

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Patent Number: 7,374,758

(Application No. 11/016,503)

**For: Modified Chimeric Polypeptides with
Improved Pharmacokinetic Properties
and Methods of Using Thereof**

Inventors: Papadopoulos, Davis and Yancopoulos

Issued: May 20, 2008

Assignee: Regeneron Pharmaceuticals, Inc.

**RECEIVED
DEC 22 2011
PATENT EXTENSION
Unit: OPOPLA**

**Office of Patent Legal Administration (via hand delivery)
Room MDW 7D55
600 Dulany Street (Madison Building)
Alexandria, VA 22314**

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. §156

Dear Sir,

Applicant, Regeneron Pharmaceuticals, Inc., hereby submits this application for extension of the term of United States Letters Patent No. 7,374,758 (the "'758 patent") under 35 U.S.C. §156 and 37 C.F.R. §1.740.

Applicant represents that it is the assignee of the entire interest in and to the '758 patent by virtue of assignment of all rights of inventors Nicholas J. Papadopoulos, Samuel Davis and George D. Yancopoulos (Papadopoulos *et al.*) to Regeneron Pharmaceuticals, Inc., as recorded in the U.S. Patent and Trademark Office on December 17, 2004, Reel 016103, Frame 0015 (a copy of which is attached in Attachment A).

05/09/2012 CKHLOK 00000009 100650 11016503
01 FC:1457 1120.00 DA

This application is based on the approval by the United States Food and Drug Administration ("FDA") of a Biologics License Application (BLA No. BL 125387/0) on November 18, 2011, for EYLEA™ (aflibercept intravitreal injection, also known as aflibercept IVT, aflibercept ophthalmic injection and aflibercept ophthalmic solution).

1. Identification of the Approved Product under 37 C.F.R. §1.740 (a)(1)

The name of the approved product is EYLEA™. The name of the active ingredient of EYLEA™ is aflibercept, also known as VEGF trap, VEGF-trap, VEGF Trap-Eye and VEGF-TRAP_{R1R2}. Aflibercept is a fusion protein consisting of (a) a vascular endothelial growth factor (VEGF) receptor component having immunoglobulin-like (Ig) domains consisting of an Ig domain 2 of a first VEGF receptor that is human Flt1 and an Ig domain 3 of a second VEGF receptor that is human Flk1; and (b) an Fc portion of human IgG1.

2. Federal Statute Governing Regulatory Approval of the Approved Product under 37 C.F.R. §1.740(a)(2)

The approved product was subject to regulatory review under, *inter alia*, the Public Health Service Act (42 U.S.C. § 201 *et seq.*, including 42 U.S.C. §262(a)) and the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355 *et seq.*).

3. Date of Approval for Commercial Marketing under 37 C.F.R. §1.740 (a)(3)

EYLEA™ was approved for commercial marketing or use under §351 of the Public Health Service Act on November 18, 2011.

4. Identification of Active Ingredient and Certifications Related to Commercial Marketing of Approved Product under 37 C.F.R. §1.740(a)(4)

- (a) The active ingredient of EYLEA™ is aflibercept, which is a recombinant fusion protein consisting of a VEGF receptor component having Ig domains consisting of an Ig domain 2 of human Flt1 and an Ig domain 3 of human Flk1, fused to the Fc domain of human IgG1.
- (b) Applicant certifies that aflibercept has not been approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act, the Public Health Service Act or the Virus-Serum-Toxin Act prior to the approval granted on November 18, 2011 to the present Applicant.

- (c) Afibercept has been approved for the treatment of patients with neovascular (wet) age-related macular degeneration. See EYLEA™ product label, provided as Attachment B.
- (d) EYLEA™, whose active ingredient is aflibercept, was approved for commercial marketing pursuant to § 351 of the Public Health Service Act (42 U.S.C. §262) under Regeneron's existing Department of Health and Human Services (DHHS) U.S. License No. 1760. See EYLEA™ approval letter, provided as Attachment C.

5. Statement Regarding Timeliness of Submission of Patent Term Extension Request [§ 1.740(a)(5)]

Applicant certifies that this application for patent term extension is being submitted within the sixty (60) day period permitted for submission specified in 35 U.S.C. § 156(d)(1), (as amended on September 16, 2011), and 37 C.F.R. § 1.720(f). The amended provisions of the America Invents Act state that the date on which a product receives permission is the next business day if permission is "transmitted after 4:30 P.M., Eastern Time, on a business day" Permission was transmitted at 5:47 P.M., Eastern Time, on Friday, November 18, 2011, a business day. The next business day is Monday, November 21, 2011. Accordingly, the last date on which this application may be submitted is January 19, 2012.

6. Complete Identification of the Patent for Which Extension Is Being Sought [§ 1.740(a)(6)]

The complete identification of the patent for which an extension is being sought is as follows:

- (a) Names of the inventors: Nicholas J. Papadopoulos, Samuel Davis and George D. Yancopoulos
- (b) Patent Number: U.S. Patent No. 7,374,758 (the "758 patent")
- (c) Date of Issue: May 20, 2008
- (d) Date of Expiration : January 17, 2021

7. Copy of the Patent for Which an Extension is Being Sought [§ 1.740(a)(7)]

A copy of the '758 patent is provided as Attachment D to the present application.

8. Copies of Disclaimers, Certificates of Correction, Receipt of Maintenance Fee Payment, or Reexamination Certificate [§ 1.740(a)(8)]

- (a) The '758 patent is subject to a terminal disclaimer, a copy of which is attached in Attachment E.
- (b) No certificate of correction has been issued for the '758 patent.
- (c) The first maintenance fee for the '758 patent was paid on November 14, 2011 (See Attachment F).
- (d) The '758 patent has not been the subject of a reexamination proceeding.

9. Statement Regarding Patent Claims Relative to Approved Product [§ 1.740(a)(9)]

The statements below are made solely to comply with the requirements of 37 C.F.R. § 1.740 (a)(9). Applicant notes that, as the M.P.E.P. acknowledges, § 1.740 (a)(9) does not require an applicant to show whether or how the listed claims would be infringed, and that this question cannot be answered without specific knowledge concerning acts performed by third parties. As such, these comments are not an assertion or an admission of Applicant as to the scope of the listed claims, or whether or how any of the listed claims would be infringed, literally or under the doctrine of equivalents, by the manufacture, use, sale, offer for sale or the importation of any product.

- (a) At least the following claims of the '758 patent claim a method of using the approved product: claims 1 and 2.
- (b) Pursuant to M.P.E.P. § 2753 and 37 C.F.R. § 1.740(a)(9), the following explanation is provided which shows how the above-listed claims of the '758 patent claim a method of using the approved product.

(1) *Description of the approved product*

The approved product is described as follows in the approved label for EYLEA™, a copy of which is provided as Attachment B.

EYLEA™ (aflibercept ophthalmic solution) is a recombinant fusion protein consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1 and specially purified and formulated as an iso-osmotic solution for intravitreal administration. Aflibercept is a dimeric glycoprotein with a protein molecular weight of 97 kilodaltons (kDa) and is glycosylated, with the glycosylation constituting an additional 15% of the total molecular mass, resulting in a total molecular weight of 115 kDa. Aflibercept is produced in Chinese hamster ovary (CHO) K1 cells by recombinant DNA technology.

EYLEA™ is a sterile, clear, and colorless to pale yellow, iso-osmotic solution. It is supplied as a preservative-free, sterile, aqueous solution in a single-use, sterile, pre-filled, glass syringe or single-use, glass vial designed to deliver 0.05 mL (50 microliters) of EYLEA™ (40 mg/mL) with 10 mM sodium phosphate, 40 mM sodium chloride, 0.03% polysorbate 20, and 5% sucrose, pH 6.2.

Aflibercept is also described in Holash *et al.* Proc. Natl. Acad. Sci. USA, August 20, 2002, Vol. 99, No. 17, pp. 11393-11398 ("Holash," Attachment G) as VEGF-Trap_{R1R2}, which has the Ig domain 2 of VEGF receptor 1 (VEGFR1; also known as Flt-1) fused to the Ig domain 3 of VEGF receptor 2 (VEGFR2; also known as Flk-1), which in turn is fused to the constant region (Fc) of human IgG1. See paragraph bridging pages 11393 and 11394 and Figure 1A. Moreover, Holash *et al.* demonstrate that aflibercept is a VEGF antagonist that binds to and inhibits the biologic activity of human vascular endothelial growth factor (VEGF) in various *in vitro* and *in vivo* assay systems.

(2) *Explanation Regarding Claims 1 and 2 Relative to Aflibercept*

As explained below, a method for using aflibercept, the active ingredient of the approved product, is covered by claims 1 and 2.

Claims 1 and 2 read as follows:

1. A method of inhibiting vascular endothelial growth factor (VEGF) activity in a mammal, comprising:
administering a pharmaceutical composition to the mammal, wherein the pharmaceutical composition comprises
(a) a VEGF antagonist, and

- (b) a pharmaceutically acceptable carrier wherein the VEGF antagonist comprises a dimeric fusion polypeptide comprising two fusion polypeptides, each fusion polypeptide comprising:
 - (i) a VEGF receptor component consisting of an immunoglobulin-like (Ig) domain 2 of a first VEGF receptor human Flt1 and Ig domain 3 of a second VEGF receptor human Flk1 or human Flt4; and
 - (ii) a multimerizing component, wherein VEGF activity is inhibited.

2. The method of claim 1, wherein the mammal is a human.

Comparison of aflibercept to Claim 1

Aflibercept inhibits the activation of VEGF receptors by binding to VEGF and thus, is a VEGF antagonist as defined by claim 1. *See* section 12.1 of EYLEA™ label, provided as Attachment B, and as shown further, below. As noted above, aflibercept is a dimeric glycoprotein comprising recombinant fusion polypeptides, each of which consists of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1. *See* section 11 of EYLEA™ label. Aflibercept includes an Ig domain 2 from VEGFR1 fused to an Ig domain 3 from VEGFR2 as described in Holash (See Attachment G, paragraph bridging pages 11393 and 11394 and Figure 1A). The '758 patent refers to VEGFR-1 as Flt1 in column 25, line 57 and refers to VEGFR-2 as Flk1 in column 26, line 12. In addition, Holash discloses that VEGFR-1 is also known as Flt-1 and VEGFR-2 is also known as Flk-1. *See* Figure 1A of Holash. Thus, aflibercept has a VEGF receptor component consisting of an Ig domain 2 of a first VEGF receptor human Flt-1 and an Ig domain 3 of a second VEGF receptor human Flk-1 as defined in claim 1.

Holash further describes VEGFR1 and VEGFR2 on page 11393, in the second paragraph, as being "highly related transmembrane tyrosine kinases that use their ectodomains to bind VEGF." The disclosure of the Flt1 and Flk1 components in the approved product and the construction of the expression vector used in making the active ingredient in the approved product is discussed in the '758 patent in Example 20, column 29, lines 41-56. The amino acid sequence of both the Flt1 and Flk1 components of the approved product are disclosed in Figures 24A-24C. Flt1 Ig domain 2 spans amino acid residues 27 through 129 and Flk1 Ig domain 3 spans amino acid residues 130 through 231 of the fusion protein.

Aflibercept comprises the Fc domain of human IgG1 fused to the extracellular domains from the VEGF receptors. *See* section 11 of EYLEA™ label, provided as Attachment B. A “multimerizing component” of the fusion protein of claim 1 can comprise an immunoglobulin domain, such as the Fc domain of IgG. *See* col. 5, lines 42-46 and col. 7, lines 25-30 of the '758 patent. Thus, aflibercept also includes a multimerizing component as defined in claim 1. The multimerizing component of the fusion protein, the Fc region of human IgG, is referenced throughout the '758 patent. The disclosure of the Fc multimerizing component in the actual product is discussed in Example 20, column 29, lines 41-56, and its amino acid sequence is disclosed in Figures 24A-24C, from amino acid residue 232 through 458.

As described in Section 11 of the EYLEA™ label (Attachment B), EYLEA™, the approved product, is a pharmaceutical composition comprising aflibercept (*i.e.* VEGF antagonist) and an iso-osmotic aqueous solution (*i.e.* pharmaceutically acceptable carrier). As explained above, aflibercept is a dimeric protein comprising two fusion polypeptides, each of which comprises a VEGF receptor component consisting of an Ig domain 2 of a first VEGF receptor human Flt1 and Ig domain 3 of a second VEGF receptor human Flk1 and a multimerizing component. Thus, the approved product is a pharmaceutical composition administered to a mammal according to the method of claim 1.

Aflibercept thus meets all of the limitations of claim 1.

Aflibercept inhibits VEGF activity, as shown in various Examples throughout the '758 patent.

For example, aflibercept was able to block the ability of VEGF to activate its receptor *in vitro*. This was shown by the ability of aflibercept to block VEGF-induced VEGFR2 phosphorylation when added at a 1.5 fold molar excess when compared with added VEGF (*See* the '758 patent, Example 23, column 31, lines 21-67 and column 32, lines 1-10. *See* also Figure 25A-C and Figure 26A-B).

Aflibercept was also effective in blocking VEGF-induced cell proliferation in cells engineered to express a chimeric VEGFR2 receptor, which mediates a strong cell proliferation response to VEGF *in vitro* (*See* the '758 patent, Example 24, column 32, lines 12-51 and also Figure 27).

Furthermore, aflibercept was also effective in inhibition of tumor growth in mice (*See* the '758 patent, Example 30, column 36, lines 48-67 and column 37, lines 1-8 and also Figure 40).

The effect of aflibercept was also studied in the female

reproductive system using two different readouts to assess the effect of aflibercept on VEGF activity in rats. These are described in Example 31 of the '758 patent, column 38, lines 24-67.

In this study, pregnant mare's serum gonadotropin (PMSG) was injected subcutaneously to induce ovulation in pre-pubertal female rats. This results in a surge of estradiol after two days, which in turn causes an induction of VEGF in the uterus. This induction results in hyperpermeability of the uterus and an increase in uterine wet weight 6 hours later. The study was done to determine if aflibercept could block the effect of VEGF on uterine wet weight. The results demonstrated that injection of aflibercept resulted in about a 50% reduction in uterine wet weight. These results are shown in Figure 41.

The other assay measured the effect of aflibercept on VEGF-induced blood vessel formation in the corpus luteum. This allows for secretion of progesterone into the blood stream in order to prepare the uterus for implantation. If aflibercept was effective at blocking VEGF activity in this model, then the animals receiving aflibercept should show a reduction in progesterone in the blood stream. Injection of aflibercept at 25 mg/kg or 5 mg/kg at one hour after injection of PMSG resulted in complete inhibition of progesterone induction at day 4 (See Figure 42A-42B).

Comparison of aflibercept to Claim 2

According to the EYLEA™ label, provided as Attachment B, the approved product is intended for the treatment of human patients. In addition, the '758 patent, at column 6, lines 43-48, lines 52-54 and lines 60-61, discloses several embodiments in which aflibercept can be used in humans. For example, lines 43-48 described a method of decreasing or inhibiting plasma leakage in a mammal comprising administering the fusion polypeptide described, including embodiments wherein the mammal is a human. Lines 52-54 describe a preferred embodiment being a method of blocking blood vessel growth in a human comprising administering the fusion polypeptide of the invention. Lines 60-61 note that preferred embodiments of the methods are wherein the mammal is a human.

Aflibercept thus meets all of the limitations of claim 2.

Conclusion

Because aflibercept meets all of the limitations of claims 1 and 2 of the '758 patent, and because aflibercept is the active ingredient of EYLEA™, claims 1 and 2 of U.S. Patent No. 7,374,758 cover a method of

using the approved product.

10. Relevant Dates Under 35 U.S.C. § 156 for Determination of Applicable Regulatory Review Period [§ 1.740(a)(10)]

(a) Patent Issue Date

The '758 patent issued on May 20, 2008.

(b) IND Effective Date [35 U.S.C. § 156(g)(1)(B)(i); 37 C.F.R. § 1.740(a)(10)(i)(A)]

The date that an exemption under § 505(i) of the Federal Food, Drug and Cosmetic Act became effective (*i.e.*, the date that an investigational new drug application ("IND") became effective) for EYLEA™ (referred to in the IND-receipt letter as "Vascular Endothelial Growth Factor Fc Protein (human, recombinant, CHO cells, Regeneron)") was June 15, 2005. The application date for this IND was May 13, 2005. The IND was assigned number BB-IND # 12462. A copy of the letter from the FDA reflecting the IND's number, date of submission and date of receipt is provided in Attachment H.

(c) BLA Submission Date [35 U.S.C. § 156(g)(1)(B)(i); 37 C.F.R. § 1.740(a)(10)(i)(B)]

The BLA was submitted on February 17, 2011 and received by the FDA on February 18, 2011, as shown in Attachment I. The BLA was assigned number BL 125387/0.

(d) BLA Issue Date [35 U.S.C. § 156(g)(1)(B)(ii); 37 C.F.R. § 1.740(a)(10)(i)(C)]

The FDA approved biologic license application 125387/0 authorizing the marketing of EYLEA™ on November 18, 2011. EYLEA™ was approved under Department of Health and Human Services (DHHS) U.S. License No. 1760. A copy of the approval letter from the FDA is provided as Attachment C.

**11. Summary of Significant Events During Regulatory Review Period
[§1.740(a)(11)]**

Pursuant to 37 C.F.R. § 1.740(a)(11), the following provides a brief description of the activities of Regeneron Pharmaceuticals, Inc., before the FDA in relation to the regulatory review of EYLEA™. The brief description lists the significant events that occurred during the regulatory review period for the approved product. In several instances, communications to or from the FDA are referenced. Pursuant to 37 C.F.R. § 1.740(a)(11), 21 C.F.R. § 60.20(a), and M.P.E.P. § 2753, copies of such communications are not provided in this application, but can be obtained from records maintained by the FDA.

On May 13, 2005 Regeneron submitted to FDA an investigational new drug application for a recombinant fusion protein (originally vascular endothelial growth factor Fc protein, now aflibercept) consisting of (a) a vascular endothelial growth factor (VEGF) receptor component having immunoglobulin-like (Ig) domains consisting of an Ig domain 2 of a first VEGF receptor that is human Flt1 and an Ig domain 3 of a second VEGF receptor that is human Flk1; and (b) an Fc portion of human IgG1. The fusion protein was developed as a potential new therapeutic for intravitreal administration for treating (wet) age-related macular degeneration.

On June 15, 2005, BB-IND #12462 became effective via a communication mailed to Regeneron on May 24, 2005, (see Attachment H). According to the FDA, initiation of trials could begin 30 days after May 16, 2005.

From approximately July 2005 until approximately June 2008, a series of U.S. Phase I and II clinical trials were conducted. In addition, an extension trial is ongoing as of the date of this application.

Between approximately July 2007 and July 2011, Phase III clinical trials were conducted. In addition, an extension trial is ongoing as of the date of this application.

On June 1, 2009, representatives of Regeneron and CBER participated in a Type C meeting to seek agency agreement on the pharmacology/toxicology program for aflibercept.

On September 15, 2009, representatives of Regeneron and CBER participated in a Type C meeting to review the status and development of pre-filled syringes as the intended container-closure device to support commercialization of aflibercept.

On September 8, 2010, representatives of Regeneron and CBER participated in a Type B pre-BLA submission meeting to discuss and review clinical results of trials conducted prior to that date.

On September 27, 2010, representatives of Regeneron and CBER participated in a Type B pre-BLA submission meeting to discuss information and requirements for the chemical, manufacturing and control (CMC) chapter of the BLA.

On April 15, 2011, FDA granted priority review for aflibercept for AMD.

On November 18, 2011, FDA approved BLA No. BL 125387/0, issuing marketing authorization for EYLEA™. (See Attachment C.)

12. Statement Concerning Eligibility for and Duration of Extension Sought Under 35 U.S.C. § 156 [37 C.F.R § 1.740(a)(12)]

(a) In the opinion of the Applicant, U.S. Patent No. 7,374,758 is eligible for an extension under § 156 because:

(i) one or more claims of the '758 patent claim a method of using the approved product;

(ii) the term of the '758 patent has not been previously extended on the basis of § 156;

(iii) the '758 patent has not expired;

(iv) no other patent has been extended pursuant to § 156 on the basis of the regulatory review process associated with the approved product, EYLEA™ ;

(v) there is an eligible period of regulatory review by which the patent may be extended pursuant to § 156;

(vi) the applicant for marketing approval exercised due diligence within the meaning of § 156(d)(3) during the period of regulatory review;

(vii) the present application has been submitted within the 60-day period following the approval date of the approved product, pursuant to § 156(c); and

(viii) this application otherwise complies with all requirements of 35 U.S.C. § 156 and applicable rules and procedures.

(b) The period by which the term of the '758 patent is requested by Applicant to be extended is 775 days.

(c) The requested period of extension of term for the '758 patent corresponds to the regulatory review period that is eligible for extension pursuant to §156, based on the facts and circumstances of the regulatory review associated with the approved product EYLEA™. The period was determined as follows.

(i) The relevant dates for calculating the regulatory review period, based on the events discussed in the section above, are the following.

Exemption under FDCA § 505(i) became effective June 15, 2005 (30 days after FDA receipt of the IND on May 16, 2005)

Patent was granted May 20, 2008

Biologics License Application (BLA) under PHSA § 351 was filed February 17, 2011

BLA was approved November 18, 2011

(ii) The '758 patent was granted during the period specified in §156(g)(1)(B)(i) (*i.e.*, the period from the date of the grant of the exemption under § 505(i) of the FDCA until the date of submission of the BLA). Pursuant to § 156(b) and (c)(2), the calculated regulatory review period includes a component equal to half of the number of days within that period that are after the grant of the patent (1/2 of 1002, or 501 days).

(iii) Because the patent was granted before the start of the period specified in § 156(g)(1)(B)(ii) (*i.e.*, the period from the date of submission of the BLA until the date of approval), the regulatory review period under § 156(b) includes a component equal to the total number of days in that period (274 days).

(iv) The '758 patent will expire on January 17, 2021 taking into account the Terminal Disclaimer (*See* Attachment E).

(v) Taking into account the 501 days specified in (ii) above, which accounts for the time period between the date of grant of the exemption under § 505(i) of the FDCA until the date of submission of the BLA), and further pursuant to § 156(b) and (c)(2), whereby the calculated regulatory review period includes a component equal to half of the number of days within that period that are after the grant of the patent, plus the 274 days specified in the regulatory review period under § 156(b) and noted in (iii) above, which includes the number of days from the date of submission of the BLA until the date of approval, the total number of days to be included for consideration of patent term extension is believed to be 775 days.

(vi) The date of approval of the approved product is November 18, 2011.

(vii) The date that is fourteen years from the date of approval of the approved product is November 18, 2025.

(viii) The addition of 775 days to the time of patent expiry (which includes the period disclaimed via the terminal disclaimer) brings the projected patent expiry date to March 3, 2023. The date until the end of the fourteen-year period specified in §156(c)(3) is November 18, 2025. Accordingly, the projected date of expiration taking into account the 775 day patent term extension does not exceed the date projected to be 14 years beyond the date of BLA approval. As such, the period by which the patent may be extended is not limited by the fourteen-year rule of § 156(c)(3).

(ix) The '758 patent issued after the effective date of Public Law No. 98-417. As such, the two- or three -year limit of 35 U.S.C. § 156(g)(6)(C) does not apply.

13. Statement Pursuant to 37 C.F.R. § 1.740(a)(13)

Pursuant to 37 C.F.R. § 1.740(a)(13), Applicant acknowledges its duty to disclose to the Director of the PTO and to the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought, particularly as that duty is defined in 37 C.F.R. § 1.765. In furtherance of this duty, Applicant wishes to inform the Director and Secretary that concurrently with the present Application for Extension of Patent Term Under 35 U.S.C. §156 Applicant has filed an Application for Extension of Patent Term Under 35 U.S.C. §156 (plus two copies) in connection with U.S. Patent Nos. 7,374,757 and 7,070,959.

14. Applicable Fee [§ 1.740(a)(14)]

Please deduct all fees necessary pursuant to 37 C.F.R. §1.20(j) corresponding to the fee for a patent term extension application under 35 U.S.C. § 156 from deposit account no. 18-0650. Please deduct any additional fee or fees deemed necessary in excess of this amount from our deposit account no. 18-0650.

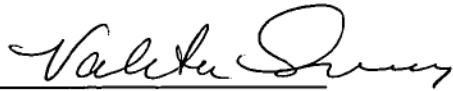
15. Name and Address for Correspondence [§ 1.740(a)(15)]

Please direct all inquiries, questions, and communications regarding this application for term extension to :

Valeta Gregg, Ph.D., J.D.
Vice President and Assistant General Counsel, Patents
Regeneron Pharmaceuticals, Inc.
777 Old Saw Mill River Rd.
Tarrytown, NY 10591-6707
Tel. 914-847-1077
Fax 914-847-7705
email: valeta.gregg@regeneron.com

Two additional copies of this application are enclosed, in compliance with 37 C.F.R. § 1.740(b).

Sincerely,



Attorney/Agent for Applicant

Registration No. 35,127

Dated: 21 Dec 2011

Index of Attachments

Attachment A – Copy of Assignment of U.S. Patent No. 7,374,758

Attachment B – Copy of EYLEA™ Product Label

Attachment C – Copy of EYLEA™ BLA Approval letter from the FDA

Attachment D – Copy of U.S. Patent No. 7,374,758

Attachment E – Copy of Terminal Disclaimer for U.S. Patent No. 7,374,758

Attachment F – Copy of Maintenance Fee Statement for U.S. Patent No. 7,374,758

Attachment G – Copy of Holash *et al.* PNAS 99(17):11393-11398 (2002)

Attachment H – Copy of IND-receipt letter from FDA

Attachment I – Copy of BLA Submission acknowledgement letter from FDA

ATTACHMENT A

Copy of Assignment of U.S. Patent No.

7,374,758

12-17-04

12-30-2004

Form PTO-1595

(Rev. 10/02)

OMB No. 0651-0027 (exp. 8/30/2005)

Tab settings

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U.S. DEPARTMENT OF COMMERCE
U.S. Patent and Trademark Office

16018 U.S. PTO

11/016503



121704

To the Honorable Commissioner of Patents and Trademarks: Please record the attached original documents or copy thereof.

1. Name of conveying party(ies):

Nicholas J. Papadopoulos

Samuel Davis

George D. Yancopoulos

2. Name and address of receiving party(ies)

Name: Regeneron Pharmaceuticals, Inc.

Internal Address: _____

Additional name(s) of conveying party(ies) attached? ☐ Yes ☒ No

3. Nature of conveyance:



Assignment



Merger



Security Agreement



Change of Name



Other _____

Street Address: 777 Old Saw Mill River RoadCity: Tarrytown State: NY Zip: 10591Execution Date: February 4, 2002Additional name(s) & address(es) attached? ☐ Yes ☒ No

4. Application number(s) or patent number(s):

If this document is being filed together with a new application, the execution date of the application is: 2/4/02

A. Patent Application No.(s) _____

B. Patent No.(s) _____

Additional numbers attached? ☐ Yes ☒ No

5. Name and address of party to whom correspondence concerning document should be mailed:

Name: Valeta GreggInternal Address: Regeneron Pharmaceuticals, IncStreet Address: 777 Old Saw Mill River RoadCity: Tarrytown State: NY Zip: 105916. Total number of applications and patents involved: 17. Total fee (37 CFR 3.41).....\$ 40.00

Enclosed



Authorized to be charged to deposit account

8. Deposit account number:

18-0650

DO NOT USE THIS SPACE

9. Signature.

Valeta Gregg

Name of Person Signing

Valeta Gregg
SignatureDecember 17, 2004

Date

Total number of pages including cover sheet, attachments, and documents: 4

Mail documents to be recorded with required cover sheet information to:

Commissioner of Patents & Trademarks, Box Assignments
Washington, D.C. 20231

12/29/2004 6TON11

00000175 180650

11016503

01 FC:0021

40.00 BA

PATENT
REEL: 016103 FRAME: 0015

ASSIGNMENT

WHEREAS, We, Nicholas J. Papadopoulos, residing at 59 Heritage Lane, Lagrangeville, New York 12540, a citizen of the United States of America, Samuel Davis, residing at 332 W. 88th Street #B2, New York, New York 10024, a citizen of the United States of America, and George D. Yancopoulos, residing at 1519 Baptist Church Road, Yorktown Heights, New York 10598, a citizen of the United States of America (HEREINAFTER CALLED "ASSIGNORS") are inventor(s) of the invention(s) disclosed and/or claimed in the following patent application:

USSN 10/009,852 filed December 6, 2001,

which is the national stage filing of PCT/US00/14142 filed June 8, 1999;

WHEREAS, REGENERON PHARMACEUTICALS INC., a corporation organized and existing under the laws of the State of New York, with offices at 777 Old Saw Mill River Road, Tarrytown, New York 10591-6707, U.S.A. (HEREINAFTER called "ASSIGNEE") is desirous of acquiring our entire right, title and interest in, to, and under the said applications;

NOW, THEREFORE, in consideration of the sum of One Dollar (\$1.00) to us in hand paid, and other good and valuable consideration, the receipt of which is hereby acknowledged, We, the said ASSIGNORS, have sold, assigned, transferred and set over, and by these presents do hereby sell, assign, transfer and set over unto the said ASSIGNEE, its successors, legal representatives, and assigns, our entire right, title and interest for all countries in and to any and all inventions which are disclosed and claimed, and any and all inventions which are disclosed but not claimed in the above-described United States applications, and in and to said United States Applications and all divisions, renewals, continuations, and continuations-in-part thereof, and all Patents of the United States which may be granted thereon and all reissues and extensions thereof; and all applications for industrial property protection, including, without limitation, all applications for patents utility models, and designs which may hereafter be filed for said inventions in any country or countries foreign to the United States, together with the right to file such applications and the right to claim for the same the priority rights derived from said United States applications under the Patent Laws of the United States, the International Convention of 1883 and later modifications thereof, under the Patent Cooperation Treaty, under the European Patent Convention, or under any other available international agreement or under the domestic laws of the country in which any such application is filed, as may be applicable; and all forms of industrial property protection, including, without limitation, patents, utility models, inventors' certificates and designs which may be granted for said inventions in any country or countries foreign to the United States and all extensions, renewals and reissues thereof;

AND WE HEREBY authorize and request the Commissioner of Patents and Trademarks of the United States and any Official of any country or countries foreign to the United States

PATENT
REEL: 016103 FRAME: 0016

whose duty it is to issue patents or other evidence or forms of industrial property protection on applications as aforesaid, to issue the same to the said ASSIGNEE, their successors, legal representatives and assigns, in accordance with this instrument;

AND WE HEREBY covenant and agree that WE have full right to convey the entire interest HEREINAFTER assigned, and that WE have not executed, and will not execute, any agreement in conflict herewith;

AND WE HEREBY further covenant and agree that WE will communicate to the said ASSIGNEE, its successors, legal representatives and assigns, any facts known to us respecting said inventions, and testify in any legal proceeding, sign all lawful papers, execute all divisional, continuing, continuation-in-part, reissue and foreign applications, make all rightful oaths, and generally do everything possible to aid the said ASSIGNEE, its successors, legal representatives and assigns, to obtain and enforce proper protection for said inventions in all countries.

IN TESTIMONY HEREOF, WE hereunto set our hands and seals the day and year set opposite our signatures.

Dated: 2/4/02

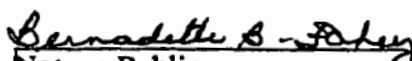

Nicholas J. Papadopoulos L.S.

State of New York

S.S.

County of Westchester

On this 4th day February 2002, before me, Bernadette B. Fahey, a Notary Public in and for the State and County aforesaid, personally appeared Nicholas J. Papadopoulos, to me known, and to me known to be the person of that name, who signed and sealed the foregoing instrument, and acknowledged the same to be his free act and deed.


Notary Public

BERNADETTE B. FAHEY
Notary Public, State of New York
No. 01FA6035879
Qualified in Putnam County
Commission Expires January 10, 2006

PATENT
REEL: 016103 FRAME: 0017

Dated: 2/4/02

Samuel Davis L.S.
Samuel Davis

State of New York

S.S.

County of Westchester

On this 4th day February 2002, before me,
Bernadette B. Fahey, a Notary Public in and for the State and County aforesaid, personally
appeared Samuel Davis, to me known, and to me known to be the person of that name, who
signed and sealed the foregoing instrument, and acknowledged the same to be his free act and
deed.

BERNADETTE B. FAHEY
Notary Public, State of New York
No. 01FA6035879
Qualified in Putnam County
Commission Expires January 10, 2006

Bernadette B. Fahey
Notary Public

Dated: 2/4/02

George D. Yancopoulos L.S.
George D. Yancopoulos

State of New York

S.S.

County of Westchester

On this 4th day February 2002, before me,
Bernadette B. Fahey, a Notary Public in and for the State and County aforesaid, personally
appeared George D. Yancopoulos, to me known, and to me known to be the person of that name,
who signed and sealed the foregoing instrument, and acknowledged the same to be his free act
and deed.

Bernadette B. Fahey
Notary Public

BERNADETTE B. FAHEY
Notary Public, State of New York
No. 01FA6035879
Qualified in Putnam County
Commission Expires January 10, 2006

ATTACHMENT B

Copy of EYLEA™

Product Label

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use EYLEA safely and effectively. See full prescribing information for EYLEA.

EYLEA™ (aflibercept) Injection
For Intravitreal Injection
Initial U.S. Approval: 2011

INDICATIONS AND USAGE

EYLEA is indicated for the treatment of patients with Neovascular (Wet) Age-Related Macular Degeneration (AMD). (1)

DOSAGE AND ADMINISTRATION

- For ophthalmic intravitreal injection only. (2.1)
- The recommended dose for EYLEA is 2 mg (0.05 mL) administered by intravitreal injection every 4 weeks (monthly) for the first 3 months, followed by 2 mg (0.05 mL) via intravitreal injection once every 8 weeks (2 months). (2.2)
- Although EYLEA may be dosed as frequently as 2 mg every 4 weeks (monthly), additional efficacy was not demonstrated when EYLEA was dosed every 4 weeks compared to every 8 weeks. (2.2)

DOSAGE FORMS AND STRENGTHS

40 mg/mL solution for intravitreal injection in a single-use vial (3)

CONTRAINDICATIONS

- Ocular or periocular infection (4.1)
- Active intraocular inflammation (4.2)
- Hypersensitivity (4.3)

WARNINGS AND PRECAUTIONS

- Endophthalmitis and retinal detachments may occur following intravitreal injections. Patients should be instructed to report any symptoms suggestive of endophthalmitis or retinal detachment without delay and should be managed appropriately. (5.1)
- Increases in intraocular pressure have been seen within 60 minutes of an intravitreal injection. (5.2)

ADVERSE REACTIONS

The most common adverse reactions (≥5%) reported in patients receiving EYLEA were conjunctival hemorrhage, eye pain, cataract, vitreous detachment, vitreous floaters, and increased intraocular pressure. (6.2)

To report SUSPECTED ADVERSE REACTIONS, contact Regeneron at 1-855-395-3248 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 11/2011

FULL PRESCRIBING INFORMATION: CONTENTS***1 INDICATIONS AND USAGE****2 DOSAGE AND ADMINISTRATION****2.1 General Dosing Information****2.2 Dosing****2.3 Preparation for Administration****2.4 Administration****3 DOSAGE FORMS AND STRENGTHS****4 CONTRAINDICATIONS****4.1 Ocular or Periocular Infections****4.2 Active Intraocular Inflammation****4.3 Hypersensitivity****5 WARNINGS AND PRECAUTIONS****5.1 Endophthalmitis and Retinal Detachments****5.2 Increase in Intraocular Pressure****5.3 Thromboembolic Events****6 ADVERSE REACTIONS****6.1 Injection Procedure****6.2 Clinical Studies Experience****6.3 Immunogenicity****8 USE IN SPECIFIC POPULATIONS****8.1 Pregnancy****8.3 Nursing Mothers****8.4 Pediatric Use****8.5 Geriatric Use****11 DESCRIPTION****12 CLINICAL PHARMACOLOGY****12.1 Mechanism of Action****12.2 Pharmacodynamics****12.3 Pharmacokinetics****13 NONCLINICAL TOXICOLOGY****13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility****13.2 Animal Toxicology and/or Pharmacology****14 CLINICAL STUDIES****16 HOW SUPPLIED/STORAGE AND HANDLING****17 PATIENT COUNSELING INFORMATION**

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

EYLEA is indicated for the treatment of patients with Neovascular (Wet) Age-Related Macular Degeneration (AMD).

2 DOSAGE AND ADMINISTRATION

2.1 General Dosing Information

FOR OPHTHALMIC INTRAVITREAL INJECTION ONLY. EYLEA must only be administered by a qualified physician.

2.2 Dosing

The recommended dose for EYLEA is 2 mg (0.05 mL or 50 microliters) administered by intravitreal injection every 4 weeks (monthly) for the first 12 weeks (3 months), followed by 2 mg (0.05 mL) via intravitreal injection once every 8 weeks (2 months). Although EYLEA may be dosed as frequently as 2 mg every 4 weeks (monthly), additional efficacy was not demonstrated when EYLEA was dosed every 4 weeks compared to every 8 weeks [*see Clinical Studies (14)*].

2.3 Preparation for Administration

EYLEA should be inspected visually prior to administration. If particulates, cloudiness, or discoloration are visible, the vial must not be used.

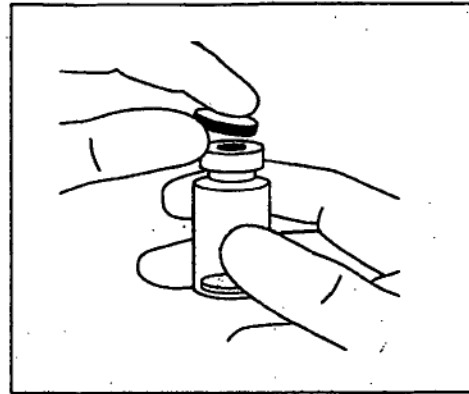
Using aseptic technique, the intravitreal injection should be performed with a 30-gauge x ½-inch injection needle.

Vial

The glass vial is for single use only.

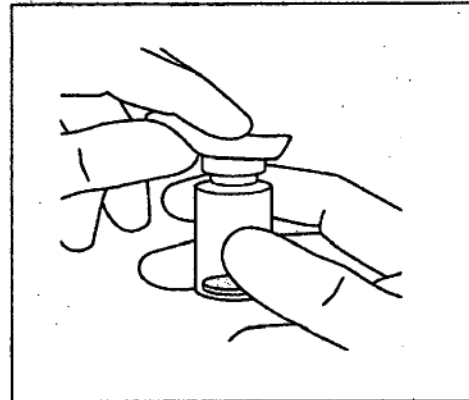
1. Remove the protective plastic cap from the vial (see Figure 1).

Figure 1:



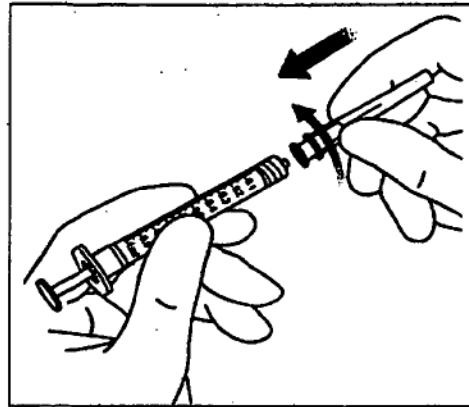
2. Clean the top of the vial with an alcohol wipe (see Figure 2).

Figure 2:



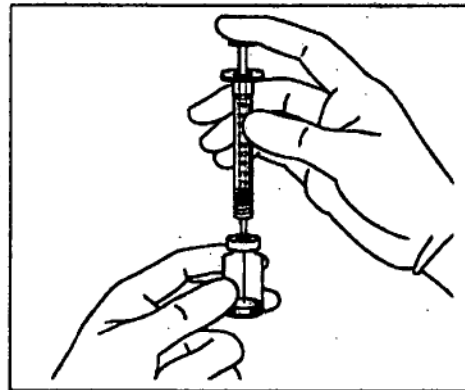
3. Remove the 19-gauge x 1½-inch, 5-micron, filter needle from its pouch and remove the 1-mL syringe supplied in the carton from its pouch. Attach the filter needle to the syringe by twisting it onto the Luer lock syringe tip (see Figure 3).

Figure 3:



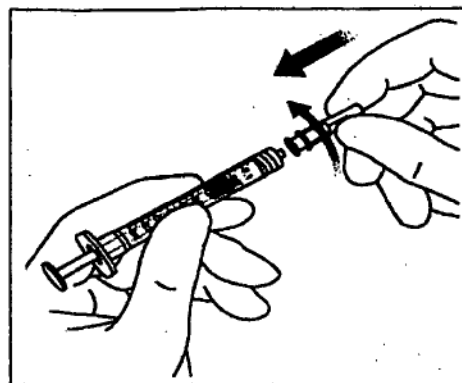
4. Push the filter needle into the center of the vial stopper until the needle touches the bottom edge of the vial.
5. Using aseptic technique withdraw all of the EYLEA vial contents into the syringe, keeping the vial in an upright position, slightly inclined to ease complete withdrawal (see Figure 4).

Figure 4:



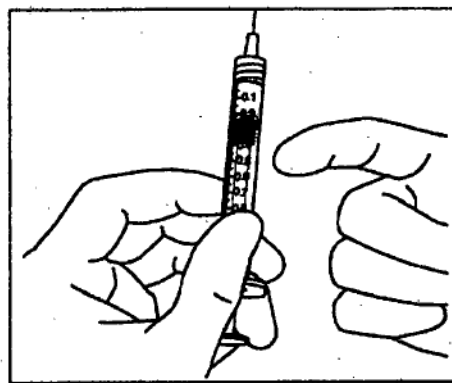
6. Ensure that the plunger rod is drawn sufficiently back when emptying the vial in order to completely empty the filter needle.
7. Remove the filter needle from the syringe and properly dispose of the filter needle.
Note: Filter needle is **not** to be used for intravitreal injection.
8. Remove the 30-gauge x ½-inch injection needle from the plastic pouch and attach the injection needle to the syringe by firmly twisting the injection needle onto the Luer lock syringe tip (see Figure 5).

Figure 5:



9. When ready to administer EYLEA, remove the plastic needle shield from the needle.
10. Holding the syringe with the needle pointing up, check the syringe for bubbles. If there are bubbles, gently tap the syringe with your finger until the bubbles rise to the top (see Figure 6).

Figure 6:



11. To eliminate all of the bubbles and to expel excess drug, SLOWLY depress the plunger so that the plunger tip aligns with the line that marks 0.05 mL on the syringe (see Figures 7 and 8).

Figure 7:

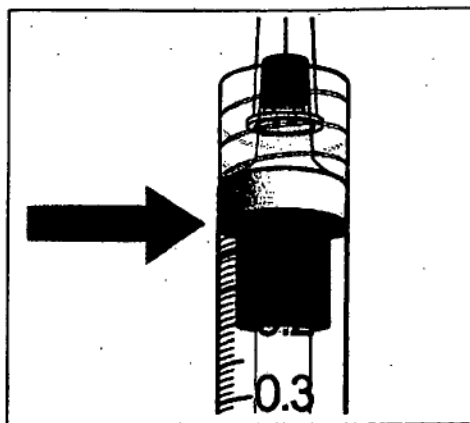
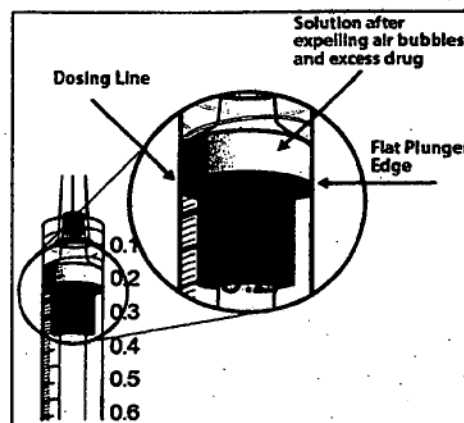


Figure 8:



2.4 Administration

The intravitreal injection procedure should be carried out under controlled aseptic conditions, which include surgical hand disinfection and the use of sterile gloves, a sterile drape, and a sterile eyelid speculum (or equivalent). Adequate anesthesia and a topical broad-spectrum microbicide should be given prior to the injection.

Immediately following the intravitreal injection, patients should be monitored for elevation in intraocular pressure. Appropriate monitoring may consist of a check for perfusion of the optic nerve head or tonometry. If required, a sterile paracentesis needle should be available.

Following intravitreal injection, patients should be instructed to report any symptoms suggestive of endophthalmitis or retinal detachment (e.g., eye pain, redness of the eye, photophobia, blurring of vision) without delay [*see Patient Counseling Information (17)*].

Each vial should only be used for the treatment of a single eye. If the contralateral eye requires treatment, a new vial should be used and the sterile field, syringe, gloves, drapes, eyelid speculum, filter, and injection needles should be changed before EYLEA is administered to the other eye.

After injection, any unused product must be discarded.

No special dosage modification is required for any of the populations that have been studied (e.g., gender, elderly).

3 DOSAGE FORMS AND STRENGTHS

Single-use, glass vial designed to provide 0.05 mL of 40 mg/mL solution for intravitreal injection.

4 CONTRAINDICATIONS

4.1 Ocular or Periocular Infections

EYLEA is contraindicated in patients with ocular or periocular infections.

4.2 Active Intraocular Inflammation

EYLEA is contraindicated in patients with active intraocular inflammation.

4.3 Hypersensitivity

EYLEA is contraindicated in patients with known hypersensitivity to aflibercept or any of the excipients in EYLEA.

5 WARNINGS AND PRECAUTIONS

5.1 Endophthalmitis and Retinal Detachments

Intravitreal injections, including those with EYLEA, have been associated with endophthalmitis and retinal detachments [*see Adverse Reactions (6.1)*]. Proper aseptic injection technique must always be used when administering EYLEA. Patients should be instructed to report any symptoms suggestive of endophthalmitis or retinal detachment without delay and should be managed appropriately [*see Dosage and Administration (2.4) and Patient Counseling Information (17)*].

5.2 Increase in Intraocular Pressure

Acute increases in intraocular pressure have been seen within 60 minutes of intravitreal injection, including with EYLEA [*see Adverse Reactions (6.1)*]. Sustained increases in intraocular pressure have also been reported after repeated intravitreal dosing with VEGF inhibitors. Intraocular pressure and the perfusion of the optic nerve head should be monitored and managed appropriately [*see Dosage and Administration (2.4)*].

5.3 Thromboembolic Events

There is a potential risk of arterial thromboembolic events (ATEs) following intravitreal use of VEGF inhibitors, including EYLEA. ATEs are defined as nonfatal stroke, nonfatal myocardial infarction, or vascular death (including deaths of unknown cause). The incidence in the VIEW1 and VIEW2 wet AMD studies during the first year was 1.8% (32 out of 1824) in the combined group of patients treated with EYLEA [*see Clinical Studies (14)*].

6 ADVERSE REACTIONS

The following adverse reactions are discussed in detail in other sections of the labeling:

- Endophthalmitis and retinal detachments [see *Warnings and Precautions (5.1)*]
- Increased intraocular pressure [see *Warnings and Precautions (5.2)*]
- Thromboembolic events [see *Warnings and Precautions (5.3)*]

The most common adverse reactions ($\geq 5\%$) reported in patients receiving EYLEA were conjunctival hemorrhage, eye pain, cataract, vitreous detachment, vitreous floaters, and increased intraocular pressure.

6.1 Injection Procedure

Serious adverse reactions related to the injection procedure have occurred in $<0.1\%$ of intravitreal injections with EYLEA including endophthalmitis, traumatic cataract, and increased intraocular pressure.

6.2 Clinical Studies Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

The data described below reflect exposure to EYLEA in 1824 patients with wet AMD, including 1223 patients treated with the 2-mg dose, in 2 double-masked, active-controlled clinical studies (VIEW1 and VIEW2) for 12 months [see *Clinical Studies (14)*].

Table 1: Most Common Adverse Reactions ($\geq 1\%$) in Phase 3 wet AMD studies

Adverse Reactions	EYLEA (N=1824)	Active Control (ranibizumab) (N=595)
Conjunctival hemorrhage	25%	28%
Eye pain	9%	9%
Cataract	7%	7%
Vitreous detachment	6%	6%
Vitreous floaters	6%	7%
Intraocular pressure increased	5%	7%
Conjunctival hyperemia	4%	8%
Corneal erosion	4%	5%

Adverse Reactions	EYLEA (N=1824)	Active Control (ranibizumab) (N=595)
Detachment of the retinal pigment epithelium	3%	3%
Injection site pain	3%	3%
Foreign body sensation in eyes	3%	4%
Lacrimation increased	3%	1%
Vision blurred	2%	2%
Retinal pigment epithelium tear	2%	1%
Injection site hemorrhage	1%	2%
Eyelid edema	1%	2%
Corneal edema	1%	1%

Less common serious adverse reactions reported in <1% of the patients treated with EYLEA were retinal detachment, retinal tear, and endophthalmitis. Hypersensitivity has also been reported in less than 1% of the patients treated with EYLEA.

6.3 Immunogenicity

As with all therapeutic proteins, there is a potential for an immune response in patients treated with EYLEA. The immunogenicity of EYLEA was evaluated in serum samples. The immunogenicity data reflect the percentage of patients whose test results were considered positive for antibodies to EYLEA in immunoassays. The detection of an immune response is highly dependent on the sensitivity and specificity of the assays used, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to EYLEA with the incidence of antibodies to other products may be misleading.

In the phase 3 studies, the pre-treatment incidence of immunoreactivity to EYLEA was 1% to 3% across treatment groups. After dosing with EYLEA for 52 weeks, antibodies to EYLEA were detected in a similar percentage range of patients. There were no differences in efficacy or safety between patients with or without immunoreactivity.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C. Aflibercept produced embryo-fetal toxicity when administered during organogenesis in pregnant rabbits at intravenous doses of 3 to 60 mg/kg. A series of external, visceral, and skeletal malformations were observed in the fetuses. The maternal No Observed

Adverse Effect Level (NOAEL) was 3 mg/kg, whereas the fetal NOAEL was below 3 mg/kg. At this dose, the systemic exposures based on C_{max} and AUC for free aflibercept were approximately 2900 times and 600 times higher, respectively, when compared to corresponding values observed in humans after an intravitreal dose of 2 mg.

There are no adequate and well-controlled studies in pregnant women. EYLEA should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

8.3 Nursing Mothers

It is unknown whether aflibercept is excreted in human milk. Because many drugs are excreted in human milk, a risk to the breastfed child cannot be excluded. EYLEA is not recommended during breastfeeding. A decision must be made whether to discontinue nursing or to discontinue treatment with EYLEA, taking into account the importance of the drug to the mother.

8.4 Pediatric Use

The safety and effectiveness of EYLEA in pediatric patients have not been established.

8.5 Geriatric Use

In the clinical studies, approximately 89% (1616/1817) of patients randomized to treatment with EYLEA were ≥ 65 years of age and approximately 63% (1139/1817) were ≥ 75 years of age. No significant differences in efficacy or safety were seen with increasing age in these studies.

11 DESCRIPTION

EYLEA (aflibercept) is a recombinant fusion protein consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1 formulated as an iso-osmotic solution for intravitreal administration. Aflibercept is a dimeric glycoprotein with a protein molecular weight of 97 kilodaltons (kDa) and contains glycosylation, constituting an additional 15% of the total molecular mass, resulting in a total molecular weight of 115 kDa. Aflibercept is produced in recombinant Chinese hamster ovary (CHO) cells.

EYLEA is a sterile, clear, and colorless to pale yellow solution. EYLEA is supplied as a preservative-free, sterile, aqueous solution in a single-use, glass vial designed to deliver 0.05 mL (50 microliters) of EYLEA (40 mg/mL in 10 mM sodium phosphate, 40 mM sodium chloride, 0.03% polysorbate 20, and 5% sucrose, pH 6.2).

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Vascular endothelial growth factor-A (VEGF-A) and placental growth factor (PlGF) are members of the VEGF family of angiogenic factors that can act as mitogenic, chemotactic, and vascular permeability factors for endothelial cells. VEGF acts via two receptor tyrosine kinases, VEGFR-1 and VEGFR-2, present on the surface of endothelial cells. PlGF binds only to VEGFR-1, which is also present on the surface of leucocytes. Activation of these receptors by VEGF-A can result in neovascularization and vascular permeability.

Aflibercept acts as a soluble decoy receptor that binds VEGF-A and PlGF, and thereby can inhibit the binding and activation of these cognate VEGF receptors.

12.2 Pharmacodynamics

In the phase 3 studies anatomic measures of disease activity improved similarly in all treatment groups from baseline to week 52. Anatomic data were not used to influence treatment decisions.

12.3 Pharmacokinetics

EYLEA is administered intravitreally to exert local effects in the eye. In patients with wet AMD, following intravitreal administration of EYLEA, a fraction of the administered dose is expected to bind with endogenous VEGF in the eye to form an inactive aflibercept: VEGF complex. Once absorbed into the systemic circulation, aflibercept presents in the plasma as free aflibercept (unbound to VEGF) and a more predominant stable inactive form with circulating endogenous VEGF (i.e., aflibercept: VEGF complex).

Absorption/Distribution

Following intravitreal administration of 2 mg per eye of EYLEA to patients with wet AMD, the mean C_{max} of free aflibercept in the plasma was 0.02 mcg/mL (range: 0 to 0.054 mcg/mL) and was attained in 1 to 3 days. The free aflibercept plasma concentrations were undetectable two weeks post-dosing in all patients. Aflibercept did not accumulate in plasma when administered as repeated doses intravitreally every 4 weeks. It is estimated that after intravitreal administration of 2 mg to patients, the mean maximum plasma concentration of free aflibercept is more than 100 fold lower than the concentration of aflibercept required to half-maximally bind systemic VEGF.

The volume of distribution of free aflibercept following intravenous (I.V.) administration of aflibercept has been determined to be approximately 6L.

Metabolism/Elimination

Aflibercept is a therapeutic protein and no drug metabolism studies have been conducted. Aflibercept is expected to undergo elimination through both target-mediated disposition via binding to free endogenous VEGF and metabolism via proteolysis. The terminal elimination half-life ($t_{1/2}$) of free aflibercept in plasma was approximately 5 to 6 days after I.V. administration of doses of 2 to 4 mg/kg aflibercept.

Specific Populations

Renal Impairment

Pharmacokinetic analysis of a subgroup of patients (n=492) in one Phase 3 study, of which 43% had renal impairment (mild n=120, moderate n=74, and severe n=16), revealed no differences with respect to plasma concentrations of free aflibercept after intravitreal administration every 4 or 8 weeks. No dose adjustment based on renal impairment status is needed.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No studies have been conducted on the mutagenic or carcinogenic potential of aflibercept. Effects on male and female fertility were assessed as part of a 6-month study in monkeys with intravenous administration of aflibercept at doses ranging from 3 to 30 mg/kg. Absent or irregular menses associated with alterations in female reproductive hormone levels and changes in sperm morphology and motility were observed at all dose levels. In addition, females showed decreased ovarian and uterine weight accompanied by compromised luteal development and reduction of maturing follicles. These changes correlated with uterine and vaginal atrophy. A No Observed Adverse Effect Level (NOAEL) was not identified. Based on C_{max} and AUC for free aflibercept observed at the lowest dose used of 3 mg/kg, the systemic exposures were approximately 4900 times and 1500 times higher, respectively, than the exposure observed in humans after an intravitreal dose of 2 mg. All changes were reversible.

13.2 Animal Toxicology and/or Pharmacology

Erosions and ulcerations of the respiratory epithelium in nasal turbinates in monkeys treated with aflibercept intravitreally were observed at intravitreal doses of 2 or 4 mg/eye. At the NOAEL of 0.5 mg/eye in monkeys, the systemic exposure was 42 times and 56 times higher based on C_{max} and AUC, respectively, than the exposure observed in humans after an intravitreal dose of 2 mg. Similar effects were not seen in clinical studies [see *Clinical Studies (14)*].

14 CLINICAL STUDIES

The safety and efficacy of EYLEA were assessed in two randomized, multi-center, double-masked, active-controlled studies in patients with wet AMD. A total of 2412 patients were treated and evaluable for efficacy (1817 with EYLEA) in the two studies (VIEW1 and VIEW2).

In each study, patients were randomly assigned in a 1:1:1:1 ratio to 1 of 4 dosing regimens:

1) EYLEA administered 2 mg every 8 weeks following 3 initial monthly doses (EYLEA 2Q8); 2) EYLEA administered 2 mg every 4 weeks (EYLEA 2Q4); 3) EYLEA 0.5 mg administered every 4 weeks (EYLEA 0.5Q4); and 4) ranibizumab administered 0.5 mg every 4 weeks (ranibizumab 0.5 mg Q4). Patient ages ranged from 49 to 99 years with a mean of 76 years.

In both studies, the primary efficacy endpoint was the proportion of patients who maintained vision, defined as losing fewer than 15 letters of visual acuity at week 52 compared to baseline. Data are available through week 52. Both EYLEA 2Q8 and EYLEA 2Q4 groups were shown to have efficacy that was clinically equivalent to the ranibizumab 0.5 mg Q4 group.

Detailed results from the analysis of the VIEW1 and VIEW2 studies are shown in Table 2 and Figure 9 below.

Table 2: Efficacy Outcomes at Week 52 (Full Analysis Set with LOCF) in VIEW1 and VIEW2 Studies

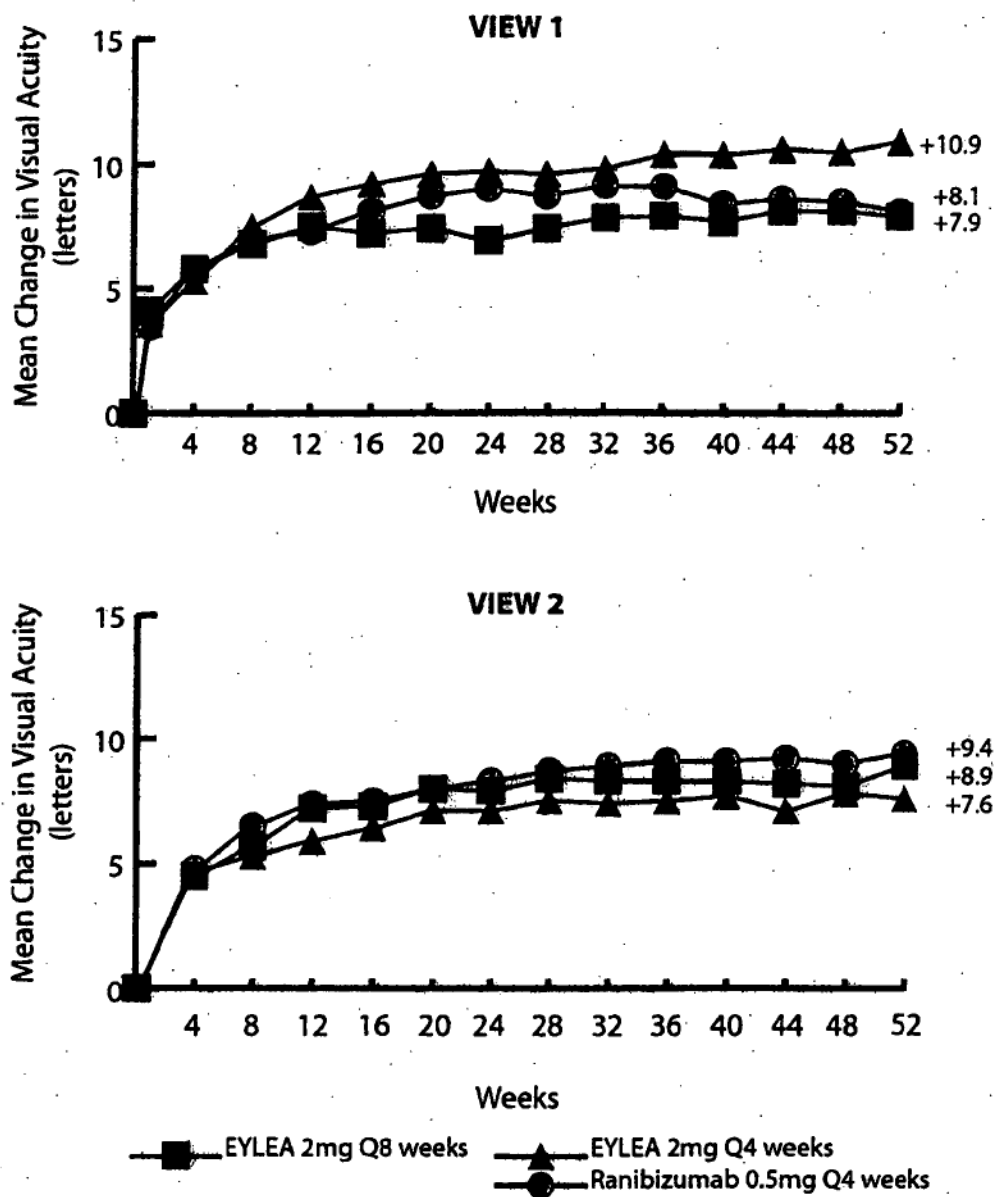
	VIEW1			VIEW2		
	EYLEA 2 mg Q8 weeks ^a	EYLEA 2 mg Q4 weeks	ranibizu- mab 0.5 mg Q4 weeks	EYLEA 2 mg Q8 weeks ^a	EYLEA 2 mg Q4 weeks	ranibizu- mab 0.5 mg Q4 weeks
Full Analysis Set	N=301	N=304	N=304	N=306	N=309	N=291
Efficacy Outcomes						
Proportion of patients who maintained visual acuity (%) (<15 letters of BCVA loss)	94%	95%	94%	95%	95%	95%
Difference ^b (%) (95.1% CI)	0.6 (-3.2, 4.4)	1.3 (-2.4, 5.0)		0.6 (-2.9, 4.0)	-0.3 (-4.0, 3.3)	
Mean change in BCVA as measured by ETDRS letter score from Baseline	7.9	10.9	8.1	8.9	7.6	9.4
Difference ^b in LS mean (95.1% CI)	0.3 (-2.0, 2.5)	3.2 (0.9, 5.4)		-0.9 (-3.1, 1.3)	-2.0 (-4.1, 0.2)	
Number of patients who gained at least 15 letters of vision from Baseline (%)	92 (31%)	114 (38%)	94 (31%)	96 (31%)	91 (29%)	99 (34%)
Difference ^b (%) (95.1% CI)	-0.4 (-7.7, 7.0)	6.6 (-1.0, 14.1)		-2.6 (-10.2, 4.9)	-4.6 (-12.1, 2.9)	

BCVA = Best Corrected Visual Acuity; CI = Confidence Interval; ETDRS = Early Treatment Diabetic Retinopathy Study; LOCF = Last Observation Carried Forward (baseline values are not carried forward); 95.1% confidence intervals were presented to adjust for safety assessment conducted during the study.

^a After treatment initiation with 3 monthly doses

^b EYLEA group minus the ranibizumab group

Figure 9: Mean Change in Visual Acuity from Baseline to Week 52 in VIEW1 and VIEW2 Studies



16 HOW SUPPLIED/STORAGE AND HANDLING

Each Vial is for single eye use only. EYLEA is supplied in the following presentation [see *Dosage and Administration* (2.3) and (2.4)].

NDC NUMBER	CARTON TYPE	CARTON CONTENTS
61755-005-02	Vial	one single-use, sterile, 3-mL, glass vial containing a 0.278 mL fill of 40 mg/mL EYLEA one 19-gauge x 1½-inch, 5-micron, filter needle for withdrawal of the vial contents one 30-gauge x ½-inch injection needle for intravitreal injection one 1-mL syringe for administration one package insert

Storage

EYLEA should be refrigerated at 2°C to 8°C (36°F to 46°F). Do Not Freeze. Do not use beyond the date stamped on the carton and container label. Protect from light. Store in the original carton until time of use.

17 PATIENT COUNSELING INFORMATION

Patients may experience temporary visual disturbances after an intravitreal injection with EYLEA and the associated eye examinations [*see Adverse Reactions (6)*]. Patients should be advised not to drive or use machinery until visual function has recovered sufficiently.

In the days following EYLEA administration, patients are at risk of developing endophthalmitis or retinal detachment. If the eye becomes red, sensitive to light, painful, or develops a change in vision, the patient should seek immediate care from an ophthalmologist [*see Warnings and Precautions (5.1)*].

REGENERON

Manufactured by:

Regeneron Pharmaceuticals, Inc.

777 Old Saw Mill River Road

Tarrytown, NY 10591-6707

U.S. License Number 1760

EYLEA[™] is a trademark of Regeneron Pharmaceuticals, Inc.

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All rights reserved.

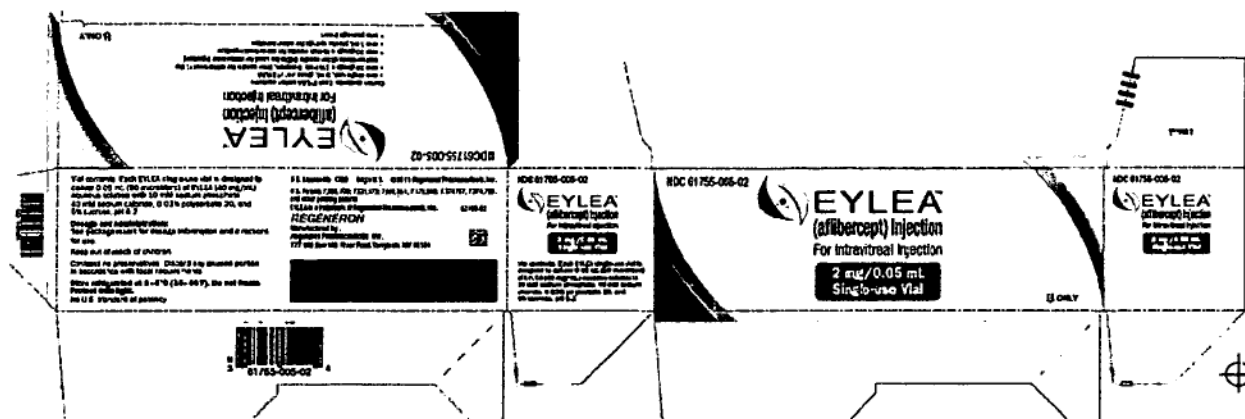
V1.0

Issue Date: November /2011

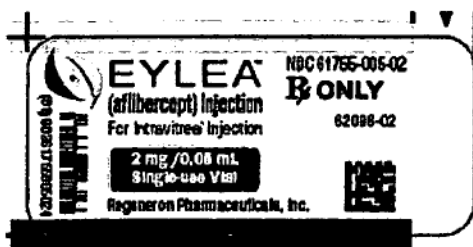
Initial U.S. Approval: 2011

Regeneron U.S. Patents 7,306,799; 7,531,173; 7,608,261; 7,070,959; 7,374,757; 7,374,758, and other pending patents

Carton Label



Vial Label



ATTACHMENT C

Copy of EYLEA™ BLA Approval Letter from the FDA

Subject: Approval Letter for BLA 125387

Date: Friday, November 18, 2011 5:47 PM

From: Puglisi, Michael <Michael.Puglisi@fda.hhs.gov>

To: Laura Pologe <Laura.Pologe@regeneron.com>

Hi Laura,

Here it is. Congratulations. Please confirm receipt. The hard copy will be mailed on Monday.

Mike



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

Our STN: BL 125387/0

BLA APPROVAL
November 18, 2011

Regeneron Pharmaceuticals, Inc.
Attention: Laura Pologe, Ph.D.
Associate Director, Regulatory Affairs
777 Old Saw Mill River Road
Tarrytown, New York 10591-6707

Dear Dr. Pologe:

Please refer to your Biologics License Application (BLA) dated February 17, 2011, received February 18, 2011, submitted under section 351 of the Public Health Service Act for Eylea (aflibercept).

We acknowledge receipt of your amendments dated February 28, March 10, 18, and 24, April 1, 8, 11 (two), 13 (two), and 29, May 11, 16, 23, and 27, June 3, 7, 9, 16, 20, and 28, July 8, 18, and 19, August 1, 5, 10, 12, and 31, September 1, 7, 12, 20, and 26, October 7, 21, 24, and 27, and November 1, 9, 11, and 17, 2011.

We have approved your BLA for aflibercept effective this date. You are hereby authorized to introduce or deliver for introduction into interstate commerce, aflibercept under your existing Department of Health and Human Services U.S. License No. 1760. Aflibercept is indicated for treatment of neovascular (wet) age-related macular degeneration.

Under this license, you are approved to manufacture aflibercept drug substance intermediate, drug substance, and formulated bulk at (b)(4). The final formulated drug product will be manufactured at (b)(4). The final formulated drug product will be labeled and packaged at (b)(4). You may label your product with the proprietary name Eylea and market it in a (b)(4) single-use vial containing 0.278 mL of 40 mg/mL aflibercept, as part of a final packaged product containing the aflibercept single-use vial, a 19-gauge x 1½-inch 5-micron filter needle, a 30-gauge x ½-inch needle and a 1-mL plastic syringe.

The dating period for aflibercept shall be 15 months from the date of manufacture when stored at 2 - 8°C. The date of manufacture shall be defined as the (b)(4). The expiration date for the packaged product, (aflibercept single-use vials, syringe, needle and filter needle) shall be dependent on the shortest expiration date of any component. (b)(4)

(b)(4)

Results of ongoing stability should be submitted to the annual report.

You are not currently required to submit samples of future lots of aflibercept to the Center for Drug Evaluation and Research (CDER) for release by the Director, CDER, under 21 CFR 610.2. We will continue to monitor compliance with 21 CFR 610.1, requiring completion of tests for conformity with standards applicable to each product prior to release of each lot.

Any changes in the manufacturing, testing, packaging, or labeling of aflibercept, or in the manufacturing facilities, will require the submission of information to your biologics license application for our review and written approval, consistent with 21 CFR 601.12.

We are approving this application for use as recommended in the enclosed agreed-upon labeling text.

CONTENT OF LABELING

As soon as possible, but no later than 14 days from the date of this letter, submit, via the FDA automated drug registration and listing system (eLIST), the content of labeling [21.601.14(b)] in structured product labeling (SPL) format, as described at <http://www.fda.gov/ForIndustry/DataStandards/StructuredProductLabeling/default.htm>, that is identical to the enclosed labeling (text for the package insert). Information on submitting SPL files using eLIST may be found in the guidance for industry titled "SPL Standard for Content of Labeling Technical Qs and As" at <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072392.pdf>. For administrative purposes, please designate this submission "**Product Correspondence – Final SPL for approved BLA STN 125387.**"

The SPL will be accessible via publicly available labeling repositories.

CARTON AND IMMEDIATE CONTAINER LABELS

Submit final printed carton and container labels that are identical to the enclosed carton and immediate container labels submitted on November 17, 2011, as soon as they are available, but no more than 30 days after they are printed. Please submit these labels electronically according to the guidance for industry titled "Providing Regulatory Submissions in Electronic Format – Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (June 2008)". Alternatively, you may submit 12 paper copies, with 6 of the copies individually mounted on heavy-weight paper or similar material. For administrative purposes, designate this submission "**Product Correspondence – Final Printed Carton and Container Labels for approved BLA STN 125387.**" Approval of this submission by FDA is not required before the labeling is used.

Marketing the product(s) with final printed labeling (FPL) that is not identical to the approved labeling text may render the product misbranded and an unapproved new drug.

REQUIRED PEDIATRIC ASSESSMENTS

Under the Pediatric Research Equity Act (PREA) (21 U.S.C. 355c), all applications for new active ingredients, new indications, new dosage forms, new dosing regimens, or new routes of administration are required to contain an assessment of the safety and effectiveness of the product for the claimed indication(s) in pediatric patients unless this requirement is waived, deferred, or inapplicable. We are waiving the pediatric study requirement for this application because the product treats a disease that does not exist in pediatric age groups.

POSTMARKETING REQUIREMENTS UNDER 505(o)

Section 505(o)(3) of the Federal Food, Drug, and Cosmetic Act (FDCA) authorizes FDA to require holders of approved drug and biological product applications to conduct postmarketing studies and clinical trials for certain purposes, if FDA makes certain findings required by the statute.

We have determined that an analysis of spontaneous postmarketing adverse events reported under subsection 505(k)(1) of the FDCA will not be sufficient to identify an unexpected serious risk of corneal endothelial cell decompensation following the intravitreal administration of Eylea (aflibercept).

Furthermore, the new pharmacovigilance system that FDA is required to establish under section 505(k)(3) of the FDCA will not be sufficient to assess this serious risk.

Finally, we have determined that only a clinical trial (rather than a nonclinical or observational study) will be sufficient to identify an unexpected serious risk of corneal endothelial cell decompensation following the intravitreal administration of Eylea (aflibercept).

Therefore, based on appropriate scientific data, FDA has determined that you are required to conduct the following:

1. Provide clinical information from a 1-year (minimum) clinical trial evaluating the adverse effects, if any, on the corneal endothelium following administration of aflibercept.

The timetable you submitted on October 24, 2011, states that you will conduct this trial according to the following schedule:

Final Protocol Submission:	March 2012
Trial Completion:	November 2015
Final Report Submission:	May 2016

Submit the protocol to your IND 12462, with a cross-reference letter to this BLA. Submit all final report(s) to your BLA. Prominently identify the submission with the following wording in

bold capital letters at the top of the first page of the submission, as appropriate: **“Required Postmarketing Protocol Under 505(o)”**, **“Required Postmarketing Final Report Under 505(o)”**, **“Required Postmarketing Correspondence Under 505(o)”**.

Section 505(o)(3)(E)(ii) of the FDCA requires you to report periodically on the status of any study or clinical trial required under this section. This section also requires you to periodically report to FDA on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Section 506B of the FDCA, as well as 21 CFR 601.70 requires you to report annually on the status of any postmarketing commitments or required studies or clinical trials.

FDA will consider the submission of your annual report under section 506B and 21 CFR 601.70 to satisfy the periodic reporting requirement under section 505(o)(3)(E)(ii) provided that you include the elements listed in 505(o) and 21 CFR 601.70. We remind you that to comply with 505(o), your annual report must also include a report on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Failure to submit an annual report for studies or clinical trials required under 505(o) on the date required will be considered a violation of FDCA section 505(o)(3)(E)(ii) and could result in enforcement action.

POSTMARKETING COMMITMENTS NOT SUBJECT TO THE REPORTING REQUIREMENTS UNDER SECTION 506B

We remind you of your postmarketing commitments:

2. To conduct three drug product hold time studies of the 40 mg/mL vial presentation filled at [REDACTED] (b)(4) site. Material will be held at commercial scale, and microbiological samples (total viable count, bacterial endotoxin) will be taken at the end of the hold times. The completed validation report will be submitted as a CBE-0 supplement.

The timetable you submitted on November 11, 2011, states that you will conduct this study according to the following schedule:

Final Report Submission: November 2012

3. To conduct three drug product hold time studies for the 40 mg/mL vial presentation filled at the [REDACTED] (b)(4) site. These studies will include t=0 and end of hold samples for product quality (pH, purity by size exclusion, purity by nrSDS-PAGE, charge variant distribution by IEF, isoaspartate, and potency of aflibercept) evaluation. The completed validation report will be provided as a CBE-0 supplement.

The timetable you submitted on November 11 2011, states that you will conduct this study according to the following schedule:

Final Report Submission: June 2012

4. To confirm (b)(4) by the aflibercept (b)(4) process. The (b)(4) study will be performed under protocol on three lots of drug substance produced at the commercial scale. (b)(4) will be measured with a validated analytical test method for determining (b)(4). The completed method validation and final reports will be submitted in the 2012 annual report by January 2013.

The timetable you submitted on November 11, 2011, states that you will conduct this study according to the following schedule:

Final Report Submission: January 2013

5. To re-evaluate the release and shelf-life specifications for aflibercept drug product after 30 commercial manufacturing runs to reflect increased manufacturing experience. The revisions to the quality control system, the corresponding data from the 30 commercial manufacturing runs, and the analysis and statistical plan used to evaluate the specifications and any changes to specifications will be provided in a PAS within 60 days after completion of the 30th lot manufactured using the commercial process or by December, 2014, whichever occurs first.

The timetable you submitted on November 11, 2011, states that you will conduct this study according to the following schedule:

Final Report Submission: December 2014

6. To re-evaluate the release and shelf-life specifications for aflibercept drug substance after 30 commercial manufacturing runs to reflect increased manufacturing experience. The revisions to the quality control system, the corresponding data from the 30 commercial manufacturing runs, and the analysis and statistical plan used to evaluate the specifications and any changes to specifications will be provided in a PAS by within 60 days after completion of the 30th lot manufactured using the commercial process or by June, 2013, whichever occurs first.

The timetable you submitted on November 11, 2011, states that you will conduct this study according to the following schedule:

Final Report Submission: June 2013

7. To re-evaluate the release and shelf-life specifications for aflibercept drug substance intermediate after 30 commercial manufacturing runs to reflect increased manufacturing experience. The revisions to the quality control system, the corresponding data from the 30 commercial manufacturing runs, and the analysis and statistical plan used to evaluate the specifications and any changes to specifications will be provided in a PAS within 60 days after completion of the 30th lot manufactured using the commercial process or by June, 2014, whichever occurs first.

The timetable you submitted on November 11, 2011, states that you will conduct this study according to the following schedule:

Final Report Submission: June 2014

8. To re-evaluate the release and shelf-life specifications for aflibercept formulated bulk after 30 commercial manufacturing runs to reflect increased manufacturing experience. The revisions to the quality control system, the corresponding data from the 30 commercial manufacturing runs, and the analysis and statistical plan used to evaluate the specifications and any changes to specifications will be provided in a PAS within 60 days after completion of the 30th lot manufactured using the commercial process or by June, 2013, whichever occurs first.

The timetable you submitted on November 11, 2011, states that you will conduct this study according to the following schedule:

Final Report Submission: June 2013

Submit clinical protocols to your IND 12462 for this product. Submit nonclinical and chemistry, manufacturing, and controls protocols and all final reports to this BLA. In addition, under 21 CFR 601.70 you should include a status summary of each commitment in your annual progress report of postmarketing studies and clinical trials to this BLA. The status summary should include expected summary completion and final report submission dates, any changes in plans since the last annual report, and, for clinical studies/trials, number of patients entered into each study/trial. All submissions, including supplements, relating to these postmarketing commitments should be prominently labeled **"Postmarketing Commitment Protocol," "Postmarketing Commitment Final Report,"** or **"Postmarketing Commitment Correspondence."**

REPORTING REQUIREMENTS

You must submit adverse experience reports under the adverse experience reporting requirements for licensed biological products (21 CFR 600.80). You should submit postmarketing adverse experience reports to:

Food and Drug Administration
Center for Drug Evaluation and Research
Central Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

Prominently identify all adverse experience reports as described in 21 CFR 600.80.

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive

copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at

<http://www.fda.gov/Safety/MedWatch/HowToReport/ucm166910.htm>.

You must submit distribution reports under the distribution reporting requirements for licensed biological products (21 CFR 600.81).

You must submit reports of biological product deviations under 21 CFR 600.14. You should promptly identify and investigate all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA-3486 to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Compliance Risk Management and Surveillance
5901-B Ammendale Road
Beltsville, MD 20705-1266

Biological product deviations, sent by courier or overnight mail, should be addressed to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Compliance Risk Management and Surveillance
10903 New Hampshire Avenue, Bldg. 51, Room 4206
Silver Spring, MD 20903

PROMOTIONAL MATERIALS

You may request advisory comments on proposed introductory advertising and promotional labeling. To do so, submit, in triplicate, a cover letter requesting advisory comments, the proposed materials in draft or mock-up form with annotated references, and the package insert to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Drug Marketing, Advertising, and Communications
5901-B Ammendale Road
Beltsville, MD 20705-1266

You must submit final promotional materials, and the package insert, at the time of initial dissemination or publication, accompanied by a Form FDA 2253. For instruction on completing the Form FDA 2253, see page 2 of the Form. For more information about submission of promotional materials to the Division of Drug Marketing, Advertising, and Communications (DDMAC), see <http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm090142.htm>.

All promotional claims must be consistent with and not contrary to approved labeling. You should not make a comparative promotional claim or claim of superiority over other products unless you have substantial evidence to support that claim.

POST-ACTION FEEDBACK MEETING

New molecular entities and new biologics qualify for a post-action feedback meeting. Such meetings are used to discuss the quality of the application and to evaluate the communication process during drug development and marketing application review. The purpose is to learn from successful aspects of the review process and to identify areas that could benefit from improvement. If you would like to have such a meeting with us, call the Regulatory Project Manager for this application.

If you have any questions, call Michael Puglisi, Regulatory Project Manager, at (301) 796-0791.

Sincerely,

/ Edward Cox, M.D., M.P.H./

Edward Cox, M.D., M.P.H.

Director

Office of Antimicrobial Products

Center for Drug Evaluation and Research

ENCLOSURES:

Content of Labeling

Carton and Container Labeling

ATTACHMENT D

Copy of U.S. Patent 7,374,758



US007374758B2

(12) **United States Patent**
Papadopoulos et al.(10) **Patent No.: US 7,374,758 B2**(45) **Date of Patent: *May 20, 2008**(54) **MODIFIED CHIMERIC POLYPEPTIDES WITH IMPROVED PHARMACOKINETIC PROPERTIES AND METHODS OF USING THEREOF**(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 489 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **11/016,503**(22) Filed: **Dec. 17, 2004**(65) **Prior Publication Data**

US 2005/0245447 A1 Nov. 3, 2005

Related U.S. Application Data

(62) 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.**
A61K 38/18 (2006.01)
C07K 14/71 (2006.01)
C12N 15/62 (2006.01)(52) **U.S. Cl.** **424/134.1; 424/192.1; 514/2; 514/23; 530/350; 536/23.4**(58) **Field of Classification Search** None
See application file for complete search history.(56) **References Cited****U.S. PATENT DOCUMENTS**

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WO	WO99/03996	1/1999

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Primary Examiner—Christine J Saoud*Assistant Examiner*—Jon M Lockard(74) *Attorney, Agent, or Firm*—Valeta Gregg, Esq.(57) **ABSTRACT**

Modified chimeric polypeptides with improved pharmacokinetics are disclosed. Specifically, modified chimeric Flt1 receptor polypeptides that have been modified in such a way as to improve their pharmacokinetic profile are disclosed. Also disclosed are methods of making and using the modified polypeptides including but not limited to using the modified polypeptides to decrease or inhibit plasma leakage and/or vascular permeability in a mammal.

3 Claims, 55 Drawing Sheets

Fig.1.

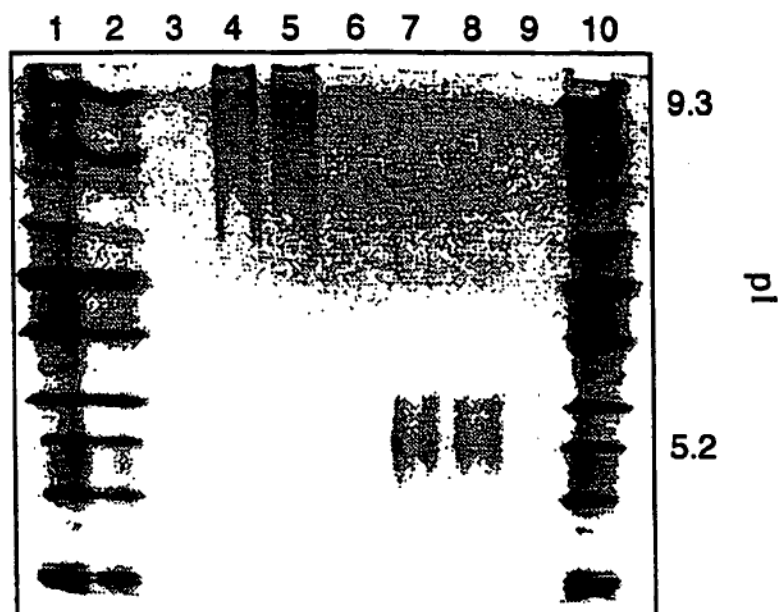


Fig.2.

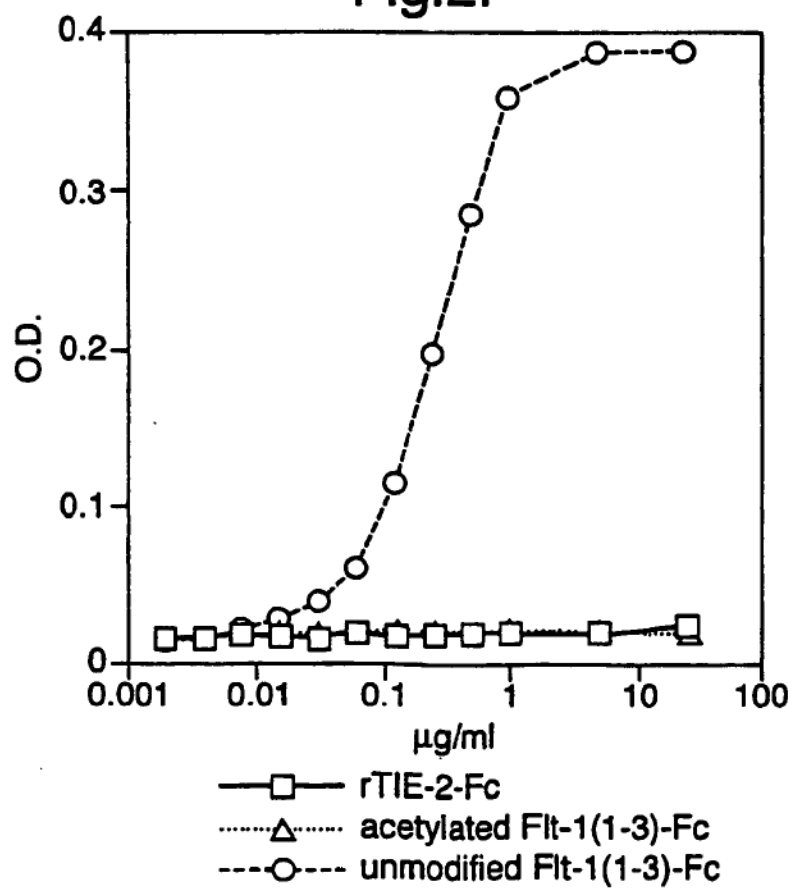
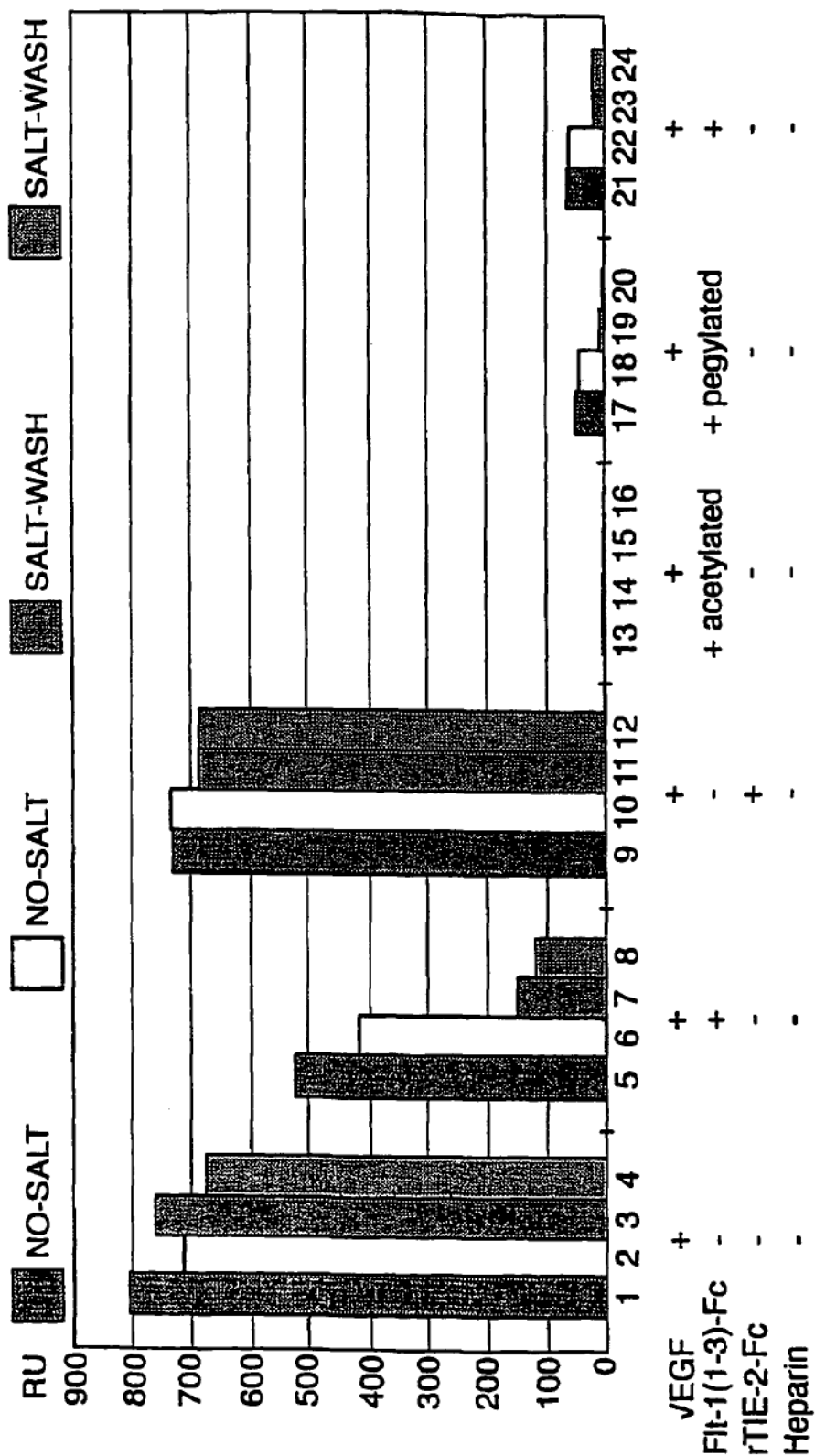


Fig.3.



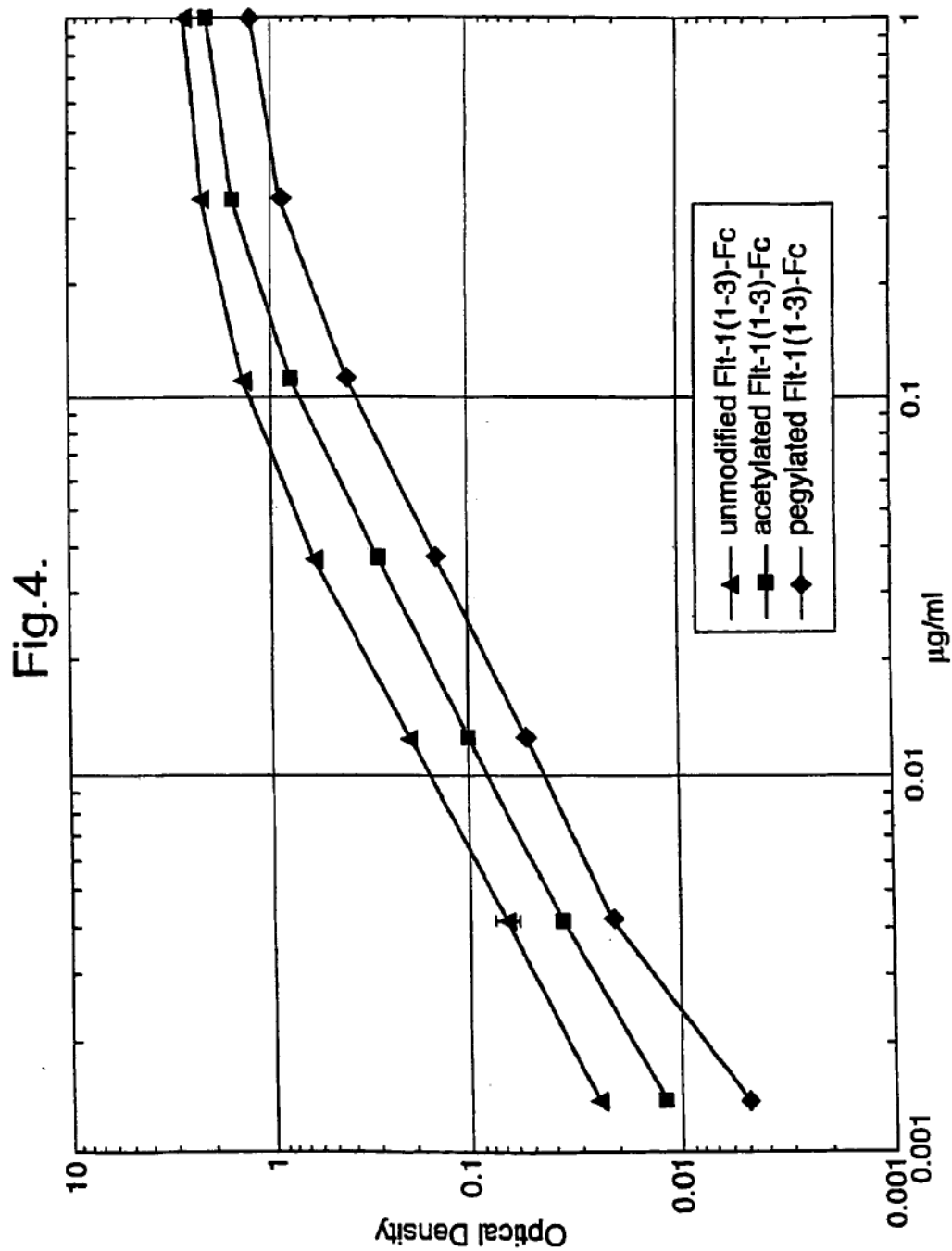


Fig. 5.

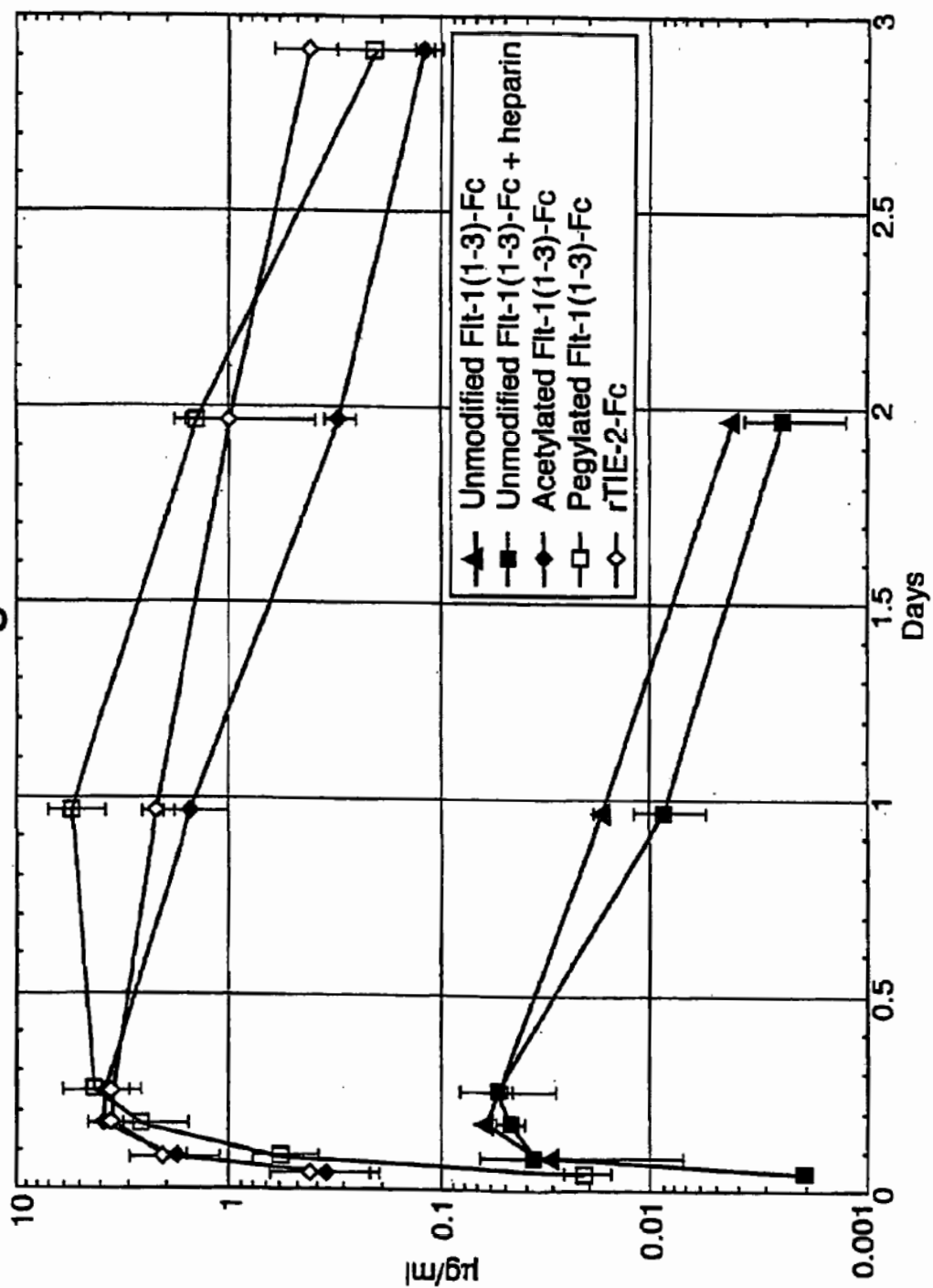


Fig.6A.

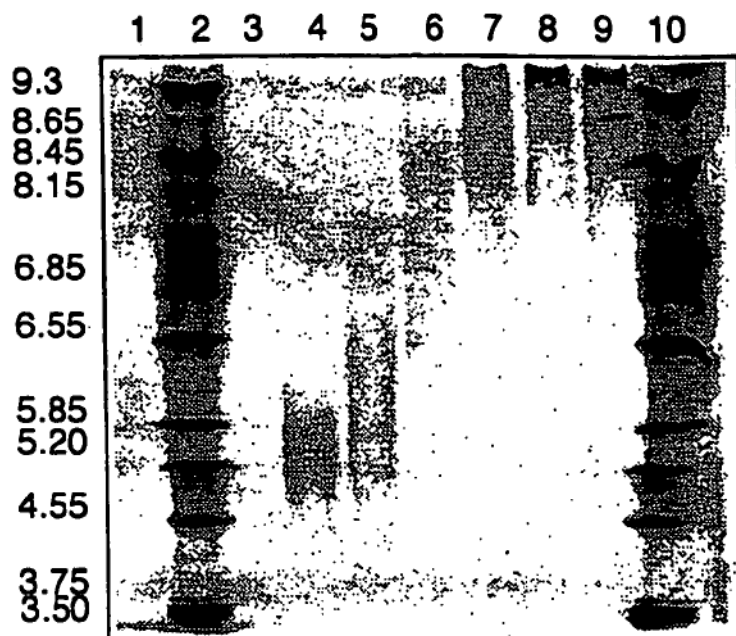


Fig.6B.

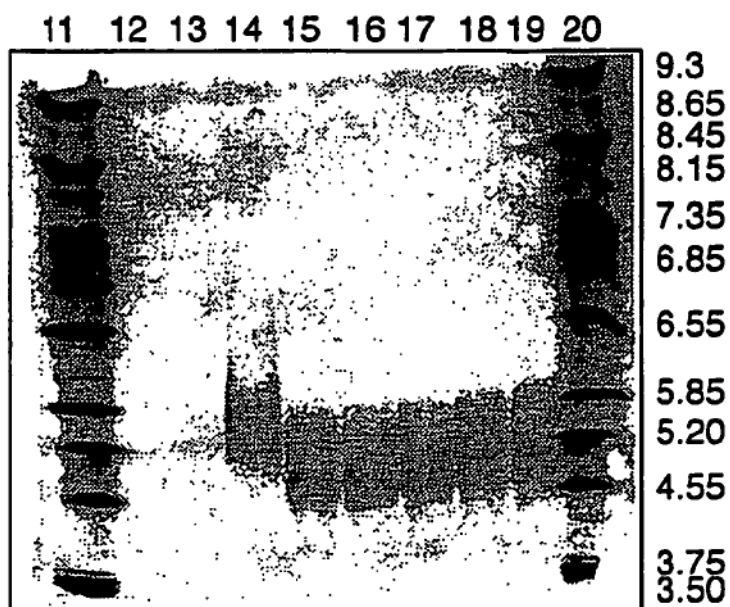
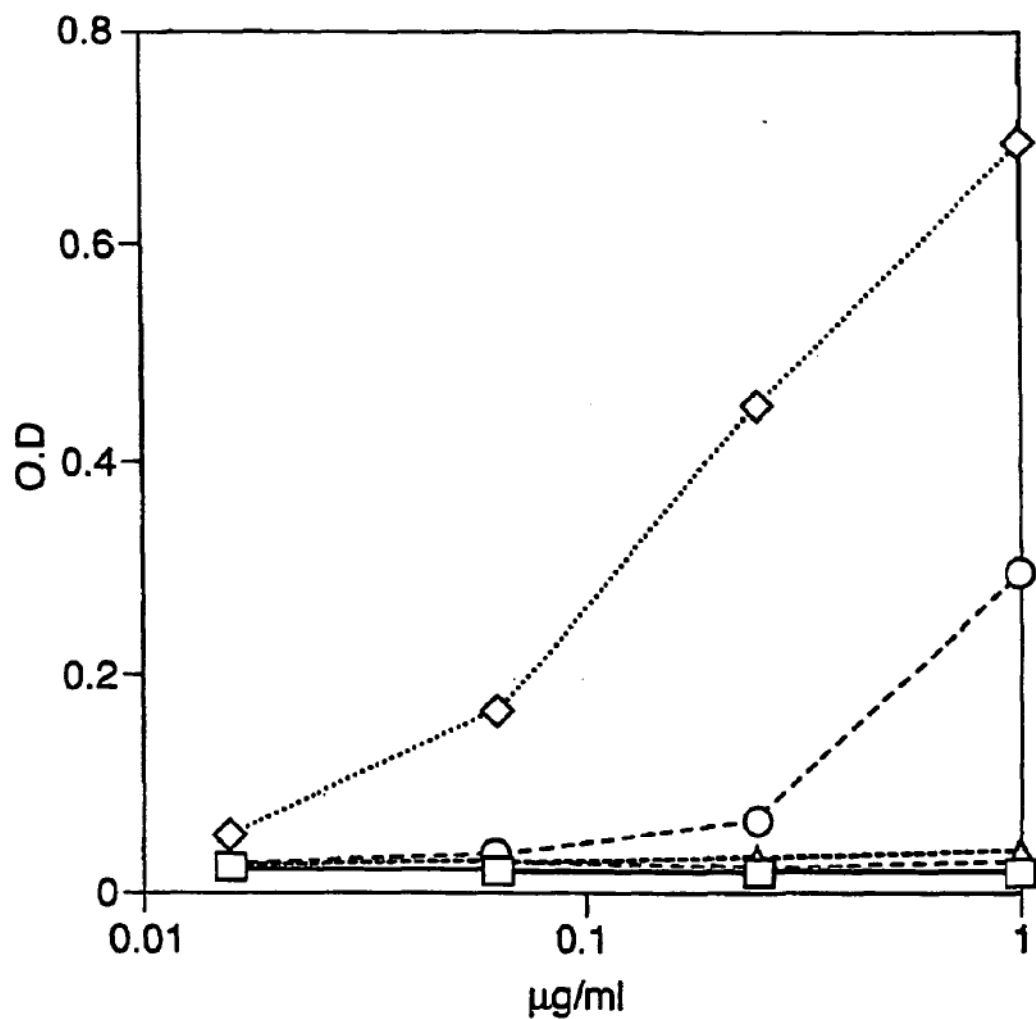


Fig.7.



- rTIE-2-Fc
-◇..... unmodified Flt-1(1-3)-Fc
- - -○- - - acetylated Flt-1(1-3)-Fc (10X)
- . -△- . - acetylated Flt-1(1-3)-Fc (20X)
- - -▣- - - acetylated Flt-1(1-3)-Fc (30X)

Fig.8.

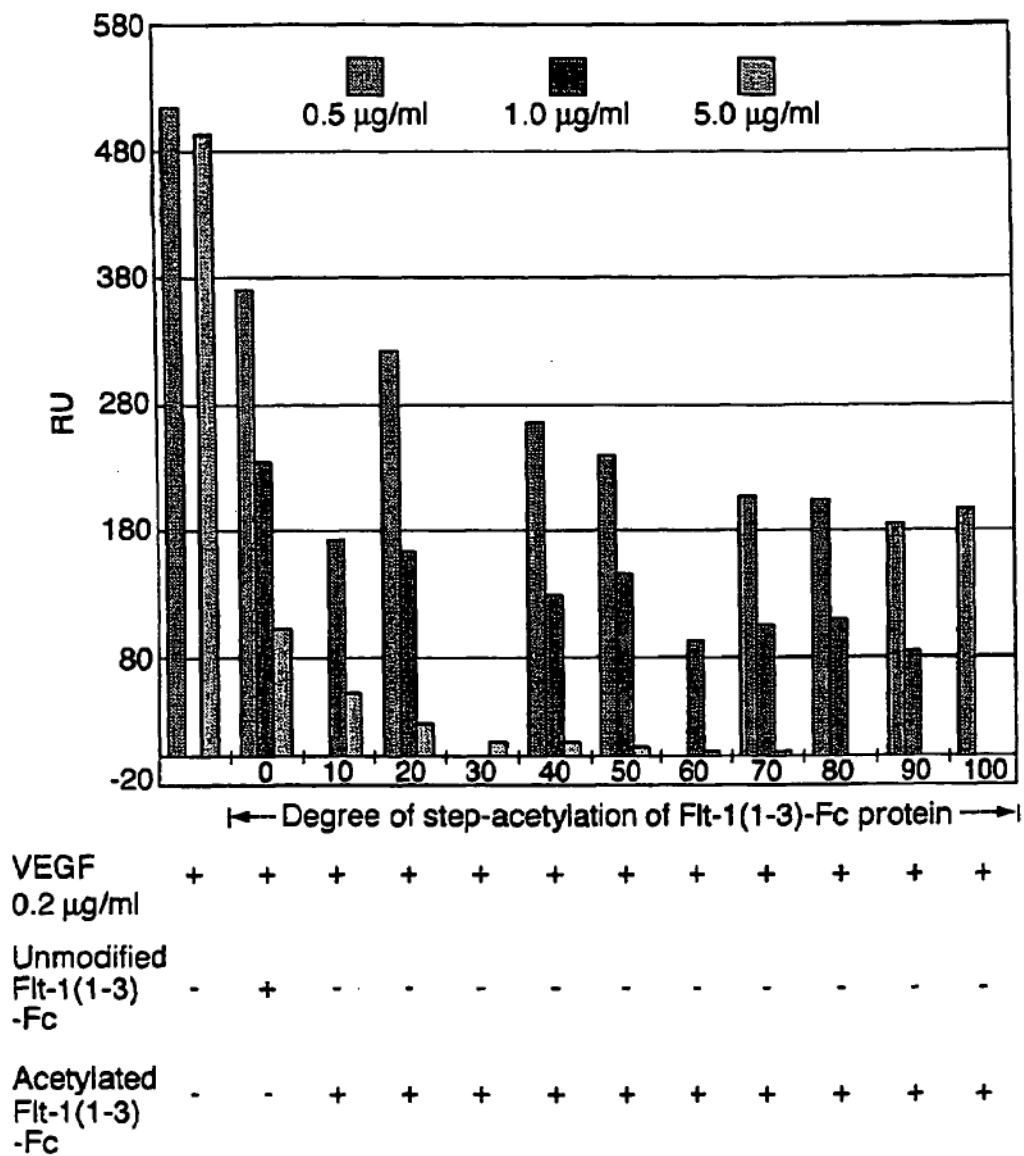


Fig. 9.

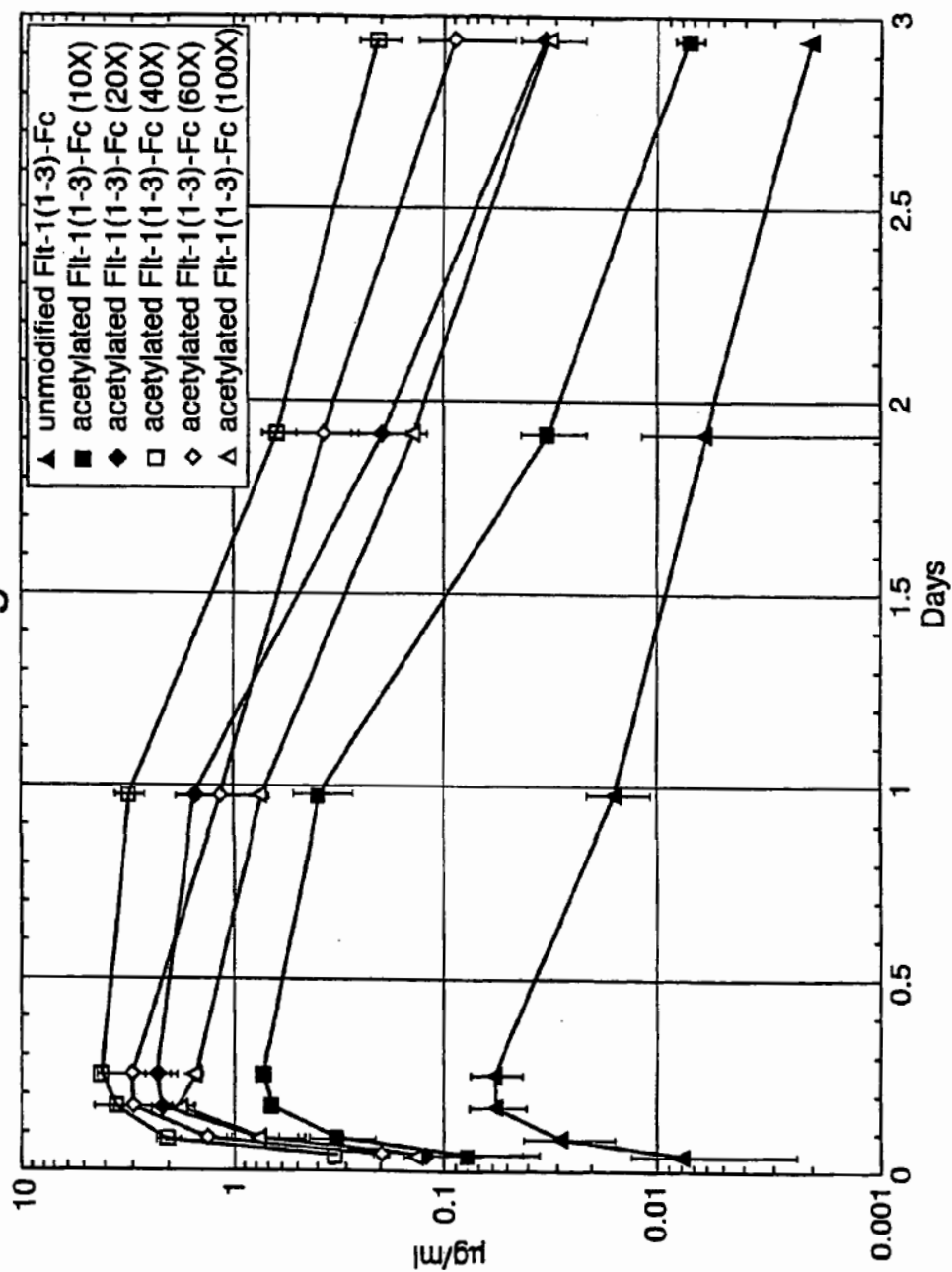


Fig.10A.

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TAC CAG TCG ATG ACC CTG TGG CCC CAG GAC GAC ACG GCG GAC GAG TCG ACA GAC GAA GAG
Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser Cys Leu Leu Leu>

70 80 90 100 110 120
* * * * *
ACA GGA TCT AGT TCA GGT TCA AAA TTA AAA GAT CCT GAA CTG AGT TTA AAA GGC ACC CAG
TGT CCT AGA TCA AGT OCA AGT TTT AAT TTT CTA GGA CTT GAC TCA AAT TTT CCG TGG GTC
Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro Glu Leu Ser Leu Lys Gly Thr Gln>

130 140 150 160 170 180
* * * * *
CAC ATC ATG CAA GCA GGC CAG ACA CTG CAT CTC CAA TGC AGG GGG GAA GCA GCC CAT AAA
GTG TAG TAC GTT CGT CCG GTC TGT GAC GTA GAG GTT ACG TOC CCC CTT CGT CCG GTA TTT
His Ile Met Gln Ala Gly Gln Thr Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys>

190 200 210 220 230 240
* * * * *
TGG TCT TTG CCT GAA ATG GTG AGT AAG GAA AGC GAA AAG CTG AGC ATA ACT AAA TCT GCC
ACC AGA AAC GGA CTT TAC CAC TCA TTC CTT TCG CTT TOC GAC TCG TAT TGA TTT AGA CCG
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* * * * *
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ACA CCT TCT TTA CCG TTT GTT AAG ACG TCA TGA AAT TGG AAC TTG TGT CGA GTT CGT TTG
Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr Ala Gln Ala Asn>

310 320 330 340 350 360
* * * * *
CAC ACT GGC TTC TAC AGC TGC AAA TAT CTA GCT GTA CCT ACT TCA AAG AAG AAG GAA ACA
GTG TGA CCG AAG ATG TCG ACG TTT ATA GAT CGA CAT GGA TGA AGT TTC TTC TTC CTT TGT
His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val Pro Thr Ser Lys Lys Lys Glu Thr>

370 380 390 400 410 420
* * * * *
GAA TCT GCA ATC TAT ATA TTT ATT AGT GAT ACA GGT AGA CCT TTC GTA GAG ATG TAC AGT
CTT AGA CGT TAG ATA TAT AAA TAA TCA CTA TGT CCA TCT GGA AAG CAT CTC TAC ATG TCA
Glu Ser Ala Ile Tyr Ile Phe Ile Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser>

430 440 450 460 470 480
* * * * *
GAA ATC CCC GAA ATT ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CCG GTT
CTT TAG GGG CTT TAA TAT GTG TAC TGA CTT CCT TCC CTC GAG CAG TAA GGG ACG GCC CAA
Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val>

490 500 510 520 530 540
* * * * *
ACG TCA CCT AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC CCT GAT
TGC AGT GGA TTG TAG TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG GGA CTA
Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp>

Fig.10B.

550 560 570 580 590 600
* * * * *
GGA AAA CGC ATA ATC TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG TAC AAA
CCT TTT GCG TAT TAG ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC ATG TTT
Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys>

610 620 630 640 650 660
* * * * *
GAA ATA GGG CTT CTG ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT
CTT TAT CCC GAA GAC TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT TTG ATA
Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr>

670 680 690 700 710 720
* * * * *
CTC ACA CAT CGA CAA ACC AAT ACA ATC ATA GAT GTC CAA ATA AGC ACA CCA CGC CCA GTC
GAG TGT GTA GCT GTT TGG TTA TGT TAG TAT CTA CAG GTT TAT TCG TGT GGT GCG GGT CAG
Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val>

730 740 750 760 770 780
* * * * *
AAA TTA CTT AGA GGC CAT ACT CTT GTC CTC AAT TGT ACT GCT ACC ACT CCC TTG AAC ACG
TTT AAT GAA TCT CCG GTA TGA GAA CAG GAG TTA ACA TGA CGA TGG TGA GGG AAC TTG TGC
Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr Pro Leu Asn Thr>

790 800 810 820 830 840
* * * * *
AGA GTT CAA ATG ACC TGG AGT TAC CCT GAT GAA AAA AAT AAG AGA GCT TCC GTA AGG CGA
TCT CAA GTT TAC TGG ACC TCA ATG GGA CTA CTT TTT TTA TTC TCT CGA AGG CAT TCC GCT
Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys Asn Lys Arg Ala Ser Val Arg Arg>

850 860 870 880 890 900
* * * * *
CGA ATT GAC CAA AGC AAT TCC CAT GCC AAC ATA TTC TAC AGT GTT CTT ACT ATT GAC AAA
GCT TAA CTG GTT TCG TTA AGG GTA CCG TTG TAT AAG ATG TCA CAA GAA TGA TAA CTG TTT
Arg Ile Asp Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys>

910 920 930 940 950 960
* * * * *
ATG CAG AAC AAA GAC AAA GGA CTT TAT ACT TGT CGT GTA AGG AGT GGA CCA TCA TTC AAA
TAC GTC TTG TTT CTG TTT CCT GAA ATA TGA ACA GCA CAT TCC TCA CCT GGT AGT AAG TTT
Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys>

970 980 990 1000 1010 1020
* * * * *
TCT GTT AAC ACC TCA GTG CAT ATA TAT GAT AAA GCA GGC CCG GGC GAG CCC AAA TCT TGT
AGA CAA TTG TGG AGT CAC GTA TAT ATA CTA TTT CGT CCG GGC CCG CTC GGG TTT AGA ACA
Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Gly Pro Gly Glu Pro Lys Ser Cys>

1030 1040 1050 1060 1070 1080
* * * * *
GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC
CTG TTT TGA GTG TGT ACG GGT GGC ACG GGT CGT GGA CTT GAG GAC CCC CCT GGC AGT CAG
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val>

Fig.10C.

1090 1100 1110 1120 1130 1140
* * * * *
TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA
AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG GAG TAC TAG AGG GCC TGG GGA CTC CAG TGT
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr>
1150 1160 1170 1180 1190 1200
* * * * *
TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC
ACG CAC CAC CAC CTG CAC TGG GTG CTT CTG GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp>
1210 1220 1230 1240 1250 1260
* * * * *
GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC
CCG CAC CTC CAC GTA TTA CCG TTC TGT TTC GGC GCC CTC CTC GTC ATG TTG TCG TCG ATG
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr>
1270 1280 1290 1300 1310 1320
* * * * *
CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG
GCA CAC CAG TCG CAG GAG TGG CAG GAC GTG GTC CTG ACC GAC TTA CCG TTC CTC ATG TTC
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys>
1330 1340 1350 1360 1370 1380
* * * * *
TGC AAG GTC TCC AAC AAA GCC CTC CCA GGC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
ACG TTC CAG AGG TTG TTT CCG GAG GGT CCG GGG TAG CTC TTT TGG TAG AGG TTT CCG TTT
Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys>
1390 1400 1410 1420 1430 1440
* * * * *
GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG
CCC GTC GGG GCT CTT GGT GTC CAC ATG TGG GAC GGG GGT AGG GCC CTA CTC GAC TGG TTC
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys>
1450 1460 1470 1480 1490 1500
* * * * *
AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG
TTG GTC CAG TCG GAC TGG ACG GAC CAG TTT CCG AAG ATA GGG TCG CTG TAG CCG CAC CTC
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu>
1510 1520 1530 1540 1550 1560
* * * * *
TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC
ACC CTC TCG TTA CCC GTC GGC CTC TTG TTG ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser>
1570 1580 1590 1600 1610 1620
* * * * *
GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG
CTG CCG AGG AAG AAG GAG ATG TGG TTC GAG TGG CAC CTG TTC TCG TCC ACC GTC GTC CCC
Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly>

Fig.10D.

```
          1630          1640          1650          1660          1670          1680
      *      *      *      *      *      *      *      *      *      *
AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC
TTG CAG AAG AGT ACG AGG CAC TAC GTA CTC CGA GAC GTG TTG GTG ATG TGC GTC TTC TCG
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser>

          1690          1700
      *      *      *      *
CTC TCC CTG TCT CCG GGT AAA TGA
GAG AGG GAC AGA GGC CCA TTT ACT
Leu Ser Leu Ser Pro Gly Lys ***>
```

Fig.11.

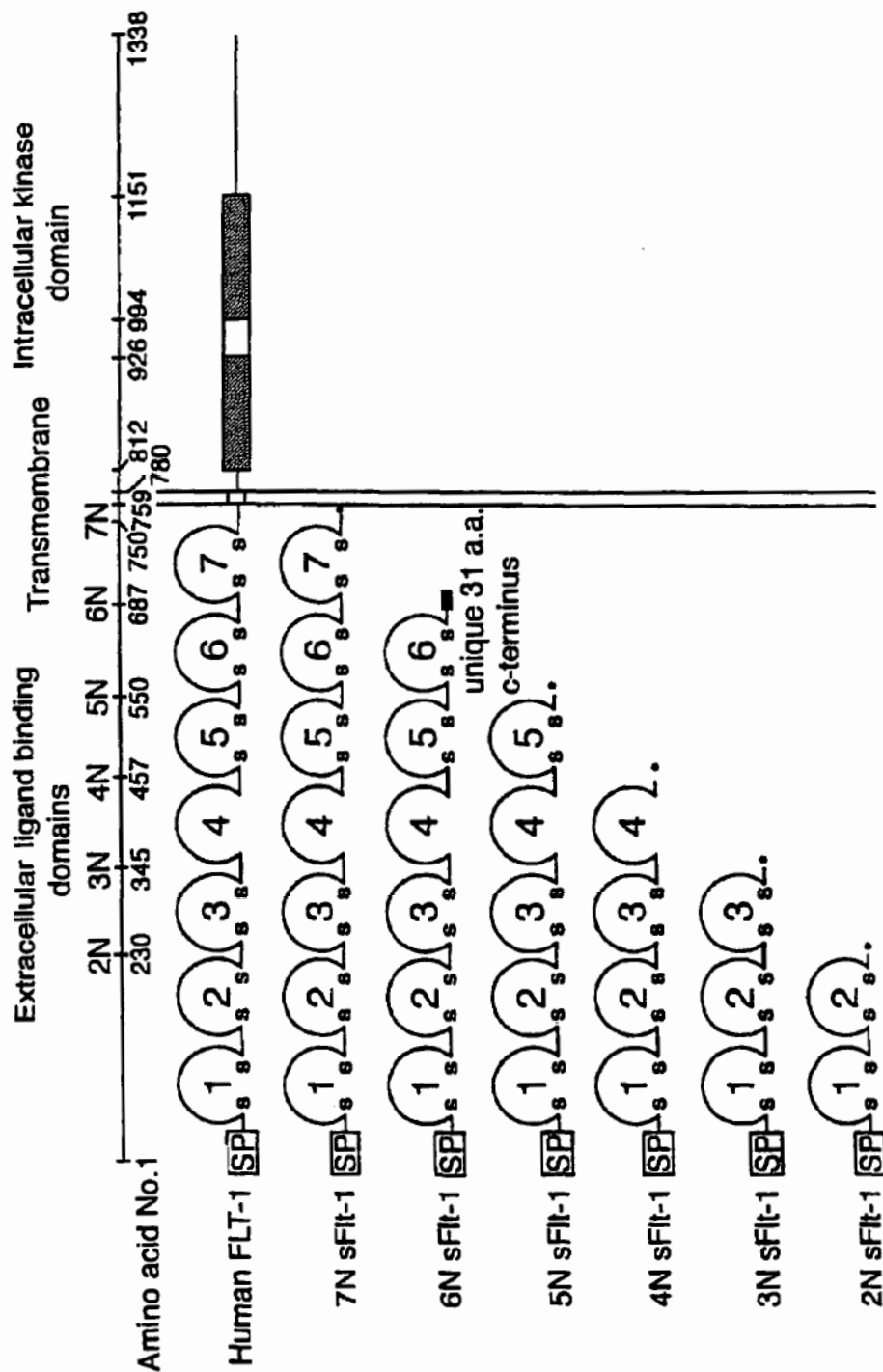


Fig.12A.

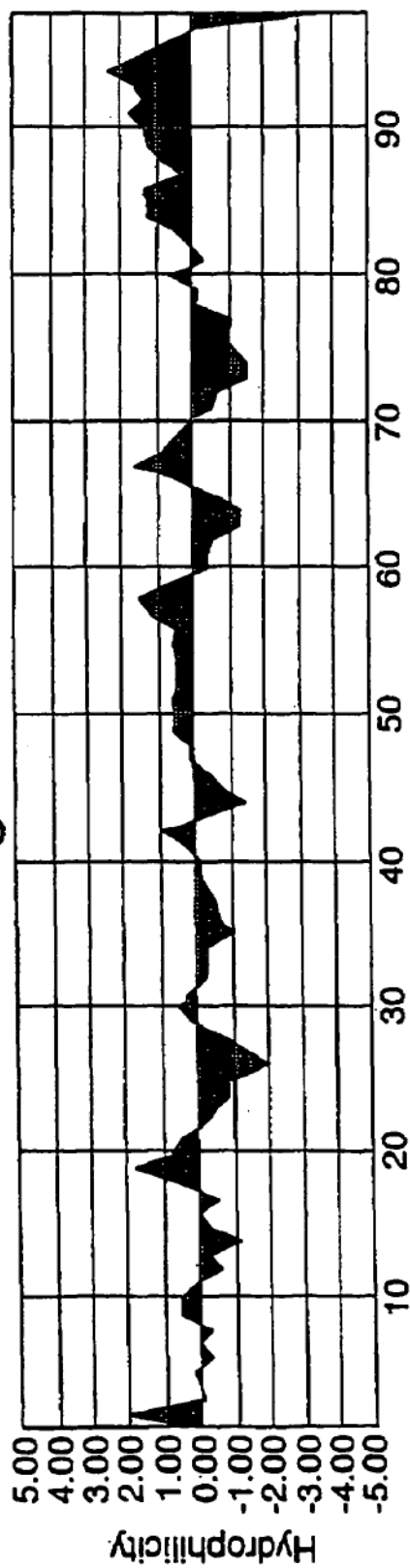


Fig.12B.

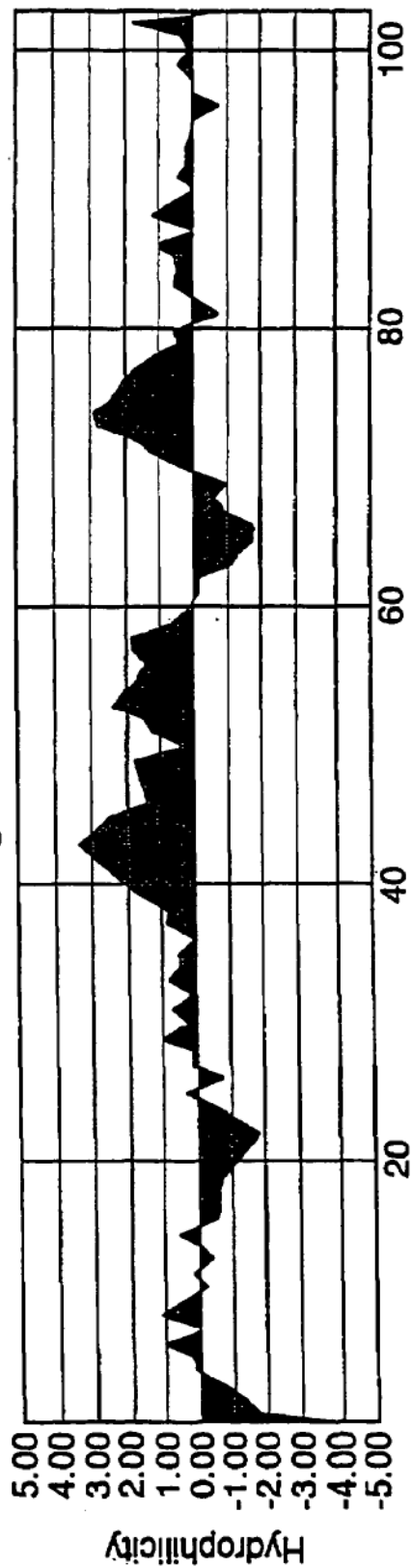


Fig.13A.

10 20 30 40 50 60
* * * * *
ATG GTC AGC TAC TGG GAC ACC GGG GTC CTG CTG TGC GCG CTG CTC AGC TGT CTG CTT CTC
TAC CAG TCG ATG ACC CTG TGG CCC CAG GAC GAC ACG GCG GAC GAG TCG ACA GAC GAA GAG
Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser Cys Leu Leu Leu>

70 80 90 100 110 120
* * * * *
ACA GGA TCT AGT TCA GGT TCA AAA TTA AAA GAT CCT GAA CTG AGT TTA AAA GGC ACC CAG
TGT CCT AGA TCA AGT CCA AGT TTT AAT TTT CTA GGA CTT GAC TCA AAT TTT CCG TGG GTC
Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro Glu Leu Ser Leu Lys Gly Thr Gln>

130 140 150 160 170 180
* * * * *
CAC ATC ATG CAA GCA GGC CAG ACA CTG CAT CTC CAA TGC AGG GGG GAA GCA GCC CAT AAA
GTG TAG TAC GTT CGT CCG GTC TGT GAC GTA GAG GTT ACG TCC CCC CTT CGT CCG GTA TTT
His Ile Met Gln Ala Gly Gln Thr Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys>

190 200 210 220 230 240
* * * * *
TGG TCT TTG CCT GAA ATG GTG AGT AAG GAA AGC GAA AGG CTG AGC ATA ACT AAA TCT GCC
ACC AGA AAC GGA CTT TAC CAC TCA TTC CTT TCG CTT TCC GAC TCG TAT TGA TTT AGA CCG
Trp Ser Leu Pro Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala>

250 260 270 280 290 300
* * * * *
TGT GGA AGA AAT GGC AAA CAA TTC TGC AGT ACT TTA ACC TTG AAC ACA GCT CAA GCA AAT
ACA CCT TCT TTA CCG TTT GTT AAG ACG TCA TGA AAT TGG AAC TTG TGT CGA GTT CGT TTG
Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr Ala Gln Ala Asn>

310 320 330 340 350 360
* * * * *
CAC ACT GGC TTC TAC AGC TGC AAA TAT CTA GCT GTA CCT ACT TCA AAG AAG AAG GAA ACA
GTG TGA CCG AAG ATG TCG ACG TTT ATA GAT CGA CAT GGA TGA AGT TTC TTC TTC CTT TGT
His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val Pro Thr Ser Lys Lys Lys Glu Thr>

370 380 390 400 410 420
* * * * *
GAA TCT GCA ATC TAT ATA TTT ATT AGT GAT ACA GGT AGA CCT TTC GTA GAG ATG TAC AGT
CTT AGA CGT TAG ATA TAT AAA TAA TCA CTA TGT CCA TCT GGA AAG CAT CTC TAC ATG TCA
Glu Ser Ala Ile Tyr Ile Phe Ile Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser>

430 440 450 460 470 480
* * * * *
GAA ATC CCC GAA ATT ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CCG GTT
CTT TAG GGG CTT TAA TAT GTG TAC TGA CTT CCT TCC CTC GAG CAG TAA GGG ACG GCC CAA
Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val>

490 500 510 520 530 540
* * * * *
ACG TCA CCT AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC CCT GAT
TGC AGT GGA TTG TAG TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG GGA CTA
Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp>

Fig.13B.

550 560 570 580 590 600
* * * * *
GGA AAA CGC ATA ATC TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG TAC AAA
CCT TTT GCG TAT TAG ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC ATG TTT
Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys>

610 620 630 640 650 660
* * * * *
GAA ATA GGG CTT CTG ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT
CTT TAT CCC GAA GAC TGG ACA CTT CGT TGT CAG TTA CCG GTA AAC ATA TTC TGT TTG ATA
Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr>

670 680 690 700 710 720
* * * * *
CTC ACA CAT CGA CAA ACC AAT ACA ATC ATA GAT GTC CAA ATA AGC ACA CCA CCG CCA GTC
GAG TGT GTA GCT GTT TGG TTA TGT TAG TAT CTA CAG GTT TAT TCG TGT GGT GCG GGT CAG
Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val>

730 740 750 760 770 780
* * * * *
AAA TTA CTT AGA GGC CAT ACT CTT GTC CTC AAT TGT ACT GCT ACC ACT CCC TTG AAC ACG
TTT AAT GAA TCT CCG GTA TGA GAA CAG GAG TTA ACA TGA CGA TGG TGA GGG AAC TTG TGC
Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr Pro Leu Asn Thr>

790 800 810 820 830 840
* * * * *
AGA GTT CAA ATG ACC TGG AGT TAC OCT GAT GAA ATT GAC CAA AGC AAT TCC CAT GCC AAC
TCT CAA GTT TAC TGG ACC TCA ATG GGA CTA CTT TAA CTG GTT TCG TTA AGG GTA CCG TTG
Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Ile Asp Gln Ser Asn Ser His Ala Asn>

850 860 870 880 890 900
* * * * *
ATA TTC TAC AGT GTT CTT ACT ATT GAC AAA ATG CAG AAC AAA GAC AAA GGA CTT TAT ACT
TAT AAG ATG TCA CAA GAA TGA TAA CTG TTT TAC GTC TTG TTT CTG TTT CCT GAA ATA TGA
Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr>

910 920 930 940 950 960
* * * * *
TGT CGT GTA AGG AGT GGA CCA TCA TTC AAA TCT GTT AAC ACC TCA GTG CAT ATA TAT GAT
ACA GCA CAT TCC TCA CCT GGT AGT AAG TTT AGA CAA TTG TGG AGT CAC GTA TAT ATA CTA
Cys Arg Val Arg Ser Gly Pro Ser Phe Lys Ser Val Asn Thr Ser Val His Ile Tyr Asp>

970 980 990 1000 1010 1020
* * * * *
AAA GCA GGC CCG GGC GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA
TTT CGT CCG GGC CCG CTC GGG TTT AGA ACA CTG TTT TGA GTG TGT ACG GGT GGC ACG GGT
Lys Ala Gly Pro Gly Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro>

1030 1040 1050 1060 1070 1080
* * * * *
GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC
CGT GGA CTT GAG GAC CCC CCT GGC AGT CAG AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr>

Fig.13C.

1090 1100 1110 1120 1130 1140
* * * * *
CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC
GAG TAC TAG AGG GCC TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC TCG GTG CTT CTG
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp>

1150 1160 1170 1180 1190 1200
* * * * *
CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG
GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA TTA CCG TTC TGT TTC
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys>

1210 1220 1230 1240 1250 1260
* * * * *
CCG CCG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC
GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA CAC CAG TCG CAG GAG TGG CAG GAC GTG
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His>

1270 1280 1290 1300 1310 1320
* * * * *
CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC
GTC CTG ACC GAC TTA CCG TTC CTC ATG TTC ACG TTC CAG AGG TTG TTT CCG GAG GGT CCG
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala>

1330 1340 1350 1360 1370 1380
* * * * *
CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC
GGG TAG CTC TTT TGG TAG AGG TTT CCG TTT CCC GTC GGG GCT CTT GGT GTC CAC ATG TGG
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr>

1390 1400 1410 1420 1430 1440
* * * * *
CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA
GAC GGG GGT AGG GCC CTA CTC GAC TGG TTC TTG GTC CAG TCG GAC TGG ACG GAC CAG TTT
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys>

1450 1460 1470 1480 1490 1500
* * * * *
GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC
CCG AAG ATA GGG TCG CTG TAG CCG CAC CTC ACC CTC TCG TTA CCC GTC GGC CTC TTG TTG
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn>

1510 1520 1530 1540 1550 1560
* * * * *
TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC
ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG CCG AGG AAG AAG GAG ATG TCG TTC GAG
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu>

1570 1580 1590 1600 1610 1620
* * * * *
ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG
TGG CAC CTG TTC TCG TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG CAC TAC GTA CTC
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu>

Fig.13D.

	1630		1640		1650		1660		1670								
	*	*	*	*	*	*	*	*	*								
GCT	CTG	CAC	AAC	CAC	TAC	ACG	CAG	AAG	AGC	CTC	TCC	CTG	TCT	CCG	GGT	AAA	TGA
CGA	GAC	GTG	TTG	GTG	ATG	TGC	GTC	TTC	TCG	GAG	AGG	GAC	AGA	GGC	CCA	TTT	ACT
Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	***>

Fig.14A.

10 20 30 40 50 60
* * * * *
ATG GTC AGC TAC TGG GAC ACC GGG GTC CTG CTG TGC GCG CTG CTC AGC TGT CTG CTT CTC
TAC CAG TCG ATG ACC CTG TGG CCC CAG GAC GAC ACG CGC GAC GAG TCG ACA GAC GAA GAG
Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser Cys Leu Leu Leu>

70 80 90 100 110 120
* * * * *
ACA GGA TCT AGT TCC GGA GGT AGA CCT TTC GTA GAG ATG TAC AGT GAA ATC CCC GAA ATT
TGT CCT AGA TCA AGG CCT CCA TCT GGA AAG CAT CTC TAC ATG TCA CTT TAG GGG CTT TAA
Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile>

130 140 150 160 170 180
* * * * *
ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT ACG TCA CCT AAC ATC
TAT GTG TAC TGA CTT CCT TCC CTC GAG CAG TAA GGG ACG GCC CAA TGC AGT GGA TTG TAG
Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile>

190 200 210 220 230 240
* * * * *
ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC CCT GAT GGA AAA CGC ATA ATC
TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG GGA CTA CCT TTT GCG TAT TAG
Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile>

250 260 270 280 290 300
* * * * *
TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG TAC AAA GAA ATA GGG CTT CTG
ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC ATG TTT CTT TAT CCC GAA GAC
Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu>

310 320 330 340 350 360
* * * * *
ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT CTC ACA CAT CGA CAA
TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT TTG ATA GAG TGT GTA GCT GTT
Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln>

370 380 390 400 410 420
* * * * *
ACC AAT ACA ATC ATA GAT GTC CAA ATA AGC ACA CCA CGC CCA GTC AAA TTA CTT AGA GGC
TGG TTA TGT TAG TAT CTA CAG GTT TAT TCG TGT GGT GCG GGT CAG TTT AAT GAA TCT CCG
Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val Lys Leu Leu Arg Gly>

430 440 450 460 470 480
* * * * *
CAT ACT CTT GTC CTC AAT TGT ACT GCT ACC ACT CCC TTG AAC ACG AGA GTT CAA ATG ACC
GTA TGA GAA CAG GAG TTA ACA TGA CGA TGG TGA GGG AAC TTG TGC TCT CAA GTT TAC TGG
His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr Pro Leu Asn Thr Arg Val Gln Met Thr>

490 500 510 520 530 540
* * * * *
TGG AGT TAC CCT GAT GAA ATT GAC CAA AGC AAT TCC CAT GCC AAC ATA TTC TAC AGT GTT
ACC TCA ATG GGA CTA CTT TAA CTG GTT TCG TTA AGG GTA CGG TTG TAT AAG ATG TCA CAA
Trp Ser Tyr Pro Asp Glu Ile Asp Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val>

Fig.14B.

550 560 570 580 590 600
* * * * *
CTT ACT ATT GAC AAA ATG CAG AAC AAA GAC AAA GGA CTT TAT ACT TGT CGT GTA AGG AGT
GAA TGA TAA CTG TTT TAC GTC TTG TTT CTG TTT CCT GAA ATA TGA ACA GCA CAT TCC TCA
Leu Thr Ile Asp Lys Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser>

610 620 630 640 650 660
* * * * *
GGA CCA TCA TTC AAA TCT GTT AAC ACC TCA GTG CAT ATA TAT GAT AAA GCA GGC CCG GGC
CCT GGT AGT AAG TTT AGA CAA TTG TGG AGT CAC GTA TAT ATA CTA TTT CGT CCG GGC CCG
Gly Pro Ser Phe Lys Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Gly Pro Gly>

670 680 690 700 710 720
* * * * *
GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG
CTC GGG TTT AGA ACA CTG TTT TGA GTG TGT ACG GGT GGC ACG GGT CGT GGA CTT GAG GAC
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu>

730 740 750 760 770 780
* * * * *
GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CCG
CCC CCT GGC AGT CAG AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG GAG TAC TAG AGG GGC
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg>

790 800 810 820 830 840
* * * * *
ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC
TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC TCG GTG CTT CTG GGA CTC CAG TTC AAG
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe>

850 860 870 880 890 900
* * * * *
AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CCG GAG GAG CAG
TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA TTA CCG TTC TGT TTC GGC GCC CTC CTC GTC
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln>

910 920 930 940 950 960
* * * * *
TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT
ATG TTG TCG TGC ATG GCA CAC CAG TCG CAG GAG TGG CAG GAC GTG GTC CTG ACC GAC TTA
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn>

970 980 990 1000 1010 1020
* * * * *
GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC
CCG TTC CTC ATG TTC ACG TTC CAG AGG TTG TTT CCG GAG GGT CCG GGG TAG CTC TTT TGG
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr>

1030 1040 1050 1060 1070 1080
* * * * *
ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CCG
TAG AGG TTT CCG TTT CCC GTC GGG GCT CTT GGT GTC CAC ATG TGG GAC GGG GGT AGG GCC
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg>

Fig.14C.

1090	1100	1110	1120	1130	1140
* * *	* * *	* * *	* * *	* * *	* * *
GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC					
CTA CTC GAC TGG TTC TTG GTC CAG TCG GAC TGG ACG GAC CAG TTT CCG AAG ATA GGG TCG					
Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser>					
1150	1160	1170	1180	1190	1200
* * *	* * *	* * *	* * *	* * *	* * *
GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT					
CTG TAG CGG CAC CTC ACC CTC TCG TTA CCC GTC GGC CTC TTG TTG ATG TTC TGG TGC GGA					
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro>					
1210	1220	1230	1240	1250	1260
* * *	* * *	* * *	* * *	* * *	* * *
CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC					
GGG CAC GAC CTG AGG CTG CCG AGG AAG AAG GAG ATG TCG TTC GAG TGG CAC CTG TTC TCG					
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser>					
1270	1280	1290	1300	1310	1320
* * *	* * *	* * *	* * *	* * *	* * *
AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC					
TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG CAC TAC GTA CTC CGA GAC GTG TTG GTG					
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His>					
1330	1340	1350			
* * *	* * *	* * *			
TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA					
ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC CCA TTT ACT					
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys ***>					

Fig.15A.

10 20 30 40 50 60
* * * * *
ATG GTC AGC TAC TGG GAC ACC GGG GTC CTG CTG TGC GCG CTG CTC AGC TGT CTG CTT CTC
TAC CAG TCG ATG ACC CTG TGG CCC CAG GAC GAC ACG CGC GAC GAG TCG ACA GAC GAA GAG
Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser Cys Leu Leu Leu>

70 80 90 100 110 120
* * * * *
ACA GGA TCT AGT TCC GGA GGT AGA CCT TTC GTA GAG ATG TAC AGT GAA ATC CCC GAA ATT
TGT CCT AGA TCA AGG CCT CCA TCT GGA AAG CAT CTC TAC ATG TCA CTT TAG GGG CTT TAA
Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile>

130 140 150 160 170 180
* * * * *
ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT ACG TCA CCT AAC ATC
TAT GTG TAC TGA CTT OCT TCC CTC GAG CAG TAA GGG ACG GCC CAA TGC AGT GGA TTG TAG
Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile>

190 200 210 220 230 240
* * * * *
ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC CCT GAT GGA AAA CGC ATA ATC
TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG GGA CTA OCT TTT GCG TAT TAG
Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile>

250 260 270 280 290 300
* * * * *
TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG TAC AAA GAA ATA GGG CTT CTG
ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC ATG TTT CTT TAT CCC GAA GAC
Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu>

310 320 330 340 350 360
* * * * *
ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT CTC ACA CAT CGA CAA
TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT TTG ATA GAG TGT GTA GCT GTT
Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln>

370 380 390 400 410 420
* * * * *
ACC AAT ACA ATC ATA GAT GTC CAA ATR AGC ACA CCA CGC CCA GTC AAA TTA CTT AGA GGC
TGG TTA TGT TAG TAT CTA CAG GTT TAT TCG TGT GGT GCG GGT CAG TTT AAT GAA TCT CCG
Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val Lys Leu Leu Arg Gly>

430 440 450 460 470 480
* * * * *
CAT ACT CTT GTC CTC AAT TGT ACT GCT ACC ACT CCC TTG AAC ACG AGA GTT CAA ATG ACC
GTA TGA GAA CAG GAG TTA ACA TGA CGA TGG TGA GGG AAC TTG TGC TCT CAA GTT TAC TGG
His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr Pro Leu Asn Thr Arg Val Gln Met Thr>

490 500 510 520 530 540
* * * * *
TGG AGT TAC CCT GAT GAA AAA AAT AAG AGA GCT TCC GTA AGG CGA CGA ATT GAC CAA AGC
ACC TCA ATG GGA CTA CTT TTT TTA TTC TCT CGA AGG CAT TCC GCT GCT TAA CTG GTT TCG
Trp Ser Tyr Pro Asp Glu Lys Asn Lys Arg Ala Ser Val Arg Arg Arg Ile Asp Gln Ser>

Fig.15B.

550 560 570 580 590 600
* * * * *
AAT TCC CAT GCC AAC ATA TTC TAC AGT GTT CTT ACT ATT GAC AAA ATG CAG AAC AAA GAC
TTA AGG GTA CCG TTG TAT AAG ATG TCA CAA GAA TGA TAA CTG TTT TAC GTC TTG TTT CTG
Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys Asp>

610 620 630 640 650 660
* * * * *
AAA GGA CTT TAT ACT TGT CGT GTA AGG AGT GGA CCA TCA TTC AAA TCT GTT AAC ACC TCA
TTT CCT GAA ATA TGA ACA GCA CAT TCC TCA CCT GGT AGT AAG TTT AGA CAA TTG TGG AGT
Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys Ser Val Asn Thr Ser>

670 680 690 700 710 720
* * * * *
GTG CAT ATA TAT GAT AAA GCA GGC CCG GGC GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA
CAC GTA TAT ATA CTA TTT CGT CCG GGC CCG CTC GGG TTT AGA ACA CTG TTT TGA GTG TGT
Val His Ile Tyr Asp Lys Ala Gly Pro Gly Glu Pro Lys Ser Cys Asp Lys Thr His Thr>

730 740 750 760 770 780
* * * * *
TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA
ACG GGT GGC ACG GGT CGT GGA CTT GAG GAC CCC CCT GGC AGT CAG AAG GAG AAG GGG GGT
Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro>

790 800 810 820 830 840
* * * * *
AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC
TTT GGG TTC CTG TGG GAG TAC TAG AGG GCC TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp>

850 860 870 880 890 900
* * * * *
GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT
CAC TCG GTG CTT CTG GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His>

910 920 930 940 950 960
* * * * *
AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC
TTA CCG TTC TGT TTC GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA CAC CAG TCG CAG
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val>

970 980 990 1000 1010 1020
* * * * *
CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC
GAG TGG CAG GAC GTG GTC CTG ACC GAC TTA CCG TTC CTC ATG TTC ACG TTC CAG AGG TTG
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn>

1030 1040 1050 1060 1070 1080
* * * * *
AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA
TTT CCG GAG GGT CCG GGG TAG CTC TTT TGG TAG AGG TTT CCG TTT CCC GTC GGG GCT CTT
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu>

Fig.15C.

```

      1090      1100      1110      1120      1130      1140
      *        *        *        *        *        *
CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG
GGT GTC CAC ATG TGG GAC GGG GGT AGG GGC CTA CTC GAC TGG TTC TTG GTC CAG TCG GAC
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu>

      1150      1160      1170      1180      1190      1200
      *        *        *        *        *        *
ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GGC GTG GAG TGG GAG AGC AAT GGG
TGG ACG GAC CAG TTT CCG AAG ATA GGG TCG CTG TAG CCG CAC CTC ACC CTC TCG TTA CCC
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly>

      1210      1220      1230      1240      1250      1260
      *        *        *        *        *        *
CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC
GTC GGC CTC TTG TTG ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG CCG AGG AAG AAG
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe>

      1270      1280      1290      1300      1310      1320
      *        *        *        *        *        *
CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC
GAG ATG TCG TTC GAG TGG CAC CTG TTC TCG TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys>

      1330      1340      1350      1360      1370      1380
      *        *        *        *        *        *
TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG
AGG CAC TAC GTA CTC CGA GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro>

      *
GGT AAA TGA
CCA TTT ACT
Gly Lys ***>

```

Fig.16A.

10 20 30 40 50 60
* * * * *
ATG GTC AGC TAC TGG GAC ACC GGG GTC CTG CTG TGC GCG CTG CTC AGC TGT CTG CTT CTC
TAC CAG TCG ATG ACC CTG TGG CCC CAG GAC GAC ACG CGC GAC GAG TCG ACA GAC GAA GAG
Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser Cys Leu Leu>

70 80 90 100 110 120
* * * * *
ACA GGA TCT AGT TCA GGT TCA AAA TTA AAA GAT CCT GAA CTG AGT TTA AAA GGC ACC CAG
TGT CCT AGA TCA AGT CCA AGT TTT AAT TTT CTA GGA CTT GAC TCA AAT TTT CCG TGG GTC
Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro Glu Leu Ser Leu Lys Gly Thr Gln>

130 140 150 160 170 180
* * * * *
CAC ATC ATG CAA GCA GGC CAG ACA CTG CAT CTC CAA TGC AGG GGG GAA GCA GCC CAT AAA
GTG TAG TAC GTT CGT CCG GTC TGT GAC GTA GAG GTT ACG TCC CCC CTT CGT CCG GTA TTT
His Ile Met Gln Ala Gly Gln Thr Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys>

190 200 210 220 230 240
* * * * *
TGG TCT TTG CCT GAA ATG GTG AGT AAG GAA AGC GAA AGG CTG AGC ATA ACT AAA TCT GCC
ACC AGA AAC GGA CTT TAC CAC TCA TTC CTT TCG CTT TCC GAC TCG TAT TGA TTT AGA CCG
Trp Ser Leu Pro Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala>

250 260 270 280 290 300
* * * * *
TGT GGA AGA AAT GGC AAA CAA TTC TGC AGT ACT TTA ACC TTG AAC ACA GCT CAA GCA AAC
ACA CCT TCT TTA CCG TTT GTT AAG ACG TCA TGA AAT TGG AAC TTG TGT CGA GTT CGT TTG
Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr Ala Gln Ala Asn>

310 320 330 340 350 360
* * * * *
CAC ACT GGC TTC TAC AGC TGC AAA TAT CTA GCT GTA CCT ACT TCA AAG AAG AAG GAA ACA
GTG TGA CCG AAG ATG TCG ACG TTT ATA GAT CGA CAT GGA TGA AGT TTC TTC TTC CTT TGT
His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val Pro Thr Ser Lys Lys Lys Glu Thr>

370 380 390 400 410 420
* * * * *
GAA TCT GCA ATC TAT ATA TTT ATT AGT GAT ACA GGT AGA CCT TTC GTA GAG ATG TAC AGT
CTT AGA CGT TAG ATA TAT AAA TAA TCA CTA TGT CCA TCT GGA AAG CAT CTC TAC ATG TCA
Glu Ser Ala Ile Tyr Ile Phe Ile Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser>

430 440 450 460 470 480
* * * * *
GAA ATC CCC GAA ATT ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CCG GTT
CTT TAG GGG CTT TAA TAT GTG TAC TGA CTT CCT TCC CTC GAG CAG TAA GGG ACG CCC CAA
Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val>

490 500 510 520 530 540
* * * * *
ACG TCA CCT AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC CCT GAT
TGC AGT GGA TTG TAG TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG GGA CTA
Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp>

Fig.16B.

550 560 570 580 590 600
* * * * *
GGA AAA CGC ATA ATC TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG TAC AAA
CCT TTT GCG TAT TAG ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC ATG TTT
Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys>

610 620 630 640 650 660
* * * * *
GAA ATA GGG CTT CTG ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT
CTT TAT CCC GAA GAC TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT TTG ATA
Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr>

670 680 690 700 710 720
* * * * *
CTC ACA CAT CGA CAA ACC AAT ACA ATC ATA GAT GTC CAA ATA AGC ACA CCA CGC CCA GTC
GAG TGT GTA GCT GTT TGG TTA TGT TAG TAT CTA CAG GTT TAT TCG TGT GGT GCG GGT CAG
Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val>

730 740 750 760 770 780
* * * * *
AAA TTA CTT AGA GGC CAT ACT CTT GTC CTC AAT TGT ACT GCT ACC ACT CCC TTG AAC ACG
TTT AAT GAA TCT CCG GTA TGA GAA CAG GAG TTA ACA TGA CGA TGG TGA GGG AAC TTG TGC
Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr Pro Leu Asn Thr>

790 800 810 820 830 840
* * * * *
AGA GTT CAA ATG ACC TGG AGT TAC CCT GAT GAA AAA AAT AAG AAC GCT TCC GTA AGG CGA
TCT CAA GTT TAC TGG ACC TCA ATG GGA CTA CTT TTT TTA TTC TTG CGA AGG CAT TCC GCT
Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys Asn Lys Asn Ala Ser Val Arg Arg>

850 860 870 880 890 900
* * * * *
CGA ATT GAC CAA AGC AAT TCC CAT GGC AAC ATA TTC TAC AGT GTT CTT ACT ATT GAC AAA
GCT TAA CTG GTT TCG TTA AGG GTA CCG TTG TAT AAG ATG TCA CAA GAA TGA TAA CTG TTT
Arg Ile Asp Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys>

910 920 930 940 950 960
* * * * *
ATG CAG AAC AAA GAC AAA GGA CTT TAT ACT TGT CGT GTA AGG AGT GGA CCA TCA TTC AAA
TAC GTC TTG TTT CTG TTT CCT GAA ATA TGA ACA GCA CAT TCC TCA CCT GGT AGT AAG TTT
Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys>

970 980 990 1000 1010 1020
* * * * *
TCT GTT AAC ACC TCA GTG CAT ATA TAT GAT AAA GCA GGC CCG GGC GAG CCC AAA TCT TGT
AGA CAA TTG TGG AGT CAC GTA TAT ATA CTA TTT CGT CCG GGC CCG CTC GGG TTT AGA ACA
Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Gly Pro Gly Glu Pro Lys Ser Cys>

1030 1040 1050 1060 1070 1080
* * * * *
GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC
CTG TTT TGA GTG TGT ACG GGT GGC ACG GGT CGT GGA CTT GAG GAC CCC CCT GGC AGT CAG
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val>

Fig.16C.

1090 1100 1110 1120 1130 1140
* * * * *
TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCG CGG ACC CCT GAG GTC ACA
AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG GAG TAC TAG AGG GCC TGG GGA CTC CAG TGT
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr>

1150 1160 1170 1180 1190 1200
* * * * *
TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC
ACG CAC CAC CAC CTG CAC TCG GTG CTT CTG GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp>

1210 1220 1230 1240 1250 1260
* * * * *
GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC
CCG CAC CTC CAC GTA TTA CCG TTC TGT TTC GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr>

1270 1280 1290 1300 1310 1320
* * * * *
CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG
GCA CAC CAG TCG CAG GAG TGG CAG GAC GTG GTC CTG ACC GAC TTA CCG TTC CTC ATG TTC
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys>

1330 1340 1350 1360 1370 1380
* * * * *
TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
ACG TTC CAG AGG TTG TTT CGG GAG GGT CGG GGG TAG CTC TTT TGG TAG AGG TTT CGG TTT
Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys>

1390 1400 1410 1420 1430 1440
* * * * *
GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG
CCC GTC GGG GCT CTT GGT GTC CAC ATG TGG GAC GGG GGT AGG GCC CTA CTC GAC TGG TTC
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys>

1450 1460 1470 1480 1490 1500
* * * * *
AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG
TTG GTC CAG TCG GAC TGG ACG GAC CAG TTT CCG AAG ATA GGG TCG CTG TAG CGG CAC CTC
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu>

1510 1520 1530 1540 1550 1560
* * * * *
TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC
ACC CTC TCG TTA CCC GTC GGC CTC TTG TTG ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser>

1570 1580 1590 1600 1610 1620
* * * * *
GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG
CTG CCG AGG AAG AAG GAG ATG TCG TTC GAG TGG CAC CTG TTC TCG TCC ACC GTC GTC CCC
Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly>

Fig.16D.

```

      1630      1640      1650      1660      1670      1680
      *        *        *        *        *        *        *
AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC
TTG CAG AAG AGT ACG AGG CAC TAC GTA CTC CGA GAC GTG TTG GTG ATG TGC GTC TTC TCG
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser>

      1690      1700
      *        *        *        *
CTC TCC CTG TCT CCG GGT AAA TGA
GAG AGG GAC AGA GGC CCA TTT ACT
Leu Ser Leu Ser Pro Gly Lys ***>
```

Fig.17.

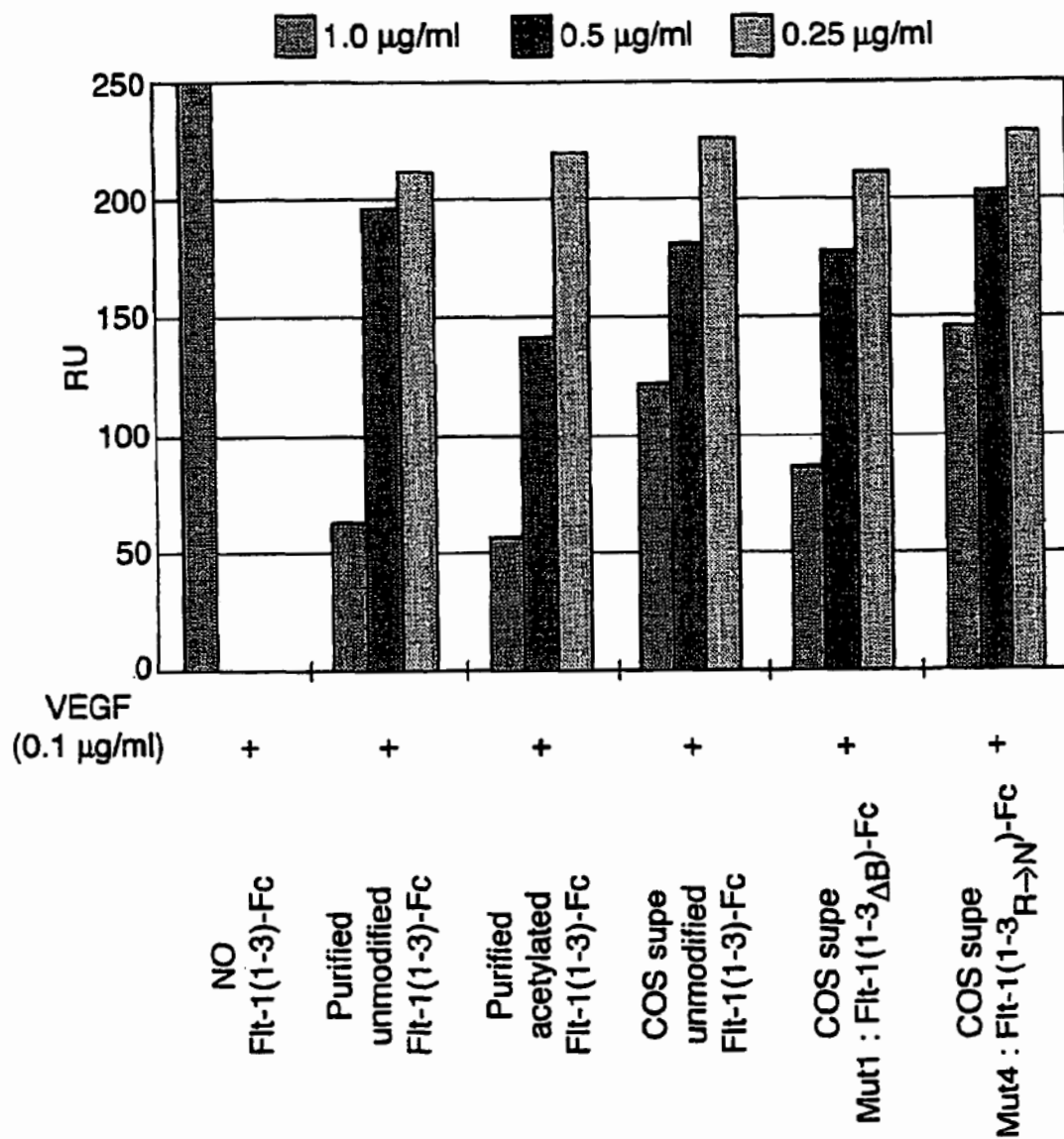


Fig.18.

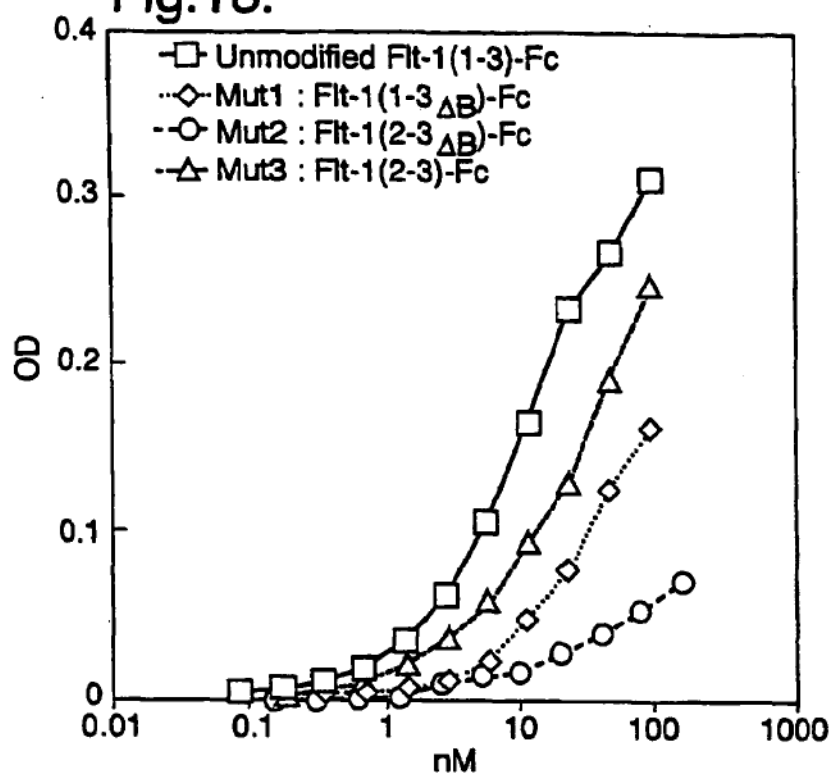


Fig.19.

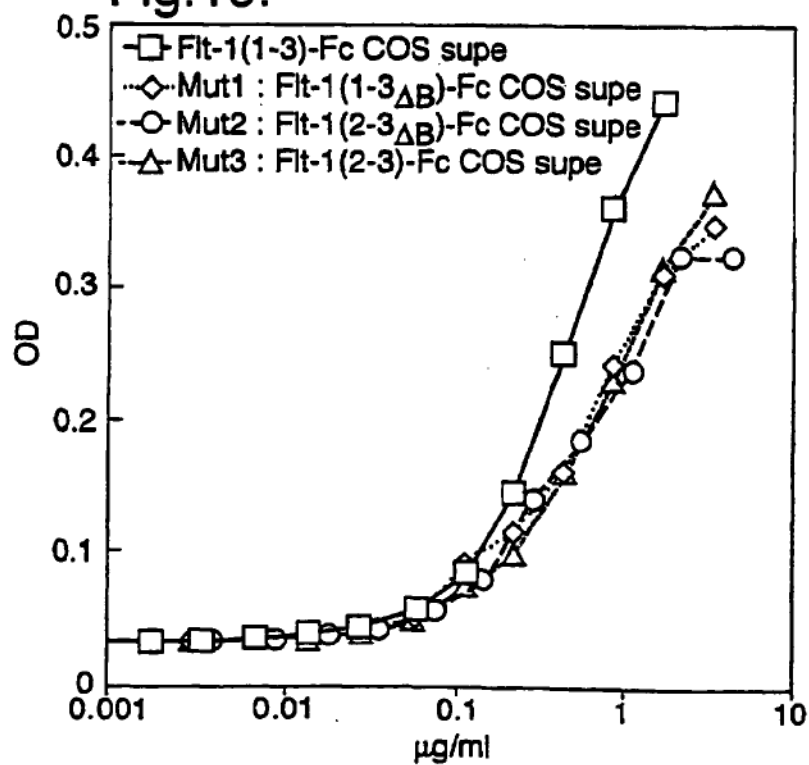


Fig.20.

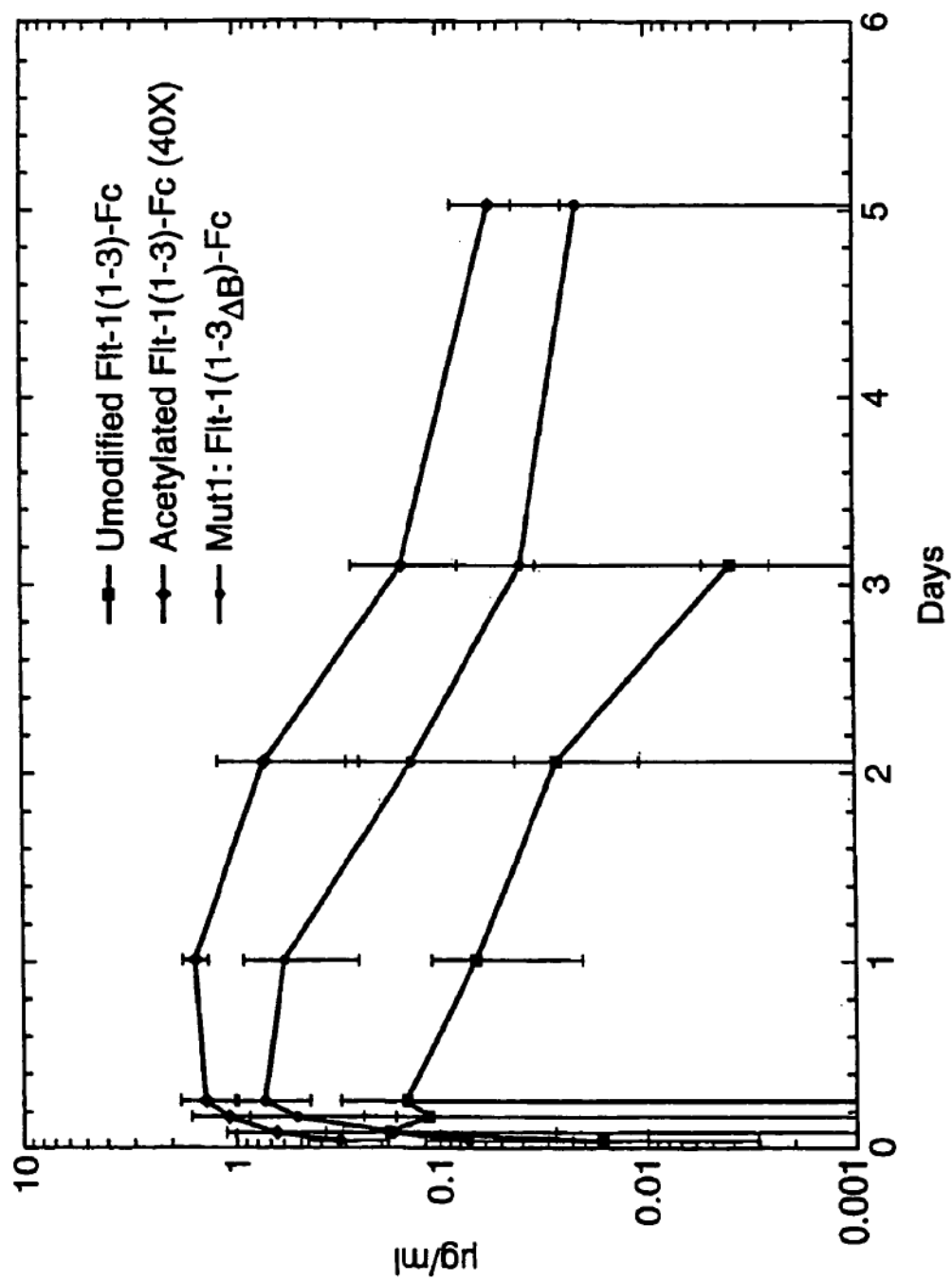


Fig.21A.

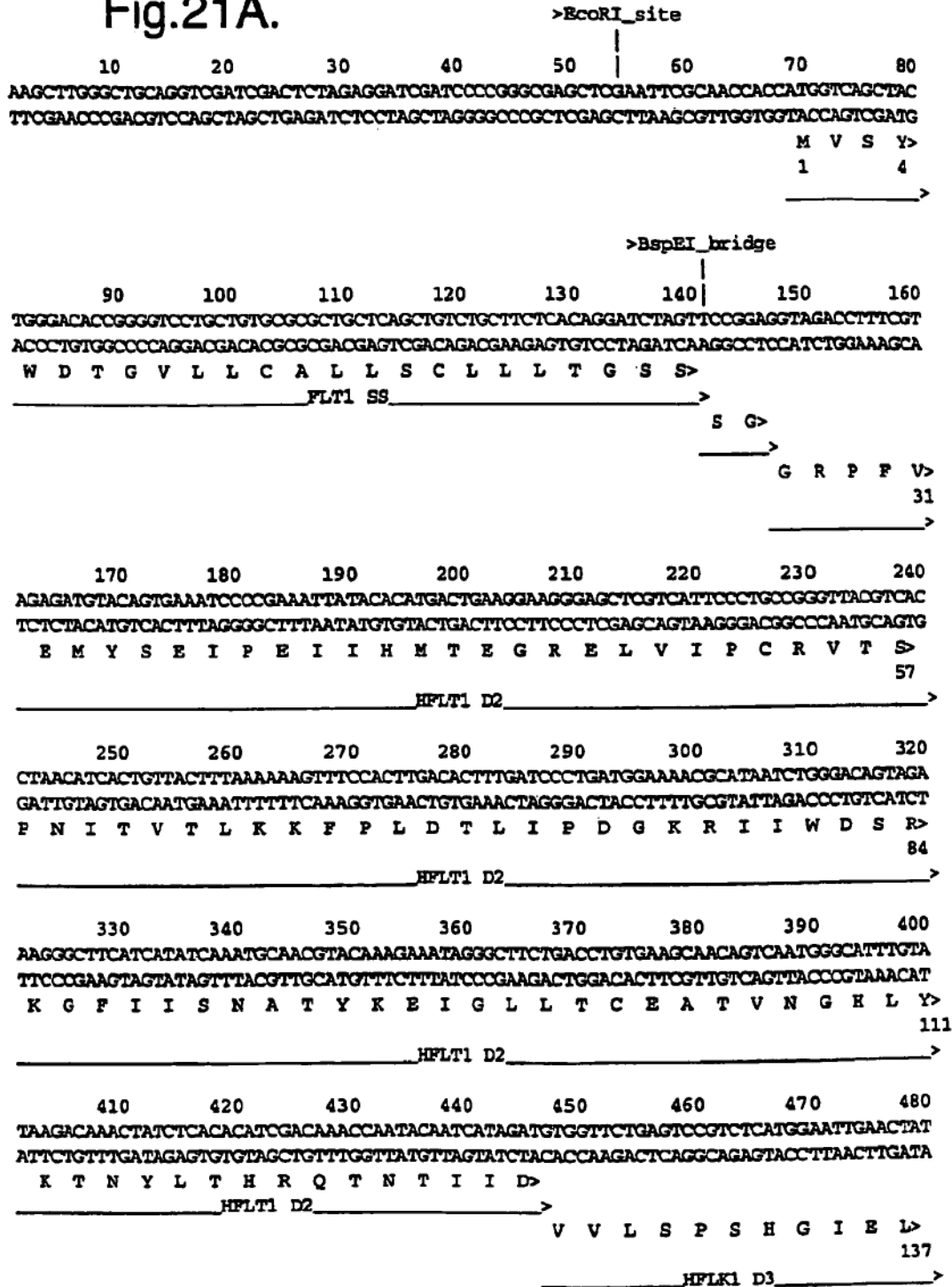


Fig.21B.

490 500 510 520 530 540 550 560
CTGTGGAGAAAAGCTTGTCTTAAATGTACAGCAAGAACTGAACTAAATGTGGGGATTGACTTCAACTGGGAATACCT
GACAACTCTTTTCGAACAGAATTTAACATGTGGTCTTGAAGTTGATTACACCCCTAACTGAAGTTGACCCCTATGGGA
S V G E K L V L N C T A R T E L N V G I D F N W E Y P>
164
HFLK1 D3>

570 580 590 600 610 620 630 640
TCTTGAAGCATCAGCTAAGAACTTGTAAACCGAGACCTAAAAACCCAGTCTGGGAGTGAGATGAAGAAATTTTGAG
AGAAGCTTGTAGTGTATTCTTTGAACATTTGGCTCTGGATTCTTGGGTCAGACCCCTACTCTACTTCTTTAAAAACTC
S S K H Q H K K L V N R D L K T Q S G S E M K K F L S>
191
HFLK1 D3>

650 660 670 680 690 700 710 720
CACTTAACATATAGATGGTGTAAACCCGAGTGACCAAGGATTGTACACCTGTGCAGCATCCAGTGGGCTGATGACCAAGA
GTGGAAATTGATATCTAACACATGGGCTCACTGGTCTCTAACATGTGGACACGTCGTAGGTCACCCGACTACTGGTCT
T L T I D G V T R S D Q G L Y T C A A S S G L M T K>
217
HFLK1 D3>

>Srf_Bridge_

730 740 750 760 770 780 790 800
AGAACAGCACATTGTCTAGGGTCCATGAAAAGGGCCCGGGGACAAAACCTCACACATGCCACCGTGGCCAGCACCTGAA
TCTTGTCTGTAAACAGTCCAGGTACTTTTCCCGGGCCCGCTGTCTTGTAGTGTGTACGGGTGGCACGGGTCTGGACTT
K N S T F V R V H E K>
HFLK1 D3>

G P G>

D K T H T C P P C P A P E>
244
FCAC1 (A)>

810 820 830 840 850 860 870 880
CTCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTCA
GAGGACCCCTTGGCAGTCAGAGGAGAGGGGGGTTTGGGTTCTGTGGGAGTACTAGAGGGCTGGGGACTCCAGTG
L L G G P S V F L F P P K P K D T L M I S R T P E V T>
271
FCAC1 (A)>

890 900 910 920 930 940 950 960
ATGGTGGTGGTGGACGTGAGCCAGAGACCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATG
TACGCACCAACCTGCACTCGGTGCTTCTGGGACTCCAGTTCAGTTGACCATGCACCTGCCGCACTCCAGTATTAC
C V V V D V S H E D P E V K F N W Y V D G V E V H N>
297
FCAC1 (A)>

970 980 990 1000 1010 1020 1030 1040
CCAAGACAAAGCGGGGAGGAGCAGTACAACAGCAGTACCGTGTGGTCAAGCTCTCACCGTCTGCACCAAGGACTGG
GGTCTGTCTTGGCGCCCTCTCGTCAATGTGTGTCATGTCACACCAAGTCCGAGGAGTGGCAGGAGTGGTCTGACC
A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W>
324
FCAC1 (A)>

Fig.21C.

1050 1060 1070 1080 1090 1100 1110 1120
CTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAGCCCTCCAGCCCCATCGAGAAACCATCTCCAAAGCCAA
GACTTACCGTTCTCATGTTCACGTTCAGAGGTGTGTTCCGGAGGGTCCGGGGTAGCTCTTTTGGTAGAGGTTTGGGTT
L N G K E Y K C K V S N K A L P A P I E K T I S K A K>
351
FCAC1 (A) >

>A>C_A_allotype
|
>G>T_A_allotype
| |
1130 1140 1150 1160 1170 1180 1190 1200
AGGGCAGCCCCGAGAACCACAGGTGTACACCTGCCGCCATCCCGGATGAGCTGACCAAGAACCAGGTTCAGCCTGACCT
TCCCGTCCGGGCTCTTGGTGTCCACATGTGGGACGGGGTAGGGCCCTACTCGACTGGTCTTGGTCCAGTCCGACTGGA
G Q P R E P Q V Y T L P P S R D E L T K N Q V S L T>
377
FCAC1 (A) >

1210 1220 1230 1240 1250 1260 1270 1280
GCCTGGTCAAAGGCTTCTATCCAGCGACATCCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACC
CGGACCAGTTTCCGAAGATAGGGTCGCTGTAGCGGCACCTCACCTCTCGTTACCCGTCGGCCCTCTGTGTGATGTCTGG
C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T>
404
FCAC1 (A) >

>T>C
|
1290 1300 1310 1320 1330 1340 1350 1360
ACGCCTCCCGTGTCTGGACTCCGACGGCTCCTTCTTCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGG
TGGGAGGGCAGACCTGAGGCTGCCGAGGAAGAAGGAGATATCGTTCGAGTGGCACCTGTTCTCGTCCACCGTGTGCC
T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G>
431
FCAC1 (A) >

1370 1380 1390 1400 1410 1420 1430 1440
GAACGCTCTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTA
CTTGCAAGAGATACGAGGCACTACGTACTCCGAGACGTGTGTTGATGTGCGTCTTCTCGGAGAGGGACAGAGGCCCAT
N V F S C S V M H E A L H N H Y T Q K S L S L S P G>
457
FCAC1 (A) >

>NotI_site
|
1450
AATGAGGGGGCGC
TTACTGGCGGGCG
K *>
458
>

Fig.22A.

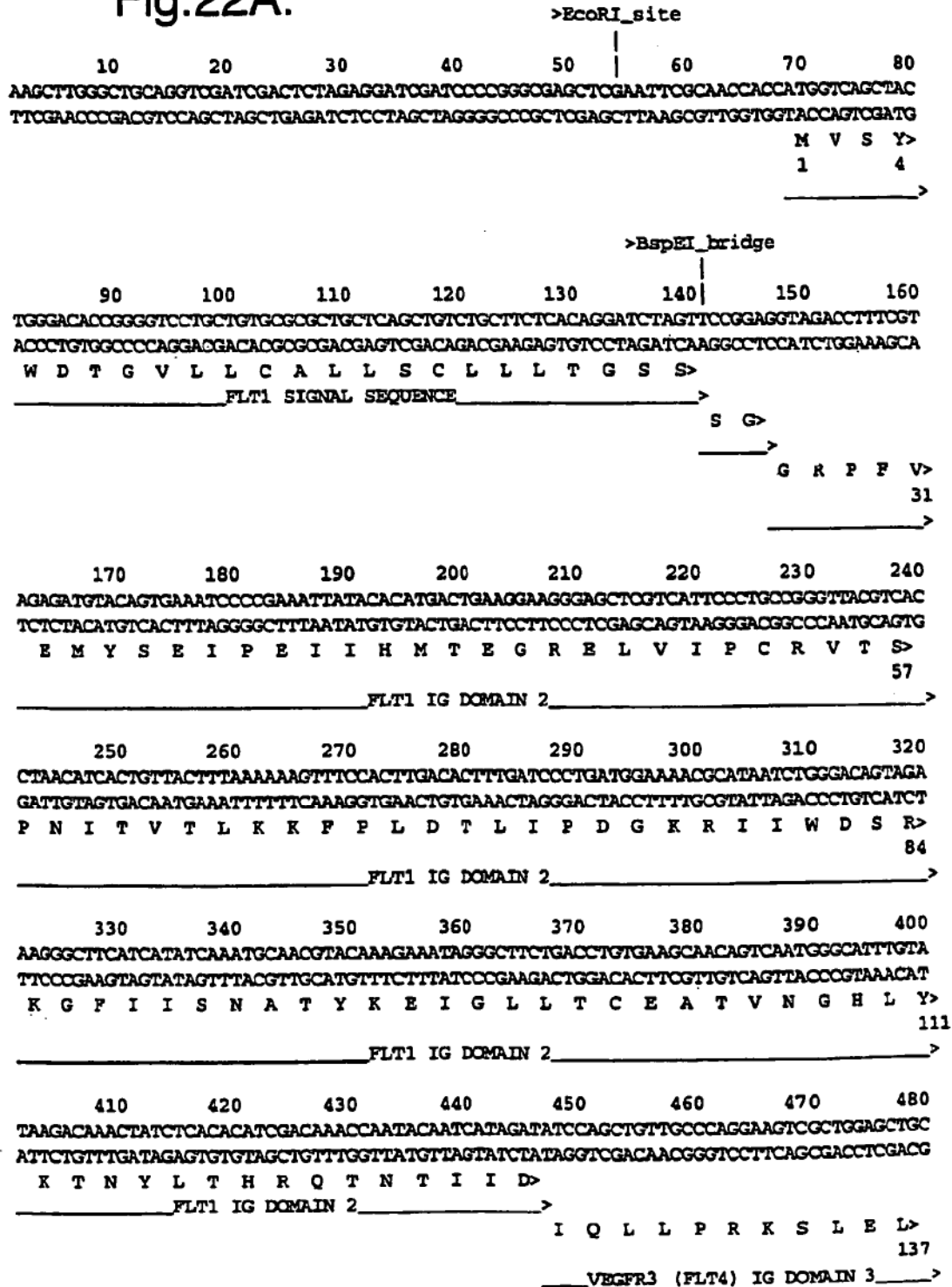


Fig.22B.

490 500 510 520 530 540 550 560
TGGTAGGGGAGAAGCTGGTCCTCAACTGCACCGTGTGGGCTGAGTTTAACTCAGGTGTCACCTTTGACTGGGACTACCCA
ACCATCCCCCTCTCGACCAGGAGTTGACGTGGGCACACCGACTCAAATTGAGTCCACAGTGGAAACTGACCGTGTATGGGT
L V G E K L V L N C T V W A E F N S G V T F D W D Y P>
164
____VEGFR3 (FLT4) IG DOMAIN 3____>

570 580 590 600 610 620 630 640
GGGAAGCAGGCAGAGCGGGGTAAGTGGGTGCCCGAGCGAGCTCCCAACAGACCCACACAGAACTCTCCAGCATCCTGAC
CCCTTCGTCCGTCTCGCCCCATTCAACCAAGGGCTCGCTGCGAGGGGTGTCTGGGTGTGTCTTGAGAGGTCGTAGGACTG
G K Q A E R G K W V P E R R S Q Q T H T E L S S I L T>
191
____VEGFR3 (FLT4) IG DOMAIN 3____>

650 660 670 680 690 700 710 720
CATCCACAACGTCAGCCAGCACGACCTGGGCTCGTATGTGTGCAAGGCCAACACCGGCATCCAGCGATTTCCGGAGAGCA
GTAGGTGTGTCAGTCCGTCTGCTGGACCGAGCATACACAGCTTCCGGTTGTGTCCTAGGTGCTAAAGCCCTCTCGT
I H N V S Q H D L G S Y V C K A N N G I Q R F R E S>
217
____VEGFR3 (FLT4) IG DOMAIN 3____>

730 740 750 760 770 780 790 800
COGAGGTCAATTGTGCATGAAATGGCCCGGGCGACAAAACCTCACACATGCCACCGTGGCCAGCACCTGAACCTCTGGGG
GGCTCCAGTAACACGTACTTTTACCGGGCCCGCTGTTTGAAGTGTGTACGGGTGGCACGGGTGCTGGACTTGAGGACCCC
T E V I V H E N>
____VEGFR3 (FLT4) IG____>
G P G>
____>
D K T H T C P P C P A P E L L G>
244
____FCAC1 - A ALLOTYP E____>

810 820 830 840 850 860 870 880
GGACCGTCAGTCTTCCTCTTCCCCCAAAACCAAGGACACCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGT
CCTGGCAGTCAGAGGAGGAGGGGGGTTTGGGTTCCTGTGGGAGTACTAGAGGGCCCTGGGACTCCAGTGTACGCAACA
G P S V F L F P P K P K D T L M I S R T P E V T C V V>
271
____FCAC1 - A ALLOTYP E____>

890 900 910 920 930 940 950 960
GGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAA
CCACCTGCACTCGGTGCTTCTGGGACTCCAGTTCAAGTTGACCATGCACCTGCGGCACCTCCACGTATTACGGTTCTGTT
V D V S H E D P E V K P N W Y V D G V E V H N A K T>
297
____FCAC1 - A ALLOTYP E____>

970 980 990 1000 1010 1020 1030 1040
AGCCGGGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTTCAGCGTCTCTCACCGTCTCTGCACCAGGACTGGCTGAATGGC
TCGGGCGCCCTCTCTCGTCATGTTGTGTGTCATGGCACACAGTCCGAGGAGTGGCAGGACGTGGTCTCTGACCGACTTACCG
K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G>
324
____FCAC1 - A ALLOTYP E____>

Fig.22C.

```

      1050      1060      1070      1080      1090      1100      1110      1120
AAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCC
TTCTCATGTTTCACGTTCCAGAGGTGTGTTTCGGGAGGGTCGGGGGTAGCTCTTTTGGTAGAGGTTTCGGTTTCCCGTCGG
K E Y K C K V S N K A L P A P I E K T I S K A K G Q P>
                                                                351
_____FCAC1 - A ALLOTYPE_____>

                                >A>C_A_allotype
                                |
                                >G>T_A_allotype
                                |
                                |
      1130      1140      1150      1160      1170      1180      1190      1200
CCGAGAACACAGGTGTACACCCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCTGGTCA
GGCTCTTGGTGTCCACATGTGGGACGGGGGTAGGGCCCTACTCGACTGGTTCTTGGTCCAGTCCGACTGGACGGACCAAGT
R E P Q V Y T L P P S R D E L T K N Q V S L T C L V>
                                                                377
_____FCAC1 - A ALLOTYPE_____>

      1210      1220      1230      1240      1250      1260      1270      1280
AAGGCTTCTATCCAGCGACATGCGCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCAAGCCTCCC
TTCCGAAGATAGGGTCGCTGTAGCGGCACCTCACCTCTCGTTACCCGTCGGCCTCTTGTGTGATGTTCTGGTGGGAGGG
K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P>
                                                                404
_____FCAC1 - A ALLOTYPE_____>

                                >T>C
                                |
      1290      1300      1310      1320      1330      1340      1350      1360
GTGCTGGACTCCGACGGCTCCTTCTTCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT
CACGACCTGAGGCTGCCGAGGAAGAAGGAGATATCGTTGAGTGGCACCTGTCTCGTCCACCGTCGTCCCTTGCAGAA
V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F>
                                                                431
_____FCAC1 - A ALLOTYPE_____>

                                >NotI_site
                                |
      1370      1380      1390      1400      1410      1420      1430      1440
CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGAGCGG
GAGTACGAGGCACTACGTACTCCGAGACGTGTGTTGATGTGCGTCTTCTCGGAGAGGGACAGAGGCCCATTTACTCGCC
S C S V M H E A L H N H Y T Q K S L S L S P G K ">
                                                                455
_____FCAC1 - A ALLOTYPE_____>

CCGC
GGCG
```

Fig.23.

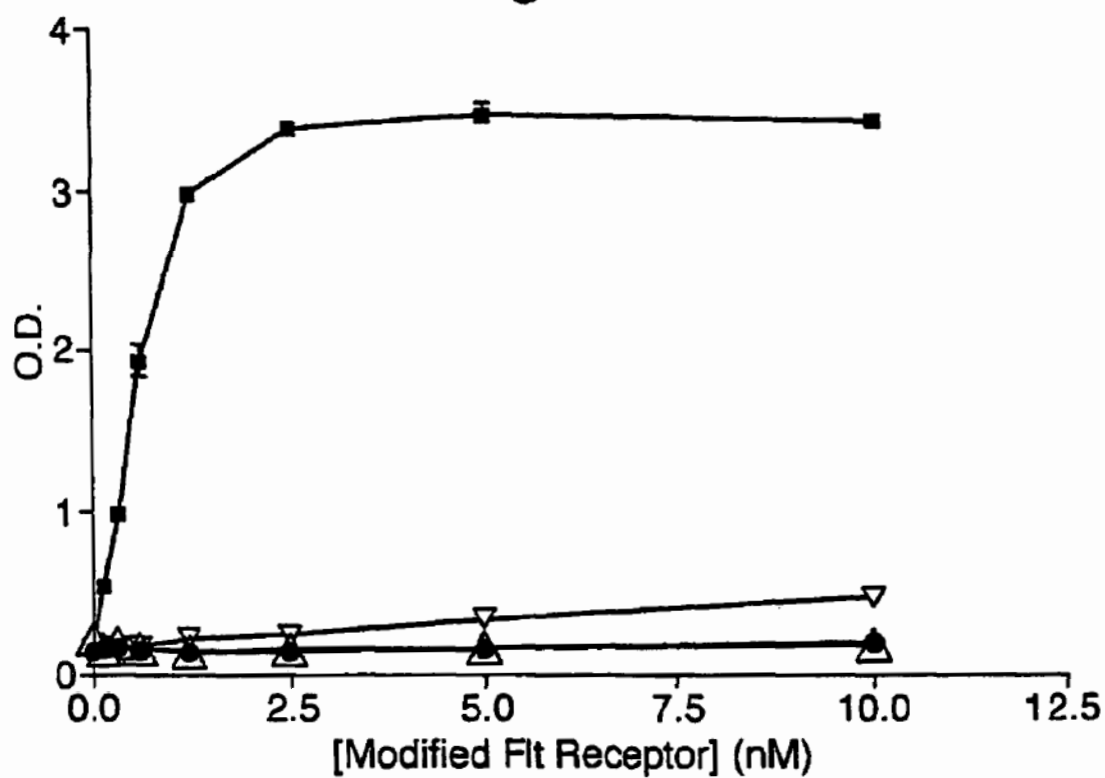


Fig.24A.

```

      10      20      30      40      50      60
      *      *      *      *      *      *
ATG GTC AGC TAC TGG GAC ACC GGG GTC CTG CTG TGC GCG CTG CTC AGC TGT CTG CTT CTC
TAC CAG TCG ATG ACC CTG TGG CCC CAG GAC GAC ACG CGC GAC GAG TCG ACA GAC GAA GAG
M V S Y W D T G V L L C A L L S C L L L>
1_____5_____hFLT1 SIGNAL SEQUENCE_____15_____20>

      70      80      90      100      110      120
      *      *      *      *      *      *
ACA GGA TCT AGT TCC GGA AGT GAT ACC GGT AGA CCT TTC GTA GAG ATG TAC AGT GAA ATC
TGT CCT AGA TCA AGG CCT TCA CTA TGG CCA TCT GGA AAG CAT CTC TAC ATG TCA CTT TAG
T G S S S G>
21_hFLT1 SIGNAL SEQ_26>

      S D T G R P F V E M Y S E I>
      27_____30_____hFLT1 IG DOMAIN 2_____40>

      130      140      150      160      170      180
      *      *      *      *      *      *
CCC GAA ATT ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT ACG TCA
GGG CTT TAA TAT GTG TAC TGA CTT CCT TCC CTC GAG CAG TAA GGG ACG GGC CAA TGC AGT
P E I I H M T E G R E L V I P C R V T S>
41_____45_____hFLT1 IG DOMAIN 2_____55_____60>

      190      200      210      220      230      240
      *      *      *      *      *      *
CCT AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC CCT GAT GGA AAA
GGA TTG TAG TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG GGA CTA CCT TTT
P N I T V T L K K F P L D T L I P D G K>
61_____65_____hFLT1 IG DOMAIN 2_____75_____80>

      250      260      270      280      290      300
      *      *      *      *      *      *
CGC ATA ATC TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG TAC AAA GAA ATA
GGG TAT TAG ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC ATG TTT CTT TAT
R I I W D S R K G F I I S N A T Y K E I>
81_____85_____hFLT1 IG DOMAIN 2_____95_____100>

      310      320      330      340      350      360
      *      *      *      *      *      *
GGG CTT CTG ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT CTC ACA
CCC GAA GAC TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT TTG ATA GAG TGT
G L L T C E A T V N G H L Y K T N Y L T>
101_____105_____hFLT1 IG DOMAIN 2_____115_____120>

      370      380      390      400      410      420
      *      *      *      *      *      *
CAT CGA CAA ACC AAT ACA ATC ATA GAT GTG GTT CTG AGT CCG TCT CAT GGA ATT GAA CTA
GTA GCT GTT TGG TTA TGT TAG TAT CTA CAC CAA GAC TCA GGC AGA GTA CCT TAA CTT GAT
H R Q T N T I I D>
121_____hFLT1 IG DOMAIN 2_____129>

      V V L S P S H G I E L>
      130_____hFLKL IG DOMAIN 3_____140>

```

Fig.24B.

```

      430      440      450      460      470      480
      *      *      *      *      *      *
TCT GTT GGA GAA AAG CTT GTC TTA AAT TGT ACA GCA AGA ACT GAA CTA AAT GTG GGG ATT
AGA CAA CCT CTT TTC GAA CAG AAT TTA ACA TGT CGT TCT TGA CTT GAT TTA CAC CCC TAA
S V G E K L V L N C T A R T E L N V G I>
141_____145_____hFLK1 IG DOMAIN 3_____155_____160>

      490      500      510      520      530      540
      *      *      *      *      *      *
GAC TTC AAC TGG GAA TAC OCT TCT TCG AAG CAT CAG CAT AAG AAA CTT GTA AAC CGA GAC
CTG AAG TTG ACC CTT ATG GGA AGA AGC TTC GTA GTC GTA TTC TTT GAA CAT TTG GCT CTG
D F N W E Y P S S K H Q H K K L V N R I>
161_____165_____hFLK1 IG DOMAIN 3_____175_____180>

      550      560      570      580      590      600
      *      *      *      *      *      *
CTA AAA ACC CAG TCT GGG AGT GAG ATG AAG AAA TTT TTG AGC ACC TTA ACT ATA GAT GGT
GAT TTT TGG GTC AGA CCC TCA CTC TAC TTC TTT AAA AAC TCG TGG AAT TGA TAT CTA CCA
L K T Q S G S E M K K F L S T L T I D G>
181_____185_____hFLK1 IG DOMAIN 3_____195_____200>

      610      620      630      640      650      660
      *      *      *      *      *      *
GTA ACC CGG AGT GAC CAA GGA TTG TAC ACC TGT GCA GCA TCC AGT GGG CTG ATG ACC AAG
CAT TGG GGC TCA CTG GTT OCT AAC ATG TGG ACA CGT CGT AGG TCA CCC GAC TAC TGG TTC
V T R S D Q G L Y T C A A S S G L M T K>
201_____205_____hFLK1 IG DOMAIN 3_____215_____220>

      670      680      690      700      710      720
      *      *      *      *      *      *
AAG AAC AGC ACA TTT GTC AGG GTC CAT GAA AAG GAC AAA ACT CAC ACA TGC CCA CCG TGC
TTC TTG TCG TGT AAA CAG TCC CAG GTA CTT TTC CTG TTT TGA GTG TGT ACG GGT GGC ACG
K N S T F V R V H E K>
221_____hFLK1 IG DOMAIN 3_____231>
      D K T H T C P P C>
      232_____hFCAC1 A_____240>

      730      740      750      760      770      780
      *      *      *      *      *      *
CCA GCA OCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC OCC CCA AAA CCC AAG GAC
GGT CGT GGA CTT GAG GAC CCC OCT GGC AGT CAG AAG GAG AAG GGG GGT TTT GGG TTC CTG
P A P E L L G G P S V F L F P P K P K I>
241_____245_____hFCAC1 A_____255_____260>

      790      800      810      820      830      840
      *      *      *      *      *      *
ACC CTC ATG ATC TCC CGG ACC OCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA
TGG GAG TAC TAG AGG GCC TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC TCG GTG CTT
T L M I S R T P E V T C V V V D V S H E>
261_____265_____hFCAC1 A_____275_____280>

      850      860      870      880      890      900
      *      *      *      *      *      *
GAC OCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA
CTG GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA TTA CCG TTC TGT
D P E V K F N W Y V D G V E V H N A K T>
281_____285_____hFCAC1 A_____295_____300>

```

Fig.24C.

910 920 930 940 950 960
* * * * *
AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG
TTC GGC GCC CTC CTC GTC ATG TTG TGC TGC ATG GCA CAC CAG TCG CAG GAG TGG CAG GAC
K P R E E Q Y N S T Y R V V S V L T V L>
301_____305_____hFCAC1 A _____315_____320>

970 980 990 1000 1010 1020
* * * * *
CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA
GTG GTC CTG ACC GAC TTA CCG TTC CTC ATG TTC ACG TTC CAG AGG TTG TTT CCG GAG GGT
H Q D W L N G K E Y K C K V S N K A L P>
321_____325_____hFCAC1 A _____335_____340>

1030 1040 1050 1060 1070 1080
* * * * *
GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC
CGG GGG TAG CTC TTT TGG TAG AGG TTT CCG TTT CCC GTC GGG GCT CTT GGT GTC CAC ATG
A P I E K T I S K A K G Q P R E P Q V Y>
341_____345_____hFCAC1 A _____355_____360>

1090 1100 1110 1120 1130 1140
* * * * *
ACC CTG CCC CCA TCC CCG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC
TGG GAC GGG GGT AGG GCC CTA CTC GAC TGG TTC TTG GTC CAG TCG GAC TGG ACG GAC CAG
T L P P S R D E L T K N Q V S L T C L V>
361_____365_____hFCAC1 A _____375_____380>

1150 1160 1170 1180 1190 1200
* * * * *
AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC
TTT CCG AAG ATA GGG TCG CTG TAG CCG CAC CTC ACC CTC TCG TTA CCC GTC GGC CTC TTG
K G F Y P S D I A V E W E S N G Q P E N>
381_____385_____hFCAC1 A _____395_____400>

1210 1220 1230 1240 1250 1260
* * * * *
AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG
TTG ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG CCG AGG AAG AAG GAG ATG TCG TTC
N Y K T T P P V L D S D G S F P L Y S K>
401_____405_____hFCAC1 A _____415_____420>

1270 1280 1290 1300 1310 1320
* * * * *
CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT
GAG TGG CAC CTG TTC TCG TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG CAC TAC GTA
L T V D K S R W Q Q G N V F S C S V M H>
421_____425_____hFCAC1 A _____435_____440>

1330 1340 1350 1360 1370
* * * * *
GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA
CTC CGA GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC CCA TTT ACT
E A L H N H Y T Q K S L S L S P G K *>
441_____445_____hFCAC1 A _____455_____458>

Fig.25A.

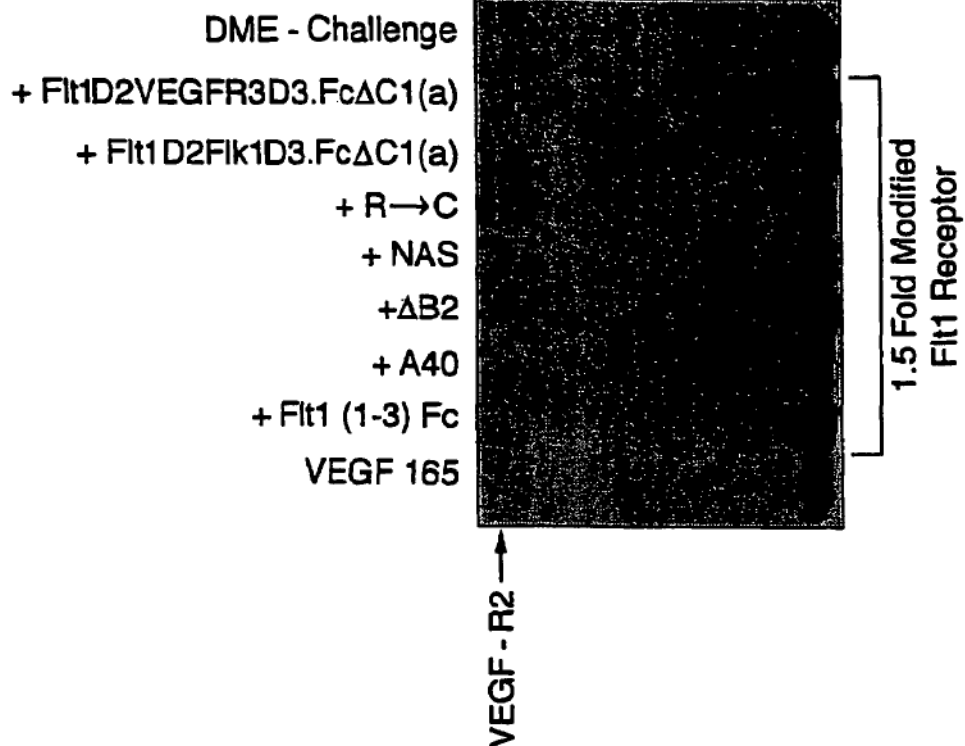


Fig.25B.

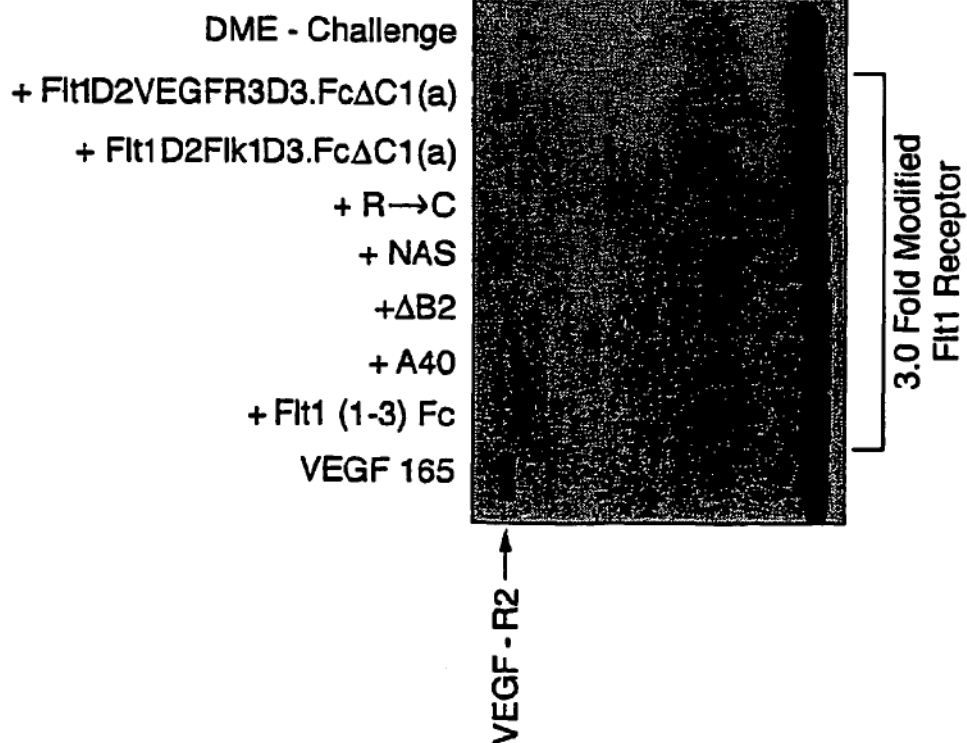


Fig.25C.

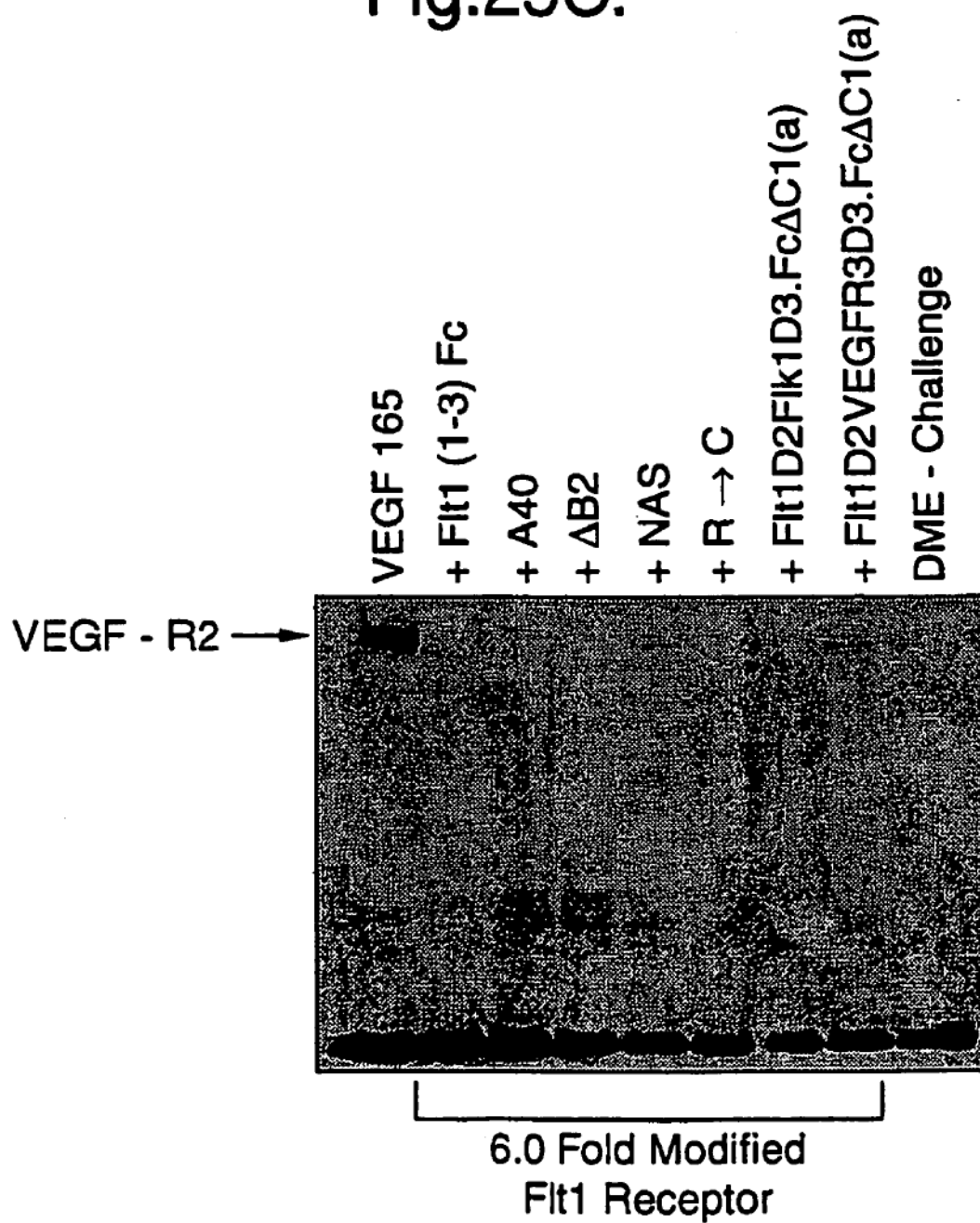


Fig.26A.

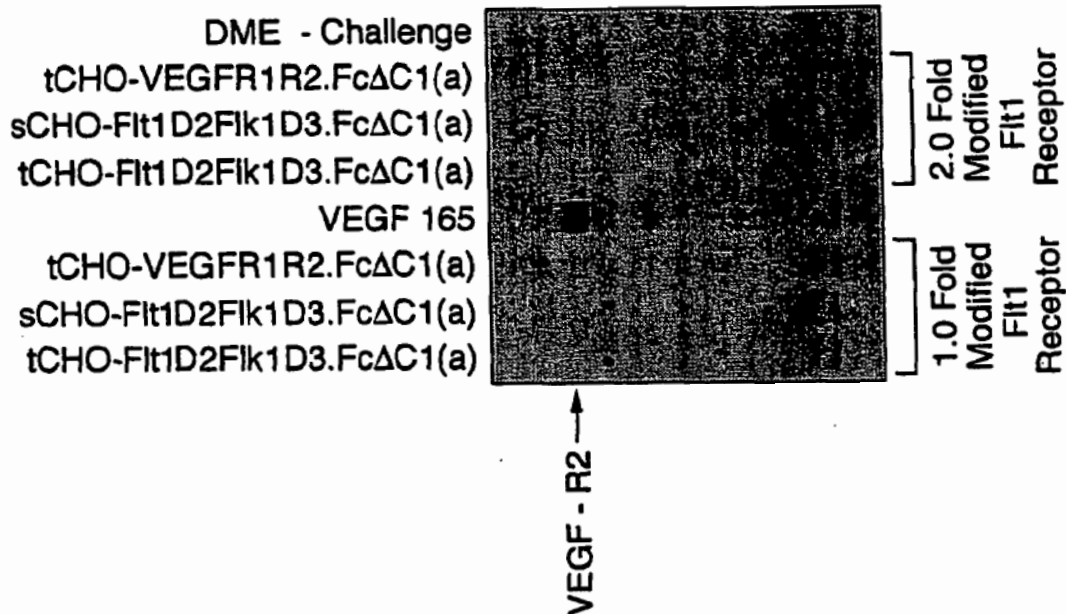


Fig.26B.

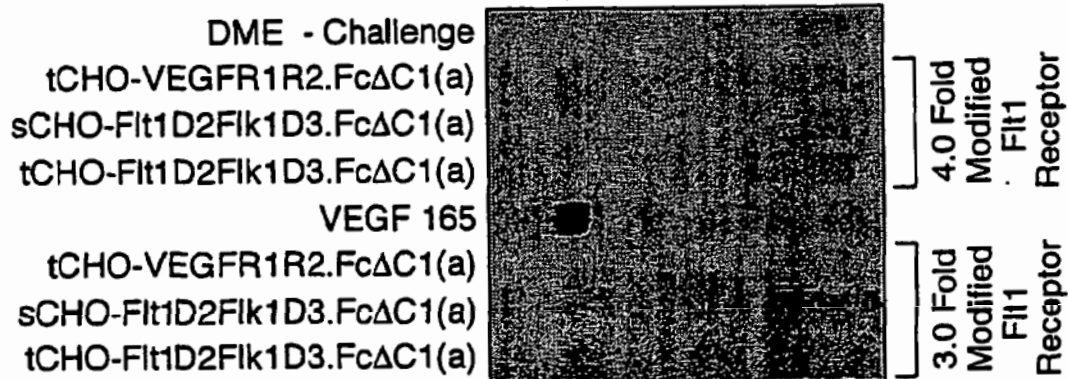


Fig.27.

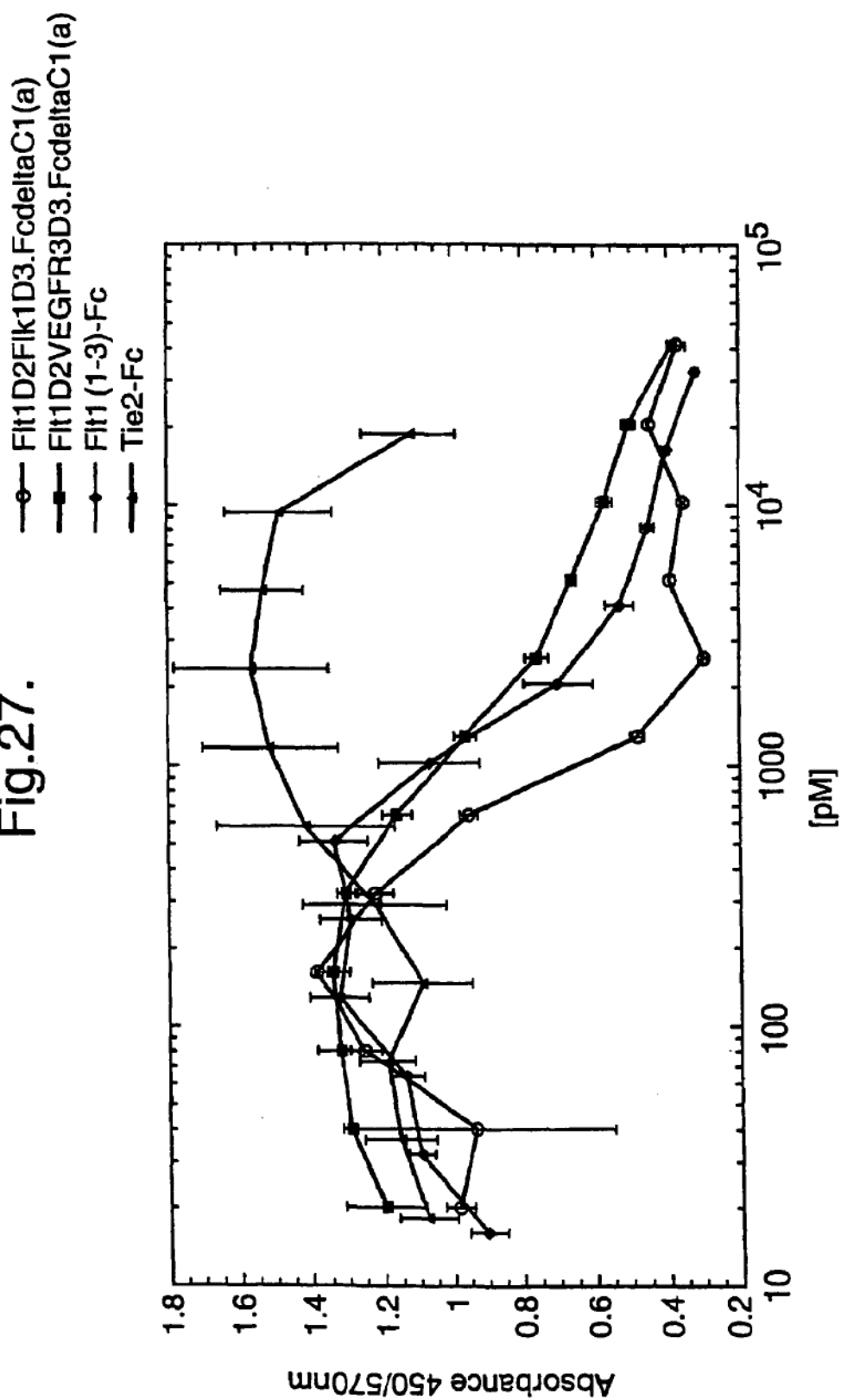


Fig.28.

Binding Stoichiometry of hVEGF165 to Flt1D2Flk1D3.FcΔC1(a) & VEGFR1R2-FcΔC1(a)		
hVEGF165 (nM)	VEGF/Flt1D2Flk1D3.FcΔC1(a)	VEGF/VEGFR1R2-FcΔC1(a)
1	0.93	0.98
10	0.97	0.94
50	1	0.99
Average ± StDev	0.96 ± 0.03	0.97 ± 0.02

Fig.29.

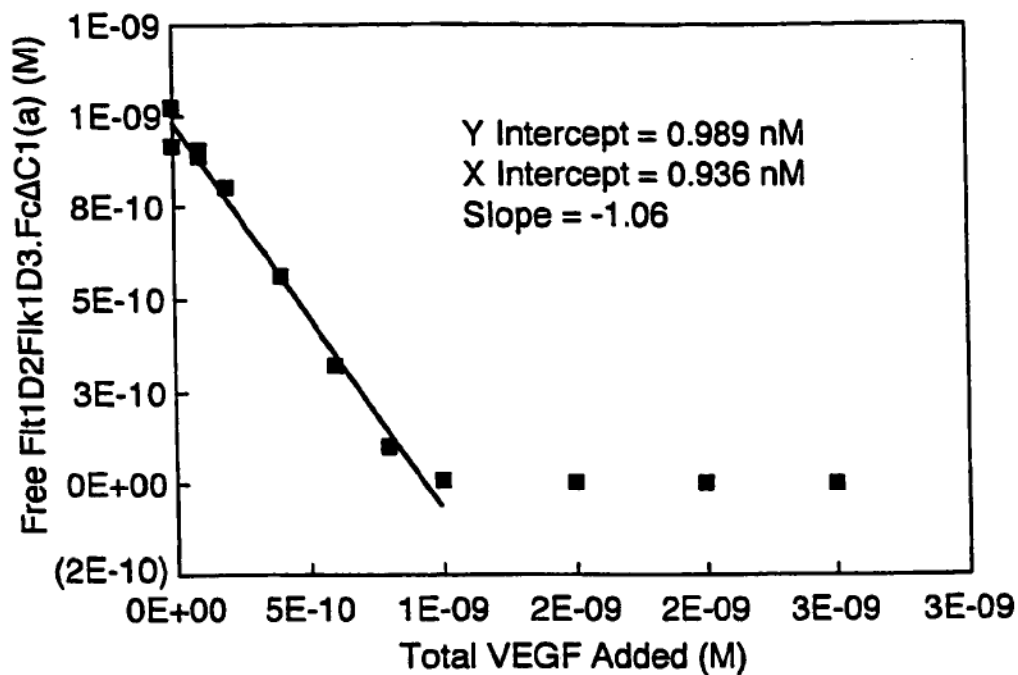


Fig.30.

