REYES & REYES
A B O G A D O S

Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO
E. S. D.

REF: **EXPEDIENTE 11-130-832**

TÍTULO SOLICITADO: **"ANTICUERPOS ANTI-HER".**

PUBLICACIÓN: **Gaceta 643 de 30 de abril 2012, número de publicación 487**

SOLICITANTE: **GENENTECH, INC.**

PRIORIDAD: **US 61/210,562 de 20/03/2009**

APODERADO: **JUAN PABLO CADENA SARMIENTO**

TRÁMITE: **REMISION DE INFORMACION**

JOSÉ LUIS REYES VILLAMIZAR, mayor de edad, identificado como aparece al pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional número 44655 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad TECNOQUIMICAS S.A., en desarrollo de lo dispuesto por los artículos 42 y siguientes de la Decisión 486 de 2000 de la Comunidad Andina de Naciones, me permito solicitarle, muy respetuosamente, dar curso a la siguiente

I. PETICIÓN

Sírvase negar el beneficio patentario a la solicitud contenida en el expediente **11-130-832**, por incumplir los requisitos materiales de patentabilidad establecidos por el Título II, Capítulo I de la Decisión 486 de 2000, con base en los fundamentos de hecho y de derecho que me permito exponer a continuación.

II. COMPLEMENTO DE INFORMACION

La solicitud colombiana en trámite 11-130-832 proporciona anticuerpos anti-HER3[1] multiespecíficos, composiciones que los comprenden y métodos para usarlos, así

[1] Un receptor tirosina quinasa de la familia EGFR

como también se proporcionan anticuerpos multiespecíficos contra EGFR/HER3.

Según lo anterior, la solicitud no presenta **claridad**, **concisión** ni **soporte en la descripción** para todas las moléculas de anticuerpo reclamadas, además establece una cantidad de secuencias que obedecen a varios grupos inventivos, por lo que la solicitud no presenta **unidad de invención**. En cuanto a las reivindicaciones, la solicitud reclama métodos de tratamiento, métodos de diagnóstico y usos que no pueden ser patentables bajo nuestra jurisdicción nacional.

Con relación a los criterios de patentabilidad, la solicitud no cumple los criterios para otorgársele el privilegio de patente de invención, particularmente en materia de **Novedad** y **Nivel Inventivo,** si se tiene en cuenta la materia revelada entre otros, en documentos solos o en combinación, tales como los que se mencionan a continuación:

1. La publicación internacional WO 2006/091209 A2 de 31/08/2006 titulado: "*Bispecific binding agents for modulating biological activity*" por Nielsen, U. y Schoeberl, B.
2. La publicación internacional WO 2007/042289 A2 de 19/04/2007 Titulado: *"NanobodiesTM and polypeptides against EGFR and IGF-IR"* por Laeremans, et al.

CARENCIA DE REQUISITOS DE PATENTABILIDAD, CLARIDAD Y EXCEPCIONES.

La solicitud presenta un pliego de 37 reivindicaciones que obedecen a un anticuerpo multiespecífico que comprende un dominio de unión al antígeno que se une específicamente a EGFR y a HER3, donde la toxicidad del anticuerpo es menor que la toxicidad de un antagonista de EGFR.

Así, la solicitud en sus reclamaciones involucra una materia ampliada irracionalmente al reclamar cualquier anticuerpo con un dominio de unión al antígeno, sin especificar dicho dominio de unión por su secuencia, estructura, o por el certificado de depósito del hibridoma que produce el anticuerpo de la invención. Por otra parte, no existe parámetro ni punto de comparación para determinar técnicamente que la toxicidad del anticuerpo de la invención sea menor que la toxicidad de un antagonista de EGFR, sin definir técnicamente ninguno de

los dos anticuerpos. En ese orden de ideas, del gran número de posibles compuestos reclamados mediante la inclusión de una característica que no limita la funcionalidad ni la estructura de la molécula, tales reivindicaciones no pueden ser consideradas claras ni con pleno sustento en la descripción.

En este caso, los anticuerpos reclamados se definen erróneamente por su función de unirse a un antígeno de forma multiespecífica, entendiéndose que dicho objeto inventivo se basa en el descubrimiento de una función adicional de un anticuerpo que se une a dos factores (EGFR y HER3) que incluso pertenecen a la misma familia y sobre los cuales podría actuar cualquier anticuerpo con diferencias en la afinidad (ver a continuación el aparte de la misma solicitud internacional pág. 34). De esta forma, cualquier anticuerpo, que ni siquiera han sido evaluado o contemplado en la solicitud, cumpliría las características funcionales reclamadas quedando cubierto por la amplitud de la protección.

> In some embodiments, the affinity of the multispecific antibody for one of its target HER receptors is greater than for its other target HER receptor or receptors. In one embodiment, the affinity of the multispecific antibody for one target HER receptor is at least 2, 3, 4, 5, 8, 10, 12, 15, 18, 20, 22, 25, 30, 35, 40, 50, 100-fold greater than its affinity for another target HER receptor. In one embodiment, the multispecific antibody specifically binds to EGFR and another HER receptor and its binding affinity for the other HER receptor is at least 2, 3, 4, 5, 8, 10, 12, 15, 18, 20, 22, 25, 30, 35, 40, 50, or 100-fold greater than its affinity for EGFR. In one embodiment, the multispecific antibody specifically binds to EGFR and HER3 and its binding affinity for HER3 is at least 2, 3, 4, 5, 8, 10, 12, 15, 18, 20, 22, 25, 30, 35, 40, 50, or 100-fold greater than its binding affinity for EGFR. In another

Por otra parte, es útil mencionar que la solicitud incumple los requisitos de claridad y definición al no presentar en su capítulo reivindicatorio las características técnicas esenciales; de la lectura del mismo, se observa un gran número de reivindicaciones principales que no definen el anticuerpo por su secuencia, por el hibridoma o línea celular que lo produce, en consecuencia, un técnico medio versado en la materia no podría llevar a cabo la invención de forma inequívoca reproduciendo el anticuerpo y su efectividad, concluyendo que no existe soporte en la descripción para la mayoría de las reivindicaciones.

Ahora bien, al plantearse las reivindicaciones de forma tal que cualquier anticuerpo multiespecífico anti cumple con las características que solucionen el problema técnico, no puede pensarse que exista una ventaja técnica experimental para solo un anticuerpo multiespecífico evaluado, por lo tanto, cualquier anticuerpo

que presentara un dominio que se una a EGFR y a HER3 destruiría la novedad de la solicitud haciendo irrelevante el argumento de una ventaja sorprendente encontrada con un anticuerpo determinado con una secuencia determinada.

Teniendo esto en mente, se puede entender más fácilmente el análisis de patentabilidad sobre la base de los documentos referenciados anteriormente, tal como se verá a continuación.

CARENCIA DE NOVEDAD Y NIVEL INVENTIVO DE LOS ANTICUERPOS RECLAMADOS

Como primera instancia se pone de presente que la reivindicación principal menciona *"un anticuerpo multiespecífico CARACTERIZADO porque comprende un dominio de unión al antígeno que se une específicamente a EGFR y a HER3, donde la toxicidad del anticuerpo es menor que la toxicidad de un antagonista de EGFR".*

Es de notar que las únicas características técnicas se remiten a que el anticuerpo presenta un dominio que se une específicamente a EGFR y a HER3, pero no define la estructura de dicho dominio, ni como se une a los receptores de HER mencionados, dando lugar a una amplitud común cuando se caracteriza la materia por una funcionalidad en lugar de definirla por la estructura. Cabe mencionar además que solo hasta la reivindicación 11 se observa una definición estructural del anticuerpo que se pretende reclamar; éste hecho corrobora que la invención no puede ser resuelta por medio de un anticuerpo determinado, sino por cualquiera que presente la funcionalidad de unirse específicamente a EGFR y HER3 y que el anticuerpo de la reivindicación 11 es solo una modalidad particular de la invención que la ejemplifica.

Ahora bien, el documento WO 2006/091209 describe un anticuerpo multiespecífico (anti-EGFR/ErbB3 bsBA) que comprende un dominio de unión al antígeno que se une específicamente a EGFR y HER3 (ver aparte a continuación de la página 3, líneas 25 – 30, y ejemplos 8 y 9, páginas 67-71), describiendo además sus usos farmacéuticos y médicos en el tratamiento del cáncer:

25 embodiments, the target cell is a cancer cell. In some embodiments, the first target molecule is a tumor-associated antigen, cytokine receptor, or growth factor receptor. In some embodiments, the first target molecule is a tyrosine kinase receptor selected from the group consisting of EGFR and ErbB2. In some embodiments, the second target molecule is ErbB3 (HER3), insulin-like growth factor-1 receptor (IGF1-R), any of FGF receptors 1-4, HGF
30 receptor, insulin receptor, either of PDGF receptors α and beta, C-KIT, or ErbB4. In some

Adicionalmente la anterioridad reclama métodos para modular la actividad biológica de moléculas diana en una célula determinada mediante la provisión de agentes de unión biespecíficos (reivindicación 1) que pueden estar determinados por un primer receptor de tirosin quinasa (reivindicación 6) y un segundo agente dirigido a una molecula como ErbB3 (HER3) (reivindicacion 16).

En este sentido, el documento WO 2006/091209 ya había anticipado las características técnicas de la invención mostrando anticuerpos (agentes) biespecificos dirigidos hacia EGFR y HER3, en consecuencia, la novedad es objetada únicamente con este documento, toda vez que se mencionan las características técnicas del anticuerpo, tal como se reclama en reivindicaciones de la solicitud en trámite.

Por su parte, el documento WO 2007/042289 describe un anticuerpo multiespecífico que comprende un dominio de unión al antígeno que se une específicamente al EGFR y HER3. En el caso de la anterioridad, en la página 94 (ver aparte de la solicitud internacional en el siguiente párrafo) se mencionan los motivos de unión a los que se hace referencia, teniendo en cuenta que pueden ser proteínas, polipéptidos, fragmentos de proteínas, etc.; además se describen los motivos, (o dominios de unión) como polipéptidos inferiores a 15 kDa los cuales también pueden comprender nanocuerpos.

A binding moiety according to the present invention can be any peptide or nucleotide containing moiety having a known binding affinity for at least one antigen. The moiety can be a protein, a polypeptide, a protein fragment (such as an antibody fragment) or one or more subunit(s) of any protein. A typical example of a binding moiety would be an enzyme, a receptor or a transport protein. It can also be a carrier protein such as albumin or an antibody. The binding moiety can also be, or include, a sequence of DNA or RNA.

In a preferred embodiment, one or more of the binding moieties on the bispecific or multispecific polypeptide of the invention is a polypeptide below 15 kDa. In an even more preferred embodiment, one or more of the binding moieties on the bispecific or multispecific polypeptide of the invention is a VH, a VHH, a domain antibody, a single domain antibody, a "dAb" or a Nanobody. In a most preferred embodiment, the binding moieties on the bispecific or multispecific polypeptide of the invention are Nanobodies.

Dichos nanocuerpos son polipéptidos dirigidos contra EGFR y HER3 (ver páginas 94, líneas 31-33).

En la solicitud además se describen los usos farmacéuticos y médicos de dichos péptidos y anticuerpos en el tratamiento del cáncer.

Therefore, in a preferred embodiment, the invention also provides a bispecific polypeptide comprising or essentially consisting of a Nanobody directed against EGFR and a Nanobody directed against another member of the EGFR family. The polypeptide of the invention may comprise or essentially consist of a Nanobody directed against EGFR and a Nanobody directed against HER2. In another embodiment, the polypeptide of the invention may comprise or essentially consist of a Nanobody directed against EGFR and a Nanobody directed against HER3. In another embodiment, the polypeptide of the invention may

Teniendo en cuenta los documentos presentados en este escrito, la solicitud colombiana en trámite no cumple con el criterio de novedad ya que todas las características técnicas esenciales reveladas allí, ya habían sido contempladas en dichas anterioridades.

Por otra parte, en el caso de los anticuerpos presentados como modalidad particular en las reivindicaciones y definidos por secuencias presentes en los CDRs, cabe también mencionar que los anticuerpos aunque presentan un sitio activo definido por sus CDRs, las evaluaciones desarrolladas en la solicitud en trámite, obedecen únicamente a ensayos de toxicidad comparándolas con los anticuerpos comerciales tipo Cetuximab (ver ejemplo 19 de la solicitud) y no para demostrar un efecto técnico mayor en actividad de los sitios activos o CDRs.

Según estos estudios, la toxicidad del anticuerpo de la solicitud DL11f que fue diseñado bajo estrictas condiciones y seleccionado por sus propiedades, demuestra una toxicidad inferior a Cetuximab, sin embargo, en toxicidad e inmunogenicidad el total del anticuerpo puede determinar el efecto deseado, y no únicamente las CDRs ya que éstas en términos generales determinan la unión del anticuerpo al receptor o antígeno.

De esta forma, las secuencias reclamadas no dan una idea de la característica esencial de la solicitud de tener baja toxicidad; en este caso, la solicitud falla en reclamar un único anticuerpo, sobre el cual se demuestre su baja toxicidad.

Ahora, en el caso en el que se estableciera que los anticuerpos reclamados por sus secuencias CDRs presentan novedad, es necesario mencionar que dichos anticuerpos así reclamados no permiten establecer su superioridad en baja toxicidad que en últimas obedecería al problema planteado al compararse con un anticuerpo comercial como Cetuximab. En este caso, únicamente podría demostrársele nivel inventivo a un anticuerpo completo, debido a que la toxicidad del mismo se relaciona con toda su estructura y no sólo a los CDRs en cuestión. Debido a que este no es el caso, la solicitud no solo carece de nivel inventivo, sino que sus modalidades particulares no pueden demostrar un efecto técnico real en baja toxicidad y las mismas tampoco demuestran nivel inventivo.

Por último, en vista de que no se ha indicado en las reivindicaciones un anticuerpo como el mencionado, es decir, descrito por toda su estructura y secuencia para lograr el efecto técnico deseado de baja toxicidad con relación a uno semejante tipo Cetuximab, la inclusión de una característica adicional en la estructura del anticuerpo obedecería a una ampliación de materia, por lo que la solicitud en estudio no puede ser objeto de concesión.

Se concluye entonces que un anticuerpo multiespecífico que comprende un dominio de unión al antígeno que se une específicamente a EGFR y a HER3, donde la toxicidad del anticuerpo es menor que la toxicidad de un antagonista de EGFR es bien conocida en el estado de la técnica y no define las características que logran esa baja toxicidad.

Debido a que las características técnicas de la invención han sido anticipadas por los documentos mencionados, solos o en combinación, los anticuerpos reclamados carecen de **Novedad y Nivel Inventivo**.

Por último, encarando el mencionado problema técnico y partiendo de las enseñanzas de los documentos citados, la persona versada en la materia no requeriría un proceso inventivo para desarrollar cualquier anticuerpo multiespecífico que se une a EGFR y a HER3 como el reclamado.

III. PRUEBAS

Ruego que se tengan como tales, sin perjuicio de que el Despacho considere oficiosamente, en particular de las allegadas por el suscrito a los expedientes mencionados en este escrito, las siguientes:

- El documento de patente WO 2006/091209 A2 de 31/08/2006 titulado: "*Bispecific binding agents for modulating biological activity*" por Nielsen, U. y Schoeberl, B.Portada, páginas 3 y 67 a 71 y reivindicaciones.
- El documento de patente WO 2007/042289 A2 de 19/04/2007 Titulado: *"NanobodiesTM and polypeptides against EGFR and IGF-IR"* por Laeremans, et al.Portada, página 94 y reivindicaciones.

Señor Superintendente,

JOSÉ LUIS REYES VILLAMIZAR
C.C. 79'152.473 de Usaquén
T.P.A. 44.655 del C.S.J.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
31 August 2006 (31.08.2006)

PCT

(10) International Publication Number
WO 2006/091209 A2

(51) International Patent Classification:
A61K 39/395 (2006.01)

(21) International Application Number:
PCT/US2005/015638

(22) International Filing Date: 5 May 2005 (05.05.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/655,836 23 February 2005 (23.02.2005) US

(71) Applicant *(for all designated States except US)*: **MERRIMACK PHARMACEUTICALS, INC.** [US/US]; 101 Binney Street, Cambridge, Massachusetts 02142 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **NIELSEN, Ulrik, B.** [DK/US]; 19 Kenney Street, Jamaica Plain, Massachusetts 02130 (US). **SCHOEBERL, Birgit, M.** [DE/US]; 71 Spring Street, Cambridge, Massachusetts 02141 (US).

(74) Agents: **HYMAN, Laurence, J.** et al.; Townsend And Townsend And Crew Llp, Two Embarcadero Center, 8th Floor, San Francisco, California 94111-3834 (US).

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

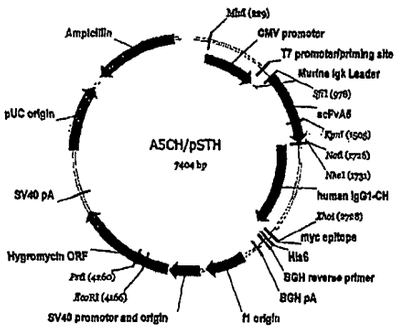
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

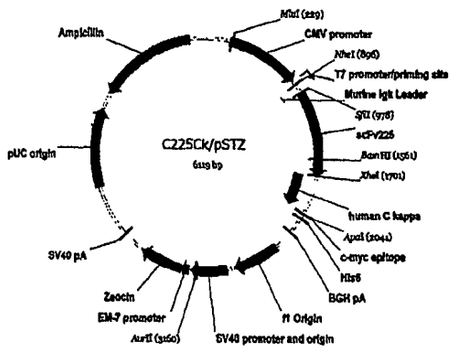
Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: BISPECIFIC BINDING AGENTS FOR MODULATING BIOLOGICAL ACTIVITY





(57) Abstract: Methods for improving the specific binding ability of bispecific binding compositions are described. The bispecific binding compositions are able to target cells by a high affinity targeting domain to a target cell surface marker and a low affinity binding domain that binds specifically to a second cell surface marker, wherein the binding of each domain to its respective cell surface marker increases or decreases, as desired, the biological activity of the respective cell surface markers. The invention further provides bispecific binding agents for use in the methods, as well as uses for the agents.

WO 2006/091209 A2

side effects due to binding of the agent to normal cells expressing the target molecule. For example, the HER2 (erbB2) receptor that is the target for the FDA-approved immunotherapeutic agent Herceptin®, is overexpressed at levels some 10 to 100 times more than the expression of the HER2 receptor in non-cancer cells. Nonetheless, a percentage of patients develop cardiac arrhythmia and other adverse side effects due to binding of Herceptin® to normal cells.

[0009] Thus, it would be desirable to increase the therapeutic window of immunotherapeutic agents by developing bsBAs with an improved ability to bind to diseased cells without binding to normal cells.

BRIEF SUMMARY OF THE INVENTION

[0010] The present invention provides methods for modulating biological activity or activities of target molecules on a target cell. The methods comprise providing a bispecific binding agent having a first binding domain having a Kd for a first target molecule on the surface of the cell of at least 10^{-7} M and a second binding domain having an affinity for a second target molecule on the surface of said cell that is at least 10 times lower than the Kd of the first binding domain; wherein the first and the second target molecules each have a biological activity, which activity may be the same or different and, contacting the bispecific binding agent with the target cell under conditions that permit the first and second binding domains to bind to the first and second target molecules, respectively, wherein the binding of the first and second target molecules modulates the biological activity or biological activities of the target molecueles. In some embodiments, the bispecific binding agent comprises two antibodies. In some embodiments, the antibodies are diabodies, two single chain Fvs connected directly or by a linker, disulfide stabilized Fvs, or combinations thereof. In some embodiments, the target cell is a cancer cell. In some embodiments, the first target molecule is a tumor-associated antigen, cytokine receptor, or growth factor receptor. In some embodiments, the first target molecule is a tyrosine kinase receptor selected from the group consisting of EGFR and ErbB2. In some embodiments, the second target molecule is ErbB3 (HER3), insulin-like growth factor-1 receptor (IGF1-R), any of FGF receptors 1-4, HGF receptor, insulin receptor, either of PDGF receptors α and beta, C-KIT, or ErbB4. In some embodiments, the Kd of the first binding domain to the first target molecule is between 10^{-8} and 10^{-12} M. In some embodiments, the Kd of the second binding domain to the second

Example 8

[0217] This example describes the production of an exemplar anti-EGFR/ErbB3 bsBA having the characteristics described above.

Materials and Methods

[0218] Cell Lines. Mouse hybridoma cell line 225, the 293T cell line, and human breast cancer cell line A431 were obtained from the American Type Culture Collection (Manassas, VA). All cell lines were cultured in DMEM medium supplemented with 10% fetal bovine serum, or with low IgG fetal bovine serum (FBS) (Invitrogen), or CD293 serum-free medium (Invitrogen) as indicated and supplemented with 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin ("growth medium").

Cloning of mouse anti-EGFR antibody

[0219] RNA was extracted from cell line ATCC No. HB-8508 using a RNAeasy kit (QIAGEN) as described by the manufacturer. First-chain cDNA was synthesized using a cDNA Synthesis kit (Amersham Biosciences Corp., Piscataway, NJ) as described by the manufacturer with the RT-primer, pd(N)6, provided in the kit. The heavy chain of the C225 antibody was amplified from the cDNA using a mixture of mouse primers:

VH1: CTA GCT AGC GGG GCC ATG GCC S AGG TYC AGC TBC AGC AGT C (SEQ ID NO:1);

VH2: CTA GCT AGC GGG GCC ATG GCC C AGG TTC ACC TGC AGC ART C (SEQ ID NO:2);

VH3: CTA GCT AGC GGG GCC ATG GCC C AGG TRC AGC TGA AGG AGT C (SEQ ID NO:3);

VH4: CTA GCT AGC GGG GCC ATG GCC C AGG TCC AAC TVC AGC ARC C (SEQ ID NO:4);

VH5: CTA GCT AGC GGG GCC ATG GCC C AGA TCC AGT TGG TVC AGT C (SEQ ID NO:5);

VH6: CTA GCT AGC GGG GCC ATG GCC C AGG TGC AGC TGA AGS AST C (SEQ ID NO:6);

VH7: CTA GCT AGC GGG GCC ATG GCC G AGG TGC AGS KGG TGG AGT C (SEQ ID NO:7);

VH8: CTA GCT AGC GGG GCC ATG GCC G AAG TGA ARS TTG AGG AGT C (SEQ

12

ID NO:8);

VH9: CTA GCT AGC GGG GCC ATG GCC G AKG TSV AGC TTC AGG AGT C (SEQ ID NO:9);

VH10: CTA GCT AGC GGG GCC ATG GCC G AGG TGA ASS TGG TGG AAT C (SEQ ID NO:10);

VH11: CTA GCT AGC GGG GCC ATG GCC G AGG TGA AGC TGR TGG ART C (SEQ ID NO:11);

VH12: CTA GCT AGC GGG GCC ATG GCC G ARG TGA AGC TGR TGG AGT C (SEQ ID NO:12);

VH13: CTA GCT AGC GGG GCC ATG GCC G AAG TGC AGC TGT TGG AGA C (SEQ ID NO:13);

VH14: CTA GCT AGC GGG GCC ATG GCC G ARG TGA AGC TTC TCS AGT C (SEQ ID NO:14);

VH15: CTA GCT AGC GGG GCC ATG GCC C ARG TTA CTC TGA AAG AGT (SEQ ID NO:15);

JH1-mGAP: cc tgg ttt ccc aga acc gct ctg cgc gcc g CT CGA GAC GGT GAC CGT GGT CCC (SEQ ID NO:16);

JH2-mGAP: cc tgg ttt ccc aga acc gct ctg cgc gcc g CT CGA GAC TGT GAG AGT GGT GCC (SEQ ID NO:17);

JH3-mGAP: cc tgg ttt ccc aga acc gct ctg cgc gcc g CT CGA GAC AGT GAC CAG AGT CCC (SEQ ID NO:18);

JH4-mGAP: cc tgg ttt ccc aga acc gct ctg cgc gcc g CT CGA GAC GGT GAC TGA GGT TCC (SEQ ID NO:19).

The amplified product was then subjected to a second PCR using primers Hind3-C225-VH-5' (5'- CTA GCT AGC GGG AAG CTT CAG GTA CAA CTG CAG GAG TCA-3' (SEQ ID NO:20)) and C225-VH-3' (5'- AGA GGA AAC GGT GAC CGT GGT –3' (SEQ ID NO:21)). The light chain of the of the C225 antibody was amplified from the cDNA using a mixture of primers

VK1: TAT TCG TCG ACG GAT ATT GTG ATG ACB CAG DC (SEQ ID NO:22);

VK2: TAT TCG TCG ACG GAT RTT KTG ATG ACC CAR AC (SEQ ID NO:23);

VK3: TAT TCG TCG ACG GAA AAT GTG CTC ACC CAG TC (SEQ ID NO:24);

VK4: TAT TCG TCG ACG GAY ATT GTG ATG ACA CAG TC (SEQ ID NO:25);

VK5: TAT TCG TCG ACG GAC ATC CAG ATG ACA CAG AC (SEQ ID NO:26);

VK6: TAT TCG TCG ACG GAY ATT GTG CTS ACY CAR TC (SEQ ID NO:27);

VK7: TAT TCG TCG ACG GAC ATC CAG ATG ACY CAR TC (SEQ ID NO:28);
VK8: TAT TCG TCG ACG CAA ATT GTT CTC ACC CAG TC (SEQ ID NO:29);
JK1/2: TTT CTC GTG CGG CCG CAC GTT TKA TTT CCA GCT TGG (SEQ ID NO:30);
JK4: TTT CTC GTG CGG CCG CAC GTT TTA TTT CCA ACT TTG (SEQ ID NO:31);
JK5: TTT CTC GTG CGG CCG CAC GTT TCA GCT CCA GCT TGG (SEQ ID NO:32)).
The amplified product was then subjected to a second PCR using primers C225-VH-Link-VL-UP (ACC ACG GTC ACC GTT TCC TCT GGT GGA GGC GGT TCA GGC GGA GGT GGC TCT GGC GGT GGC GGA TCG GAC ATC CAG CTG ACC CAG TCT (SEQ ID NO:33)) and C225-VL-Xho-His6-NotI (CCC GCC TGC GGC CGC TCA GTG GTG GTG GTG GTG GTG CTC GAG TTT GAT CTC CAG CTT GGT CCC AGC ACC GAA (SEQ ID NO:34)). All PCR was performed under standard PCR methods and instrumentation. The PCR products were purified using QIAquick as described by the manufacturer and assembled into scFv C225 by mixing 100 ng of each purified heavy and light chains products and subjecting to seven cycles of PCR without primers and then another 25 cycles of PCR with primers Nco1-C225 (CAG CCG GCC ATG GCC cag gta caa ctg cag gag tc (SEQ ID NO:35)) and C225-Xho1-3' (GAT CTC GAG CTT GGT CCC AGC (SEQ ID NO:36)). The ~750 bp band was purified from agarose gel using QIAquick as described by the manufacturer and then cloned using TOPO TA Cloning Kit (Invitrogen) also as described by the manufacturer. The cloned product was transformed into *E. coli* strain XL1 using standard methods and sequenced using standard DNA sequencing technology. The Ckappa (kappa constant) was cloned from cDNA obtained from human leukocytes (obtained as described above) using primers 5'-Xho-Ck (GAG CTC GAG ATC AAA CGA ACT GTG GCT GCA CCA (SEQ ID NO:37)) and Ck-Apa1-3' (CGC GGG CCC TCA ACA CTC TCC CCT GTT GAA GC (SEQ ID NO:38)). The CH (heavy constant) was cloned using primers 5'-CH1-gamma1 (CCAAGAGCACCTCTGGGG (SEQ ID NO:39)) and HuIgG-Xho-Xba-3 (GGCCCTCTAGACTCGAGTAATTCATTTACCCGGAGACAGGG (SEQ ID NO:40)). The product of this was used as a template in a second PCR with primers (5'-Not1 Nhe1 CH1 gtg gcg gcc gct agc acc aag ggc cca tcg gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct ggg g (SEQ ID NO:41)) and HuIgG-Xho-Xba-3'
(GGCCCTCTAGACTCGAGTAATTCATTTACCCGGAGACAGGG (SEQ ID NO:42)). The products were purified using QIAquick purification kit (Qiagen) as recommended by the manufacturer, and then cloned using the TOPO TA cloning kit and the final constructs were verified by DNA sequencing. The CH product was then sub-cloned into pSecTaq2A Hygro vector (Invitrogen) at Not1 and Xho1 site to make CH/pSecTaq2A Hygro (CH/pSTH) and

the Ck into pSecTaq2A vector at Xho1 and Apa1 site to make Ck/pSecTaq2A (Ck/pSTZ). Finally, the A5 anti-ErbB3 scFv (kindly provided by Dr. James Marks, University of California at San Francisco) was sub-cloned into the CH/pSTH construct using Sfi1 and Not1 sites to form A5CH/pSTH and the C225 scFv was sub-cloned into CK/pSTZ at Sfi1 and Xho1 sites to form C225Ck/pSTZ.

Expression of the recombinant bsAb

[0220] Transient transfection of 293T Cells. The 293T cell line was co-transfected with A5CH/pSTH and C225Ck/pSTZ using FuGene™ transfection reagent (Roche Applied Science, Indianapolis, IN) as described by the manufacturer (cells were transfected in DMEM with ultra low IgG FBS, then changed into CD293 serum-free medium 1 day after transfection). Transfected cells were cultured in growth medium for 5 days before purification on Protein G (Pierce Biotechnology, Inc., Rockford, IL) as recommended by the manufacturer to form the C225-A5 Ig-scFv fusion.

Flow cytometry analysis of antibody binding

[0221] A431 cells were incubated with 1 ug/mL of C225-A5 Ig-scFv fusion for 30 mins on ice, then washed twice in wash buffer (phosphate buffered saline, 2%FBS, 0.1% Azide) and binding detected using an anti-human IgG antibody labeled with Alexa Fluor® 488 (Invitrogen) and quantitated in a FACSCalibur instrument (Becton-Dickinson, Franklin Lakes, NJ).

Testing of inhibition of growth factor induced AKT phosphorylation by bsAb

[0222] For the antibody dose-response experiments, A431 cells were incubated with varying amounts of C225-A5 Ig-scFv fusion for 30 minutes in DMEM (no serum) before stimulating the cells with 50 ng/mL heregulin-beta (R&D Systems Inc., Minneapolis, MN) or 50 ng/mL EGF (PeproTech, Inc., Rocky Hill, NJ) for five minutes. The cells were lysed in lysis buffer (20 mM Tris, pH7.4; 150 mM NaCl; 2 mM EDTA; 1 mM EGTA; 1 mM NaF; 10 mM beta-glycerophosphate; 1% Triton X-100; Protease Inhibitor Cocktail (Sigma-Aldrich Corp., St. Louis, MO); 1 mM Sodium orthovanadate; 50 μM Phenylarsine; 10 μM bpV and 100ug/ml DNase) and the reduction of growth factor stimulated phosphorylation of AKT was determined using antibody microarray assay as described in Nielsen et al. Proc Natl Acad Sci USA 100(16):9330-9335 (2003). AKT antibodies were obtained from Cell Signaling Technology (Beverly, MA).

16

Example 9

[0223] This example describes the results of tests of an exemplar anti-EGFR/ErbB3 bsBA having the characteristics described above.

Results

[0224] The bi-specific antibody C225-A5 Ig-scFv fusion was expressed by co-transfecting the two expression plamids A5CH/pSTH and C225Ck/pSTZ (described in Fig. 1) in 293T cells. The expressed C225-A5 Ig-scFv fusion was purified from the supernatant and the product evaluated for binding to cancer cell line A431 by flow cytometry. A431 cells overexpress EGF receptor, and as expected, the C225-A5 Ig-scFv fusion bound well to these cells, as shown in Fig. 2.

[0225] The expressed C225-A5-Ig-scFv was subsequently tested for inhibition of growth factor induced AKT phosphorylation in the cancer cell line A431. Cells were pre-incubated with the C225-A5 Ig-scFv fusion for 30 minutes at varying concentrations before stimulating with either heregulin (HRG) or EGF for five minutes. Cells were then lysed in buffer containing detergent and assayed for AKT phosphorylation. The results are depicted in Fig. 3. The C225-A5 Ig-scFv fusion inhibited the phosphorylation of AKT in response to EGF with an approximate IC_{50} of 2 nM. Heregulin is a ligand of the ErbB3 receptor that leads to activation of several intracellular kinases, including AKT. Despite the relatively low affinity of the anti-ErbB3 antibody A5 (about 700 nM), the C225-A5 Ig-scFv fusion inhibited heregulin induced activation with an approximate IC_{50} of 0.2 nM. This demonstrates that a "hi-lo" bispecific agent (an agent with a high affinity for a first antigen and a low affinity for a second antigen) can very potently inhibit the activity of the kinases targeted by the antibody.

[0226] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

WHAT IS CLAIMED IS:

1. A method for modulating biological activity or activities of target molecules on a target cell, comprising:

(a) providing a bispecific binding agent having a first binding domain having a dissociation constant ("Kd") for a first target molecule on the surface of said cell of at least 10^{-7} M and a second binding domain having an affinity for a second target molecule on the surface of said cell that is at least 10 times lower than the Kd of the first binding domain; wherein said first and said second target molecules each have a biological activity, which activity may be the same or different and,

(b) contacting the bispecific binding agent with the target cell under conditions that permit the first and second binding domains to bind to the first and second target molecules, respectively, wherein said binding of said first and second target molecules modulates the biological activity or biological activities of the target molecueles.

2. A method of claim 1 wherein the bispecific binding agent comprises two antibodies.

3. A method of claim 2, wherein the antibodies are diabodies, two single chain Fvs connected directly or by a linker, disulfide stabilized Fvs, or combinations thereof.

4. A method of claim 1 wherein the target cell is a cancer cell.

5. A method of claim 1 wherein the first target molecule is a tumor-associated antigen, cytokine receptor, or growth factor receptor.

6. A method of claim 1, wherein the first target molecule is a receptor which is a tyrosine kinase.

7. A method of claim 1, wherein the second target molecule is selected from the group consisting of ErbB3, ErbB4, any of FGF receptors 1-4, HGF receptor, IGF1-R, PDGF, receptors alpha and beta, and C-KIT.

8. A method of claim 1, wherein the Kd of the first binding domain to the first target molecule is between 10^{-8} and 10^{-12} M.

9. A method of claim 1, wherein the Kd of the second binding domain to the second target molecule is at least 20 times lower than the Kd of the first binding domain to the first target molecule.

10. A method for modulating biological activity or activities of target molecules on target cells in an organism having target and non-target cells, wherein the target cells have a first target molecule on their exterior and a second target molecule on their exterior surface, and wherein (i) said first and second target molecules do not share a common ligand, (ii) said first target molecule is at least 10 times more abundant on the surface of the target cells than on non-target cells that also bear the second target molecule, and (iii) said first target molecule and said second target molecule each have a biological activity, which may be the same or different, the method comprising:

(a) providing a bispecific binding agent having a first binding domain having a Kd for the first target molecule of at least 10^{-7} M and a second binding domain having a Kd for the second target molecule that is at least 10 times lower than the Kd of the first binding domain; and,

(b) contacting the bispecific binding agent with the target cells under conditions that permit the first and second binding domains to bind to the first and second target molecules, respectively,

wherein said binding of said first and said second binding domains modulates the biological activity or activities of said first and said second target molecules, respectively.

11. A method of claim 10, wherein the bispecific binding agent comprises two antibodies.

12. A method of claim 11, wherein the antibodies are diabodies, two single chain Fvs connected directly or by a linker, disulfide stabilized Fvs, or combinations thereof.

13. A method of claim 10, wherein the target cell is a cancer cell.

14. A method of claim 10, wherein the first target molecule is a tumor-associated antigen, cytokine receptor, or growth factor receptor.

15. A method of claim 10, wherein the first target molecule is a receptor which is a tyrosine kinase.

16. A method of claim 15, wherein the second target molecule is ErbB3 (HER3), insulin-like growth factor-1 receptor (IGF1-R), any of FGF receptors 1-4, HGF receptor, insulin receptor, either of PDGF receptors α and beta, C-KIT, or ErbB4.

17. A method of claim 10, wherein the Kd of the first binding domain to the first target molecule is between 10^{-8} and 10^{-12} M.

18. A method of claim 10, wherein the Kd of the second binding domain to the second target molecule is at least 20 times lower than the Kd of the first binding domain to the first target molecule.

19. A method of claim 10, wherein the Kd of the second binding domain to the second target molecule is at least 50 times lower than the Kd of the first binding domain to the first target molecule.

20. A method of claim 10, wherein said modulating is decreasing the activity of a receptor which is a tyrosine kinase.

21. A bispecific binding agent (bsBA) comprising a first binding domain having a Kd of at least 10^{-7} M for a first target molecule on a target cell and a second binding domain having a Kd for a second target molecule on a target cell which Kd is at least 10 times lower than the Kd of the first binding domain for the first target molecule, wherein (i) said first and second target molecules do not have the same natural ligand, (ii) said first target molecule and said second target molecule each have a biological activity, which may be the same or different, and (iii) said first and said second binding domains, when bound to said first and said second target molecules, modulate the biological activity or activities of the first and second target molecules, respectively.

22. A bsBA of claim 21, wherein said Kd of said second binding domain is more than 50 times lower than the Kd of the first binding domain.

23. A bsBA of claim 21, wherein said Kd of said second binding domain is 100 or more times lower than the Kd of the first binding domain.

24. A bsBA of claim 21, wherein said bsBA comprises two antibodies.

25. A bsBA of claim 24, wherein the antibodies are diabodies, two single chain Fvs connected directly or by a linker, disulfide stabilized Fvs, or combinations thereof.

26. A bsBA of claim 21, wherein the first binding domain binds to a tumor-associated antigen, cytokine receptor, or growth factor receptor.

27. A bsBA of claim 21, wherein the first binding domain binds to a receptor tyrosine kinase.

28. A bsBA of claim 21, wherein the second binding domain binds ErbB3 (HER3), insulin-like growth factor-1 receptor (IGF1-R), any of FGF receptors 1-4, HGF receptor, insulin receptor, either of PDGF receptors α and beta, C-KIT, or ErbB4.

29. A bsBA of claim 21, wherein said first binding domain binds to EGFR and said second binding domain binds ErbB3 (HER3).

30. A bsBA of claim 21, wherein the Kd of the first binding domain is between 10^{-8} and 10^{-12} M.

31. A bsBA of claim 21, wherein said first target molecule is overexpressed by at least 10 times on target cells as compared to its expression on normal cells.

32. A composition of (a) a bispecific binding agent (bsBA) comprising a first binding domain having a Kd for a first target molecule on a target cell of at least 10^{-7} M and a second binding domain having a Kd for a second target molecule on a target cell that is at least 10 times lower than the Kd of the first binding domain, wherein said first and second target molecules do not have the same natural ligand, wherein (i) said first target molecule and said second target molecule each have a biological activity, which may be the same or different, and (ii) said first and said second binding domains, when bound to said first and said second target molecules, modulate the biological activity or activities of the first and second target molecules, respectively, and, (b) a pharmaceutically acceptable carrier.

33. A composition of claim 32, wherein said Kd of said second binding domain is more than 50 times lower than the Kd of the first binding domain.

34. A composition of claim 32, wherein said Kd of said second binding domain is 100 or more times lower than the Kd of the first binding domain.

35. A composition of claim 32, wherein said bsBA comprises two antibodies.

36. A composition of claim 35, wherein said antibodies are diabodies, two single chain Fvs connected directly or by a linker, disulfide stabilized Fvs, or combinations thereof.

37. A composition of claim 32, wherein the first binding domain binds to a tumor-associated antigen, cytokine receptor, growth factor receptor or receptor tyrosine kinase.

38. A composition of claim 32, wherein said first target molecule is overexpressed by at least 10 times on target cells than on non-target cells that also bear the second target molecule

39. A use of a bispecific binding agent (bsBA) comprising a first binding domain having a Kd for a first target molecule on a target cell of at least 10^{-7} M and a second binding domain having a Kd for a second target molecule on a target cell that is at least 10 times lower than the Kd of the first binding domain, wherein said first and second target molecules do not have the same natural ligand, wherein said first target molecule and said second target molecule each have a biological activity, which may be the same or different, and said first and said second binding domains, when bound to said first and said second target molecules, modulate the biological activity or activities of the first and second target molecules, respectively, for the manufacture of a medicament.

40. A use of claim 39, wherein said Kd of said second binding domain is more than 50 times lower than the Kd of the first binding domain.

41. A use of claim 39, wherein said Kd of said second binding domain is 100 or more times lower than the Kd of the first binding domain.

42. A use of claim 39, wherein said bsBA comprises two antibodies.

43. A use of claim 42, wherein said antibodies are diabodies, two single chain Fvs connected directly or by a linker, disulfide stabilized Fvs, or combinations thereof.

44. A use of claim 39, wherein the target molecules bound by the first binding domain and by the second binding domain are independently selected from the group consisting of a tumor-associated antigen, a cytokine receptor, and a growth factor receptor, provided that the first binding domain and the second binding domain do not bind the same tumor-associated antigen, cytokine receptor, or growth factor receptor.

45. A use of claim 39, wherein the medicament is for inhibiting the proliferation of cancer cells.

46. A use of claim 39, wherein said first target molecule is overexpressed by at least 10 times on target cells than on non-target cells that also bear the second target molecule, and further

47. A kit comprising

(a) a container and

(b) a bispecific binding agent (bsBA) comprising a first binding domain having a Kd of at least 10^{-7} M for a first target molecule on a target cell and a second binding domain having a Kd for a second target molecule on a target cell which Kd is at least 10 times lower than the Kd of the first binding domain for the first target molecule, wherein (i) said first and second target molecules do not have the same natural ligand, (ii) said first target molecule and said second target molecule each have a biological activity, which may be the same or different, and (iii) said first and said second binding domains, when bound to said first and said second target molecules, modulate the biological activity or activities of the first and second target molecules, respectively.

48. A kit of claim 47, wherein said Kd of said second binding domain is more than 50 times lower than the Kd of the first binding domain.

49. A kit of claim 47, wherein said Kd of said second binding domain is 100 or more times lower than the Kd of the first binding domain.

50. A kit of claim 47, wherein said bsBA comprises two antibodies.

51. A kit of claim 47, wherein the antibodies are diabodies, two single chain Fvs connected directly or by a linker, disulfide stabilized Fvs, or combinations thereof.

52. A kit of claim 47, wherein the first binding domain binds to a tumor-associated antigen, cytokine receptor, growth factor receptor or receptor tyrosine kinase.

53. A kit of claim 47, wherein the second binding domain binds ErbB3 (HER3), insulin-like growth factor-1 receptor (IGF1-R), any of FGF receptors 1-4, HGF receptor, insulin receptor, either of PDGF receptors α and beta, C-KIT, or ErbB4.

54. A kit of claim 47, wherein said first binding domain binds to EGFR and said second binding domain binds ErbB3 (HER3).

55. A kit of claim 47, wherein the Kd of the first binding domain is between 10^{-8} and 10^{-12} M.

56. A kit of claim 47, wherein said first target molecule is overexpressed by at least 10 times on target cells as compared to its expression on normal cells.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 April 2007 (19.04.2007)

PCT

(10) International Publication Number
WO 2007/042289 A2

(51) International Patent Classification:
C07K 16/28 (2006.01) *G01N 33/563* (2006.01)
C12N 15/13 (2006.01) *A61P 35/00* (2006.01)
A61K 39/395 (2006.01) *A61P 37/00* (2006.01)

(21) International Application Number:
PCT/EP2006/009840

(22) International Filing Date: 11 October 2006 (11.10.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/725,939 11 October 2005 (11.10.2005) US

(71) Applicant *(for all designated States except US)*: **ABLYNX N.V.** [BE/BE]; Technologiepark 4, B-9052 Zwijnaarde (BE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **LAEREMANS, Toon** [BE/BE]; Krauwenberg 35, B-1653 Dworp-Beersel (BE). **DE HAARD, Hans** [NL/NL]; 't Zwint 1, NL-4436 NA Oudelande (NL). **HOOGENBOOM, Hendricus, Renerus, Jacobus, Mattheus** [NL/NL]; Hertogsingel 46, NL-6214 AE Maastricht (NL).

(74) Agent: **TAORMINO, Joseph**; Hoffmann Eitle, Arabellastrasse 4, 81925 Munich (DE).

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2007/042289 A2

(54) Title: NANOBODIES™ AND POLYPEPTIDES AGAINST EGFR AND IGF-IR

(57) Abstract: The invention relates to polypeptides and Nanobodies against Epidermal Growth Factor Receptor (EGFR) and/or Insulin Growth Factor-I Receptor (IGF-IR). The invention also relates to nucleic acids encoding such Nanobodies and polypeptides; to methods for preparing such Nanobodies and polypeptides; to host cells expressing or capable of expressing such Nanobodies or polypeptides; to compositions, and in particular to pharmaceutical compositions, that comprise such Nanobodies, polypeptides, nucleic acids and/or host cells; and to uses of such Nanobodies, polypeptides, nucleic acids, host cells and/or compositions, in particular for prophylactic, therapeutic or diagnostic purposes.

same tumor associated antigen (also called biparatopic binding moieties). In another embodiment, each binding moiety is directed against a different tumor associated antigen. Each binding moiety can be directed against a different tumor associated antigen on either a single or adjacent tumor cell. In a preferred embodiment, said at least two binding moieties have a moderate or low affinity to their individual tumor associated antigen or epitope and, accordingly, have only a reduced retention on normal tissues or cells expressing one of the tumor associated antigens. Those at least two binding moieties, however preferentially target (have a high avidity for) tumor cells that express both antigens recognized by the bispecific or multispecific polypeptide.

A binding moiety according to the present invention can be any peptide or nucleotide containing moiety having a known binding affinity for at least one antigen. The moiety can be a protein, a polypeptide, a protein fragment (such as an antibody fragment) or one or more subunit(s) of any protein. A typical example of a binding moiety would be an enzyme, a receptor or a transport protein. It can also be a carrier protein such as albumin or an antibody. The binding moiety can also be, or include, a sequence of DNA or RNA.

In a preferred embodiment, one or more of the binding moieties on the bispecific or multispecific polypeptide of the invention is a polypeptide below 15 kDa. In an even more preferred embodiment, one or more of the binding moieties on the bispecific or multispecific polypeptide of the invention is a VH, a VHH, a domain antibody, a single domain antibody, a "dAb" or a Nanobody. In a most preferred embodiment, the binding moieties on the bispecific or multispecific polypeptide of the invention are Nanobodies.

Different EGFR family members (EGFR, HER2, HER3, HER4), for example, are overexpressed on tumors in breast cancer, colon cancer, ovarian cancer, lung cancer and head and neck cancer. By simultaneous targeting two of these tumor associated antigens, or different epitopes on one of these tumor associated antigens, a much more selective and/or enhanced tumor targeting will be obtained.

Therefore, in a preferred embodiment, the invention also provides a bispecific polypeptide comprising or essentially consisting of a Nanobody directed against EGFR and a Nanobody directed against another member of the EGFR family. The polypeptide of the invention may comprise or essentially consist of a Nanobody directed against EGFR and a Nanobody directed against HER2. In another embodiment, the polypeptide of the invention may comprise or essentially consist of a Nanobody directed against EGFR and a Nanobody directed against HER3. In another embodiment, the polypeptide of the invention may comprise or essentially consist of a Nanobody directed against EGFR and a Nanobody

Gly Ser Leu Arg Leu Ser Cys Val Ala Ser Gly Arg Thr Phe Ser Arg
275 280 285

Thr Ala Met Ala Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe
290 295 300

Val Ala Thr Ile Thr Trp Asn Ser Gly Thr Thr Arg Tyr Ala Asp Ser
305 310 315 320

Val Lys Gly Arg Phe Phe Ile Ser Lys Asp Ser Ala Lys Asn Thr Ile
325 330 335

Tyr Leu Glu Met Asn Ser Leu Glu Pro Glu Asp Thr Ala Val Tyr Tyr
340 345 350

Cys Ala Ala Thr Ala Ala Ala Val Ile Thr Pro Thr Arg Gly Tyr Tyr
355 360 365

Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
370 375 380

CLAIMS

1. Nanobody against EGFR, which comprises or consists of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), in which:

CDR1 is an amino acid sequence chosen from the group consisting of:

TYTMA	[SEQ ID NO:42]
SYGMG	[SEQ ID NO:43]
GFAMG	[SEQ ID NO:44]
SNNMG	[SEQ ID NO:45]
GDVMG	[SEQ ID NO:46]
SYVVG	[SEQ ID NO:47]
DYNMA	[SEQ ID NO:48]
TYTMA	[SEQ ID NO:49]
NNAMA	[SEQ ID NO:50]
SYVMG	[SEQ ID NO:51]
SYAMG	[SEQ ID NO:52]
A	[SEQ ID NO:53]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the above amino acid sequences; in which

(i) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or from the group consisting of amino acid sequences that have 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(i) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or in which:

CDR2 is an amino acid sequence chosen from the group consisting of:

GISRSDGGTYDADSVKG	[SEQ ID NO:54]
GISWRGDSTGYADSVKG	[SEQ ID NO:55]
AISWSGGSLLYVDSVKG	[SEQ ID NO:56]
AIGWGGLETHYSDSVKG	[SEQ ID NO:57]
GFSRSTSTTHYADSVKG	[SEQ ID NO:58]
GIAWGDGITYYADSVKG	[SEQ ID NO:59]
HISWLGGRTYYRDSVKG	[SEQ ID NO:60]
GFSGSGGATYYAHSVEG	[SEQ ID NO:61]
AISWRGGSTYYADSVKG	[SEQ ID NO:62]
AINWSSGSTYYADSVKG	[SEQ ID NO:63]
TIAWDSGSTYYADSVKG	[SEQ ID NO:64]
GLSWSADSTYYADSVKG	[SEQ ID NO:65]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the above amino acid sequences; in which

(i) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(i) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or in which:

CDR3 is an amino acid sequence chosen from the group consisting of:

ASVKLVYVNPNRYSY	[SEQ ID NO:66]
AAGSAWYGTLYEYDY	[SEQ ID NO:67]
AAGSTWYGTLYEYDY	[SEQ ID NO:68]
MVGPPPRSLDYGLGNHYEYDY	[SEQ ID NO:69]
SSTRTVIYTLPRMYNY	[SEQ ID NO:70]
NSRSSWVIFTIKGQYDR	[SEQ ID NO:71]

RPGMIITTIQATYGF	[SEQ ID NO:72]
GSPYGTELPYTRIEQYAY	[SEQ ID NO:73]
ANEYWVYVNPNRYTY	[SEQ ID NO:74]
RRKSGEVVFTIPARYDY	[SEQ ID NO:75]
VYRVGAISEYSGTDYYTDEYDY	[SEQ ID NO:76]
GYQINSGNYNFKDYEYDY	[SEQ ID NO:77]
SYNVYYNNYYYPISRDEYDY	[SEQ ID NO:78]
HRRPFASVFTTTRMYDY	[SEQ ID NO:79]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the above amino acid sequences; in which

(i) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(i) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s).

2. Polypeptide of less than 15 kDa directed against IGF-IR.

3. Polypeptide according to claim 2 which is able to inhibit IGF-I interaction with IGF-IR.

4. Polypeptide according to any of claims 2 or 3 which binds IGF-IR with a binding affinity of at least $10^7 M^{-1}$.

5. Polypeptide according to any of claims 2 to 4 which is selected from a single domain antibody, a domain antibody, a "dAb", a VH, a VHH or a Nanobody.

6. Nanobody against IGF-IR.

7. Nanobody according to claim 6, which comprises or consists of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), in which:

CDR1 is an amino acid sequence chosen from the group consisting of:

FNAMG	[SEQ ID NO:94]
INVMA	[SEQ ID NO:95]
NYAMG	[SEQ ID NO:96]
RTAMA	[SEQ ID NO:97]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the above amino acid sequences; in which

(i) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or from the group consisting of amino acid sequences that have 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(i) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or in which:

CDR2 is an amino acid sequence chosen from the group consisting of:

VIISGGSTHYVDSVKG	[SEQ ID NO:98]
EITRSGRTNYVDSVKG	[SEQ ID NO:99]
AINWNSRSTYYADSVKG	[SEQ ID NO:100]
TITWNSGTTRYADSVKG	[SEQ ID NO:101]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the above amino acid sequences; in which

(i) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(i) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or in which:

CDR3 is an amino acid sequence chosen from the group consisting of:

KKFGDY	[SEQ ID NO:102]
IDGSWREY	[SEQ ID NO:103]
SHDSDYGGTNANLYDY	[SEQ ID NO:104]
TAAAVITPTRGYYNY	[SEQ ID NO:105]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the above amino acid sequences; in which

(i) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(i) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s).

8. Method for obtaining a Nanobody according to claim 6 comprising the steps of:

a) providing at least one V_{HH} domain directed against IGF-IR, by a method generally comprising the steps of (i) immunizing a mammal belonging to the Camelidae with IGF-IR or a part or fragment thereof, so as to raise an immune response and/or

antibodies (and in particular heavy chain antibodies) against IGF-IR; (ii) obtaining a biological sample from the mammal thus immunized, wherein said sample comprises heavy chain antibody sequences and/or V_{HH} sequences that are directed against IGF-IR; and (iii) obtaining (e.g isolating) heavy chain antibody sequences and/or V_{HH} sequences that are directed against IGF-IR from said biological sample; and/or by a method generally comprising the steps of (i) screening a library comprising heavy chain antibody sequences and/or V_{HH} sequences for heavy chain antibody sequences and/or V_{HH} sequences that are directed against IGF-IR or against at least one part or fragment thereof; and (ii) obtaining (e.g. isolating) heavy chain antibody sequences and/or V_{HH} sequences that are directed against IGF-IR from said library;

b) optionally subjecting the heavy chain antibody sequences and/or V_{HH} sequences against IGF-IR thus obtained to affinity maturation, to mutagenesis (e.g. random mutagenesis or site-directed mutagenesis) and/or any other technique(s) for increasing the affinity and/or specificity of the heavy chain antibody sequences and/or V_{HH} sequences for IGF-IR;

c) determining the sequences of the CDR's of the heavy chain antibody sequences and/or V_{HH} sequences against IGF-IR thus obtained; and

d) providing a Nanobody in which at least one, preferably at least two, and more preferably all three of the CDR's (i.e. CDR1, CDR2 and CDR3, and in particular at least CDR3) has a sequence that has been determined in step c).

9. Polypeptide comprising at least one Nanobody according to any of claims 1 or 6 to 7 or comprising at least a polypeptide according to any of claims 2 to 5, or an EGFR or IGF-IR binding part or fragment thereof.

10. Polypeptide comprising at least two binding moieties, wherein each of said at least two binding moieties is directed against a tumor associated antigen or epitope.

11. Polypeptide according to claim 10, wherein each of said at least two binding moieties is directed against a different tumor associated antigen.

12. Polypeptide according to claim 10, wherein each of said at least two binding moieties is directed against a different epitope on the same tumor associated antigen.

13. Polypeptide according to any of claims 10 to 12, wherein at least one of the binding moieties is selected from a VH, a VHH, a domain antibody, a single domain antibody, a "dAb" or a Nanobody.

14. Polypeptide according to any of claims 10 to 13, wherein each of the binding moieties is a Nanobody.

15. Polypeptide according to claim 10, comprising at least one Nanobody against EGFR and at least one Nanobody against an EGFR family member selected from the group consisting of HER2, HER3, and HER4.

16. Polypeptide according to claim 10, comprising at least one Nanobody against EGFR and at least one Nanobody against IGF-IR.

17. Polypeptide comprising at least two Nanobodies wherein binding of one of said at least two Nanobodies modulates the binding by the second of said at least two Nanobodies.

18. Polypeptide according to claim 17 wherein binding by the second of said at least two Nanobodies is enhanced.

19. Polypeptide according to claim 17 wherein binding by the second of said at least two Nanobodies is reduced.

20. Polypeptide according to claim 17 wherein binding by the second of said at least two Nanobodies is inhibited.

21. Nucleic acid encoding a Nanobody according to any of claims 1 or 6 to 7, or encoding a polypeptide according to any of claims 2 to 5 or 9 to 20.

22. Host cell expressing a Nanobody according to any of claims 1 or 6 to 7, or expressing a polypeptide according to any of claims 2 to 5 or 9 to 20, or comprising a nucleic acid according to claim 21.

23. Method for preparing a Nanobody according to any of claims 1 or 6 to 7, or a polypeptide according to any of claims 2 to 5 or 9 to 20, comprising the steps of:
    - the expression, in a suitable host cell or host organism or in another suitable expression system of a nucleic acid according to claim 21, optionally followed by
    - isolating and/or purifying the Nanobody or polypeptide thus obtained.

24. A composition comprising at least one Nanobody according to any of claims 1 or 6 to 7, at least one polypeptide according to any of claims 2 to 5 or 9 to 20, and/or at least one nucleic acid according to claim 21.

25. Pharmaceutical composition comprising at least one Nanobody according to any of claims 1 or 6 to 7, at least one polypeptide according to any of claims 2 to 5 or 9 to 20, and/or at least one nucleic acid according to claim 21, and optionally at least one pharmaceutically acceptable carrier.

26. Diagnostic composition comprising at least one Nanobody according to any of claims 1 or 6 to 7, at least one polypeptide according to any of claims 2 to 5 or 9 to 20, and/or at least one nucleic acid according to claim 21, and optionally at least one imaging agent.

27. Nanobody according to any of claims 1 or 6 to 7, polypeptide according to any of claims 2 to 5 or 9 to 20, nucleic acid according to claim 21 and/or pharmaceutical composition according to claim 25 for use in prophylaxis and/or therapy.

28. Method for the prevention and/or treatment of diseases or disorders associated with EGFR and/or IGF-IR comprising the step of administering a therapeutically effective amount of a Nanobody according to any of claims 1 or 6 to 7, a polypeptide according to any of claims 2 to 5 or 9 to 20 or a pharmaceutical composition according to claim 25.

29. Method according to claim 28, wherein the disease or disorder is associated with or characterised by the over-expression of EGFR and/or IGF-IR.

30. Method according to claim 29 wherein the disease or disorder is cancer or a tumor.