US 20060058234A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2006/0058234 A1**

Daly et al. (43) **Pub. Date: Mar. 16, 2006**

(54) **VEGF TRAPS AND THERAPEUTIC USES THEREOF**

(76) Inventors: **Thomas J. Daly**, New City, NY (US); **Nicholas J. Papadopoulos**, LaGrangeville, NY (US); **Margaret Karow**, Putnam Vally, NY (US)

Correspondence Address:
REGENERON PHARMACEUTICALS, INC
777 OLD SAW MILL RIVER ROAD
TARRYTOWN, NY 10591 (US)

(21) Appl. No.: **11/204,709**

(22) Filed: **Aug. 16, 2005**

Related U.S. Application Data

(60) Continuation-in-part of application No. 11/016,097, filed on Dec. 17, 2004.
Continuation-in-part of application No. 11/016,503, filed on Dec. 17, 2004, which is a division of application No. 10/009,852, filed on Dec. 6, 2001, filed as 371 of international application No. PCT/US00/14142, filed on May 23, 2000.
Continuation-in-part of application No. 10/880,021, filed on Jun. 29, 2004, which is a continuation-in-part of application No. 10/609,775, filed on Jun. 30, 2003, which is a continuation-in-part of application No. 10/009,852, filed on Dec. 6, 2001.

(60) Provisional application No. 60/138,133, filed on Jun. 8, 1999.

Publication Classification

(51) **Int. Cl.**

C07K 14/71	(2006.01)
A61K 38/17	(2006.01)
C07H 21/04	(2006.01)
C12P 21/06	(2006.01)
C12N 5/06	(2006.01)

(52) **U.S. Cl.** **514/12**; 530/350; 435/69.1; 435/320.1; 435/358; 536/23.5

(57) **ABSTRACT**

Nucleic acid molecules and multimeric proteins capable of binding vascular endothelial growth factor (VEGF). VEGF traps are disclosed which are therapeutically useful for treating VEGF-associated conditions and diseases, and are specifically designed for local administration to specific organs, tissues, and/or cells.

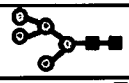
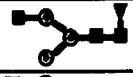
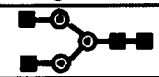
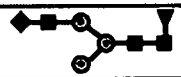
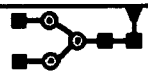
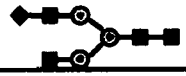
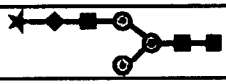
Observed Masses		Expected Masses	Monosaccharide Compositions	Proposed Sugar Chain Structures
VGT C04003 M500	VGT C04008 M500			
1356.4	1356.0	1356.1	(Man)2(Man)3(Glc)2	
1380.9	1381.6	1381.2	(Glc)(Man)3(Glc)2(Fuc)	
1397.2	1397.2	1397.3	(Gal)(Glc)(Man)3(Glc)2	
1438.6	1438.2	1438.2	(Glc)2(Man)3(Glc)2	
1526.7	1525.9	1527.2	(Glc)(Man)3(Glc)2(Fuc)2	
1543.4	1542.8	1543.4	(Gal)(Glc)(Man)3(Glc)2(Fuc)	
1559.7	1559.4	1559.4	(Gal)(Glc)(Man)(Man)3(Glc)2	
1584.6	1584.0	1584.4	(Glc)2(Man)3(Glc)2(Fuc)	
1600.5	1600.0	1600.4	(Gal)(Glc)2(Man)3(Glc)2	
1646.5	1646.6			Adduct of 1584.4
1688.5	1688.0	1688.4	(SA)(Gal)(Glc)(Man)3(Glc)2	
1721.5	1720.8	1721.5	(Gal)(Glc)(Man)2(Man)3(Glc)2	

Figure 1A

Observed Masses		Expected Masses	Monosaccharide Compositions	Proposed Sugar Chain Structures
VGT C04003 M500	VGT C04008 M500			
1746.7	1746.2	1746.5	(Gal)(Glc)2(Man)3(Glc)2(Fuc)	
1762.8	1762.6	1762.1	(Gal)2(Glc)2(Man)3(Glc)2	
1809.5	1809.1			Adduct of 1746.5
1834.8	1834.7	1834.5	(SA)(Gal)(Glc)(Man)3(Glc)2(Fuc)	
1849.9	1851.0	1850.5	(SA)(Gal)2(Glc)(Man)3(Glc)2	
1891.9	1891.1	1891.6	(SA)(Gal)(Glc)2(Man)3(Glc)2	
1908.6	1908.2	1908.6	(Gal)(Glc)2(Man)3(Glc)2(Fuc)	
1996.9	1996.5	1996.6	(SA)(Gal)2(Glc)(Man)3(Glc)2(Fuc)	
2012.8	2012.3	2012.8	(SA)(Gal) (Glc)2(Man)2(Man)3(Glc)2	
2037.7	2037.3	2037.7	(SA)(Gal)(Glc)2(Man)3(Glc)2(Fuc)	
2053.8	2053.2	2053.8	(SA)(Gal)2(Glc)2(Man)3(Glc)2	
2075.8	2074.9			Adduct of 2053.8
2116.0	2115.7			Adduct of 2053.8

Figure 1B

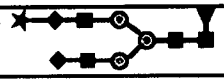
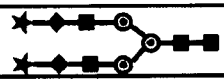
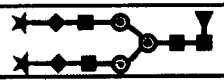
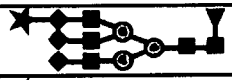
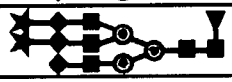
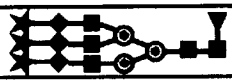
Observed Masses		Expected Masses	Monosaccharide Compositions	Proposed Sugar Chain Structures
VGT C04003 M500	VGT C04008 M500			
2200.0	2199.3	2199.9	(SA)(Gal)2(Glc)2(Man)3(Glc)2(Fuc)	
2221.5	2221.5			Na^+ Adduct of 2199.9
2262.5	2261.3			Adduct of 2199.9
2345.6	2345.6	2345.0	(SA)2(Gal)2(Glc)2(Man)3(Glc)2	
2407.0	2407.2			Adduct of 2345.0
2492.5	2492.0	2491.2	(SA)2(Gal)2(Glc)2(Man)3(Glc)2(Fuc)	
2554.3	2553.1			Adduct of 2491.2
2564.6	2564.0	2565.2	(SA)(Gal)3(Glc)3(Man)3(Glc)2(Fuc)	
2857.2	2857.3	2856.5	(SA)2(Gal)3(Glc)3(Man)3(Glc)2(Fuc)	
2918.1	2918.9			Adduct of 2856.5
3148.5	3148.5	3147.8	(SA)3(Gal)3(Glc)3(Man)3(Glc)2(Fuc)	
3211.0	3211.0			Adduct of 3147.8

Figure 1C

VEGF TRAPS AND THERAPEUTIC USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of application Ser. No. 11/016,097 filed 17 Dec. 2004 and Ser. No. 11/016,503 filed 17 Dec. 2004, which are divisionals of Ser. No. 10/009,852 filed 6 Dec. 2001, which is the National Stage of International Application No. PCT/US00/14142 filed 23 May 2000, which claims the benefit under 35 USC § 119(e) of U.S. provisional Application No. 60/138,133 filed 8 Jun. 1999; and application Ser. No. 10/880,021 filed 29 Jun. 2004, which is a continuation-in-part of Ser. No. 10/609,775 filed 30 Jun. 2003, which is a continuation-in-part of Ser. No. 10/009,852 filed 6 Dec. 2001, which is the National Stage of International Application No. PCT/US00/14142 filed 23 May 2000, which claims the benefit under 35 USC § 119(e) of U.S. provisional Application No. 60/138,133 filed 8 Jun. 1999, which applications are herein specifically incorporated by reference in their entireties.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] The invention encompasses fusion polypeptides capable of binding vascular endothelial cell growth factor (VEGF), VEGF family members, and splice variants with specifically desirable characteristics, as well as therapeutic methods of use.

[0004] 2. Brief Summary of the Invention

[0005] In a first aspect, the invention features an isolated nucleic acid molecule encoding a fusion polypeptide comprising receptor components R1-R2-F, wherein R1 is vascular endothelial cell growth factor (VEGF) receptor component Ig domain 2 of Flt-1 (Flt1D2), R2 is VEGF receptor component Ig domain 3 of Flk-1 (Flk1D3) (also known as KDR), and F is a fusion component.

[0006] In a related second aspect, the invention features a VEGF-binding fusion polypeptide comprising VEGF receptor components R1-R2-F, wherein R1, R2, and F are as defined above. The components may be connected directly to each other or connected via one or more spacer sequences. In a preferred embodiment, R1 and R2 are the only receptor components present. In a specific embodiment, the VEGF-binding fusion polypeptide is amino acids 27-129 (R1) and 130-231 (R2) of SEQ ID NO:8, or a variant thereof.

[0007] The fusion component F is selected from the group consisting of a multimerizing component, a serum protein, or a molecule capable of binding a serum protein. In a preferred embodiment, F is a multimerizing component capable of interacting with a multimerizing component on another fusion polypeptide to form a multimeric structure, e.g., a dimer or trimer. Most preferably, the F is selected from the group consisting of (i) a multimerizing component comprising a cleavable region (C-region), (ii) a truncated multimerizing component, (iii) an amino acid sequence between 1 to about 200 amino acids in length having at least one cysteine residue, (iv) a leucine zipper, (v) a helix loop [illegible] embodiment, F is a full-length or truncated immunoglobulin domain consisting of amino acids 232-458, 232-457, or 352-458 of SEQ ID NO:8.

[0008] The receptor components may be arranged in different orders, for example, R1R2F; R2R1F; R1FR2; R2FR1; FR1R2; FR2R1, etc. The components of the fusion polypeptide may be connected directly to each other, or connected via a spacer sequence.

[0009] In a third aspect, the invention features a multimeric VEGF-binding protein, comprising two or more fusion polypeptides of the invention (also called a VEGF "trap"). A VEGF trap composed of two fusion polypeptides having at least one truncated multimerizing component is termed a "truncated mini-trap." The multimeric VEGF-binding protein of the invention is capable of binding VEGF with an affinity (Kd) of at least 1×10^{-10} M, preferably at least 1×11^{-10} M, even more preferably at least 1×10^{-12} M, as measured by Biacore-based assays.

[0010] The C-region may be created in the multimerizing component by insertion, deletion, or mutation, such that an enzymatically or chemically cleavable site is created. The C-region may be created in any multimerizing component and at any position within; preferably, the C-region is created in a full-length Fc domain, or a fragment thereof, or a C_H3 domain. The C-region may be a site cleavable by an enzyme, such as, thrombin, ficin, pepsin, matrilysin, or prolidase or cleavable chemically by, for example, formic acid or $CUCl_2$.

[0011] In all embodiments of the VEGF-binding fusion polypeptides of the invention (including full length VEGF-binding fusion polypeptides, truncated VEGF-binding fusion polypeptides, etc.), a signal sequence (S) may be included at the beginning (or N-terminus) of the fusion polypeptide of the invention. The signal sequence may be native to the cell, recombinant, or synthetic.

[0012] The components of the fusion polypeptide may be connected directly to each other or be connected via spacers. In specific embodiments, one or more receptor and/or fusion partner components of the fusion polypeptide are connected directly to each other without spacers. In other embodiments, one or more receptor and/or fusion partner components are connected with spacers.

[0013] In a fourth aspect, the invention encompasses vectors comprising the nucleic acid molecules of the invention, including expression vectors comprising the nucleic acid molecule operatively linked to an expression control sequence. In a fifth aspect, the invention encompasses host-vector systems for the production of a fusion polypeptide which comprise the expression vector, in a suitable host cell; host-vector systems wherein the suitable host cell is a bacterial, yeast, insect, mammalian cell; an *E. Coli* cell, or a COS or CHO cell. Additional encompassed in a sixth aspect are VEGF-binding fusion polypeptides of the invention modified by acetylation or pegylation, and other post-translational modifications resulting from expression in a mammalian cell line. Methods for acetylating or pegylating a protein are well known in the art. In specific embodiments, the fusion polypeptide of the invention expressed in a mammalian cell line such as a CHO cell comprises amino [illegible]

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.