



US008647842B2

(12) **United States Patent**  
**Papadopoulos et al.**

(10) **Patent No.:** **US 8,647,842 B2**  
(45) **Date of Patent:** **\*Feb. 11, 2014**

(54) **METHODS FOR PRODUCING A FUSION PROTEIN CAPABLE OF BINDING VEGF**  
(75) Inventors: **Nicholas J. Papadopoulos**, Lagrangeville, NY (US); **Samuel Davis**, New York, NY (US); **George D. Yancopoulos**, Yorktown Heights, NY (US)

(73) Assignee: **Regeneron Pharmaceuticals, Inc.**, Tarrytown, NY (US)

(\* ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.  
This patent is subject to a terminal disclaimer.

(21) Appl. No.: **13/439,889**

(22) Filed: **Apr. 5, 2012**

(65) **Prior Publication Data**  
US 2013/0149744 A1 Jun. 13, 2013

**Related U.S. Application Data**  
(60) Continuation of application No. 12/885,185, filed on Sep. 17, 2010, now abandoned, which is a continuation of application No. 12/715,128, filed on Mar. 1, 2010, now Pat. No. 8,084,234, which is a continuation of application No. 12/102,681, filed on Apr. 14, 2008, now Pat. No. 7,704,500, which is a division of application No. 11/016,097, filed on Dec. 17, 2004, now Pat. No. 7,374,757, which is a division of application No. 10/009,852, filed as application No. PCT/US00/14142 on May 23, 2000, now Pat. No. 7,070,959.

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

(51) **Int. Cl.**  
*C12N 5/10* (2006.01)  
*C12N 5/16* (2006.01)  
*C12N 15/62* (2006.01)  
*C12N 15/63* (2006.01)  
*C07H 21/04* (2006.01)

(52) **U.S. Cl.**  
USPC ..... **435/69.7**; 435/252.3; 435/320.1; 435/325; 435/358; 536/23.4

(58) **Field of Classification Search**  
None  
See application file for complete search history.

(56) **References Cited**  
**U.S. PATENT DOCUMENTS**  
5,712,380 A 1/1998 Kendall et al.  
6,011,003 A 1/2000 Charnock-Jones et al.  
6,100,071 A 8/2000 Davis-Smyth et al.  
6,833,349 B2 12/2004 Xia et al.  
6,897,294 B2 5/2005 Davis-Smyth et al.  
7,070,959 B1 7/2006 Papadopoulos et al.

7,087,411 B2 8/2006 Daly et al.  
7,279,159 B2 10/2007 Daly et al.  
7,303,746 B2 12/2007 Wiegand et al.  
7,303,747 B2 12/2007 Wiegand et al.  
7,306,799 B2 12/2007 Wiegand et al.  
7,374,757 B2 5/2008 Papadopoulos et al.  
7,374,758 B2 5/2008 Papadopoulos et al.  
7,396,664 B2 7/2008 Daly et al.  
7,399,612 B2 7/2008 Daly et al.  
7,521,049 B2 4/2009 Wiegand et al.  
7,524,499 B2 4/2009 Papadopoulos et al.  
7,635,474 B2 12/2009 Daly et al.  
7,704,500 B2 4/2010 Papadopoulos et al.  
2004/0265309 A1 12/2004 Kandel et al.  
2005/0175610 A1 8/2005 Wiegand et al.  
2005/0260203 A1 11/2005 Wiegand et al.  
2005/0281822 A1 12/2005 Cedarbaum et al.  
2005/0281831 A1 12/2005 Davis-Smyth et al.  
2006/0210566 A1 9/2006 Holash et al.  
2007/0037748 A1 2/2007 Stahl et al.  
2009/0081217 A1 3/2009 Papadopoulos et al.  
2009/0155899 A1 6/2009 Papadopoulos et al.  
2009/0234103 A1 9/2009 Davis-Smyth et al.  
2010/0087632 A1 4/2010 Daly et al.  
2010/0221782 A1 9/2010 Papadopoulos et al.

**FOREIGN PATENT DOCUMENTS**

WO WO 94/21679 9/1994  
WO WO 97/44453 11/1997  
WO WO 98/13071 4/1998  
WO WO 99/03996 1/1999  
WO WO 00/75319 12/2000

**OTHER PUBLICATIONS**

Chung et al. (2008), Treatment of malignant ascites, Current Treatment Options in Oncology 9:215-233.  
Wells et al. (1990), Additivity of mutational effects in proteins, Biochemistry 29 (37):8509-8517.  
Ngo et al. (1994), Computational complexity, protein structure prediction, and the Levinthal paradox. In Merz and Le Grand (Eds.) Birkhauser; Boston, pp. 491-495.

(Continued)

*Primary Examiner* — Christine J Saoud  
*Assistant Examiner* — Jon M Lockard  
(74) *Attorney, Agent, or Firm* — Frank R. Cottingham

(57) **ABSTRACT**

The present invention provides methods for producing a fusion protein capable of binding vascular endothelial cell growth factor (VEGF). The methods of the invention comprise growing recombinant cells in suspension culture, wherein the recombinant cells contain an expression vector comprising a nucleic acid molecule encoding a fusion protein that binds VEGF, and isolating the fusion protein from the suspension culture. The fusion protein may comprise a VEGF receptor component having an immunoglobulin-like (Ig) domain 2 of a first VEGF receptor, an Ig domain 3 of a second VEGF receptor, and a multimerizing component.

**14 Claims, 55 Drawing Sheets**

(56)

## References Cited

## OTHER PUBLICATIONS

- Herley et al. (1999), Characterization of the VEGF binding site on the Flt-1 receptor, *Biochem Biophys Res Commun* 262:731-738.
- Terman et al. (1991), Identification of a new endothelial cell growth factor receptor tyrosine kinase, *Oncogene* 6:1677-1683.
- Hileman et al. (1998), Glycosaminoglycan-protein interactions: definitions of consensus sites in glycosaminoglycan binding proteins, *BioEssays* 20:156-167.
- Devries et al. (1992), The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor, *Science* 225:989-991.
- Sharifi et al. (1998), Improving monoclonal antibody pharmacokinetics via chemical modification, *Quart. J. Nucl. Med.* 42:242-249.
- Jensen-Pippo et al. (1996), Enteral bioavailability of human granulocyte colony stimulating factor conjugated with poly(ethylene glycol), *Pharm. Res.* 13(1):102-107.
- Tanaka et al. (1997), Characterization of the extracellular domain in vascular endothelial growth factor receptor-1 (Flt-1 tyrosine kinase). *Jpn. J. Cancer Res.* 88:867-876.
- Yang et al. (1995), The use of polyethylene glycol-modified interleukin-2 (PEG-IL-2) in the treatment of patients with metastatic renal cell carcinoma. . . *Cancer* 76:687-694.
- Davis-Smyth et al. (1996), The second immunoglobulin-like domain of the VEGF tyrosine kinase receptor Flt-1 determines ligand binding and may initiate . . . *EMBO J.* 15:4919-4927.
- Terman et al. (1992), Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor, *Biochem Biophys Res Commun* 187:1579-1586.
- Tsutsumi et al. (1997), PEGylation of interleukin-6 effectively increases its thrombopoietic potency, *Thrombosis and Haemostasis* 77(1):168-173.
- Dunca and Spreafico (1994), Polymer conjugates, *Drug Delivery Systems* 27(4):290-306.
- Kendall et al. (1993), Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor, *Proc. Natl. Acad. Sci. USA* 90:10705-10709.
- Kendall et al. (1996), Identification of a natural soluble form of the vascular endothelial growth factor receptor, FLT-1, and its . . . *Biochem Biophys Res Commun* 226:324-328.
- Autiero et al. (2003), Role of PIGF in the intra- and intermolecular cross talk between the VEGF receptors Flt1 and Flk1, *Nature Medicine* 9:936-943.
- Declaration of Dr. Sarah Hymowitz submitted to the European Patent Office on Oct. 7, 2009, by Genentech, Inc. during prosecution of European Patent Appl. No. 05023819.5.
- Mahdavevan et al. (1995), Structural role of extracellular domain 1 of alpha-platelet-derived growth factor (PDGF) receptor for PDGF-AA and . . . *J. Biol. Chem.* 270:27595-27600.
- Tanaka et al. (1995), Characterization of the ligand binding domain of FLT-1, . . . The 8th Annual Meeting of Japanese Molecular Biology, Nov. 21, 1995, Abstract 2P-227.
- Keyt et al. (1996), Identification of vascular endothelial growth factor determinants for binding KDR and FLT-1 receptors; generation of . . . *J. Biol. Chem.* 271:5638-5646.
- Heidaran et al. (1990), Chimeric alpha- and beta-platelet derived growth factor (PDGF) receptors define three immunoglobulin-like domains . . . *J. Biol. Chem.* 265:18741-18744.
- Park et al. (1994), Placenta growth factor; potentiation of vascular endothelial growth factor bioactivity, in vitro and in vivo, and high . . . *J. Biol. Chem.* 269:25646-25654.
- Shibuya et al. (1990), Nucleotide sequence and expression of a novel human receptor-type tyrosine kinase gene (flt) closely related to the fms family, *Oncogene* 5:519-524.
- Heidaran et al. (1995), Beta-PDGFR-IgG chimera demonstrates that human beta-PDGFR Ig-like domains 1 to 3 are sufficient for high affinity PDGF BB . . . *FASEB Journal* 9:140-145.
- Yu et al. (1994), Structural coincidence of alpha-PDGFR epitopes binding to platelet-derived growth factor-AA and a potent neutralizing . . . *J. Biol. Chem.* 269:10668-10674.
- Yu et al. (1995), Differential requirement of a motif within the carboxyl-terminal domain of alpha-platelet-derived growth factor . . . *J. Biol. Chem.* 270:7033-7036.
- USPTO Office Action issued on Mar. 25, 2005, in U.S. Appl. No. 10/009,852.
- USPTO Office Action issued on Jul. 16, 2007, in U.S. Appl. No. 11/089,803.
- USPTO Office Action issued on Oct. 17, 2007, in U.S. Appl. No. 11/016,503.
- Applicant Amendment and Remarks filed on May 16, 2005, in U.S. Appl. No. 10/009,852.
- USPTO Office Action issued on Sep. 7, 2005, in U.S. Appl. No. 10/009,852.
- Applicant Amendment and Remarks filed on Sep. 20, 2005, in U.S. Appl. No. 10/009,852.
- USPTO Notice of Allowance issued on Nov. 30, 2009, in U.S. Appl. No. 12/102,681.
- Preliminary Amendment filed on Oct. 6, 2008, in U.S. Appl. No. 12/104,894.
- Claims filed at the European Patent Office in EP Appl. No. 05023819.5 on Oct. 9, 2009, on behalf of Applicant Genentech, Inc. Communication pursuant to Article 94(3) EPC issued by the European Patent Office on Apr. 1, 2009, in EP Appl. No. 05023819.5.
- Summons to attend Oral Proceedings issued by the European Patent Office on Nov. 17, 2010, in EP Appl. No. 05023819.5.

Fig.1.

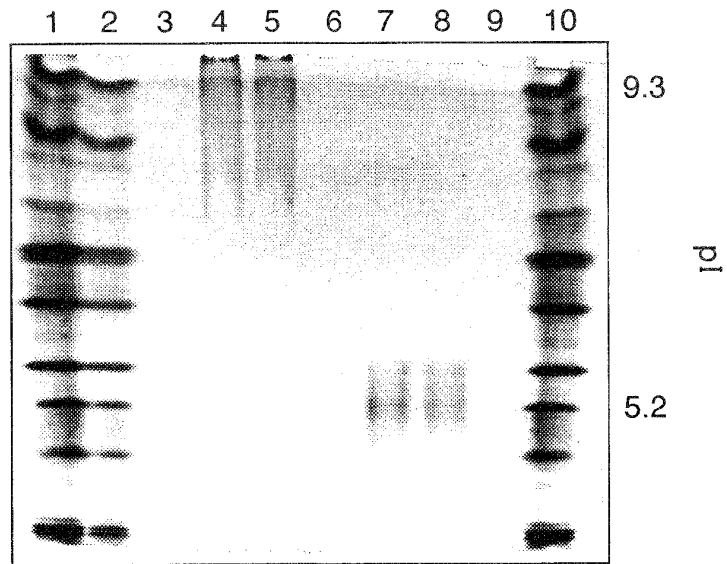


Fig.2.

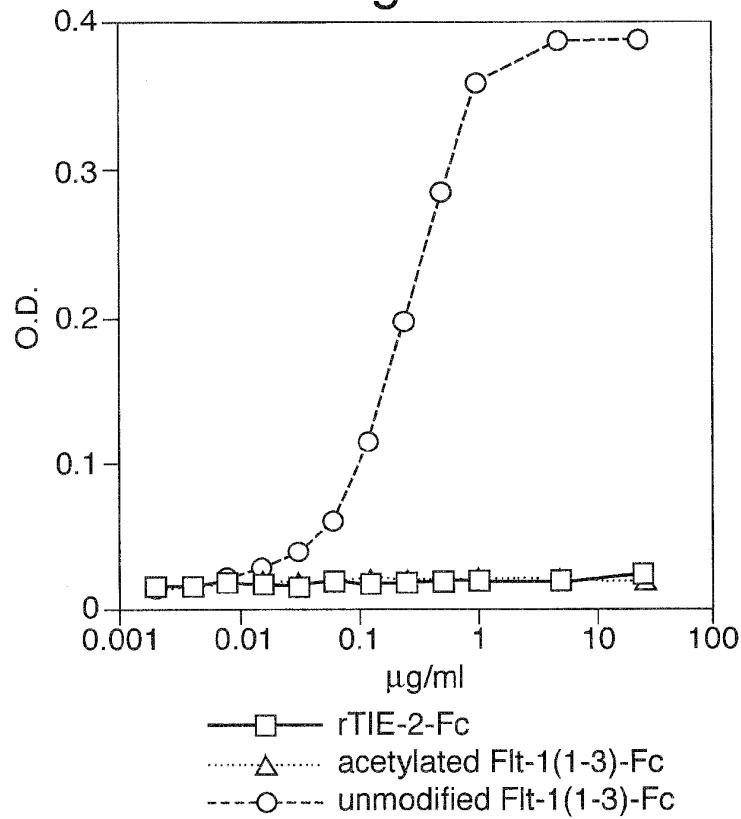


Fig.3.

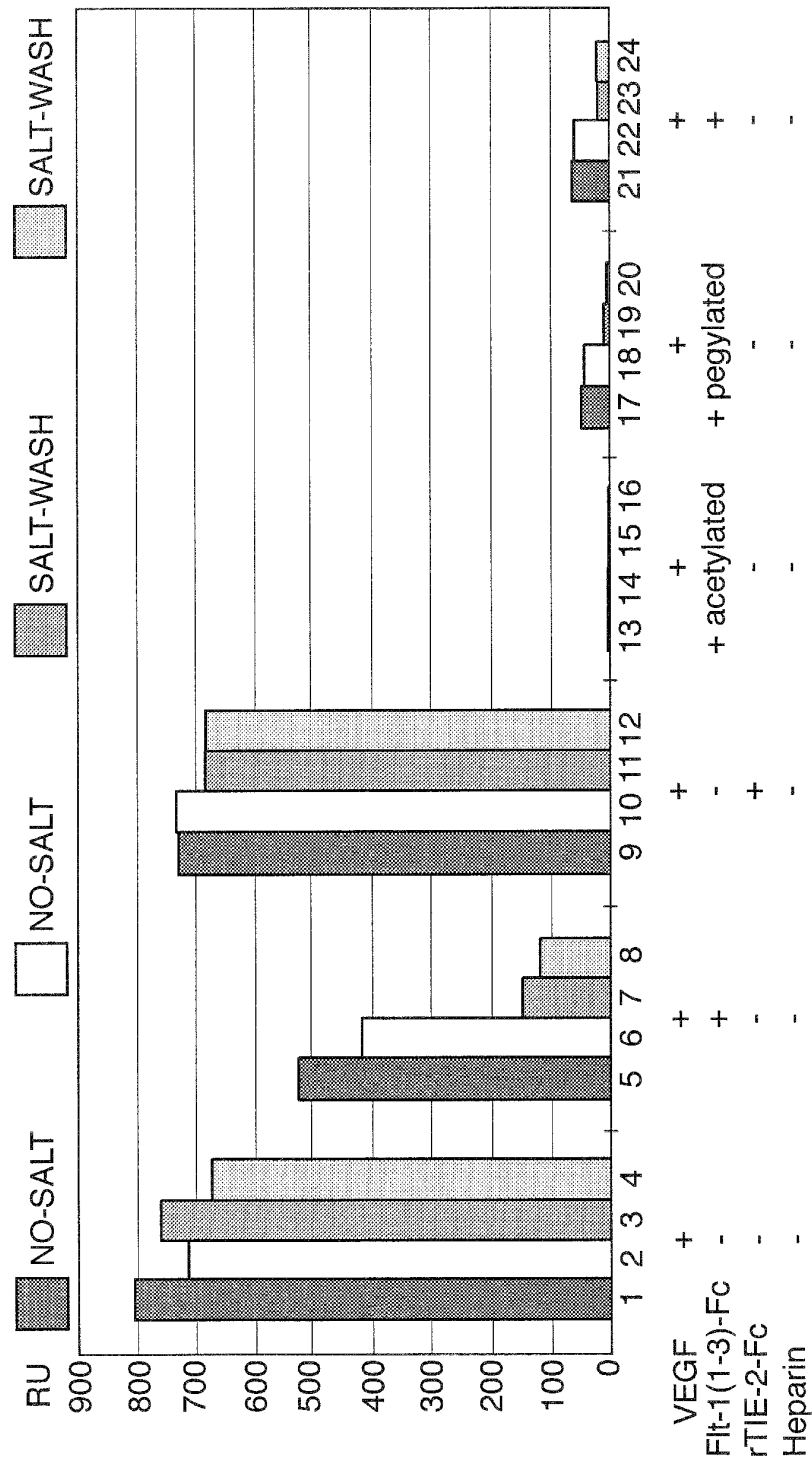
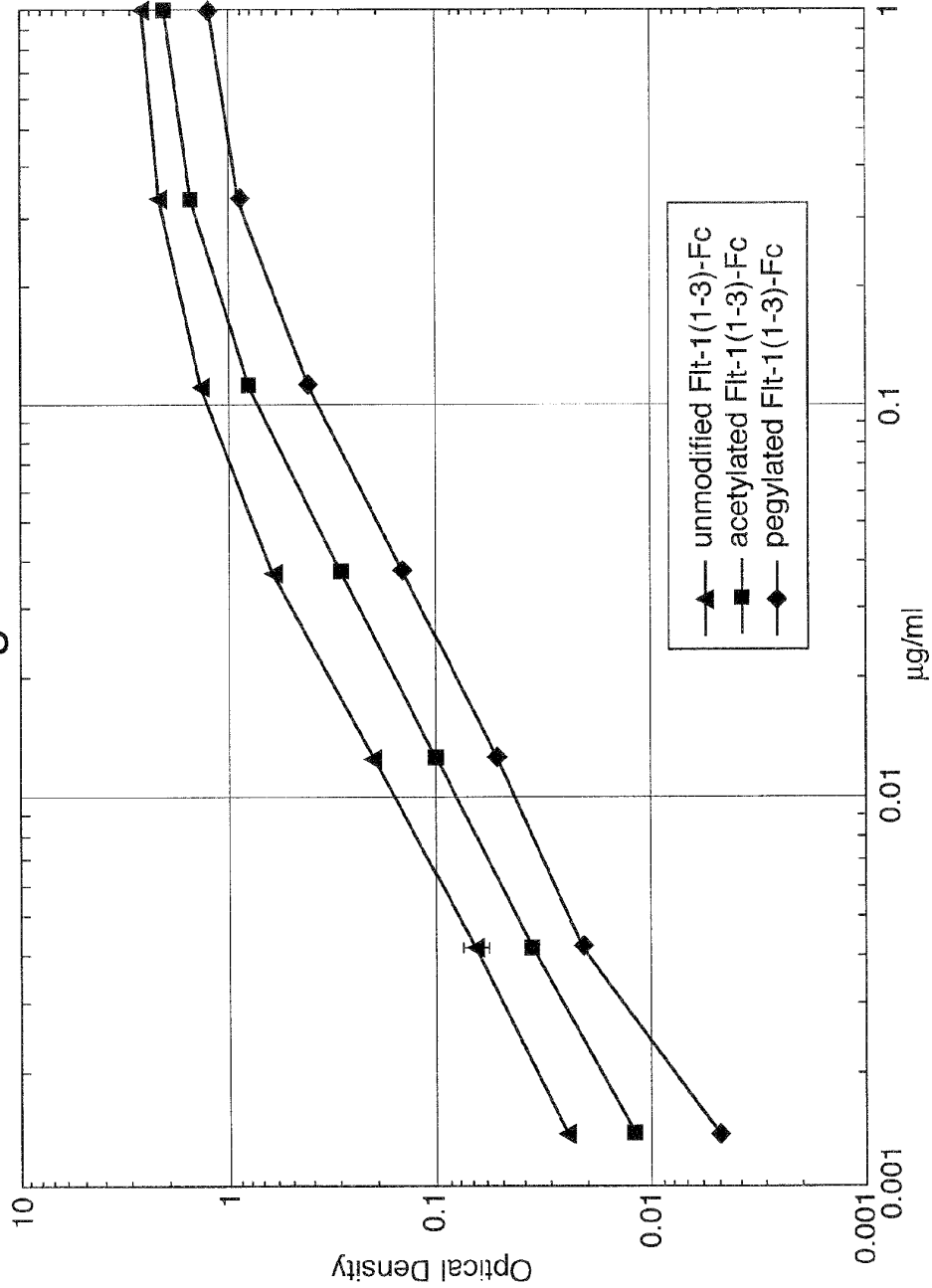


Fig.4.



# 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.