well-established front-line chemotherapy drugs (carboplatin–paclitaxel) in ovarian cancer, it was anticipated that the objective response rate would range between 60% and 80% [1–4]. The only purpose of evaluating response would be to determine if the overall level of activity would be impaired secondary to excessive delays or dose reductions due to the toxicity of this specific regimen. However, as we felt it was necessary to treat a larger population of patients for a full six cycles to assess both short-term (e.g., bone marrow suppression, diarrhea) and longer-term side effects (e.g., peripheral neuropathy, progressive worsening of diarrhea), we believed the phase 2 trial design was more appropriate in this setting than a more limited phase 1 study.

Criteria for response

Response of measurable disease

Complete response. Complete response was defined as the disappearance of all measurable disease (by physical examination or CT scan evaluation), signs, symptoms, and biochemical changes related to the tumor without clinical evidence of progression for ≥4 weeks.

Partial response. When compared to pretreatment measurements, a partial response was defined as a reduction of ≥50% in the sum of the perpendicular diameters of all measurable lesions (by physical examination or CT scan

evaluation) without appearance of new lesions for ≥4 weeks and no existing lesion enlargement.

Progressive disease. An increase of ≥25% in the sum of products of measurable lesions (by physical examination or CT scan evaluation) over the smallest sum observed (over baseline if no decrease) or reappearance of any lesion which had disappeared, or appearance of any new lesion or site. A patient was considered to have progressive disease by CA-125 criteria if all of the following conditions were satisfied [12–15].

(A) The CA-125 has more than doubled from previous values.
(B) This CA-125 value must be >130 units.
(C) This CA-125 value has been obtained on at least two separate blood specimens.

Stable disease. Stable disease was classified to be changes in the volume of disease which do not meet the criteria for either a PR or progression. Stable disease was not considered a response to the treatment program.

CA-125 Response Criteria

Two "CA-125 response" categories were considered: reductions of CA levels to (a) ≤50% or (b) ≤10% of pretreatment levels. These reductions in CA-125 antigen values had to persist for ≥2 months for the patient to be considered to have achieved a response by CA-125 criteria [16–18].

Results

Patient characteristics

A total of 30 patients were entered into this phase 2 clinical study. The median age of the patient population was 55 years (range: 40–75). Additional patient characteristics for these 30 individuals are outlined in Table 1. Three patients (10%) had measurable disease (by CT scan findings) and the other 27 had evaluable disease (by CA-125 criteria).

Twenty-three patients were previously untreated and seven patients had experienced recurrent disease, but had received no chemotherapy within the preceding 12 months. Of the seven patients who entered this phase 2 trial with recurrent disease, six had previously been treated with carboplatin and a taxane. One patient had received treatment with cisplatin, doxorubicin, and cyclophosphamide. All patients entered into this trial had a GOG performance status of 0 or 1.

Toxicity

Seventeen patients (57%) were able to complete the planned six cycles of treatment on this protocol. Three patients developed disease progression before completion of therapy, and eight patients (27%) were removed from the study due to excessive toxicity (median of three courses). Two patients requested to be removed from treatment for personal reasons unrelated to either disease progression or excessive side effects.

Table 1
Patient characteristics

Characteristic	No. of patients
No. patients entered	30
Age (years)	
Median	55
Range	40–75
Previously untreated patients	23
Recurrent disease patients (no chemotherapy for ≥12 months)	7
Prior chemotherapy regimens	
Carboplatin–paclitaxel	4
Cyclophosphamide–doxorubicin–cisplatin	1
Carboplatin–docetaxel	1
Carboplatin–paclitaxel–gemcitabine	1
Tumor type	
Epithelial ovarian cancer	20
Primary peritoneal cancer	9
Fallopian tube cancer	1
GOG performance status	
0	22
1	8

In the initial 12 patients entered into this trial, a 1 week delay in treatment was required in eight individuals due to slow recovery of neutrophils, platelets, or both blood elements. As a result, the program was modified to an every 28-day regimen, which permitted the majority of subsequently treated patients to be treated on schedule.

The clinically relevant side effects experienced by patients participating in this trial are outlined in Table 2. As anticipated, the major toxicity was bone marrow suppression, principally neutropenia. Seventeen patients (57%) developed grade 4 neutropenia, with three requiring hospitalization for management of neutropenic fever and dehydration. Three patients each experienced grade 3 (10%) or grade 4 (10%) thrombocytopenia. No patient developed abnormal bleeding as a result of the low platelet counts.

The principal nonhematologic toxicities observed in this trial were diarrhea (grade 3: 10%) and fatigue. There were no treatment-related deaths. In the small number of previously treated patients ($n = 7$), we did not observe a higher incidence of serious (grade 3–4) treatment-related toxicity compared to those individuals with no prior chemotherapy.

Responses

Objective evidence of antineoplastic activity was noted in 25 of the 30 patients entered in this trial (83%). The three patients with measurable disease achieved an objective response to the treatment program (by CT scan findings). In the 23 previously untreated patients, 19 achieved objective evidence of a response to the treatment program, as did six of seven individuals who had received prior chemotherapy.

As of the date of this report, five patients have died, with a median follow-up of the surviving patient population of 19+ months (range: 8+ to 33+ months).

In this small series we were unable to demonstrate a correlation between response and specific clinical characteristics (e.g., prior chemotherapy, stage or grade, performance status).

Table 2
Maximum toxicity experienced (*n* = 30 patients)

Toxicity	No. of patients
Hematologic	
Neutropenia	
Grade 3	6 (20%)
Grade 4	17 (57%)
Neutropenic fever	3 (10%)
Thrombocytopenia	
Grade 3	3 (10%)
Grade 4	3 (10%)
Nonhematologic	
Emesis (≥grade 3)	4 (13%)
Diarrhea	
Grade 2	2 (7%)
Grade 3	3 (10%)
Fatigue (≥grade 3)	2 (7%)

Discussion

In this trial, we have demonstrated that it was possible to safely administer a combination chemotherapy regimen containing carboplatin, paclitaxel, and irinotecan to patients with advanced ovarian cancer, both previously untreated and those who experienced a recurrence with a relatively long treatment free interval (≥12 months).

Significant, but overall manageable toxicity was observed. The major side effect of this program was neutropenia (grade 4: 57% of patients). Substantial neutropenia was also noted in a phase 2 trial of a similar treatment regimen in advanced nonsmall cell lung cancer [11].

The majority of women with advanced ovarian cancer achieve an objective response to a combination of platinum (carboplatin or cisplatin) plus taxane (paclitaxel or docetaxel)-based chemotherapy regimen [1–4]. While it is attractive to consider the possible benefits of adding a third active drug to this two-drug combination, there is legitimate concern for the potential added toxicity of such a strategy.

The side effects of the program reported here emphasize this point. However, it should also be noted that this experience has demonstrated the feasibility of administering the three drugs together at reasonable dose levels for each agent, an endpoint that has proven to be quite difficult when delivering the combination of carboplatin, paclitaxel, and topotecan [19].

While a high objective response rate was observed in this trial (83%), in the absence of data from a randomized trial it is inappropriate to comment upon the relative activity of this regimen compared to the administration of any other three-drug combination, or the use of only carboplatin and a taxane (paclitaxel or docetaxel) as primary chemotherapy of advanced ovarian cancer [1–4]. Any suggestion of a higher response rate or prolonged progression-free or overall survival in a phase 2 trial may simply reflect selection bias and the favorable natural history of a subset of patients with this malignancy.

With the substantial, but overall tolerable, toxicity noted in this trial, this regimen should not be employed outside the clinical trial setting in ovarian cancer. It might be concluded that the level of side effects observed in this trial would prohibit further investigation of the specific treatment regimen. An alternative conclusion would be that with the documented evidence of activity of irinotecan in platinum-resistant ovarian cancer [9], the three-drug combination reported here is potentially an interesting program to be examined in a randomized trial as primary therapy in this malignancy. The experience of others with this regimen will be helpful in the assessment of possible future development of this management approach.

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