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Papadopoulos et al.

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(54) **MODIFIED CHIMERIC POLYPEPTIDES WITH IMPROVED PHARMACOKINETIC PROPERTIES**

WO WO98/13071 4/1998
WO WO99/03996 1/1999

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This patent is subject to a terminal disclaimer.

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(65) **Prior Publication Data**
US 2005/0163798 A1 Jul. 28, 2005

Related U.S. Application Data

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(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; 514/12; 530/350; 536/23.4**

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

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(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.

7 Claims, 55 Drawing Sheets

Fig.1.

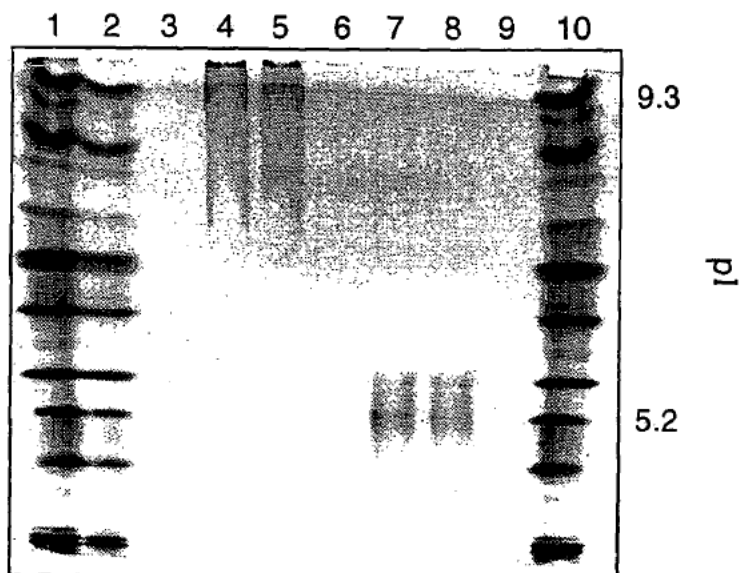


Fig.2.

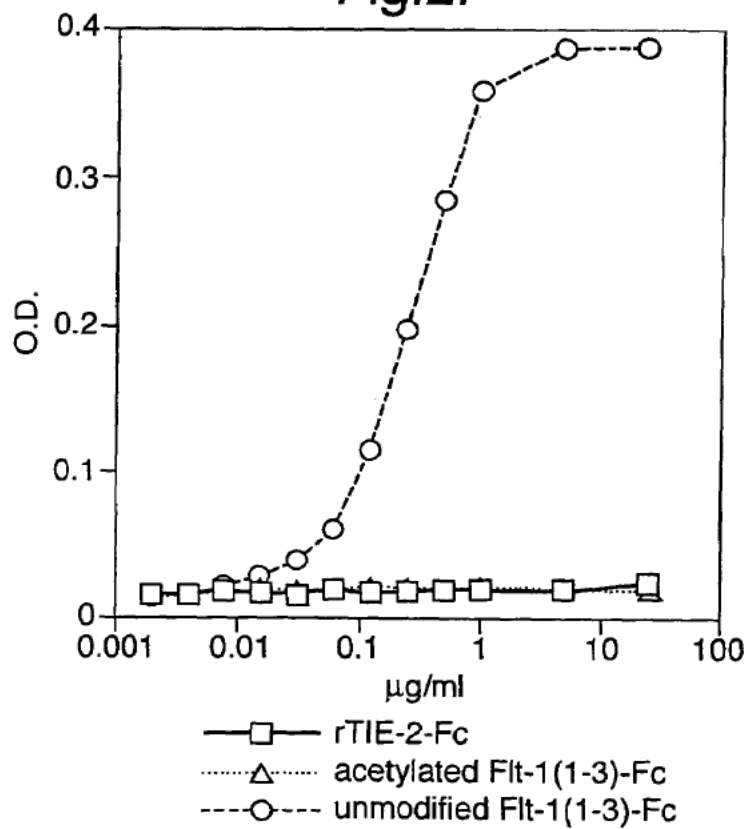


Fig.4.

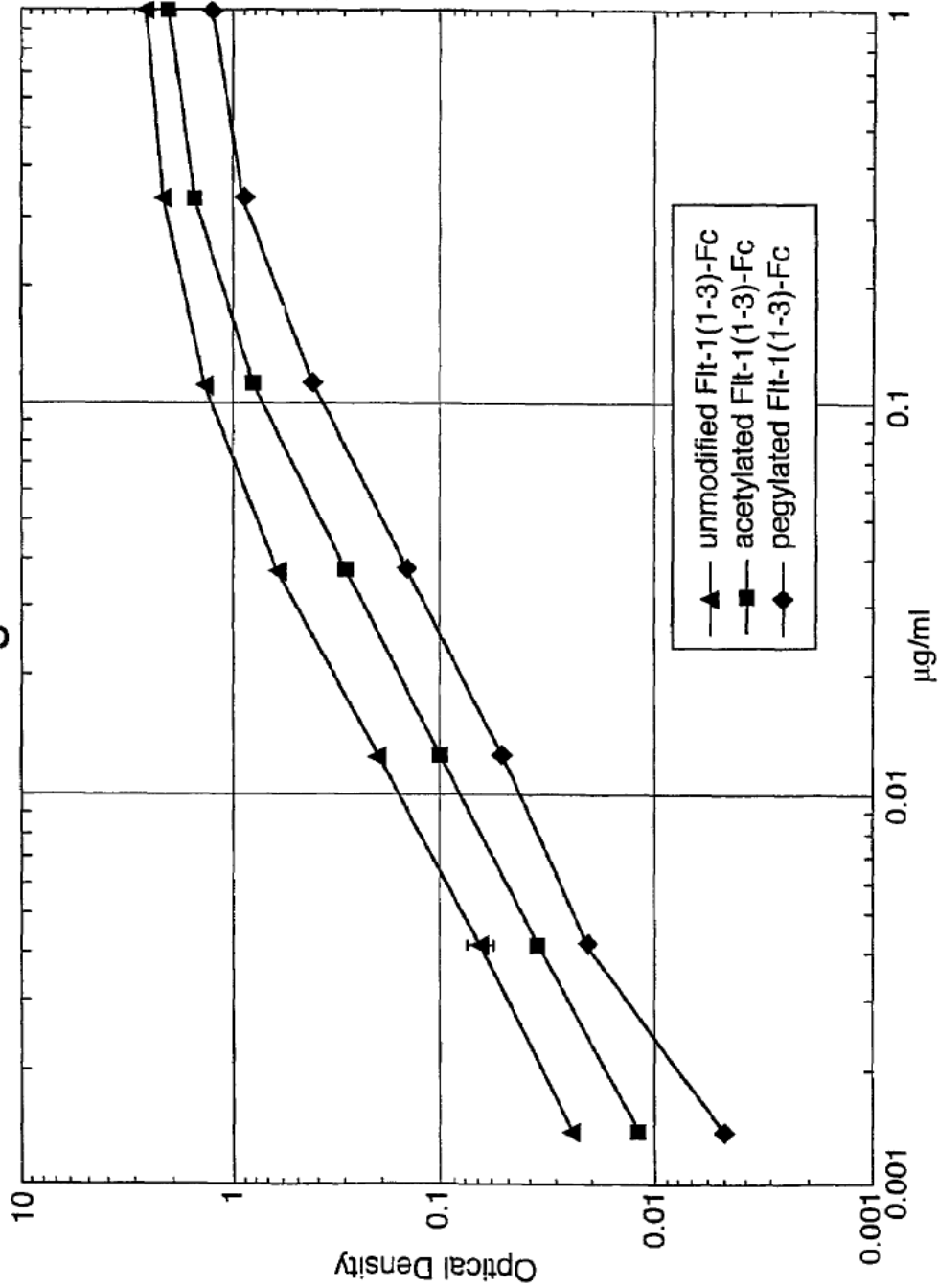


Fig.5.

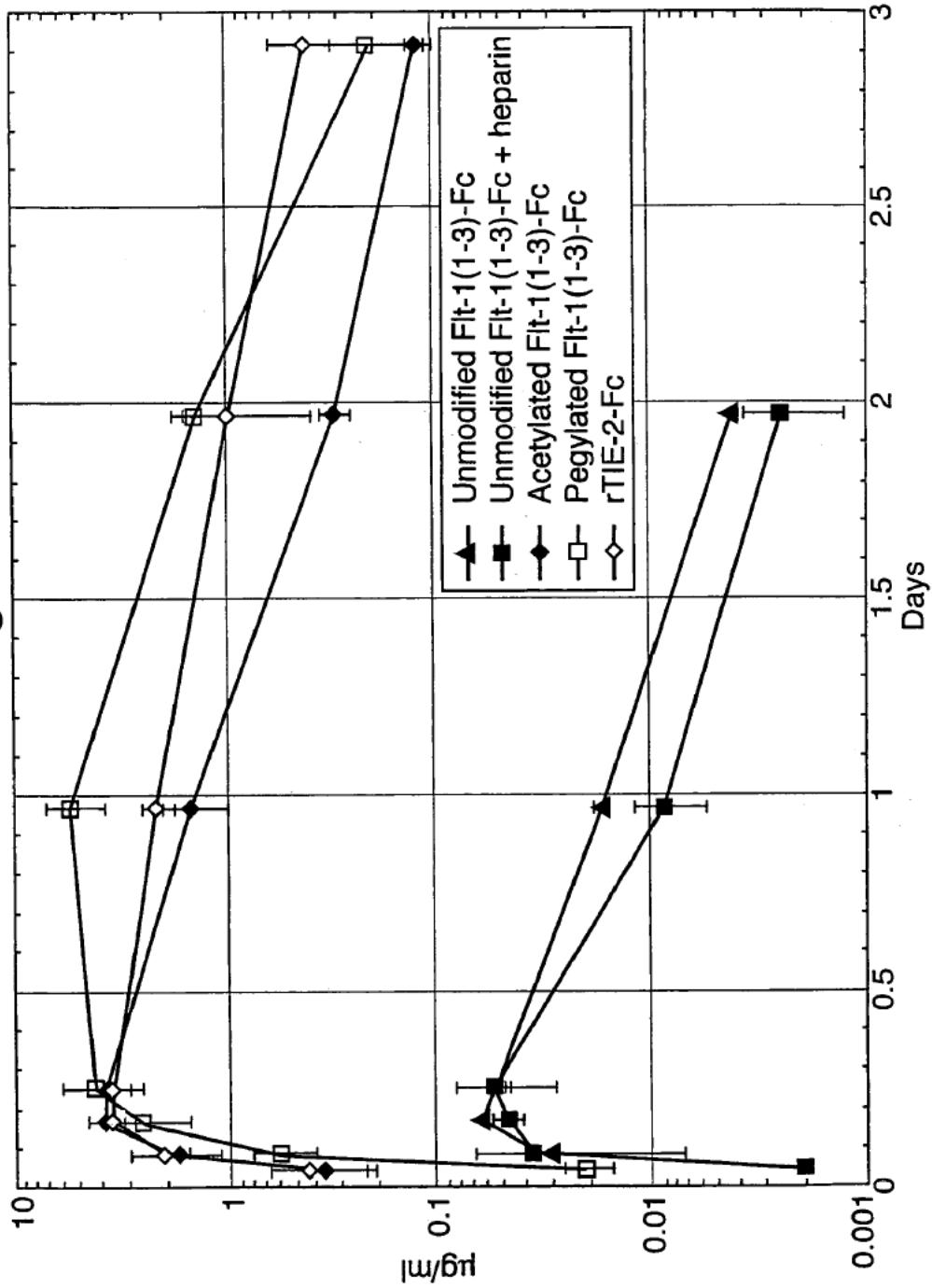


Fig.6A.

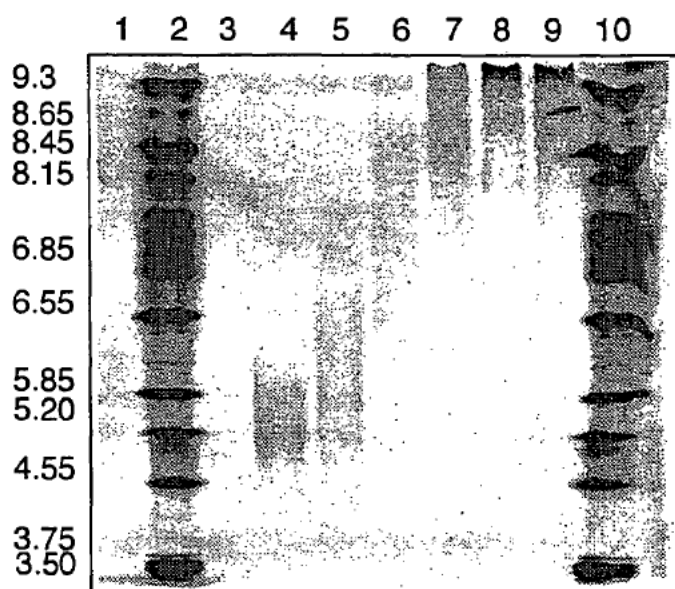


Fig.6B.

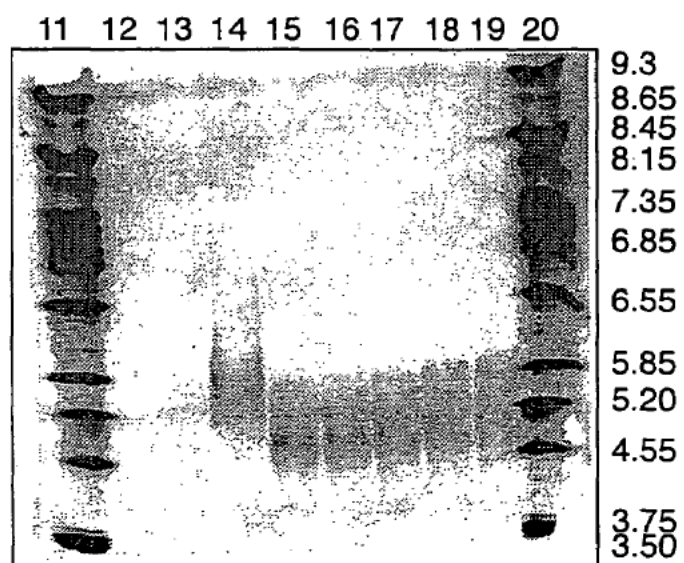
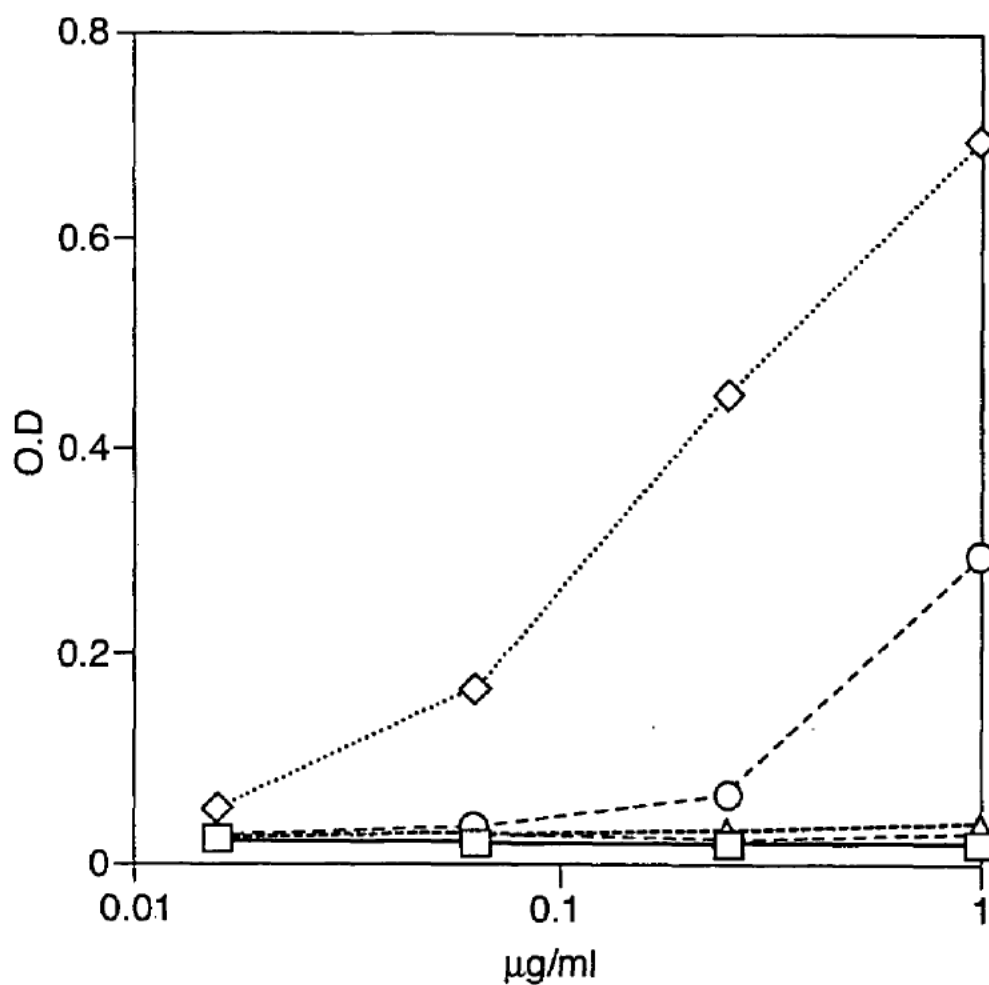


Fig.7



- 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.9.

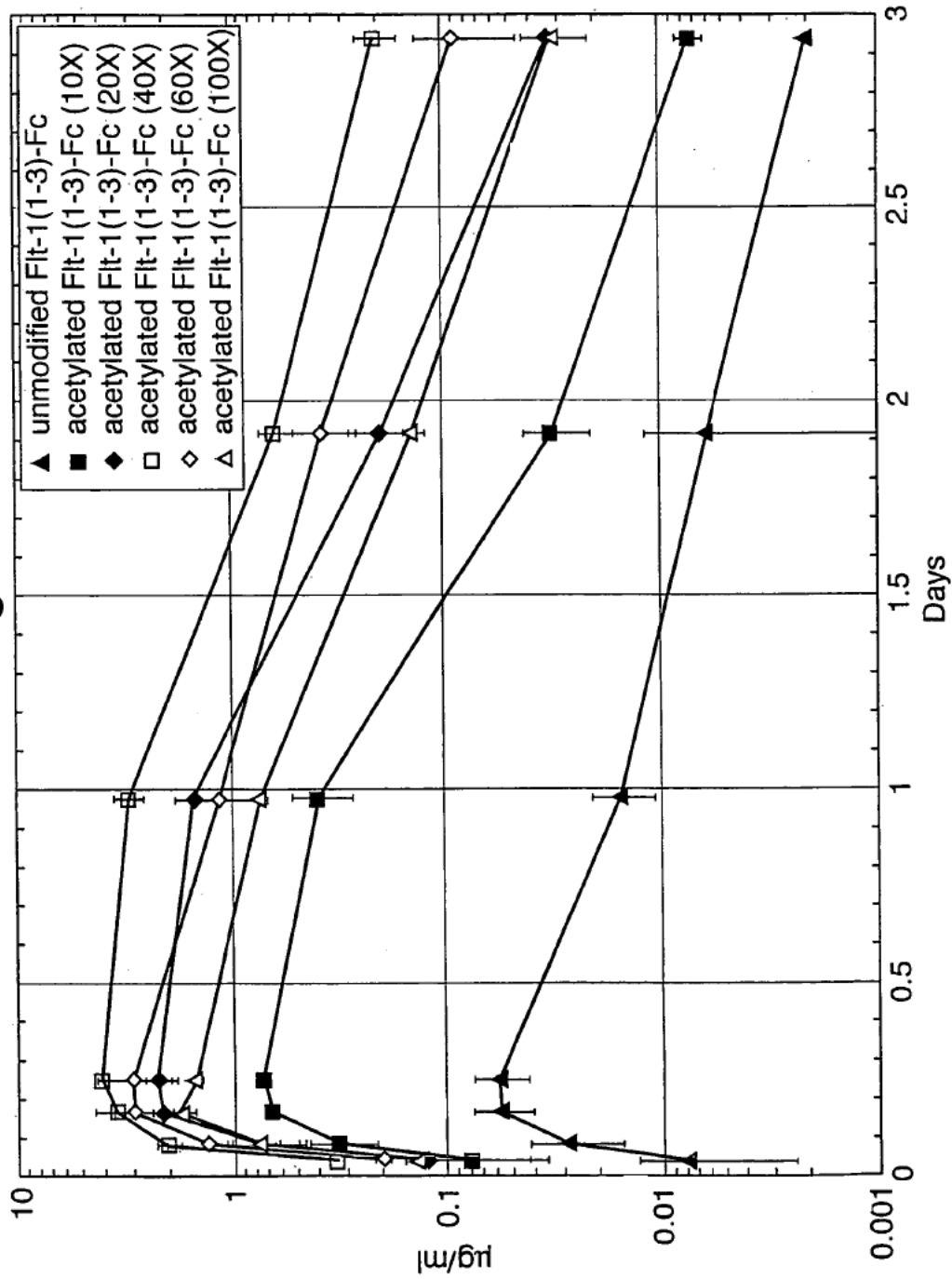


Fig.10A.

```
      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 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 CGG
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 OCT 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>
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Fig. 10B.

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      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 CGG 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>

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Fig.10C.

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      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 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 CGG 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 CAG 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>

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Fig.10D.

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      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.

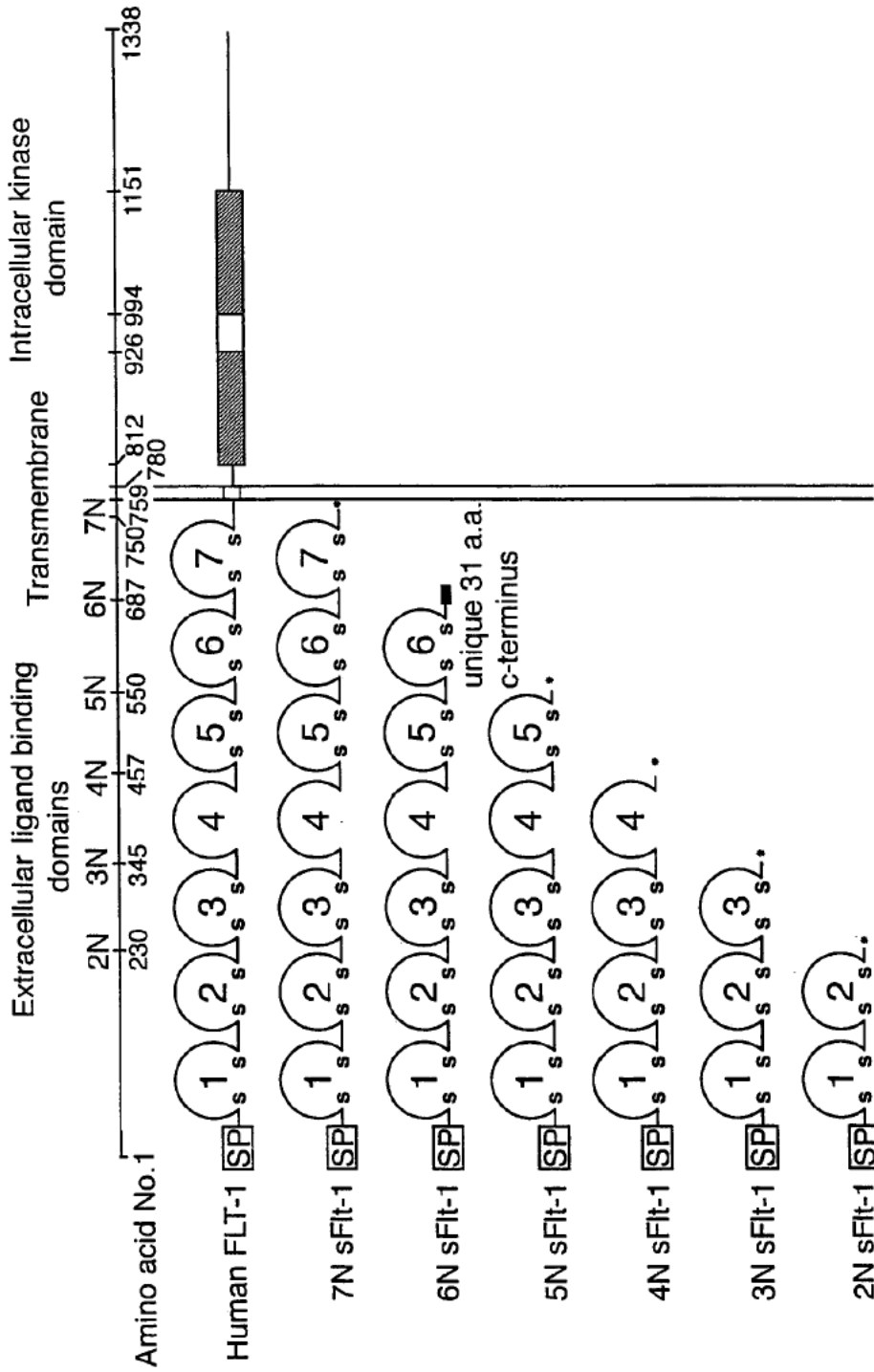


Fig.12A.

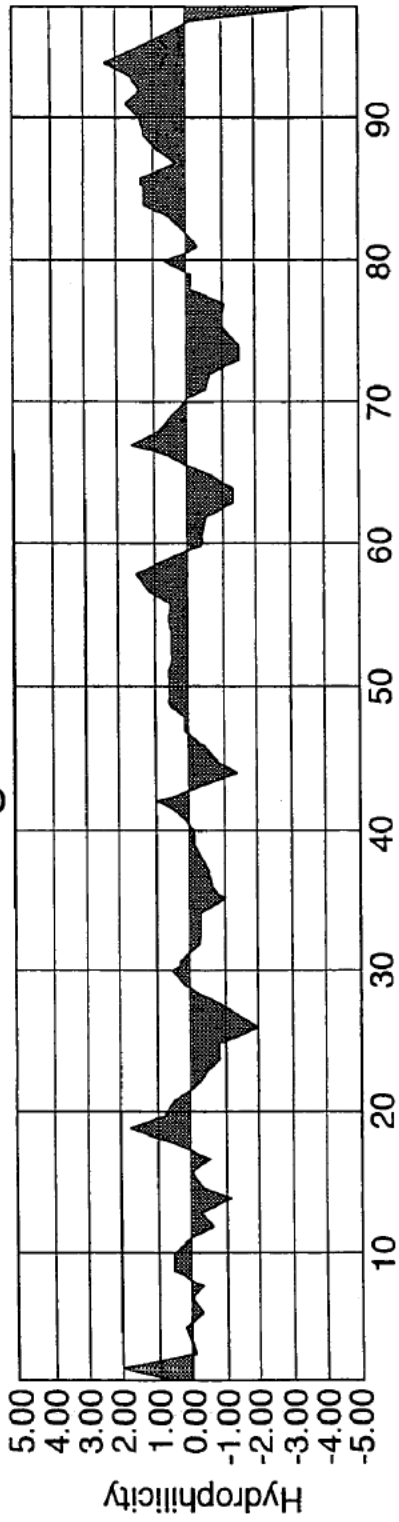


Fig.12B.

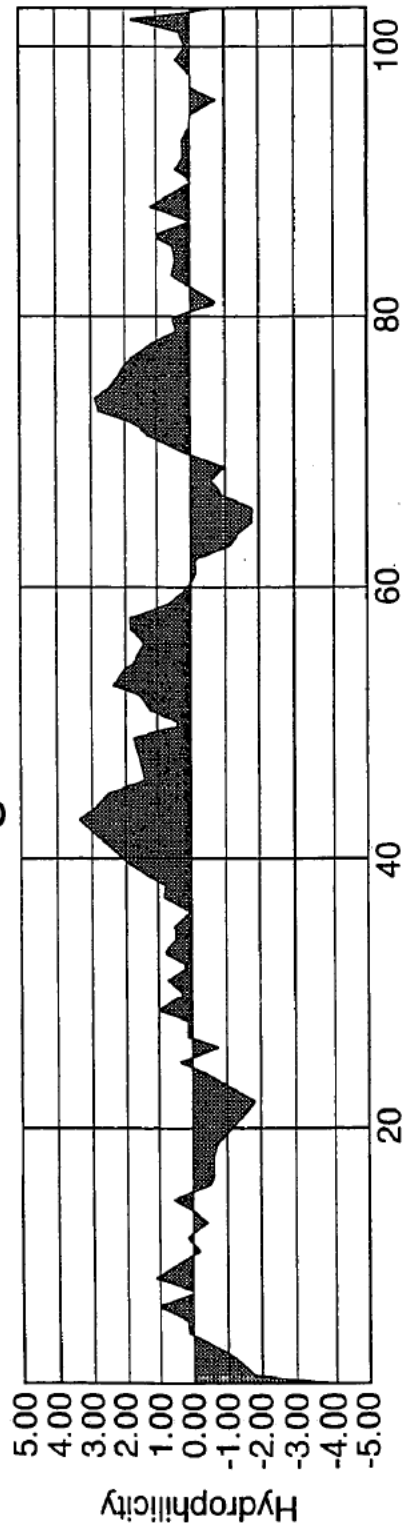


Fig.13A.

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 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 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 CGG
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 TIC 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 CGG 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 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 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 CGG TTG
Arg Val Gln Met Thr 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 CGG 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 CGG 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 CGG GAG GGT CGG
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 GCA CAA
TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT TTG ATA GAG TGT GTA CGT 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 CGG
CCC CCT GGC AGT CAG AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG GAG TAC TAG AGG GCC
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 TAG AGG 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 CGG 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 GGC 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 CGG
TAG AGG TTT CCG TTT CCC GTC GGG GCT CTT GGT GTC CAC ATG TGG GAC GGC 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 CCG 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 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 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 CGG 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 CGG 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 CGG GAG GGT CGG GGG TAG CTC TTT TGG TAG AGG TTT CGG 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 GOC 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 GCC 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 OCC 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 COG
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 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 CGG 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. 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 GCC AAC ATA TTC TAC AGT GTT CTT ACT ATT GAC AAA
GCT TAA CTG GTT TCG TTA AGG GTA CGG 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 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 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 CGG 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 CTC 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.

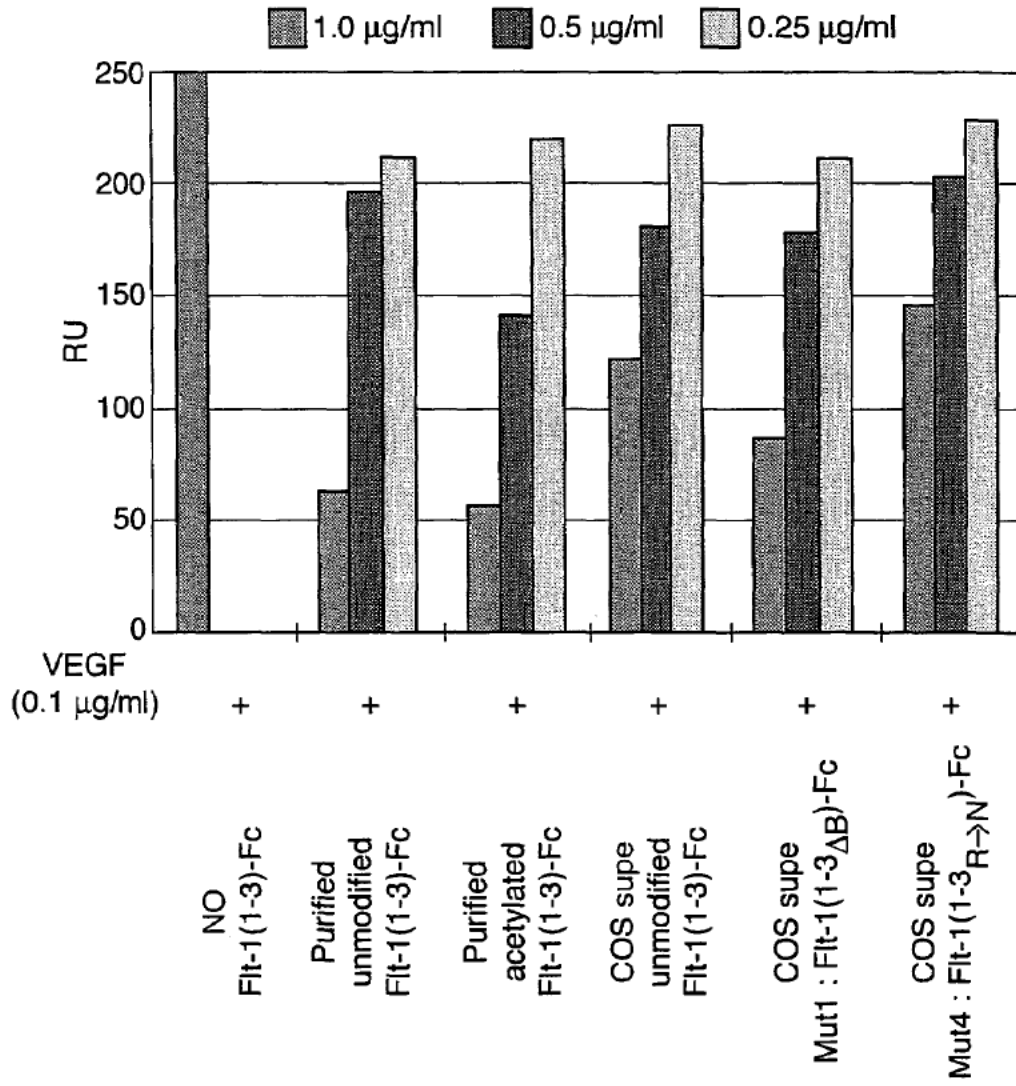


Fig.18.

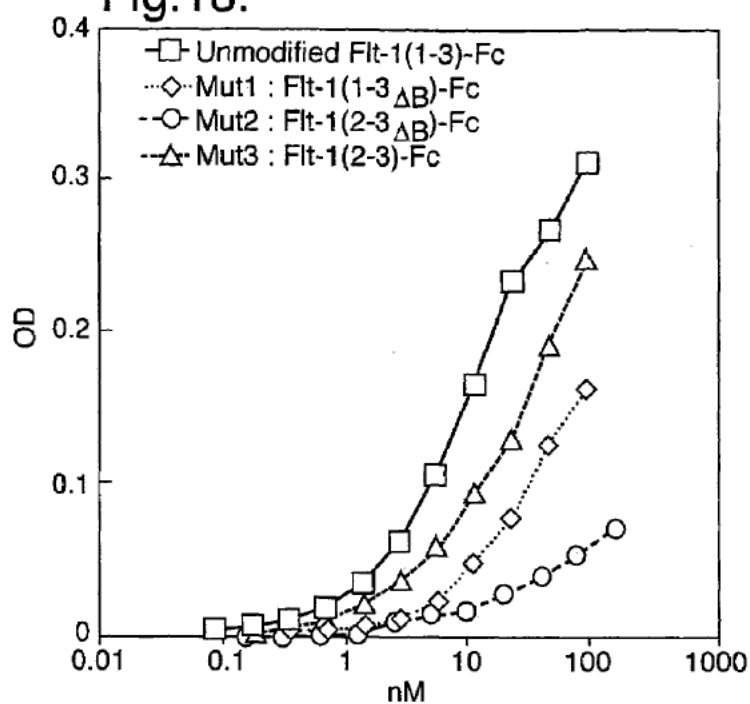


Fig.19.

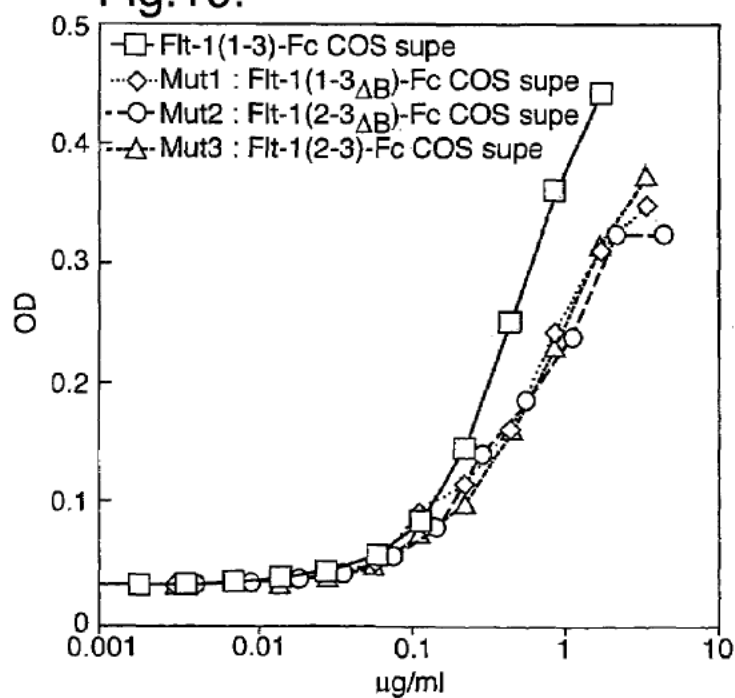


Fig.20.

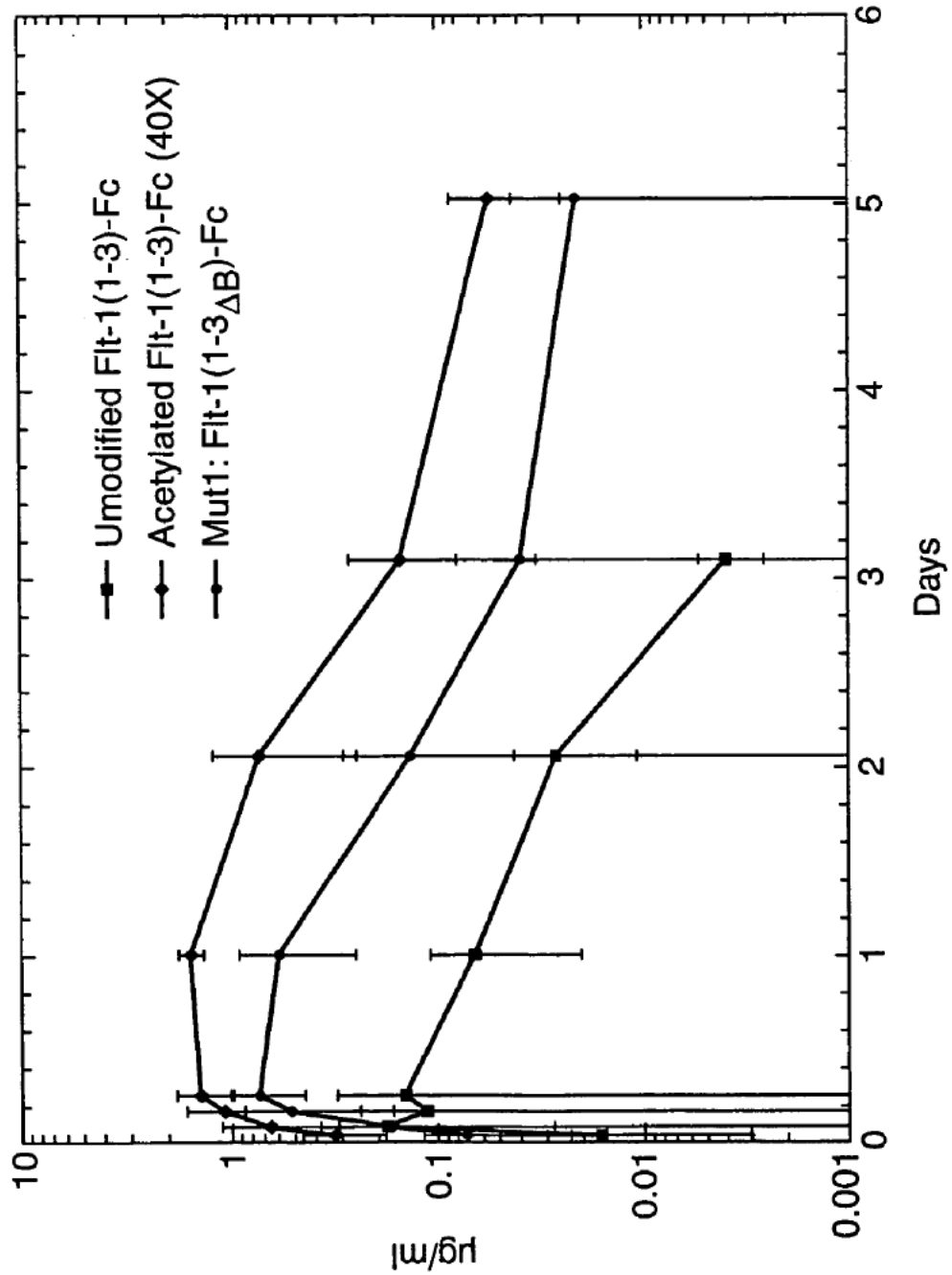


Fig.21A.

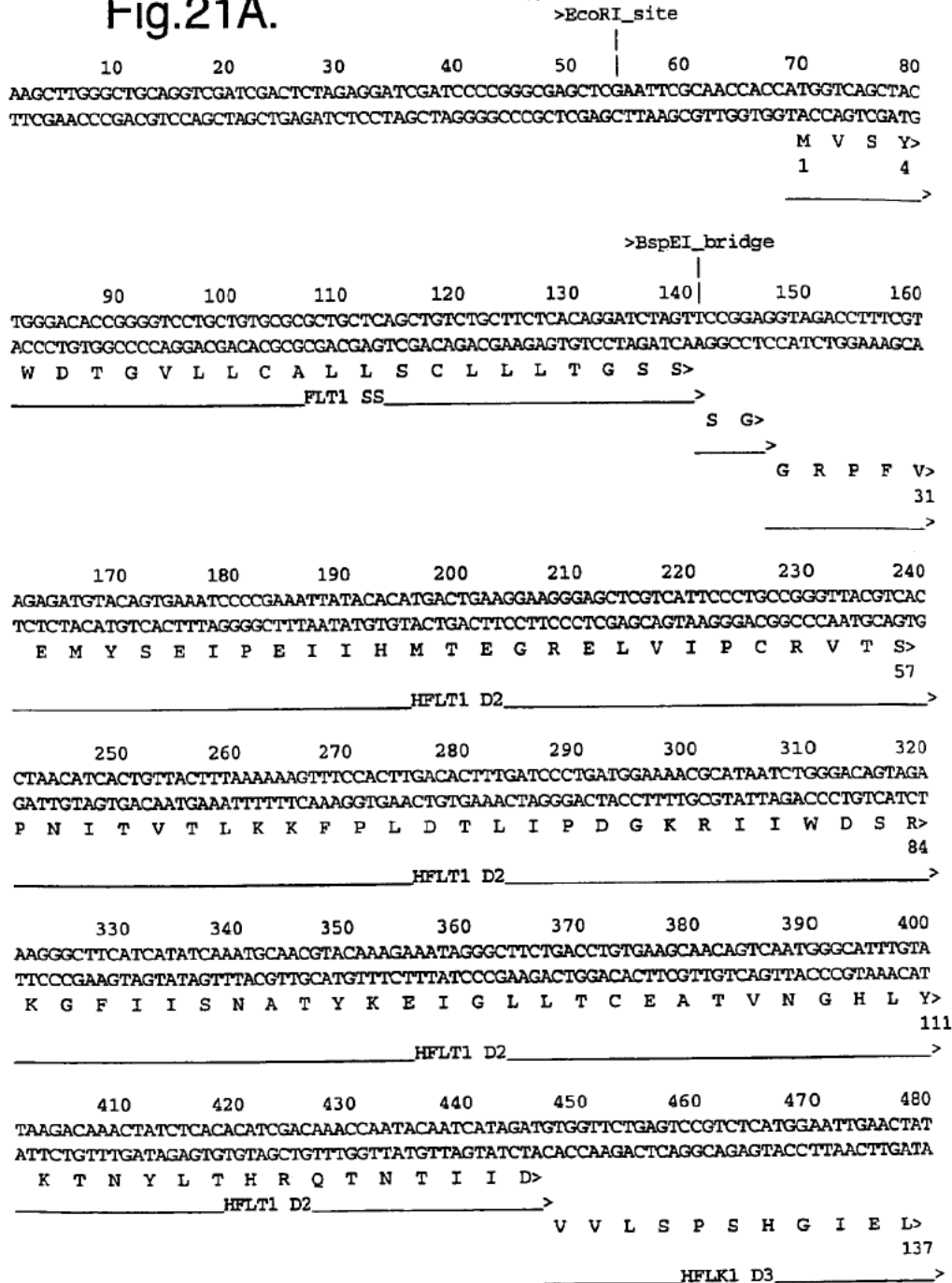


Fig.21B.

490 500 510 520 530 540 550 560
 CTGTTGGAGAAAAGCTTGTCTTAAATTGTACAGCAAGAACGAACTAAATGTGGGGATTGACTTCAACTGGGAATACCCT
 GACAACCTCTTTTCGAACAGAATTTAATCATGTGCTTCTTGACTTGATTTACACCCCTAACTGAAGTTGACCCCTTATGGGA
 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
 TCTTCGAAGCATCAGCATAAGAACTTGTAAACCGAGACCTAAAAACCCAGTCTGGGAGTGAGATGAAGAAATTTTGGAG
 AGAAGCTTCGTAGTCGTATCTTGAACATTTGGCTCTGGATTPTTGGGTCAGACCCCTCACTCTACTTCTTAAAAACTC
 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
 CACCTTAACATATAGATGGTGTAAACCGGAGTGACCAAGGATTGTACACCTGTGCAGCATCCAGTGGGCTGATGACCAAGA
 GTGGAATTGATATCTACCACATTTGGGCTCACTGGTTCCTAACATGTGGACACGTCGTAGGTACCCCGACTACTGGTTCT
 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
 AGAACAGCACATTTGTTCAGGGTCCATGAAAGGGCCCGGGCGACAAAACCTCACACATGCCACCCTGCCCAGCACCTGAA
 TCTTGTGCGTGTAAACAGTCCCAGTACTTTTCCCAGGGCCCGCTGTGTTTGTAGTGTACGGGTGGCACCGGTCGTGGACTT
 K N S T P 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
 CTCTGGGGGACCGTCAGTCTTCCCTCTTCCCCCAAACCCCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTCAC
 GAGGACCCCTTGGCAGTCAGAAAGGAGAAGGGGGTPTTGGGTTCCTGTGGGAGTACTAGAGGGCCCTGGGGACTCCAGTG
 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
 ATGCGTGGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATG
 TACGCACCCACCTGCACCTCGGTGCTTCTGGGACTCCAGTTCAAGTTGACCATGCACCTGCCGCACCTCCACGTATTAC
 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
 CCAAGACAAAGCCGGGGAGGAGCAGTACAACAGCAGCTACCGTGTGGTCCAGCGTCCCTACCGTCTGCACCAGGACTGG
 GGTCTGTGTTTCGGCGCCCTCTCTCGTCATGTTGTGCTGCATGGCACACCCAGTCCGAGGAGTGGCAGGACGTGGTCTGACC
 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
 CTGAATGGCAAGGAGTACAAGTGAAGGTCTCCAACAAGCCCTCCAGCCCCATCGAGAAAACCATCTCCAAGCCAA
 GACTTACCGTTCCCTCATGTTACAGTTCCAGAGGTTGTTTCGGGAGGGTCCGGGGTAGCTCTTTTGGTAGAGGTTCCGGTT
 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
 AGGGCAGCCCCGAGAACCACAGGTGTACACCCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTTCAGCCTGACCT
 TCCCGTCCGGGGCTCTTGGTGTCCACATGTGGGACGGGGTAGGGCCCTACTCGACTGGTTCCTGGTCCAGTCCGGACTGGA
 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
 GCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACC
 CGGACCAGTTTCCGAAGATAGGGTCGGTGTAGCGGCACCTCACCCCTCTCGTTACCCGTCGGCCTCTTGTGTGATGTTCTGG
 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
 ACGCCTCCCGTGTCTGGACTCCGACGGCTCCTTCTTCCCTATAGCAAGCTCACCCGTGGACAAGAGCAGGTGGCAGCAGGG
 TCGGAGGGCAGCACCTGAGGCTGCCGAGGAAGAAGGAGATATCGTTGAGTGGCACCTGTCTCTCGTCCACCGTCTGCC
 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
 GAACGCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTA
 CTTGCAGAAGAGTACGAGGCACTACGTACTCCGAGACGTGTTGGTGTGATGTGCGTCTTCTCGGAGAGGGACAGAGGCCCAT
 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
 AATGAGCGGCCGC
 TTAATCGCCGGCG
 K *>
 458
 _____>

Fig.22A.

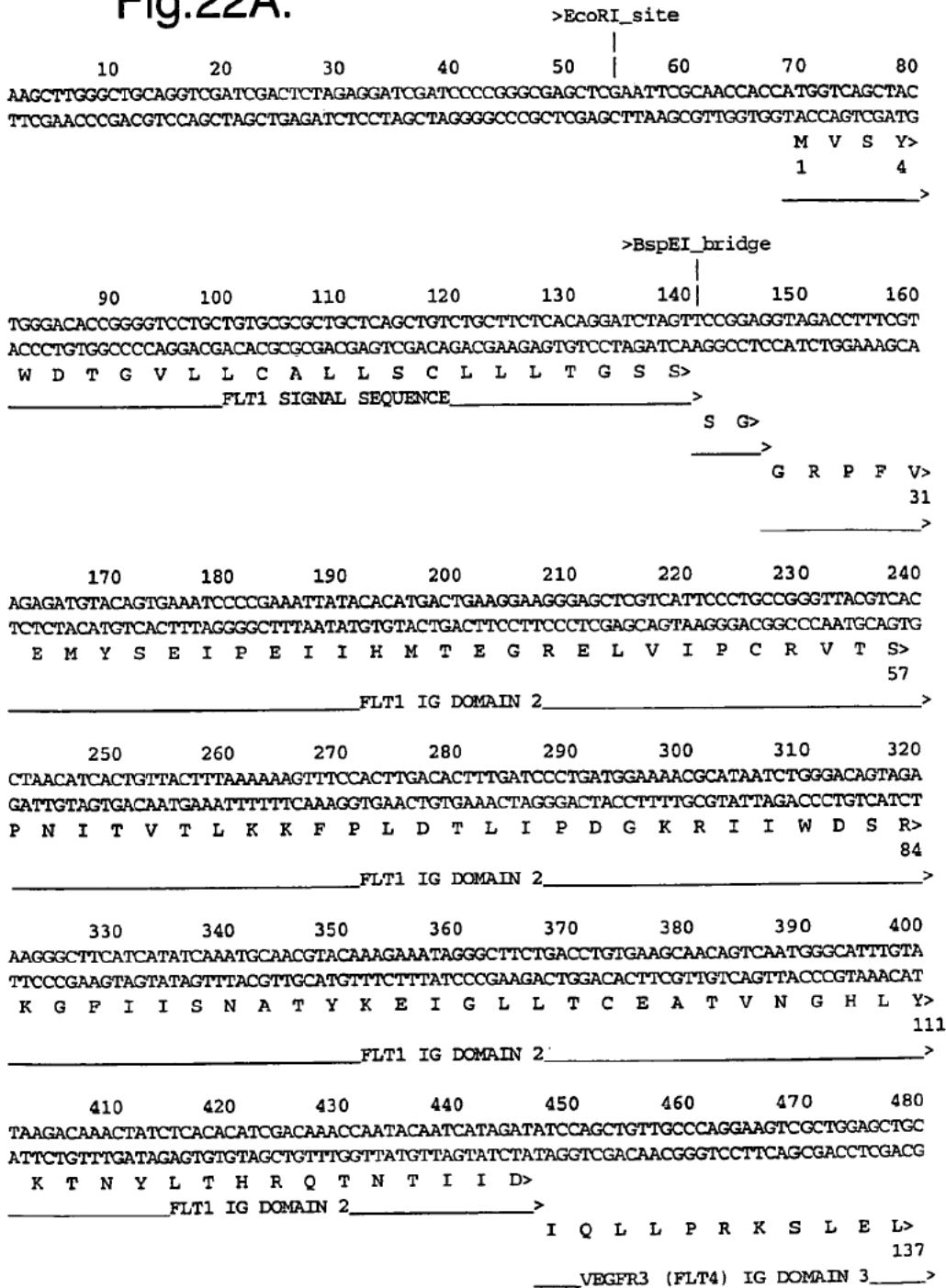


Fig.22B.

490 500 510 520 530 540 550 560
 TGGTAGGGGAGAAGCTGGTCCCTCAACTGCACCCGTGTTGGGCTGAGTTTAACTCAGGTGTCACCTTTGACTGGGACTACCCA
 ACCATCCCCTCTTCGACCAGGAGTTGACGTGGCACACCCGACTCAAATTGAGTCCACAGTGGAAACTGACCCCTGATGGGT
 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
 GGGAAGCAGGCAGAGCGGGTAAGTGGGTGCCCGAGCGACGCTCCCAACAGACCCACACAGAATCTCCAGCATCTGAC
 CCTTCGTCCGCTCTCGCCCATTCACCCACGGGCTCGCTGCGAGGGTTGCTGGGTGTGCTCTGAGAGGTCGTAGGACTG
 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
 CATCCACAACGTCAGCCAGCAGACCTGGGCTCGTATGTGTGCAAGGCCAACACGGCATCCAGCGATTTCCGGGAGAGCA
 GTAGTGTTCAGTCGGTGTGCTGGACCCGAGCATACACAGTTCCGGTTGTTGCCGTAGGTCGCTAAAGCCCTCTCGT
 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
 CCGAGGTCAATTGTGTCATGAAAATGGCCCGGGCGACAAAACCTCACACATGCCACCCTGCCAGCACCTGAACTCCTGGGG
 GGCTCCAGTAACACGTACTTTTACCGGCCCGCTGTTTGTAGTGTGTACGGGTGGCACGGGTCGTGGACTTGAGGACCCC
 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 ALLOTYPE_____>

810 820 830 840 850 860 870 880
 GGACCGTCAGTCTTCTCTCCCAAAACCAAGGACACCCCTCATGATCTCCCGGACCCTGAGGTCACATGCGTGGT
 CCTGGCAGTCAGAAGGAGAAGGGGGTTTGGGTTCCCTGTGGGAGTACTAGAGGGCCTGGGGACTCCAGTGTACGCACCA
 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 ALLOTYPE_____>

890 900 910 920 930 940 950 960
 GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTTGGACGGGCTGGAGGTGCATAATGCCAAGACAA
 CCACCTGCACTCGGTGCTTCTGGGACTCCAGTTCAAGTTGACCATGCACCTGCCGACCTCCACGTATPACGGTTCTGTT
 V D V S H E D P E V K F N W Y V D G V E V H N A K T>
 297
 _____FCAC1 - A ALLOTYPE_____>

970 980 990 1000 1010 1020 1030 1040
 AGCCCGGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCCTCACCGTCTGACACCAGGACTGGCTGAATGGC
 TCGGCGCCCTCTCTGTCATGTTGTCGTGTCATGGCACACCAAGTCCGAGGAGTGGCAGGACGTTGTTCTGACCGACTTACCG
 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 ALLOTYPE_____>

Fig.22C.

1050 1060 1070 1080 1090 1100 1110 1120
 AAGGAGTACAAGTGAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCC
 TTCTCATGTTACAGTTCCAGAGGTGTGTTTCGGGAGGGTTCGGGGTAGCTCTTTTGGTAGAGGTTTCGGTTTCCCGTCGG
 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>

FCAC1 - A ALLOTYP

351

>A>C_A_allotype

>G>T_A_allotype

1130 1140 1150 1160 1170 1180 1190 1200
 CCGAGAACCACAGGTGTACACCCCTGCCCCCATCCCGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCA
 GGCTCTTGGTGTCCACATGTGGGACGGGGTAGGGCCCTACTCGACTGGTTCTTGGTCCAGTCCGACTGGACGGACCAGT
 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>

FCAC1 - A ALLOTYP

377

1210 1220 1230 1240 1250 1260 1270 1280
 AAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGCCCTCCC
 TTCCGAAGATAGGGTCCGCTGTAGCGGCACCTCACCCCTCTCGTTACCCGTCGGCCCTCTGTGTGATGTTCTGGTGGGAGGG
 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>

FCAC1 - A ALLOTYP

404

>T>C

1290 1300 1310 1320 1330 1340 1350 1360
 GTGCTGGACTCCGACGGCTCCTTCTTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT
 CACGACCTGAGGCTGCCGAGGAAGAAGGAGATATCGTTTCGAGTGGCACCTGTTCTCGTCCACCCGTCGTCGCCCTTGCAGAA
 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>

FCAC1 - A ALLOTYP

431

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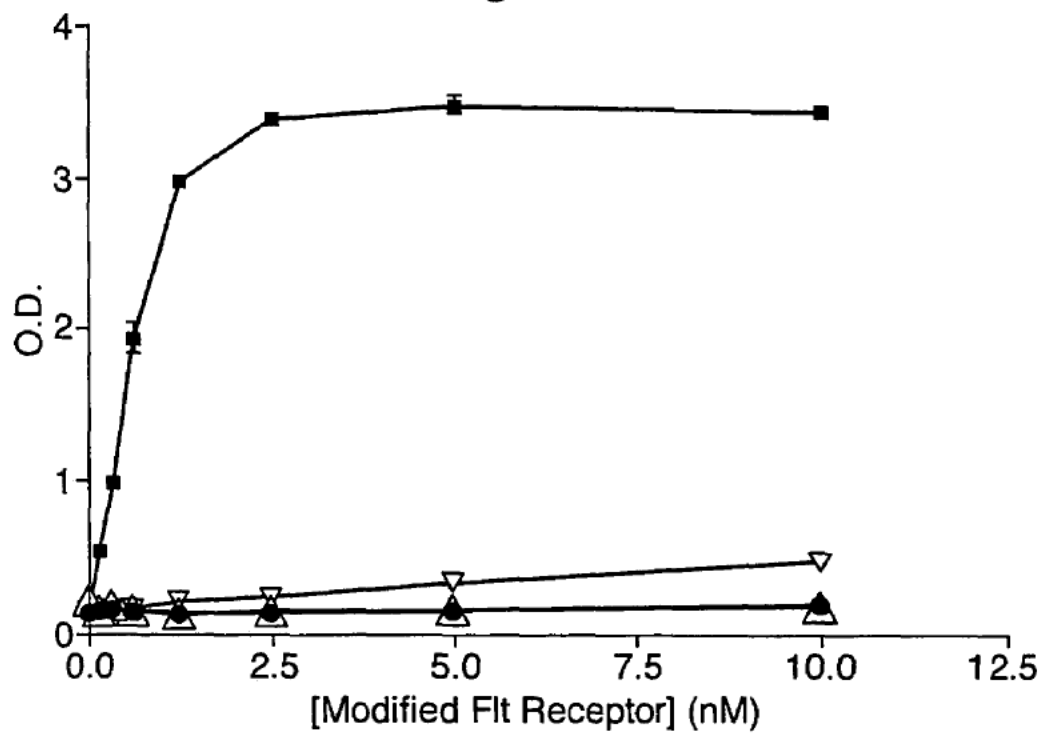
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 GAGTACGAGGCACTACGTACTCCGAGACGTGTTGGTGTGTGCGTCTTCTCGGAGAGGGACAGAGGCCCAATTACTCGCC
 S C S V M H E A L H N H Y T Q K S L S L S P G K *>

FCAC1 - A ALLOTYP

455

CCGC
GGCG

Fig.23.



- Flt1D2Flk1D3.FcdeltaC1(a)
- △ Flt1D2VEGFR3D3.FcdeltaC1(a)
- ▽ TIE2-Fc
- Flt1(1-3)-Fc

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  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 OCT 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 GCC 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
GCG 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 OGA 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_____hFLK1 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 CCT 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 GST
GAT TTT TGG GTC AGA CCC TCA CTC TAC TTC TTT AAA AAC TGG 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 GCC TCA CTG GPT CCT 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 CCT GAA CTC CTG GGG GGA COG TCA GTC TTC CTC TTC CCC 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 D>
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 CCT 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>

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Fig.24C.

```

          910          920          930          940          950          960
          *           *           *           *           *           *
AAG CCG CCG 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 TCG 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 GOC 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 GOC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC
TTT CCG AAG ATA GGG TOG 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 F 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.

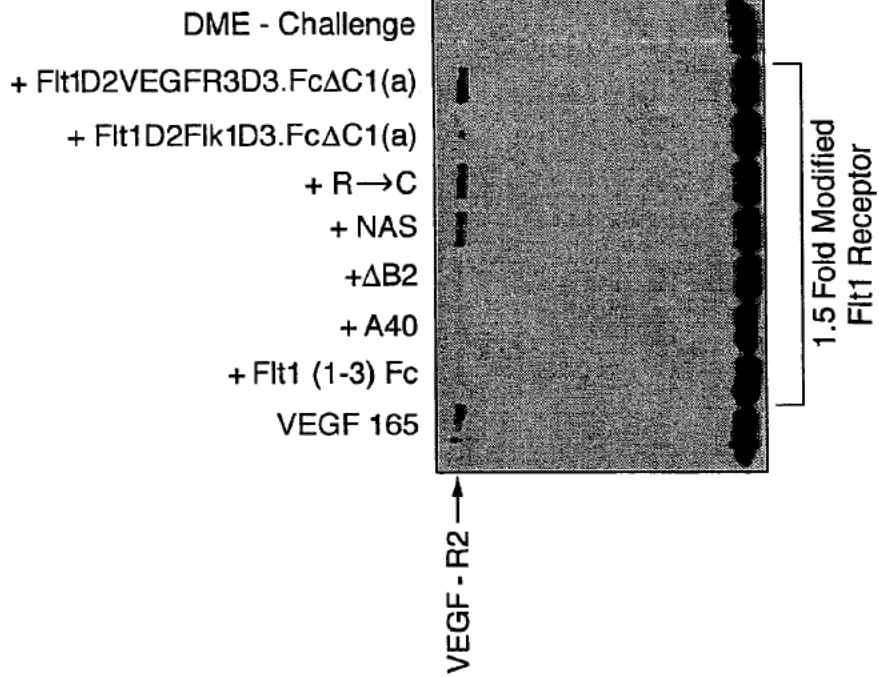


Fig.25B.

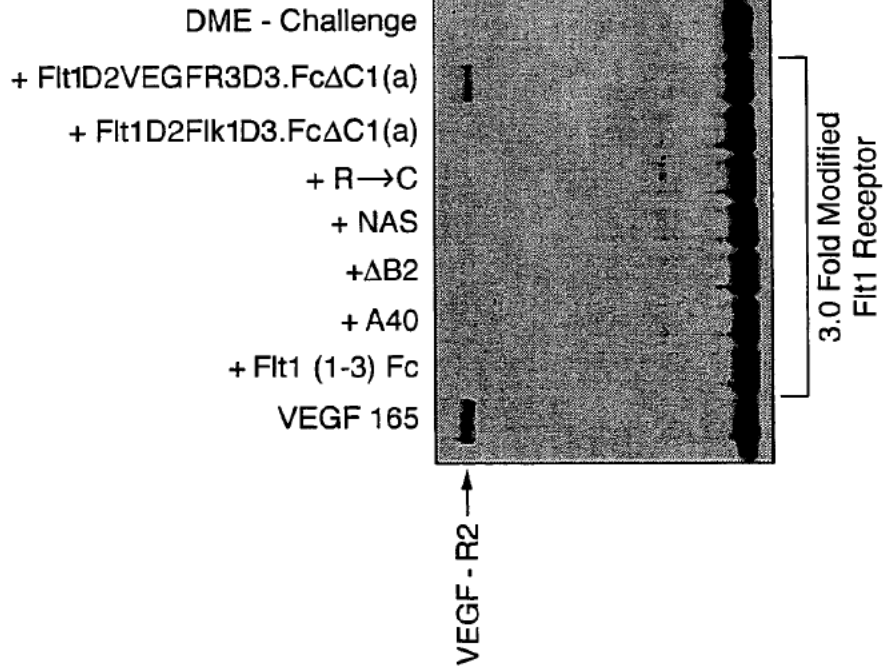


Fig.25C.

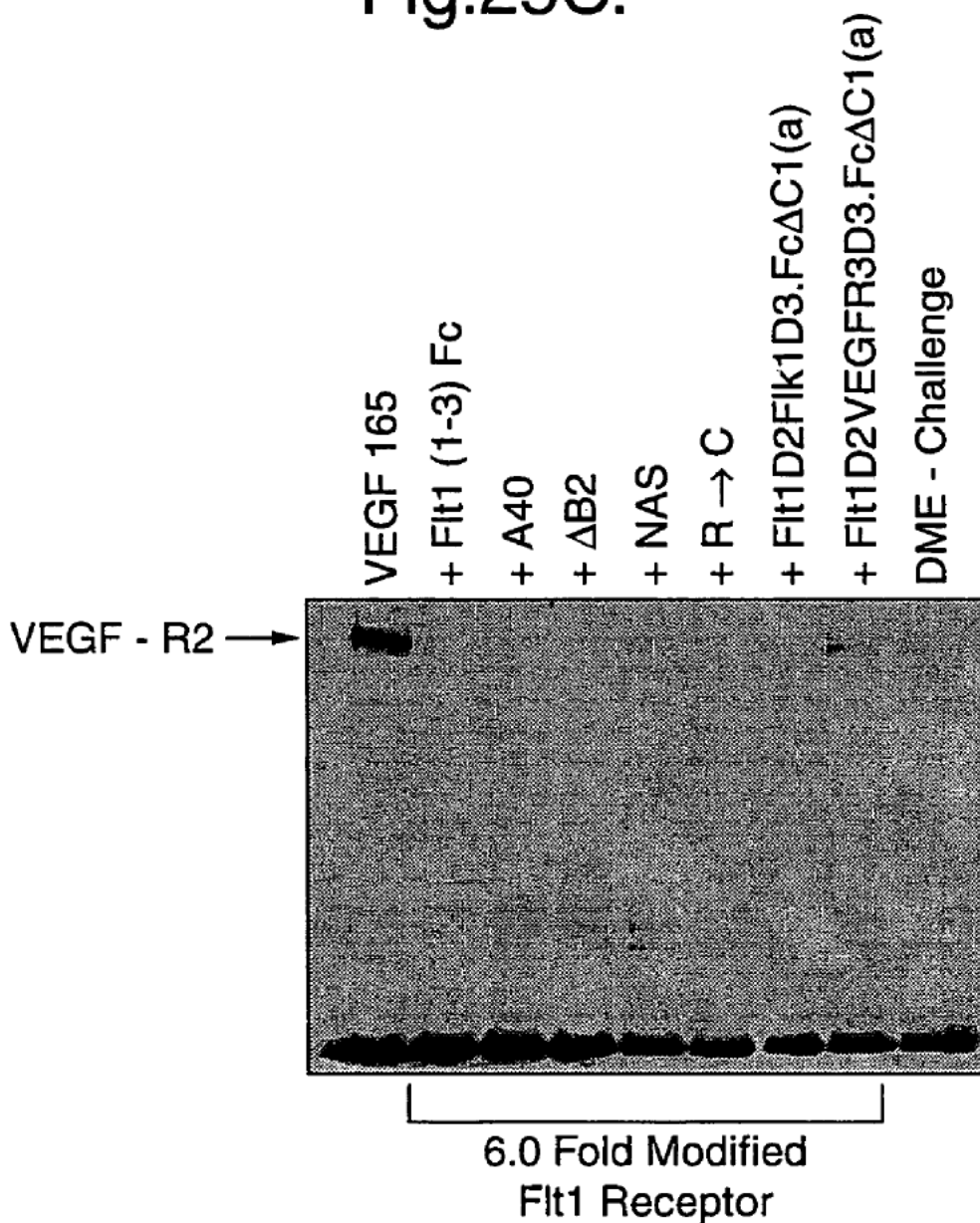


Fig.26A.

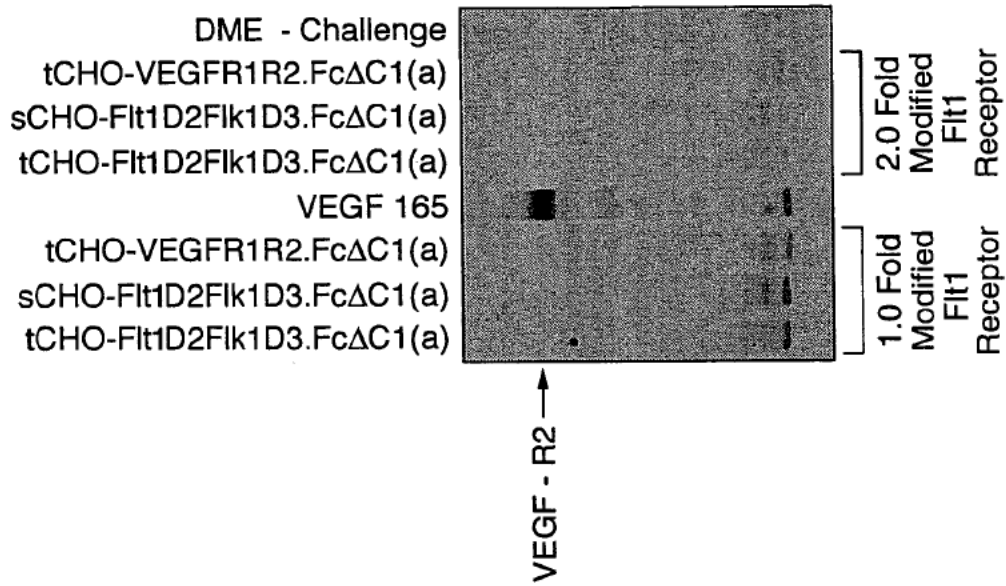


Fig.26B.

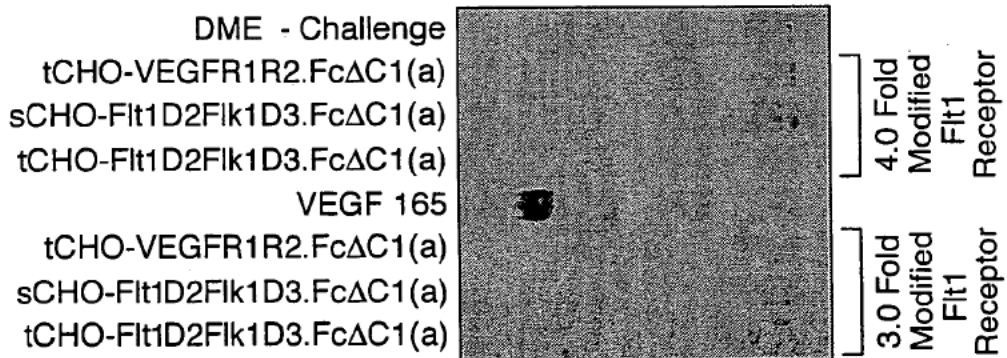


Fig.27.

- Fit1D2Flk1D3.FcdeltaC1(a)
- Fit1D2VEGFR3D3.FcdeltaC1(a)
- Fit1(1-3)-Fc
- ▲ Tie2-Fc

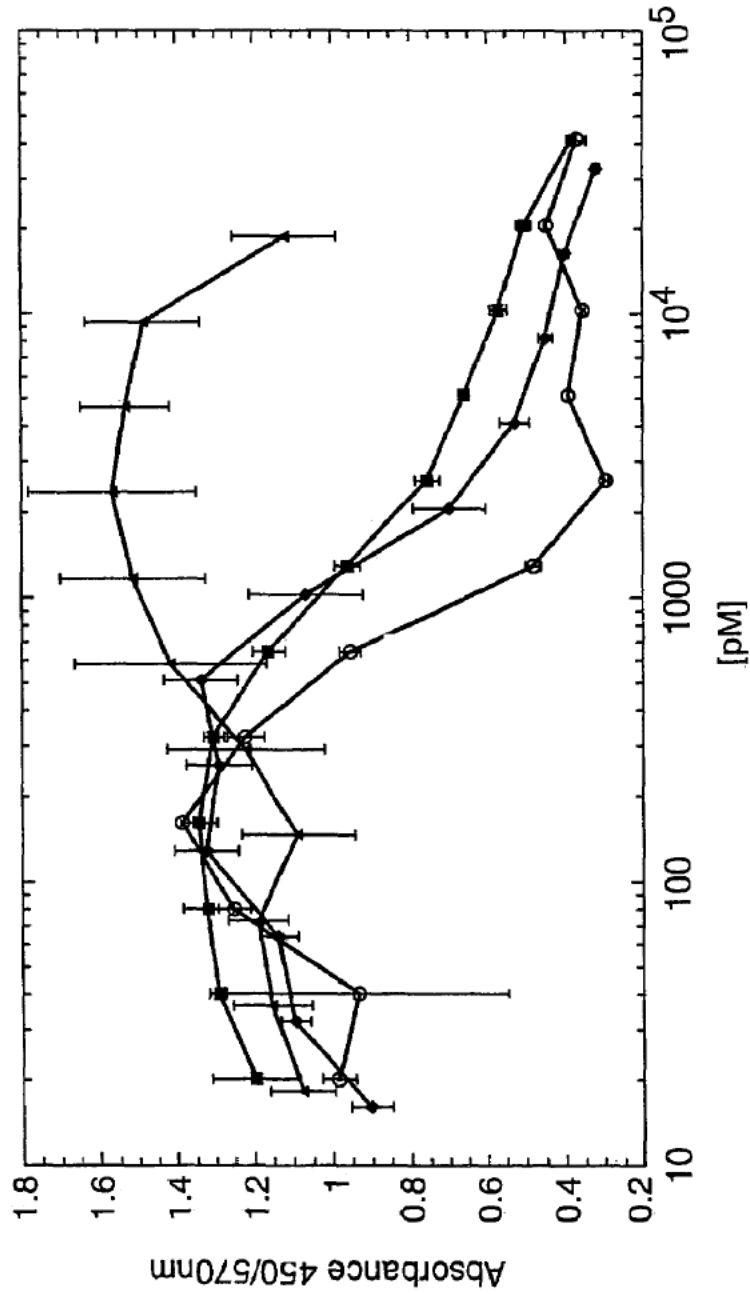


Fig.28.

Binding Stoichiometry of hVEGF165 to Fit1D2FIk1D3.FcΔC1(a) & VEGFR1R2-FcΔC1(a)		
hVEGF165 (nM)	VEGF/Fit1D2FIk1D3.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.

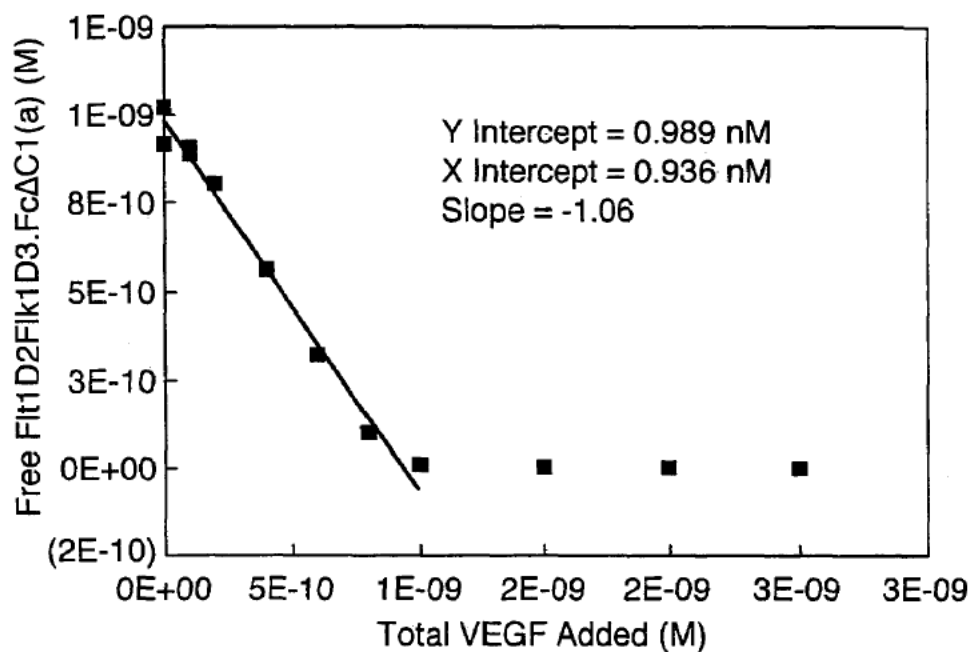


Fig.30.

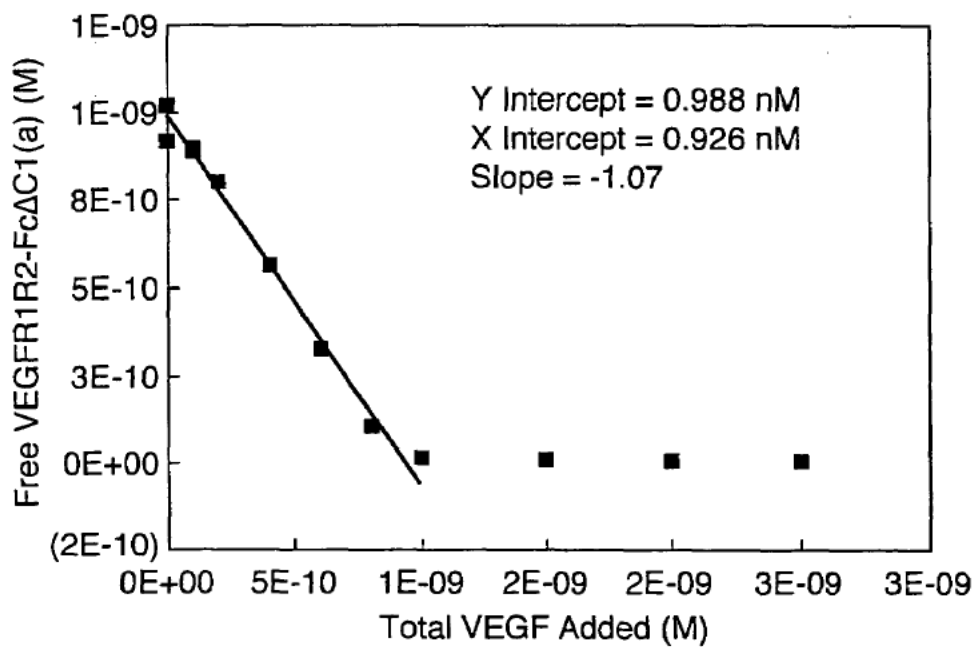


Fig.31.

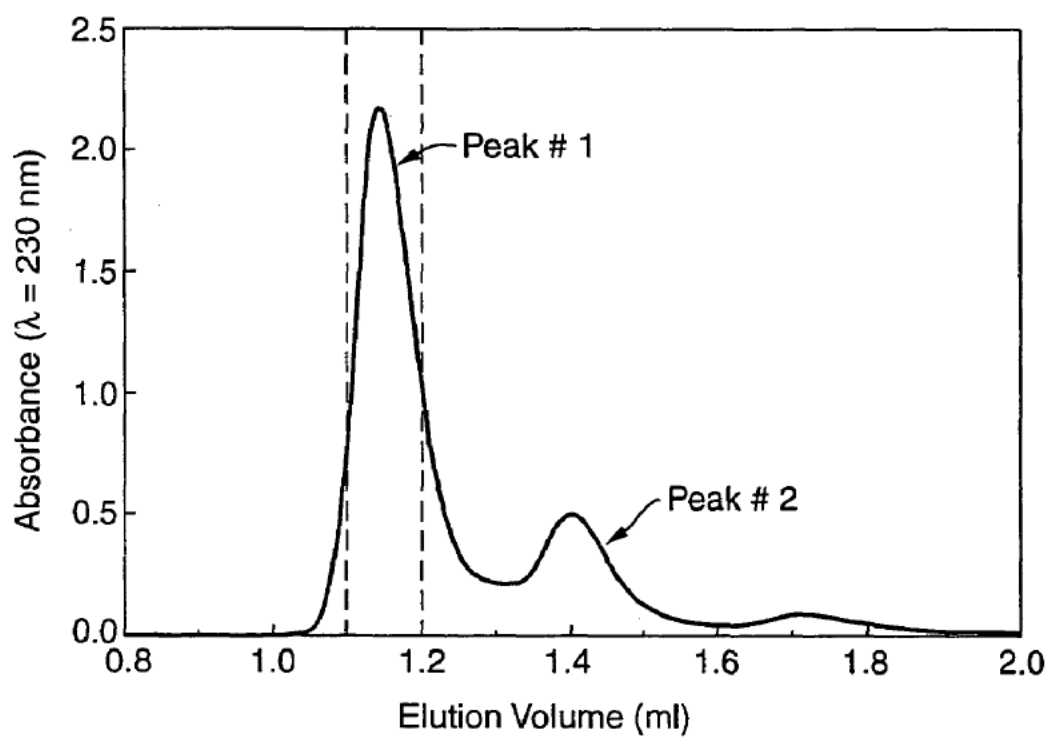


Fig.32.

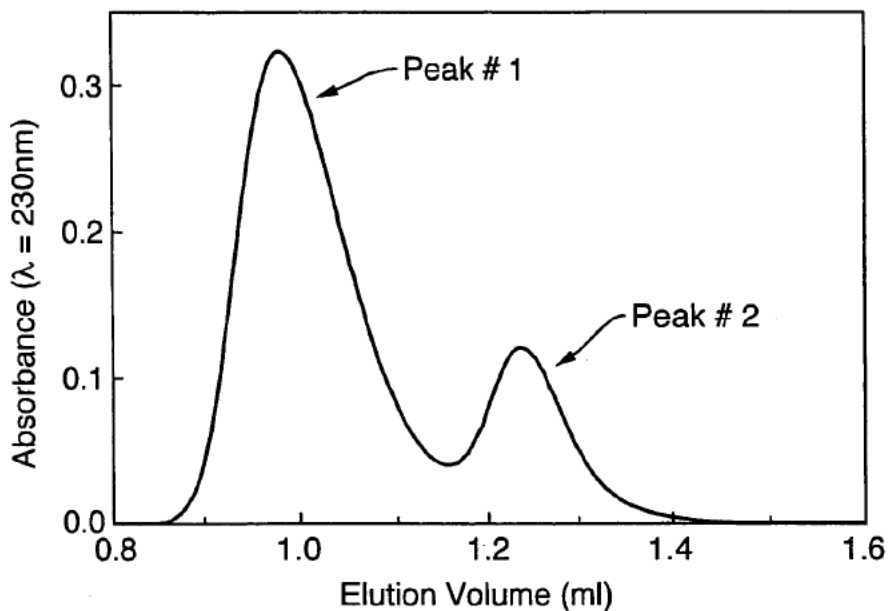


Fig.33.

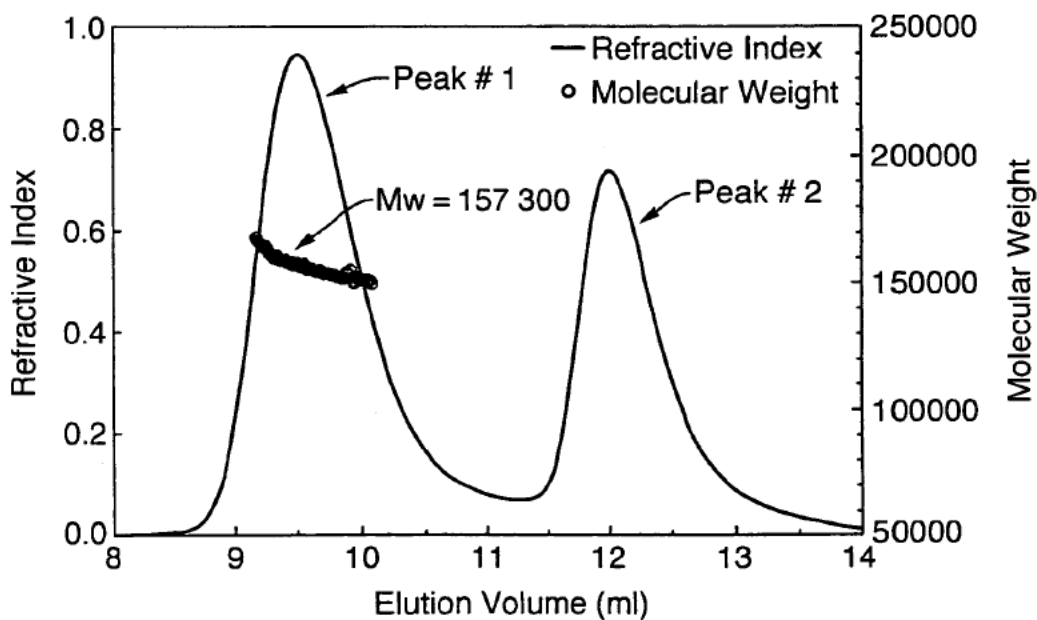


Fig.34.

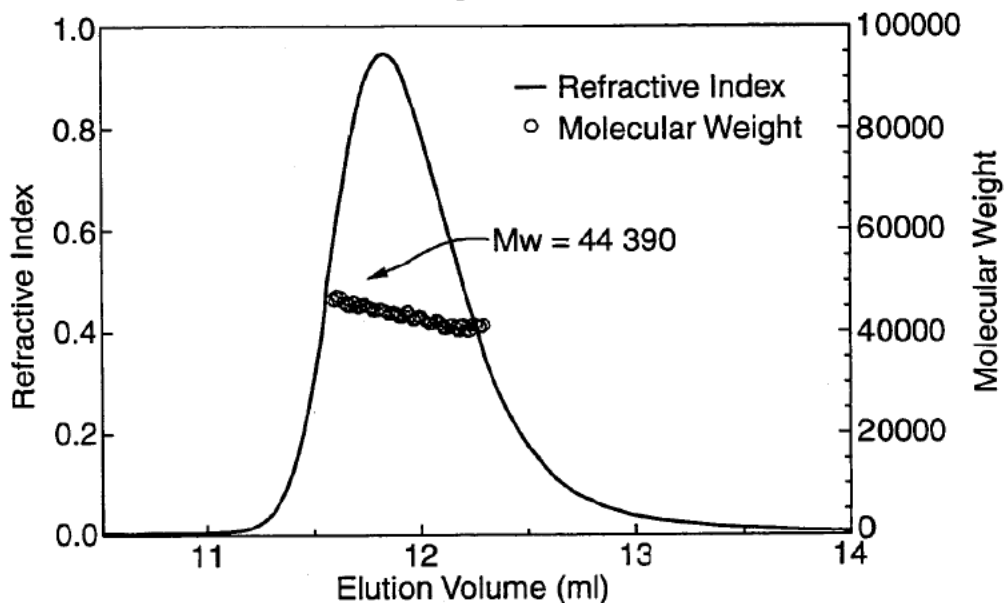


Fig.35.

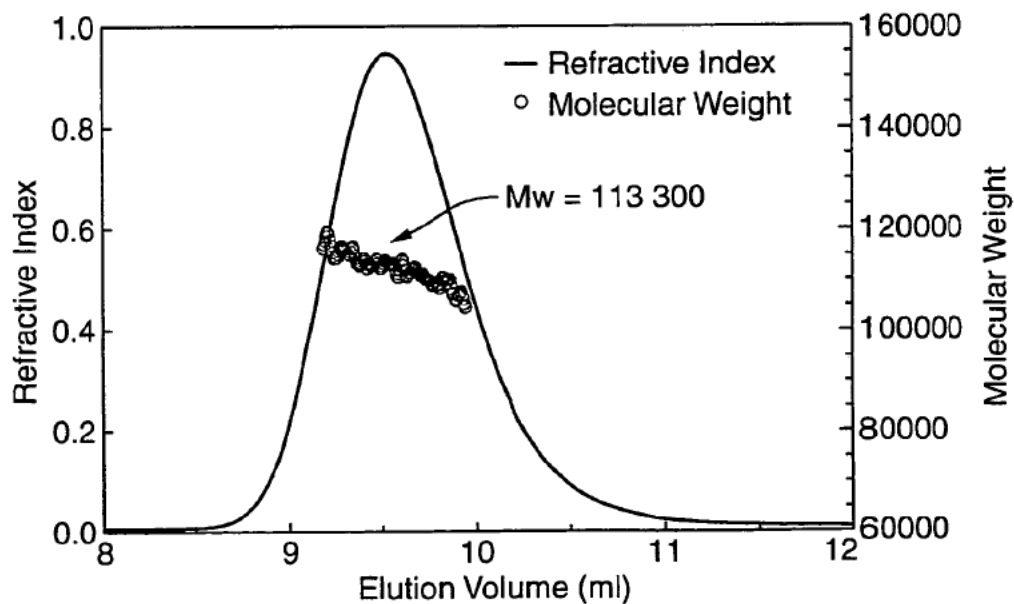


Fig.36.

50
 GRPFVEMYSEIPEIIHMTEGRELVIPCRVTS¹PNITVILKKFPLD²TLIPDG
 100
 KRJIWDSRKGFIIS³NATYKEIGLLTCEATVNGHL⁴YKTNYL⁵THRQ⁶TNTIID
 150
 VVLS⁷PSHGIELSVGEKLVLN⁸CTARTELNVGIDFNWEY⁹PSSKHQHKKLVNR
 200
 DLKTQSGSEM¹⁰KKFLSTLTIDGVTRSDQGLYTCAASSGLMTKK¹¹NSTFVRVH
 250
 EKGPGDK¹²TH¹³TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV¹⁴TCVVVD
 300
 VSHEDPEVKFNWYVDGVEVHNAKTKPREEQ¹⁵YN¹⁶STYRVVSVLTVLHQD¹⁷WLN
 350
 GKEYKCKVSNKALPAPIEK¹⁸TISKAKGQPREPQ¹⁹VY²⁰TLPPSRDELTKNQVSL
 400
 TCLVKG²¹FYPSDIAVEWESNGQPENNYK²²TPPVLDSDGSFFLYSKLTV²³DKS
 RWQQGNV²⁴FSCSVMHEALHNHY²⁵TQKSLSPGK

Fig.37.

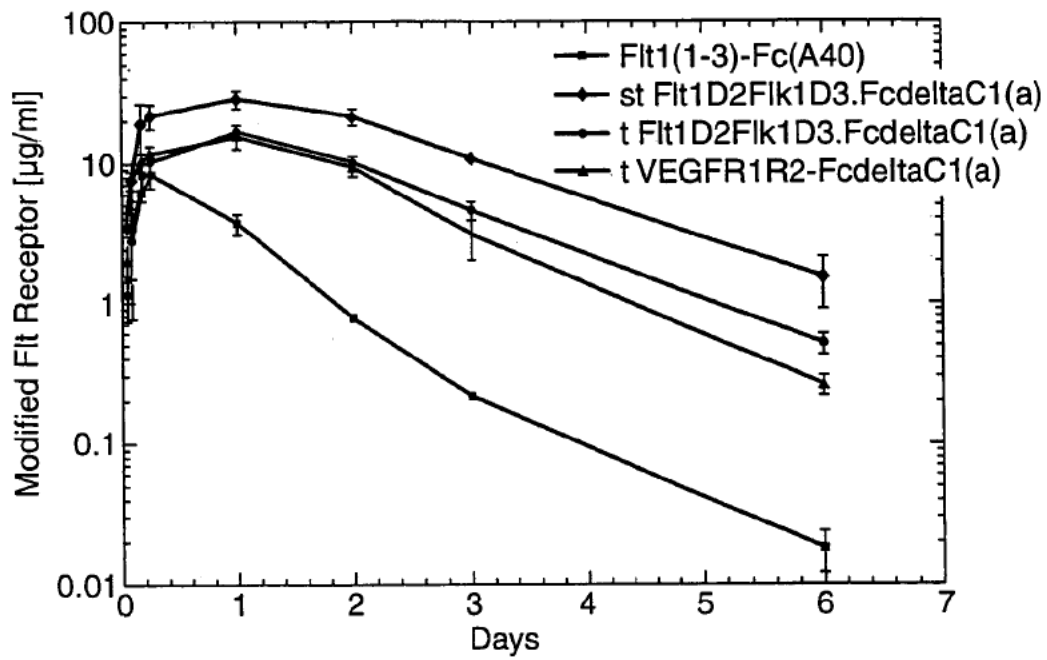
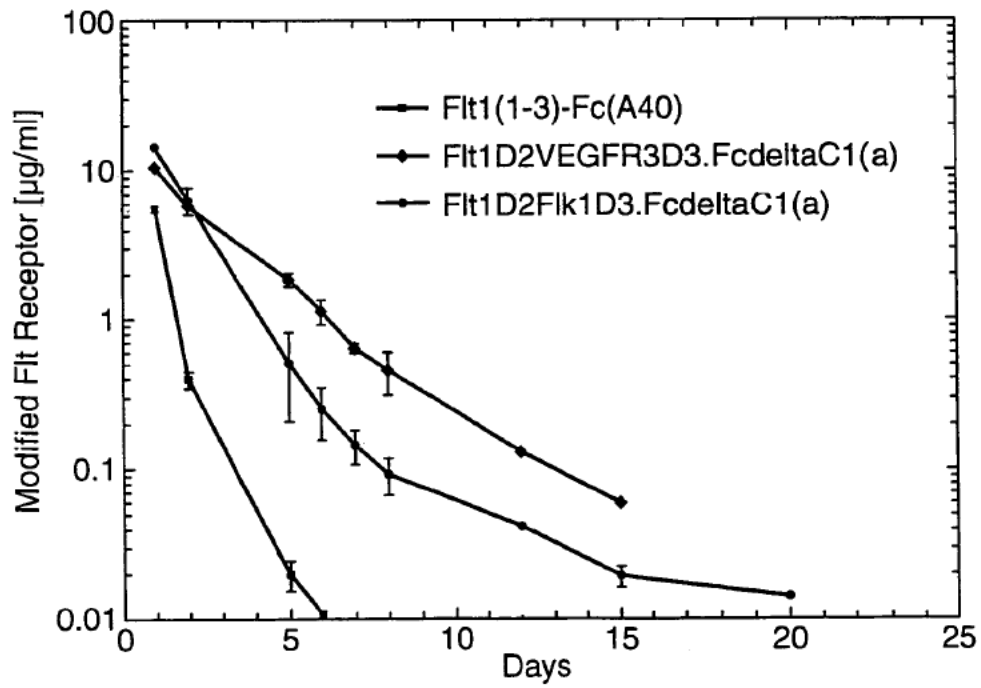


Fig.38.



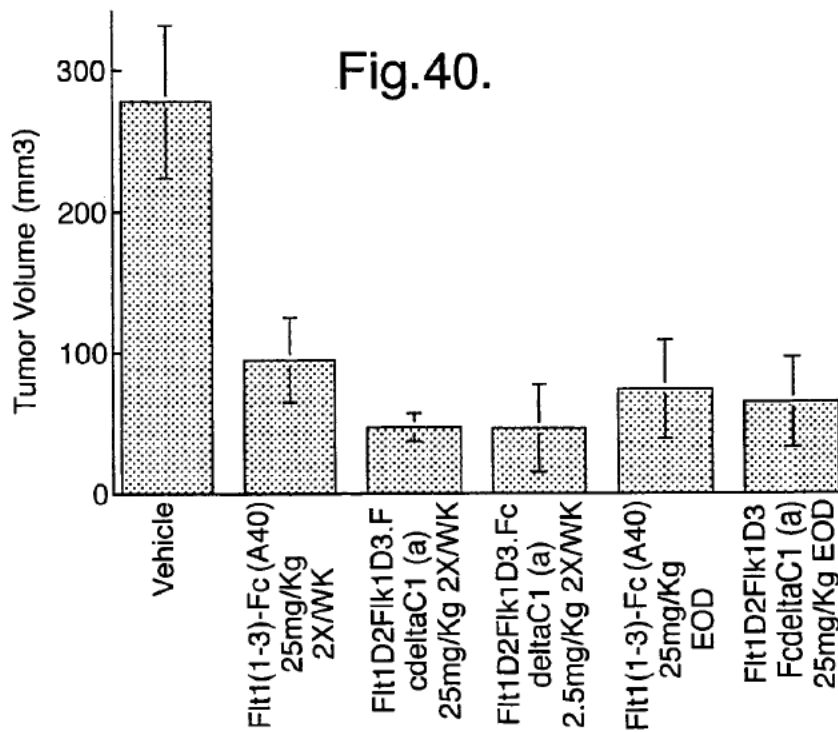
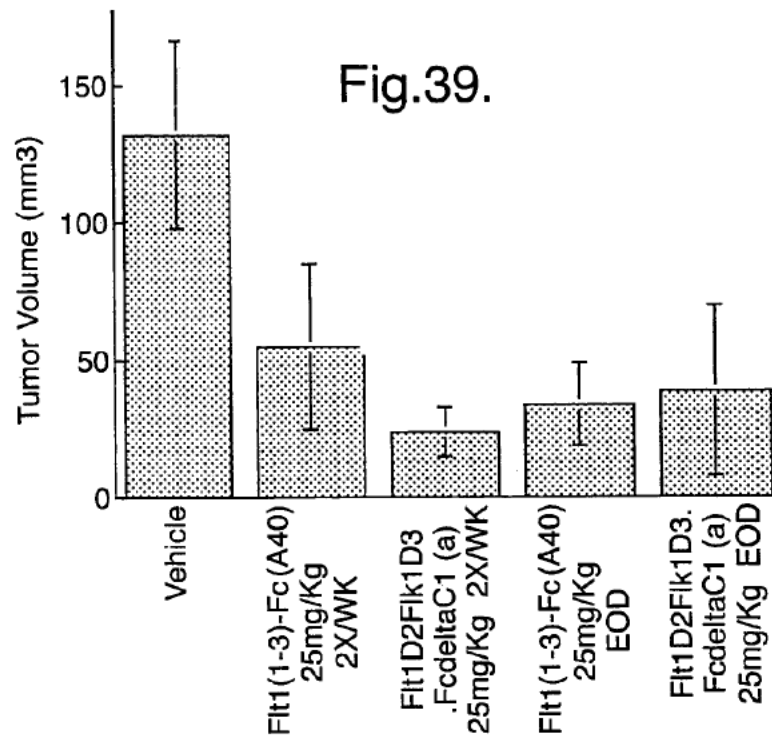


Fig.41.

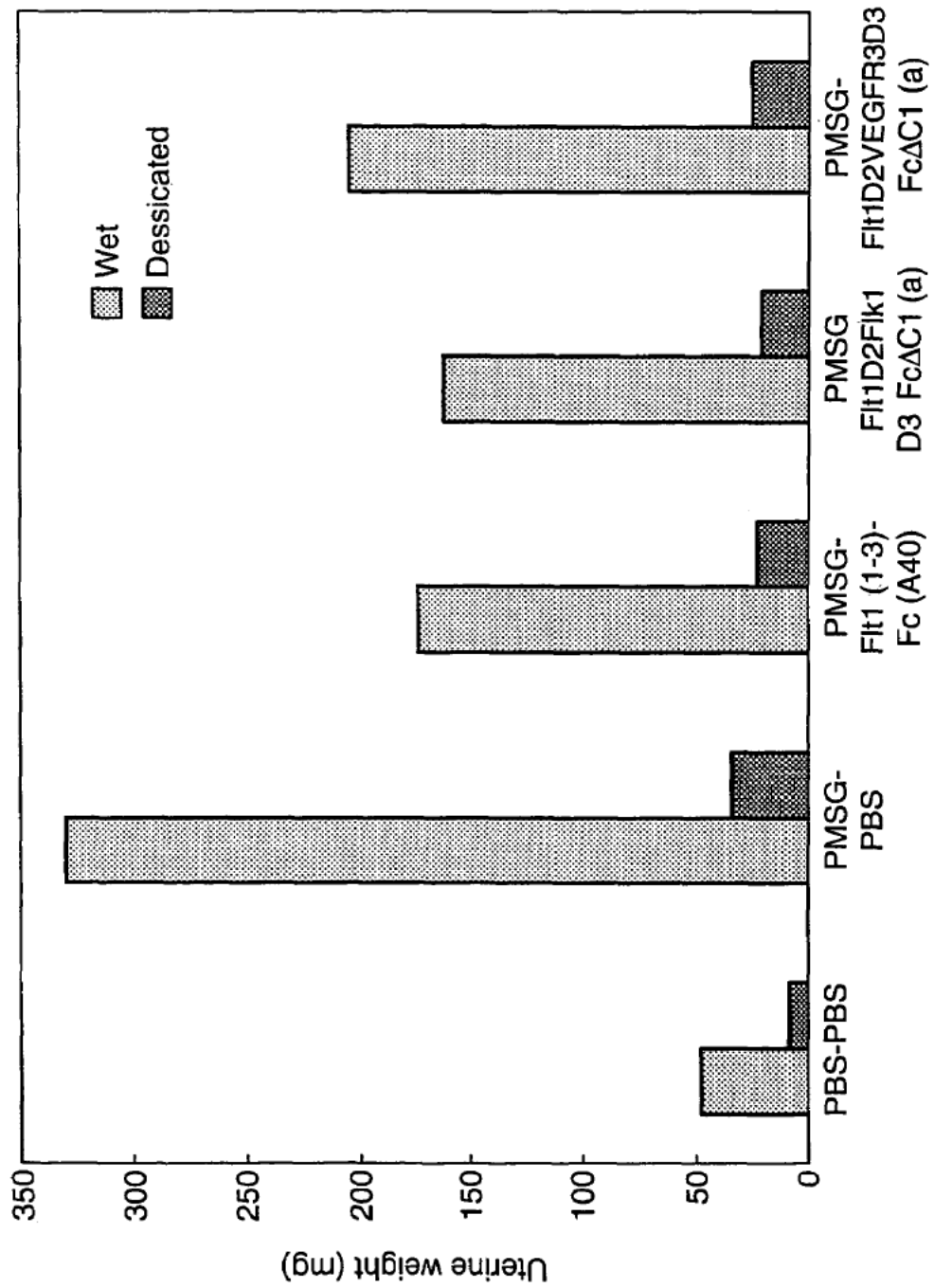


Fig.42A.

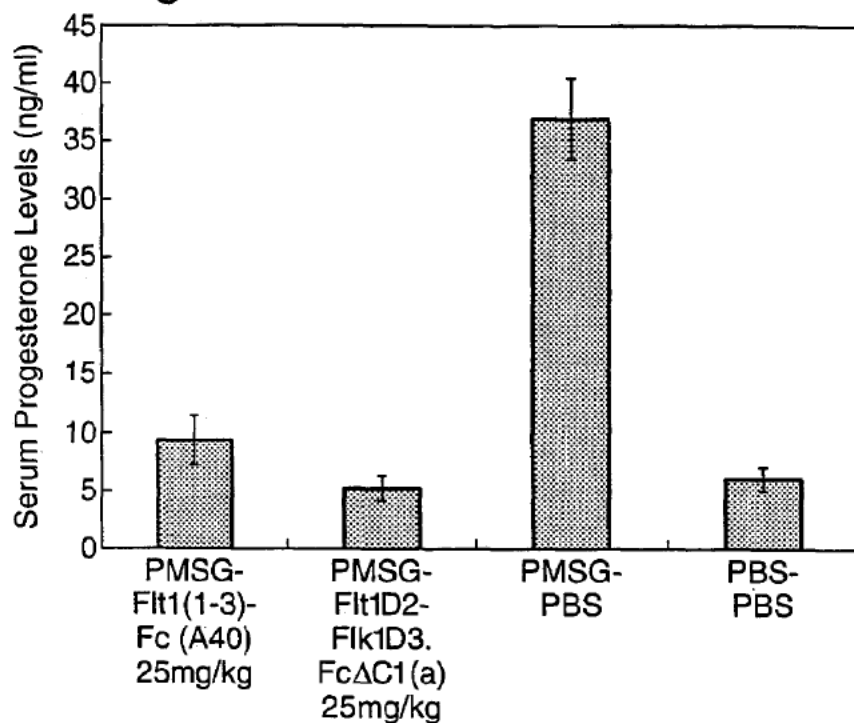
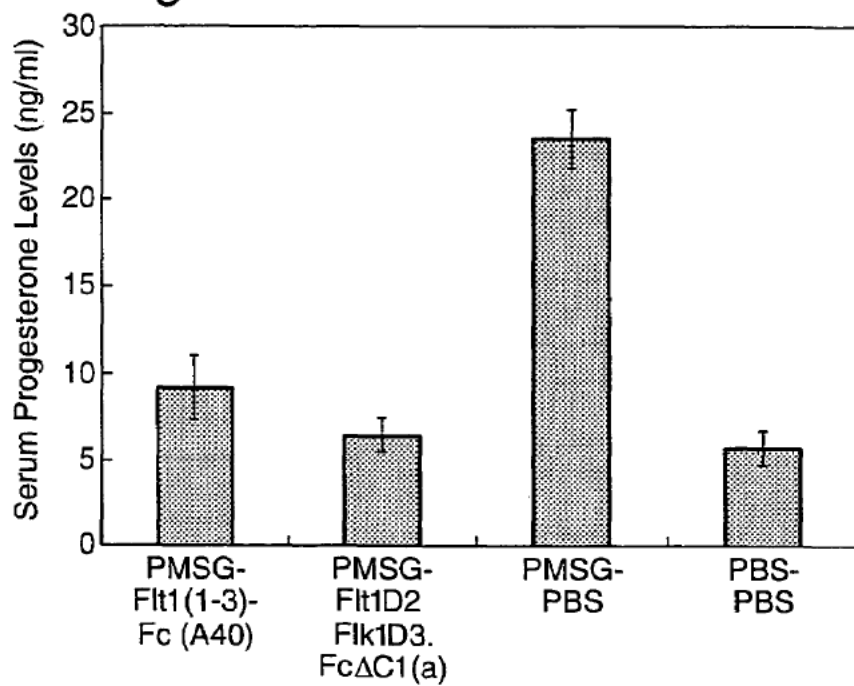


Fig.42B.



MODIFIED CHIMERIC POLYPEPTIDES WITH IMPROVED PHARMACOKINETIC PROPERTIES

This application is a divisional of U.S. patent application Ser. No. 10/009,852, filed Dec. 6, 2001, now U.S. Pat. No. 7,070,959, which is a national stage application of International Application No. PCT/US00/14142, filed May 23, 2000, which claims priority of U.S. Provisional Application No. 60/138,133, filed Jun. 8, 1999. The disclosures of these applications are herein specifically incorporated by reference in their entirety.

INTRODUCTION

The field of this invention is modified polypeptides with improved pharmacokinetics. Specifically, the field of this invention relates to Flt1 receptor polypeptides that have been modified in such a way as to improve their pharmacokinetic profile. The field of this invention also relates to 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.

BACKGROUND

The ability of polypeptide ligands to bind to cells and thereby elicit a phenotypic response such as cell growth, survival, cell product secretion, or differentiation is often mediated through transmembrane receptors on the cells. The extracellular domain of such receptors (i.e. that portion of the receptor that is displayed on the surface of the cell) is generally the most distinctive portion of the molecule, as it provides the protein with its ligand binding characteristic. Binding of a ligand to the extracellular domain generally results in signal transduction which transmits a biological signal to intracellular targets. Often, this signal transduction acts via a catalytic intracellular domain. The particular array of sequence motifs of this catalytic intracellular domain determines its access to potential kinase substrates (Mohammedi, et al., 1990, *Mol. Cell. Biol.* 11:5068-5078; Fantl, et al., 1992, *Cell* 69:413-413). Examples of receptors that transduce signals via catalytic intracellular domains include the receptor tyrosine kinases (RTKs) such as the Trk family of receptors which are generally limited to cells of the nervous system, the cytokine family of receptors including the tripartate CNTF receptor complex (Stahl & Yancopoulos, 1994, *J. Neurobiol.* 25:1454-1466) which is also generally limited to the cells of the nervous system, G-protein coupled receptors such as the β_2 -adrenergic receptor found on, for instance, cardiac muscle cells, and the multimeric IgE high affinity receptor Fc ϵ RI which is localized, for the most part, on mast cells and basophils (Sutton & Gould, 1993, *Nature* 366:421-428).

All receptors identified so far appear to undergo dimerization, multimerization, or some related conformational change following ligand binding (Schlessinger, J., 1988, *Trend Biochem. Sci.* 13:443-447; Ullrich & Schlessinger, 1990, *Cell* 61:203-212; Schlessinger & Ullrich, 1992, *Neuron* 9:383-391) and molecular interactions between dimerizing intracellular domains lead to activation of catalytic function. In some instances, such as platelet-derived growth factor (PDGF), the ligand is a dimer that binds two receptor molecules (Hart, et al., 1988, *Science*, 240:1529-1531; Heldin, 1989, *J. Biol. Chem.* 264:8905-8912) while, for example, in the case of epidermal growth factor (EGF), the

ligand is a monomer (Weber, et al., 1984, *J. Biol. Chem.* 259:14631-14636). In the case of the Fc ϵ RI receptor, the ligand, IgE, exists bound to Fc ϵ RI in a monomeric fashion and only becomes activated when antigen binds to the IgE/Fc ϵ RI complex and cross-links adjacent IgE molecules (Sutton & Gould, 1993, *Nature* 366:421-428).

Often, the tissue distribution of a particular receptor within higher organisms provides insight into the biological function of the receptor.

The RTKs for some growth and differentiation factors, such as fibroblast growth factor (FGF), are widely expressed and therefore appear to play some general role in tissue growth and maintenance. Members of the Trk RTK family (Glass & Yancopoulos, 1993, *Trends in Cell Biol.* 3:262-268) of receptors are more generally limited to cells of the nervous system, and the Nerve Growth Factor family consisting of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5), which bind the Trk RTK family receptors, promote the differentiation of diverse groups of neurons in the brain and periphery (Lindsay, R. M., 1993, in *Neurotrophic Factors*, S. E. Loughlin & J. H. Fallon, eds., pp. 257-284, San Diego, Calif., Academic Press). Fc ϵ RI is localized to a very limited number of types of cells such as mast cells and basophils. Mast cells derive from bone marrow pluripotent hematopoietic stem cell lineage, but complete their maturation in the tissue following migration from the blood stream (See Janeway & Travers, 1996, in *Immunobiology*, 2d. Edition, M. Robertson & E. Lawrence, eds., pp. 1:3-1:4, Current Biology Ltd., London, UK, Publisher) and are involved in the allergic response.

Many studies have demonstrated that the extracellular domain of a receptor provides the specific ligand binding characteristic.

Furthermore, the cellular environment in which a receptor is expressed may influence the biological response exhibited upon binding of a ligand to the receptor. For example, when a neuronal cell expressing a Trk receptor is exposed to a neurotrophin which binds to that receptor, neuronal survival and differentiation results. When the same receptor is expressed by a fibroblast, exposure to the neurotrophin results in proliferation of the fibroblast (Glass, et al., 1991, *Cell* 66:405-413).

A class of cell-derived dimeric mitogens with selectivity for vascular endothelial cells has been identified and designated vascular endothelial cell growth factor (VEGF). VEGF has been purified from conditioned growth media of rat glioma cells [Conn et al., (1990), *Proc. Natl. Acad. Sci. U.S.A.*, 87, pp 2628-2632]; and conditioned growth media of bovine pituitary follicle stellate cells [Ferrara and Henzel, (1989), *Biochem. Biophys. Res. Comm.*, 161, pp. 851-858; Gozpadarowicz et al., (1989), *Proc. Natl. Acad. Sci. U.S.A.*, 86, pp. 7311-7315] and conditioned growth medium from human U937 cells [Connolly, D. T. et al. (1989), *Science*, 246, pp. 1309-1312]. VEGF is a dimer with an apparent molecular mass of about 46 kDa with each subunit having an apparent molecular mass of about 23 kDa. VEGF has some structural similarities to platelet derived growth factor (PDGF), which is a mitogen for connective tissue cells but not mitogenic for vascular endothelial cells from large vessels.

The membrane-bound tyrosine kinase receptor, known as Flt, was shown to be a VEGF receptor [DeVries, C. et al., (1992), *Science*, 255, pp.989-991]. The Flt receptor specifically binds VEGF which induces mitogenesis. Another form of the VEGF receptor, designated KDR, is also known to bind VEGF and induce mitogenesis. The partial cDNA

sequence and nearly full length protein sequence of KDR is known as well [Terman, B. I. et al., (1991) *Oncogene* 6, pp. 1677-1683; Terman, B. I. et al., (1992) *Biochem. Biophys. Res. Comm.* 187, pp. 1579-1586].

Persistent angiogenesis may cause or exacerbate certain diseases such as psoriasis, rheumatoid arthritis, hemangiomas, angiofibromas, diabetic retinopathy and neovascular glaucoma. An inhibitor of VEGF activity would be useful as a treatment for such diseases and other VEGF-induced pathological angiogenesis and vascular permeability conditions, such as tumor vascularization. The present invention relates to a VEGF inhibitor that is based on the VEGF receptor Flt1.

Plasma leakage, a key component of inflammation, occurs in a distinct subset of microvessels. In particular, in most organs plasma leakage occurs specifically in the venules. Unlike arterioles and capillaries, venules become leaky in response to numerous inflammatory mediators including histamine, bradykinin, and serotonin. One characteristic of inflammation is the plasma leakage that results from intercellular gaps that form in the endothelium of venules. Most experimental models of inflammation indicate that these intercellular gaps occur between the endothelial cells of postcapillary and collecting venules (Baluk, P., et al., *Am. J. Pathol.* 1998 152:1463-76). It has been shown that certain lectins may be used to reveal features of focal sites of plasma leakage, endothelial gaps, and finger-like processes at endothelial cell borders in inflamed venules (Thurston, G., et al., *Am. J. Physiol.* 1996, 271: H2547-62). In particular, plant lectins have been used to visualize morphological changes at endothelial cell borders in inflamed venules of, for example, the rat trachea. Lectins, such as concanavalin A and ricin, that bind focally to inflamed venules reveal regions of the subendothelial vessel wall exposed by gaps that correspond to sites of plasma leakage (Thurston, G., et al., *Am J Physiol*, 1996, 271: H2547-62).

The properties of the microvessels are dynamic. Chronic inflammatory diseases, for example, are associated with microvascular remodeling, including angiogenesis and microvessel enlargement. Microvessels can also remodel by acquiring abnormal phenotypic properties. In a murine model of chronic airway inflammation, airway capillaries acquire properties of venules, including widened vessel diameter, increased immunoreactivity for von Willebrand factor, and increased immunoreactivity for P-selectin. In addition, these remodeled vessels leak in response to inflammatory mediators, whereas vessels in the same position in the airways of normal mice do not.

Certain substances have been shown to decrease or inhibit vascular permeability and/or plasma leakage. For example, mystixins are synthetic polypeptides that have been reported to inhibit plasma leakage without blocking endothelial gap formation (Baluk, P., et al., *J. Pharmacol. Exp. Ther.*, 1998, 284: 693-9). Also, the beta 2-adrenergic receptor agonist formoterol reduces microvascular leakage by inhibiting endothelial gap formation (Baluk, P. and McDonald, D. M., *Am. J.*

Physiol., 1994, 266:L461-8).

The angiopoietins and members of the vascular endothelial growth factor (VEGF) family are the only growth factors thought to be largely specific for vascular endothelial cells. Targeted gene inactivation studies in mice have shown that VEGF is necessary for the early stages of vascular development and that Ang-1 is required for later stages of vascular remodeling.

U.S. Pat. No. 6,011,003, issued Jan. 4, 2000, in the name of Metris Therapeutics Limited, discloses an altered, soluble

form of FLT polypeptide being capable of binding to VEGF and thereby exerting an inhibitory effect thereon, the polypeptide comprising five or fewer complete immunoglobulin domains.

U.S. Pat. No. 5,712,380, issued Jan. 27, 1998 and assigned to Merck & Co., discloses vascular endothelial cell growth factor (VEGF) inhibitors that are naturally occurring or recombinantly engineered soluble forms with or without a C-terminal transmembrane region of the receptor for VEGF.

Also assigned to Merck & Co. is PCT Publication No. WO 98/13071, published Apr. 2, 1998, which discloses gene therapy methodology for inhibition of primary tumor growth and metastasis by gene transfer of a nucleotide sequence encoding a soluble receptor protein which binds to VEGF.

PCT Publication No. WO 97/44453, published Nov. 27, 1997, in the name of Genentech, Inc., discloses novel chimeric VEGF receptor proteins comprising amino acid sequences derived from the vascular endothelial growth factor (VEGF) receptors Flt1 and KDR, including the murine homologue to the human KDR receptor FLK1, wherein said chimeric VEGF receptor proteins bind to VEGF and antagonize the endothelial cell proliferative and angiogenic activity thereof.

PCT Publication No. WO 97/13787, published Apr. 17, 1997, in the name of Toa Gosei Co., LTD., discloses a low molecular weight VEGF inhibitor usable in the treatment of diseases accompanied by neovascularization such as solid tumors. A polypeptide containing the first immunoglobulin-like domain and the second immunoglobulin-like domain in the extracellular region of a VEGF receptor FLT but not containing the sixth immunoglobulin-like domain and the seventh immunoglobulin-like domain thereof shows a VEGF inhibitory activity.

Sharifi, J. et al., 1998, *The Quarterly Jour. of Nucl. Med.* 42:242-249, disclose that because monoclonal antibodies (MAbs) are basic, positively charged proteins, and mammalian cells are negatively charged, the electrostatic interactions between the two can create higher levels of background binding resulting in low tumor to normal organ ratios. To overcome this effect, the investigators attempted to improve MAb clearance by using various methods such as secondary agents as well as chemical and charge modifications of the MAb itself.

Jensen-Pippo, et al., 1996, *Pharmaceutical Research* 13:102-107, disclose that pegylation of a therapeutic protein, recombinant human granulocyte colony stimulating factor (PEG-G-CSF), results in an increase in stability and in retention of in vivo bioactivity when administered by the intraduodenal route.

Tsutsumi, et al., 1997, *Thromb Haemost.* 77:168-73, disclose experiments wherein the in vivo thrombopoietic activity of polyethylene glycol-modified interleukin-6 (MPEG-IL-6), in which 54% of the 14 lysine amino groups of IL-6 were coupled with PEG, was compared to that of native IL-6.

Yang, et al., 1995, *Cancer* 76:687-94, disclose that conjugation of polyethylene glycol to recombinant human interleukin-2 (IL-2) results in a compound, polyethylene glycol-modified IL-2 (PEG-IL-2) that retains the in vitro and in vivo activity of IL-2, but exhibits a markedly prolonged circulating half-life.

R. Duncan and F. Spreafico, *Clin. Pharmacokinet.* 27: 290-306, 296 (1994) review efforts to improve the plasma half-life of asparaginase by conjugating polyethylene glycol.

PCT International Publication No. WO 99/03996 published Jan. 28, 1999 in the name of Regeneron Pharmaceu-

ticals, Inc. and The Regents of The University of California describes modified human noggin polypeptides having deletions of regions of basic amino acids. The modified human noggin polypeptides are described as retaining biological activity while having reduced affinity for heparin and superior pharmacokinetics in animal sera as compared to the unmodified human noggin.

SUMMARY OF THE INVENTION

The present invention is directed to VEGF antagonists with improved pharmacokinetic properties. A preferred embodiment is an isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a VEGF polypeptide comprising (a) a nucleotide sequence encoding a VEGF receptor component operatively linked to (b) a nucleotide sequence encoding a multimerizing component, wherein the VEGF receptor component is the only VEGF receptor component of the fusion polypeptide and wherein the nucleotide sequence of (a) consists essentially of a nucleotide sequence encoding the amino acid sequence of Ig domain 2 of the extracellular domain of a first VEGF receptor and a nucleotide sequence encoding the amino acid sequence of Ig domain 3 of the extracellular domain of a second VEGF receptor.

In a further embodiment, the isolated nucleic acid of the first VEGF receptor is Flt1.

In a further embodiment, the isolated nucleic acid of the second VEGF receptor is Flk1.

In yet another embodiment, the isolated nucleic acid of the second VEGF receptor is Flt4.

In another preferred embodiment, the nucleotide sequence encoding Ig domain 2 of the extracellular domain of the first VEGF receptor is upstream of the nucleotide sequence encoding Ig domain 3 of the extracellular domain of the second VEGF receptor.

In still another preferred embodiment, the nucleotide sequence encoding Ig domain 2 of the extracellular domain of the first VEGF receptor is downstream of the nucleotide sequence encoding Ig domain 3 of the extracellular domain of the second VEGF receptor.

In a preferred embodiment of the invention, the multimerizing component comprises an immunoglobulin domain.

In another embodiment, the immunoglobulin domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.

Preferred embodiments include an isolated nucleic acid molecule comprising a nucleotide sequence encoding a modified Flt1 receptor fusion polypeptide, wherein the coding region of the nucleic acid molecule consists of a nucleotide sequence selected from the group consisting of

- (a) the nucleotide sequence set forth in FIG. 13A-13D (SEQ ID NO:3);
- (b) the nucleotide sequence set forth in FIG. 14A-14C (SEQ ID NO:5);
- (c) the nucleotide sequence set forth in FIG. 15A-15C (SEQ ID NO:7);
- (d) the nucleotide sequence set forth in FIG. 16A-16D (SEQ ID NO:9);
- (e) the nucleotide sequence set forth in FIG. 21A-21C (SEQ ID NO:11);
- (f) the nucleotide sequence set forth in FIG. 22A-22C (SEQ ID NO:13);
- (g) the nucleotide sequence set forth in FIG. 24A-24C (SEQ ID NO:15); and
- (h) a nucleotide sequence which, as a result of the degeneracy of the genetic code, differs from the nucle-

otide sequence of (a), (b), (c), (d), (e), (f), or (g) and which encodes a fusion polypeptide molecule having the biological activity of the modified Flt1 receptor fusion polypeptide.

In a further embodiment of the invention, a fusion polypeptide is encoded by the isolated nucleic acid molecules described above.

A preferred embodiment is a composition capable of binding a VEGF molecule to form a nonfunctional complex comprising a multimer of the fusion polypeptide.

Also preferred is a composition wherein the multimer is a dimer.

In yet another embodiment, the composition is in a carrier.

Another embodiment is a vector which comprises the nucleic acid molecules described above, including an expression vector comprising a the nucleic acid molecules described wherein the nucleic acid molecule is operatively linked to an expression control sequence.

Other included embodiments are a host-vector system for the production of a fusion polypeptide which comprises the expression vector, in a suitable host cell; the host-vector system wherein the suitable host cell is a bacterial cell, yeast cell, insect cell, or mammalian cell; the host-vector system wherein the suitable host cell is *E. Coli*; the host-vector system wherein the suitable host cell is a COS cell; the host-vector system wherein the suitable host cell is a CHO cell.

Another embodiment of the invention is a method of producing a fusion polypeptide which comprises growing cells of the host-vector system under conditions permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.

Additional embodiments include a fusion polypeptide encoded by the nucleic acid sequence set forth in FIG. 10A-10D (SEQ ID NO:1) or FIG. 24A-24G (SEQ ID NO:15), which has been modified by acetylation or pegylation wherein the acetylation is accomplished with at least about a 100 fold molar excess of acetylation reagent or wherein acetylation is accomplished with a molar excess of acetylation reagent ranging from at least about a 10 fold molar excess to about a 100 fold molar excess or wherein the pegylation is 10K or 20K PEG.

A preferred embodiment includes a method of decreasing or inhibiting plasma leakage in a mammal comprising administering to the mammal the fusion polypeptide described above, including embodiments wherein the mammal is a human, the fusion polypeptide is acetylated or the fusion polypeptide is pegylated.

A further embodiment is a fusion polypeptide which specifically binds the VEGF receptor ligand VEGF.

A preferred embodiment of the invention is a method of blocking blood vessel growth in a human comprising administering an effective amount of the fusion polypeptide described above.

Also preferred is a method of inhibiting VEGF receptor ligand activity in a mammal comprising administering to the mammal an effective amount of the fusion polypeptide described above.

Preferred embodiments of these methods are wherein the mammal is a human.

Further embodiments of the methods of the invention include attenuation or prevention of tumor growth in a human; attenuation or prevention of edema in a human, especially wherein the edema is brain edema; attenuation or prevention of ascites formation in a human, especially wherein the ascites is ovarian cancer-associated ascites.

Preferred embodiments of the invention include a fusion polypeptide capable of binding a VEGF polypeptide comprising (a) a VEGF receptor component operatively linked to (b) a multimerizing component, wherein the VEGF receptor component is the only VEGF receptor component in the fusion polypeptide and consists essentially of the amino acid sequence of Ig domain 2 of the extracellular domain of a first VEGF receptor and the amino acid sequence of Ig domain 3 of the extracellular domain of a second VEGF receptor.

In a further embodiment of the fusion polypeptide, the first VEGF receptor is Flt1.

In yet a further embodiment of the fusion polypeptide, the second VEGF receptor is Flk1.

Still another embodiment of the fusion polypeptide is one in which the second VEGF receptor is Flt4.

Preferred embodiments include a fusion polypeptide wherein amino acid sequence of Ig domain 2 of the extracellular domain of the first VEGF receptor is upstream of the amino acid sequence of Ig domain 3 of the extracellular domain of the second VEGF receptor and a fusion polypeptide wherein the amino acid sequence of Ig domain 2 of the extracellular domain of the first VEGF receptor is downstream of the amino acid sequence of Ig domain 3 of the extracellular domain of the second VEGF receptor.

In yet another embodiment, the fusion polypeptide multimerizing component comprises an immunoglobulin domain including an embodiment wherein the immunoglobulin domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.

Preferred embodiments include a fusion polypeptide comprising an amino acid sequence of a modified Flt1 receptor, wherein the amino acid sequence selected from the group consisting of (a) the amino acid sequence set forth in FIG. 13A-13D (SEQ ID NO:4); (b) the amino acid sequence set forth in FIG. 14A-14D (SEQ ID NO:6); (c) the amino acid sequence set forth in FIG. 15A-15C (SEQ ID NO:8); (d) the amino acid sequence set forth in FIG. 16A-16D (SEQ ID NO:10); (e) the amino acid sequence set forth in FIG. 21A-21D (SEQ ID NO:12); (f) the amino acid sequence set forth in FIG. 22A-22C (SEQ ID NO:14); and (g) the amino acid sequence set forth in FIG. 24A-24C (SEQ ID NO:16).

Another preferred embodiment is a method of decreasing or inhibiting plasma leakage in a mammal comprising administering to the mammal the fusion polypeptide described above.

An alternative preferred embodiment is a method of inhibiting VEGF receptor ligand activity in a mammal comprising administering to the mammal an effective amount of the fusion polypeptide described above.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1. IEF gel analysis of unmodified and acetylated Flt1(1-3)-Fc proteins. Unmodified Flt1(1-3)-Fc protein is unable to enter the gel due to its >9.3 pl, whereas acetylated Flt1(1-3)-Fc is able to enter the gel and equilibrate at pl 5.2.

FIG. 2. Binding of unmodified Flt1(1-3)-Fc and acetylated Flt1(1-3)-Fc proteins to MATRIGEL® coated plates. Unmodified Flt1(1-3)-Fc proteins binds extensive to extracellular matrix components in Matrigel.RTM., whereas acetylated Flt1(1-3)-Fc does not bind.

FIG. 3. Binding of unmodified Flt1(1-3)-Fc, acetylated Flt1(1-3)-Fc, and pegylated Flt1(1-3)-Fc in a BIACORE™-based assay. Acetylated (columns 13-16), pegylated (columns 17-20), and heparin-treated Flt1(1-3)-Fc (columns 21-24) are each able to completely compete with the

BIACORE™ chip-bound Flt1(1-3)-Fc for VEGF binding as compared to control (columns 1-4) and irrelevant protein (columns 5-8). Unmodified Flt1(1-3)-Fc (columns 5-6) appears to only partially compete with BIACORE™ chip-bound Flt1(1-3)-Fc for VEGF binding. However, washing the bound samples with 0.5M NaCl (columns 7-8) results in a binding profile similar to the modified forms of Flt1(1-3)-Fc, indicating that the unmodified protein is exhibiting non-specific binding to the chip that can be eliminated by the salt wash.

FIG. 4. Binding of unmodified Flt1(1-3)-Fc, acetylated Flt1(1-3)-Fc, and pegylated Flt1(1-3)-Fc to VEGF in an ELISA-based assay. Both pegylated and acetylated Flt1(1-3)-Fc proteins bind to VEGF with affinities approaching that of unmodified Flt1(1-3)-Fc.

FIG. 5. Pharmacokinetic profiles of unmodified Flt1(1-3)-Fc, acetylated Flt1(1-3)-Fc, and pegylated Flt1(1-3)-Fc. Balb/c mice (23-28 g) were injected subcutaneously with 4 mg/kg of unmodified, acetylated, or pegylated Flt1(1-3)-Fc. The mice were tail bled at 1, 2, 4, 6, 24 hours, 2 days, and 3 days after injection of protein and the sera were assayed in a standard ELISA-based assay designed to detect Flt1(1-3)-Fc protein. The T_{max} for all of the Flt1(1-3)-Fc proteins was between the 6 hour and 24 hour time points. The C_{max} for the different proteins was as follows: Unmodified: 0.06 µg/ml-0.15 µg/ml; acetylated: 1.5 µg/ml-4.0 µg/ml; and pegylated: approximately 5 µg/ml.

FIG. 6A-6B. IEF gel analysis of unmodified and step-acetylated Flt1(1-3)-Fc proteins. Unmodified Flt1(1-3)-Fc protein is unable to enter the gel due to its >9.3 pl, whereas most of the step-acetylated Flt1(1-3)-Fc samples (30-100 fold excess samples) were able to migrate into the gel and equilibrate at pls ranging between 4.55-8.43, depending on the degree of acetylation.

FIG. 7. Binding of unmodified Flt1(1-3)-Fc and step-acetylated Flt1(1-3)-Fc proteins to MATRIGEL® coated plates. As with the irrelevant control protein, rTie2-Fc, step-acetylated Flt1(1-3)-Fc (20 and 30 fold excess samples) does not exhibit any binding to the Matrigel coated plate, whereas the non-acetylated Flt1(1-3)-Fc protein exhibits significant binding. The 10 fold excess sample shows reduced binding, but the degree of acetylation is not enough to completely block binding to extracellular matrix components.

FIG. 8. Binding of unmodified Flt1(1-3)-Fc and step-acetylated Flt1(1-3)-Fc in a BIACORE™-based assay. At a sub-stoichiometric ratio (0.5 µg/ml of either unmodified Flt1(1-3) or step-acetylated Flt1(1-3)-Fc vs. 0.2 µg/ml VEGF), there is not enough Flt1(1-3)-Fc (either unmodified or step-acetylated) in the solution to completely bind the VEGF. At 1.0 µg/ml, which approximates a 1:1 stoichiometric ratio, the both unmodified and step-acetylated Flt1(1-3)-Fc are better able to compete for VEGF binding, but there is still insufficient Flt1(1-3)-Fc protein (either unmodified or step-acetylated) to completely saturate the available VEGF. However, at 5.0 µg/ml, which is several times greater than a 1:1 stoichiometric ratio, both the Flt1(1-3)-Fc and the step-acetylated Flt1(1-3)-Fc proteins are able to saturate the VEGF, regardless of the degree of acetylation.

FIG. 9. Pharmacokinetic profiles of unmodified Flt1(1-3)-Fc and step-acetylated Flt1(1-3)-Fc. Balb/c mice (23-28 g) were injected subcutaneously with 4 mg/kg of unmodified or 10, 20, 40, 60 and 100 fold excess samples of step-acetylated Flt1(1-3)-Fc (3 mice for unmodified, 10, 20 and 40 fold excess samples and 2 mice for 60 and 100 fold excess samples). The mice were tail bled at 1, 2, 4, 6, 24 hours, 2 days and 3 days after injection. The sera were

assayed in an ELISA-based assay designed to detect Flt1(1-3)-Fc. The T_{max} for all of the Flt1(1-3)-Fc proteins tested was at the 6 hour time point but the C_{max} was as follows: Unmodified Flt1(1-3)-Fc: 0.06 $\mu\text{g/ml}$; 10 fold excess sample:—0.7 $\mu\text{g/ml}$, 20 fold excess sample—2 $\mu\text{g/ml}$, 40 fold excess sample—4 $\mu\text{g/ml}$, 60 fold excess sample—2 $\mu\text{g/ml}$, 100 fold excess sample—1 $\mu\text{g/ml}$.

FIG. 10A-10D. Nucleic acid (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of Flt1(1-3)-Fc.

FIG. 11. Schematic diagram of the structure of Flt1.

FIGS. 12A and 12B. Hydrophilicity analysis of the amino acid sequences of Ig domain 2 and Ig domain 3 of Flt1.

FIG. 13A-13D. Nucleic acid (SEQ ID NO:3) and deduced amino acid sequence (SEQ ID NO:4) of Mut1: Flt1(1-3 $_{\Delta B}$)-Fc.

FIG. 14A-14D. Nucleic acid (SEQ ID NO:5) and deduced amino acid sequence (SEQ ID NO:6) of Mut2: Flt1(2-3 $_{\Delta B}$)-Fc.

FIG. 15A-15C. Nucleic acid (SEQ ID NO:7) and deduced amino acid sequence (SEQ ID NO:8) of Mut3: Flt1(2-3)-Fc.

FIG. 16A-16D. Nucleic acid (SEQ ID NO:9) and deduced amino acid sequence (SEQ ID NO:10) of Mut4: Flt1(1-3 $_{R \rightarrow N}$)-Fc.

FIG. 17. Binding of unmodified Flt1(1-3)-Fc, basic region deletion mutant Flt1(1-3)-Fc, and Flt1(1-3) $_{R \rightarrow N}$ mutant proteins in a BIACORE™-based assay. At the sub-stoichiometric ratio (0.25 $\mu\text{g/ml}$ Flt1(1-3)-Fc of unmodified, acetylated or genetically modified samples vs. 01. $\mu\text{g/ml}$ VEGF), there is insufficient Flt1(1-3)-Fc protein to block binding of VEGF to the Flt1(1-3)-Fc immobilized on the BIACORE™ chip. At 0.5 $\mu\text{g/ml}$ of unmodified, acetylated or genetically modified Flt1(1-3)-Fc proteins, the stoichiometric ratio approximates 1:1 and there is an increased ability to block VEGF binding to the BIACORE™ chip. At 1.0 $\mu\text{g/ml}$ of unmodified, acetylated or genetically modified Flt1(1-3)-Fc proteins, which is approximately a 10:1 stoichiometric ratio, the Flt1(1-3)-Fc proteins are able to block binding of VEGF to the BIACORE™ chip, but they are not equivalent. Unmodified, acetylated, and Mut1: Flt1(1-3 $_{\Delta B}$)-Fc are essentially equal in their ability to block VEGF binding, whereas Mut4: Flt1(1-3 $_{R \rightarrow N}$)-Fc is somewhat less efficient at blocking binding.

FIG. 18. Binding of unmodified Flt1(1-3)-Fc, Mut1: Flt1(1-3 $_{\Delta B}$)-Fc, Mut2: Flt1(2-3 $_{\Delta B}$)-Fc, and Flt1(2-3) mutant proteins to Matrigel® coated plates. Unmodified Flt1(1-3)-Fc protein binds avidly to these wells, the Mut3: Flt1(2-3)-Fc protein binds somewhat more weakly, the Mut1: Flt1(1-3 $_{\Delta B}$)-Fc protein binds more weakly still, and the Mut2: Flt1(2-3 $_{\Delta B}$)-Fc protein shows the best profile, binding more weakly than any of the other mutant proteins. The Mut4: Flt1(1-3 $_{R \rightarrow N}$)-Fc glycosylation mutant protein shows only marginal benefit on the Matrigel assay.

FIG. 19. Binding of unmodified Flt1(1-3)-Fc, Mut1: Flt1(1-3 $_{\Delta B}$)-Fc, Mut2: Flt1(2-3 $_{\Delta B}$)-Fc, and Flt1(2-3) mutant proteins in an ELISA-based assay. At the concentrations tested, unmodified Flt1(1-3)-Fc, Mut1: Flt1(1-3 $_{\Delta B}$)-Fc, Mut2: Flt1(2-3 $_{\Delta B}$)-Fc, and Flt1(2-3) mutant proteins bind VEGF similarly.

FIG. 20. Pharmacokinetic profiles of unmodified Flt1(1-3)-Fc, Mut1: Flt1(1-3 $_{\Delta B}$)-Fc, Mut2: Flt1(2-3 $_{\Delta B}$)-Fc, and Flt1(2-3) mutant proteins. The C_{max} for these reagents was as follows: Unmodified Flt1(1-3)-Fc-0.15 $\mu\text{g/ml}$; 40 fold molar excess acetylated Flt1(1-3)-Fc-1.5 $\mu\text{g/ml}$; and Mut1: Flt1(1-3 $_{\Delta B}$)-Fc-0.7 $\mu\text{g/ml}$.

FIG. 21A-21D. Nucleotide (SEQ ID NO:11) and deduced amino acid sequence (SEQ ID NO:12) of the modified Flt1 receptor termed Flt1D2.Flk1D3.Fc Δ C1(a).

FIG. 22A-22C. Nucleotide (SEQ ID NO:13) and deduced amino acid sequence (SEQ ID NO:14) of the modified Flt1 receptor termed Flt1D2.VEGFR3D3.Fc Δ C1(a).

FIG. 23. Extracellular Matrix (ECM) Assay. The results of this assay demonstrate that the Flt1D2.Flk1D3.Fc Δ C1(a) and Flt1D2.VEGFR3D3.Fc Δ C1(a) proteins are considerably less sticky to the ECM as compared to the Flt1(1-3)-Fc protein.

FIG. 24A-24C. Nucleotide (SEQ ID NO:15) and deduced amino acid sequence (SEQ ID NO:16) of the modified Flt1 receptor termed VEGFR1R2-Fc Δ C1(a).

FIG. 25A-25C. Phosphorylation assay. At a 1.5 molar excess of either Flt1(1-3)-Fc, Flt1(1-3)-Fc (A40) or transient Flt1D2Fk1D3.Fc Δ C1(a) there is complete blockage of receptor stimulation by these three modified Flt1 receptors as compared to control media challenge. In contrast, transient Flt1D2VEGFR3D3.Fc Δ C1(a) does not show significant blockage at this molar excess, as compared with VEGF positive control challenge. Similar results are seen in FIG. 25B, where the modified Flt receptors are in a 3-fold molar excess to VEGF165 ligand. In FIG. 25C, where the modified Flt1 receptors are in a 6-fold molar excess to VEGF165 ligand, transient Flt1D2VEGFR3D3.Fc Δ C1(a) can now be shown to be partially blocking VEGF165-induced stimulation of cell-surface receptors.

FIG. 26A-26B. Phosphorylation assay. Detection by Western blot of tyrosine phosphorylated VEGFR2(Flk1) by VEGF165 ligand stimulation shows that cell-surface receptors are not phosphorylated by challenge samples which have VEGF165 preincubated with 1 and 2 fold molar excess (FIG. 26A) or 3 and 4 fold molar excess (FIG. 26B) of either transient Flt1D2Fk1D3.Fc Δ C1(a), stable Flt1D2Fk1D3.Fc Δ C1(a), or transient VEGFR1R2-Fc Δ C1(a). At all modified Flt1 receptor concentrations tested there is complete binding of VEGF165 ligand during the preincubation, resulting in no detectable stimulation of cell-surface receptors by unbound VEGF165 as compared to control media challenge.

FIG. 27. MG/R2 Cell proliferation assay. The following modified Flt receptors Flt1(1-3)-Fc, Flt1D2.Flk1D3.Fc Δ C1(a) and Flt1D2.VEGFR3D3.Fc Δ C1(a), plus an irrelevant receptor termed Tie2-Fc as a negative control, were titrated from 40 nM to 20 pM and incubated on the cells for 1 hr at 37° C. Human recombinant VEGF165 in defined media was then added to all the wells at a concentration of 1.56 nM. The negative control receptor Tie2-Fc does not block VEGF165-induced cell proliferation at any concentration whereas Flt1D2.Flk1D3.Fc Δ C1(a) blocks 1.56 nM VEGF165 with a half maximal dose of 0.8 nM. Flt1(1-3)-Fc and Flt1D2.VEGFR3D3.Fc Δ C1(a) are less effective in blocking VEGF165 in this assay with a half maximal dose of ~2 nM. VEGF165 alone gives a reading of 1.2 absorbance units and the background is 0.38 absorbance units.

FIG. 28. BIACORE™ analysis of Binding Stoichiometry. Binding 20 stoichiometry was calculated as a molar ratio of bound VEGF165 to the immobilized Flt1D2Fk1D3.Fc Δ C1(a) or VEGFR1R2-Fc Δ C1(a), using the conversion factor of 1000 RU equivalent to 1 ng/ml. The results indicated binding stoichiometry of one VEGF165 dimeric molecule per one Flt1D2Fk1D3.Fc Δ C1(a) or VEGFR1R2-Fc Δ C1(a) molecule.

FIG. 29 and FIG. 30. Size Exclusion Chromatography Stoichiometry. Flt1D2Fk1D3.Fc Δ C1(a) or VEGFR1R2-Fc Δ C1(a) at a concentration of 1 nM (estimated to be 1000 times higher than the KD of the Flt1D2Fk1D3.Fc Δ C1(a) or VEGFR1R2-Fc Δ C1(a)/VEGF165 interaction) were mixed with varied concentrations of VEGF165. After incubation,

concentrations of the free Flt1D2Flk1D3.FcΔC1(a) in solution were measured. The data shows that the addition of 1 nM VEGF165 into the Flt1D2Flk1D3.FcΔC1(a) solution completely blocks Flt1D2Flk1D3.FcΔC1(a) binding to the VEGF165 surface. This result suggested the binding stoichiometry of one VEGF165 molecule per one Flt1D2Flk1D3.FcΔC1(a) molecule.

FIG. 31. Size Exclusion Chromatography (SEC) under native conditions. Peak #1 represents the Flt1D2Flk1D3.FcΔC1(a)/VEGF165 complex and peak #2 represents unbound VEGF165. Fractions eluted between 1.1 and 1.2 ml were combined and guanidinium hydrochloride (GuHCl) was added to a final concentration 4.5M to dissociate the complex.

FIG. 32. Size Exclusion Chromatography (SEC) under dissociative conditions. To separate the components of the receptor-ligand complex and to determine their molar ratio, 50.μl of dissociated complex was loaded onto a SUPER-OSE™ 12 PC 3.2/30 equilibrated in 6M GuHCl and eluted. Peak#1 represents Flt1D2Flk1D3.FcΔC1(a) and peak#2 represents VEGF165.

FIG. 33, FIG. 34 and FIG. 35. Size Exclusion Chromatography (SEC) With On-Line Light Scattering. Size exclusion chromatography column with a MiniDawn on-line light scattering detector (Wyatt Technology, Santa Barbara, Calif.) and refractive index (RI) detectors (Shimadzu, Kyoto, Japan) was used to determine the molecular weight (MW) of the receptor-ligand complex. As shown in FIG. 33, the elution profile shows two peaks. Peak #1 represents the receptor-ligand complex and peak #2 represents the unbound VEGF165. MW was calculated from LS and RI signals. The same procedure was used to determine MW of the individual components of the receptor-ligand complex. The results of these determinations are as follows:

MW of the Flt1D2Flk1D3.FcΔC1(a)/VEGF165 complex at the peak position is 157 300 (FIG. 33), the MW of VEGF165 at the peak position is 44 390 (FIG. 34) and the MW of R1R2 at the peak is 113 300 (FIG. 35).

FIG. 36. Peptide mapping and glycosylation analysis. The disulfide structures and glycosylation sites in Flt1D2.Flk1D3.FcΔC1(a) (SEQ ID NO:12) were determined by a peptide mapping method. There are a total of ten cysteines in Flt1D2.Flk1D3.FcΔC1(a); six of them belong to the Fc region. Cys27 is disulfide bonded to Cys76. Cys121 is disulfide bonded to Cys 182. The first two cysteines in the Fc region (Cys211 and Cys214) form an intermolecular disulfide bond with the same two cysteines in another Fc chain. However, it can not be determined whether disulfide bonding is occurring between same cysteines (Cys211 to Cys211, for example) or between Cys211 and Cys211. Cys216 is disulfide bonded to Cys306. Cys 352 is disulfide bonded to Cys410.

There are five possible N-linked glycosylation sites in Flt1D2.Flk1D3.FcΔC1(a) (SEQ ID NO:12) and are found to be glycosylated to varying degrees. Complete glycosylation is observed at Asn33, Asn193, and Asn282. Partial glycosylation is observed on Asn65 and Asn120. Sites of glycosylation are highlighted by underline in the FIG.

FIG. 37. Pharmacokinetics of Flt1(1-3)-Fc (A40), Flt1D2.Flk1D3.FcΔC1(a) and VEGFR1 R2-FcΔC1(a). Balb/c mice were injected subcutaneously with 4 mg/kg of Flt1(1-3)-Fc (A40), CHO transiently expressed Flt1D2.Flk1D3.FcΔC1(a), CHO stably expressed Flt1D2.Flk1D3.FcΔC1(a), and CHO transiently expressed VEGFR1R2-FcΔC1(a). The mice were tail bled at 1, 2, 4, 6, 24 hrs, 2 days, 3 days and 6 days after injection. The sera were assayed in an ELISA designed to detect Flt1(1-3)-Fc

(A40), Flt1D2.Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a). The T_{max} for Flt1(1-3)-Fc (A40) was at 6 hrs while the T_{max} for the transient and stable Flt1D2.Flk1D3.FcΔC1(a) and the transient VEGFR1R2-FcΔC1(a) was 24 hrs. The C_{max} for Flt1(1-3)-Fc (A40) was 8 μg/ml, For both transients (Flt1D2.Flk1D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a)) the C_{max} was 18 μg/ml and the C_{max} for the stable VEGFR1R2-FcΔC1(a) was 30 μg/ml.

FIG. 38. Pharmacokinetics of Flt1(1-3)-Fc (A40), Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a). Balb/c mice were injected subcutaneously with 4 mg/kg of Flt1(1-3)-Fc (A40), CHO 5 transiently expressed Flt1D2.Flk1D3.FcΔC1(a) and CHO transiently expressed Flt1D2.VEGFR3D3.FcΔC1(a). The mice were tail bled at 1, 2, 5, 6, 7, 8, 12, 15 and 20 days after injection. The sera were assayed in an ELISA designed to detect Flt1(1-3)-Fc, Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a). Flt1(1-3)-Fc (A40) could no longer be detected in the serum after day 5 whereas Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a) were detectable for 15 days or more.

FIG. 39. The Ability of Flt1D2.Flk1D3.FcΔC1(a) to Inhibit HT-1080 Fibrosarcoma Tumor Growth In Vivo. Every other day or 2 times per week treatment of SCID mice with Flt1D2.Flk1D3.FcΔC1(a) at 25 mg/Kg significantly decreases the growth of subcutaneous HT-1080 fibrosarcoma tumors.

FIG. 40. The Ability of Flt1D2.Flk1D3.FcΔC1(a) to Inhibit C6 Glioma Tumor Growth In Vivo. Every other day or 2 times a week treatment of SCID mice with Flt1D2.Flk1D3.FcΔC1(a) significantly decreases the growth of subcutaneous C6 glioma tumors at doses as low as 2.5 mg/Kg.

FIG. 41. VEGF-Induced Uterine Hyperpermeability. PMSG injected subcutaneously (5 IU) to induce ovulation in prepubertal female rats results in a surge of estradiol after 2 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. Subcutaneous injection of Flt1(1-3)-Fc (A40), Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a) at 25 mg/kg at 1 hr after PMSG injection results in about a 50% inhibition of the increase in uterine wet weight.

FIG. 42A-42B. Assessment of Corpus Luteum Angiogenesis Using Progesterone as a Readout. PMSG was injected subcutaneously (5 IU) to induce ovulation in prepubertal female rats, resulting in a fully functioning corpus luteum containing a dense network of blood vessels that secretes progesterone into the blood stream to prepare the uterus for implantation. The induction of angiogenesis in the corpus luteum requires VEGF. Resting levels of progesterone are about 5 ng/ml and can be induced to 25-40 ng/ml after PMSG. Subcutaneous injection of Flt1(1-3)-Fc (A40) or Flt1D2.Flk1D3.FcΔC1(a) at 25 mg/kg or 5 mg/kg at 1 hr. after PMSG injection resulted in a complete inhibition of the progesterone induction on day 4.

DETAILED DESCRIPTION OF THE INVENTION

It has been a long standing problem in the art to produce a receptor based VEGF antagonist that has a pharmacokinetic profile that is appropriate for consideration of the antagonist as a therapeutic candidate. Applicants describe herein, for the first time, a chimeric polypeptide molecule, capable of antagonizing VEGF activity, that exhibits improved pharmacokinetic properties as compared to other

known receptor-based VEGF antagonists. The chimeric polypeptide molecules described herein thus provide for the first time appropriate molecules for use in therapies in which antagonism of VEGF is a desired result.

The present invention provides for novel chimeric polypeptide molecules formed by fusing a modified extracellular ligand binding domain of the Flt1 receptor to the Fc region of IgG.

The extracellular ligand binding domain is defined as the portion of a receptor that, in its native conformation in the cell membrane, is oriented extracellularly where it can contact with its cognate ligand. The extracellular ligand binding domain does not include the hydrophobic amino acids associated with the receptor's transmembrane domain or any amino acids associated with the receptor's intracellular domain. Generally, the intracellular or cytoplasmic domain of a receptor is usually composed of positively charged or polar amino acids (i.e. lysine, arginine, histidine, glutamic acid, aspartic acid). The preceding 15-30, predominantly hydrophobic or apolar amino acids (i.e. leucine, valine, isoleucine, and phenylalanine) comprise the transmembrane domain. The extracellular domain comprises the amino acids that precede the hydrophobic transmembrane stretch of amino acids. Usually the transmembrane domain is flanked by positively charged or polar amino acids such as lysine or arginine. von Heijne has published detailed rules that are commonly referred to by skilled artisans when determining which amino acids of a given receptor belong to the extracellular, transmembrane, or intracellular domains (See von Heijne, 1995, *BioEssays* 17:25-30). Alternatively, websites on the Internet have become available to provide protein chemists with information about making predictions about protein domains.

The present invention provides for the construction of nucleic acid molecules encoding chimeric polypeptide molecules that are inserted into a vector that is able to express the chimeric polypeptide molecules when introduced into an appropriate host cell. Appropriate host cells include, but are not limited to, bacterial cells, yeast cells, insect cells, and mammalian cells. Any of the methods known to one skilled in the art for the insertion of DNA fragments into a vector may be used to construct expression vectors encoding the chimeric polypeptide molecules under control of transcriptional/translational control signals. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinations (genetic recombination) (See Sambrook, et al., *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory; *Current Protocols in Molecular Biology*, Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY).

Expression of nucleic acid molecules encoding the chimeric polypeptide molecules may be regulated by a second nucleic acid sequence so that the chimeric polypeptide molecule is expressed in a host transformed with the recombinant DNA molecule. For example, expression of the chimeric polypeptide molecules described herein may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control expression of the chimeric polypeptide molecules include, but are not limited to, the long terminal repeat as described in Squinto et al., (1991, *Cell* 65:1-20); the SV40 early promoter region (Bernoist and Chambon, 1981, *Nature* 290:304-310), the CMV promoter, the M-MuLV 5' terminal repeat the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, *Cell* 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:144-1445), the regulatory

sequences of the metallothionein gene (Brinster et al., 1982, *Nature* 296:39-42); prokaryotic expression vectors such as the β -lactamase promoter (Villa-Kamaroff, et al., 1978, *Proc. Natl. Acad. Sci. U.S.A.* 75:3727-3731), or the tac promoter (DeBoer, et al., 1983, *Proc. Natl. Acad. Sci. U.S.A.* 80:21-25, see also "Useful proteins from recombinant bacteria" in *Scientific American*, 1980, 242:74-94); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADH (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, *Cell* 38:639-646; Ornitz et al., 1986, *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409; MacDonald, 1987, *Hepatology* 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, *Nature* 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, *Cell* 38:647-658; Adames et al., 1985, *Nature* 318:533-538; Alexander et al., 1987, *Mol. Cell. Biol.* 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, *Cell* 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, *Genes and Devel.* 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, *Mol. Cell. Biol.* 5:1639-1648; Hammer et al., 1987, *Science* 235:53-58); alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, *Genes and Devel.* 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, *Nature* 315:338-340; Kollias et al., 1986, *Cell* 46:89-94); myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, *Cell* 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Shani, 1985, *Nature* 314:283-286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, *Science* 234:1372-1378).

Thus, according to the invention, expression vectors capable of being replicated in a bacterial or eukaryotic host comprising chimeric polypeptide molecule-encoding nucleic acid as described herein, are used to transfect the host and thereby direct expression of such nucleic acids to produce the chimeric polypeptide molecules, which may then be recovered in a biologically active form. As used herein, a biologically active form includes a form capable of binding to VEGF.

Expression vectors containing the chimeric nucleic acid molecules described herein can be identified by three general approaches: (a) DNA-DNA hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a foreign gene inserted in an expression vector can be detected by DNA-DNA hybridization using probes comprising sequences that are homologous to the inserted chimeric polypeptide molecule sequences. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign genes in the vector. For example, if the chimeric polypeptide molecule DNA sequence is inserted within the marker gene sequence of the vector, recombinants

15

containing the insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the foreign gene product expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of the chimeric polypeptide molecules.

Cells of the present invention may transiently or, preferably, constitutively and permanently express the chimeric polypeptide molecules.

The chimeric polypeptide molecules may be purified by any technique which allows for the subsequent formation of a stable, biologically active chimeric polypeptide molecule. For example, and not by way of limitation, the factors may be recovered from cells either as soluble proteins or as inclusion bodies, from which they may be extracted quantitatively by 8M guanidinium hydrochloride and dialysis (see, for example, Builder, et al., U.S. Pat. No. 5,663,304). In order to further purify the factors, conventional ion exchange chromatography, hydrophobic interaction chromatography, reverse phase chromatography or gel filtration may be used.

In one embodiment of the invention, the nucleotide sequence encoding the first component is upstream of the nucleotide sequence encoding the second component. In another embodiment of the invention, the nucleotide sequence encoding the first component is downstream of the nucleotide sequence encoding the second component. Further embodiments of the invention may be prepared in which the order of the first, second and third fusion polypeptide components are rearranged. For example, if the nucleotide sequence encoding the first component is designated 1, the nucleotide sequence encoding the second component is designated 2, and the nucleotide sequence of the third component is designated 3, then the order of the components in the isolated nucleic acid of the invention as read from 5' to 3' may be any of the following six combinations: 1,2,3; 1,3,2; 2,1,3; 2,3,1; 3,1,2; or 3,2,1.

The present invention also has diagnostic and therapeutic utilities. In particular embodiments of the invention, methods of detecting aberrancies in the function or expression of the chimeric polypeptide molecules described herein may be used in the diagnosis of disorders. In other embodiments, manipulation of the chimeric polypeptide molecules or agonists or antagonists which bind the chimeric polypeptide molecules may be used in the treatment of diseases. In further embodiments, the chimeric polypeptide molecule is utilized as an agent to block the binding of a binding agent to its target.

By way of example, but not limitation, the method of the invention may be useful in treating clinical conditions that are characterized by vascular permeability, edema or inflammation such as brain edema associated with injury, stroke or tumor; edema associated with inflammatory disorders such as psoriasis or arthritis, including rheumatoid arthritis; asthma; generalized edema associated with burns; ascites and pleural effusion associated with tumors, inflammation or trauma; chronic airway inflammation; capillary leak syndrome; sepsis; kidney disease associated with increased leakage of protein; and eye disorders such as age related macular degeneration and diabetic retinopathy.

An amino acid sequence analysis of Flt1(1-3)-Fc revealed the presence of an unusually high number (46) of the basic amino acid residue lysine.

An IEF analysis of Flt1(1-3)-Fc showed that this protein has *pI* greater than 9.3, confirming the prediction that the protein is very basic. It was hypothesized that the basic nature of Flt1(1-3)-Fc protein was causing it to bind to

16

extracellular matrix components and that this interaction might be the cause of the extremely short detectable circulating serum half-life exhibited by Flt1(1-3)-Fc when injected into mice. In order to test this hypothesis, Flt1(1-3)-Fc protein was acetylated at the lysine residues to reduce the basic charge. Acetylated Flt1(1-3)-Fc was then tested in the assays described *infra*.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1

Expression of Flt1(1-3)-Fc Protein in CHO K1 Cells

Using standard molecular biology techniques (see e.g., *Molecular Cloning, A Laboratory Manual* (Sambrook, et al., Cold Spring Harbor Laboratory), *Current Protocols in Molecular Biology* (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY), the gene encoding Flt1(1-3)-Fc was inserted into the expression vector pEE14.1 (Lonza Biologics, plc) at a multiple cloning site downstream of the CMV promoter. CHO K1 cells were transfected with the pEE14.1/Flt1(1-3)-Fc DNA construct using lipofectamine (Gaithersburg, Md.). The transfected CHO K1 cells were grown in glutamine-free DMEM (JRH, Kansas City, Mo.) containing 25 μ M methionine sulfoximine (MSX) from Sigma Inc., St. Louis, Mo., and high recombinant protein expressors were obtained by screening the CHO K1 cell supernatants from over 100 hand-picked colony isolates using a standard immunoassay which captures and detects human Fc. The selected hand-picked clone was amplified in the presence of 100 μ M MSX followed by a second round of screening of the amplified clones. The highest producing clone had a specific productivity of recombinant Flt1(1-3)-Fc protein of 55 pg/cell/day.

The selected clone was expanded in 225 cm² T-flasks (Corning, Acton, culture media described *supra*). Cells were removed from the roller bottles by standard trypsinization and put into 3.5L of suspension medium. The suspension medium is comprised of glutamine-free ISCHO medium (Irvine Scientific, Santa Ana, Calif.) containing 5% fetal bovine serum (FBS from Hyclone Labs, Logan, Utah), 100 μ M MSX and GS supplement (JRH Scientific, Kansas City, Mo.) in a 5L Celligen bioreactor (New Brunswick Scientific, New Brunswick, N.J.) at a density of 0.3×10^6 cells/mL. After the cells reached a density of 3.6×10^6 /mL and were adapted to suspension they were transferred to a 60L bioreactor (ABEC, Allentown, Pa.) at a density of 0.5×10^6 cells/mL in 20L of ISCHO medium with 5% fetal bovine serum. After two days an additional 20L of ISCHO+5% fetal bovine serum was added to the bioreactor. The cells were allowed to grow for an additional two days reaching a final density of 3.1×10^6 cells/mL, and a final Flt1(1-3)-Fc concentration at harvest was 95 mg/L. At harvest the cells were removed by tangential flow filtration using 0.45 μ m Prostack Filters (Millipore, Inc., Bedford, Mass.).

Example 2

Purification of Flt1(1-3)-Fc Protein Obtained from CHO K1 Cells

Flt1(1-3)-Fc protein was initially purified by affinity chromatography. A Protein A column was used to bind, with high

specificity, the Fc portion of the molecule. This affinity-purified protein was then concentrated and passed over a SEC column. The protein was then eluted into the formulation buffer. The following describes these procedures in detail.

Materials and Methods

All chemicals were obtained from J. T. Baker, Phillipsburg, N.J. with the exception of PBS, which was obtained as a 10.times. concentrate from Life Technologies, Gaithersburg, Md. Protein A Fast Flow and SUPERDEX™ 200 preparation grade resins were obtained from Pharmacia, Piscataway, N.J. Equipment and membranes for protein concentration were obtained from Millipore, Bedford, Mass.

Approximately 40L of 0.45 μm -filtered CHO conditioned media containing Flt1(1-3)-Fc protein was applied to a 290 mL Protein A Fast Flow column (10 cm diameter) that had been equilibrated with PBS. The column was washed with PBS containing 350 mM NaCl and 0.02% CHAPS and the bound protein was eluted with 20 mM Citric Acid containing 10 mM Na_2HPO_4 . The single peak in the elution was collected and its pH was raised to neutrality with 1M NaOH. The eluate fractions was concentrated to approximately 9 mg/mL using 10K regenerated cellulose membranes by both tangential flow filtration and by stirred cell concentration. To remove aggregates and other contaminants, the concentrated protein was applied to a column packed with Superdex 200 preparation grade resin (10 cm \times 55 cm) and run in PBS containing 5% glycerol. The main peak fractions were pooled, sterile filtered, aliquoted and stored at -80°C .

Example 3

Acetylation of Flt1(1-3)-Fc Protein

Two milligrams of Flt1 (1-3)-Fc protein were acetylated as described in the instruction manual provided with the sulfo-NHS-acetate modification kit (Pierce Chemical Co., Rockford, Ill., Cat.#26777).

Example 4

Characterization of Acetylated Flt1(1-3)-Fc Protein

(a)IEF analysis: Flt1(1-3)-Fc and acetylated Flt1(1-3)-Fc were analyzed by standard IEF analysis. As shown in FIG. 1, Flt1(1-3)-Fc protein is not able to migrate into the gel and therefore must have a pI greater than 9.3, the highest pI in the standard. However, acetylated Flt1(1-3)-Fc is able to migrate into the gel and equilibrate at a pI of approximately 5.2. This result demonstrates that acetylation reduces the net positive charge of the protein and therefore its pI considerably.

b. Binding to Extracellular Matrix Components

To test for binding to extracellular matrix components, Flt1(1-3)-Fc and acetylated Flt1(1-3)-Fc were tested in an assay designed to mimic the interaction with extracellular matrix components. In this assay, 96-well tissue culture plates are coated with Matrigel (Biocoat MATRIGEL® matrix thin layer 96 well plate, Catalog #40607, Becton Dickinson Labware, Bedford, Mass.). The plates are incubated with varying concentrations of either Flt1(1-3)-Fc, acetylated Flt1(1-3)-Fc, or rTie2-Fc (an irrelevant control) protein are added to the wells. The plates are incubated for 1-2 hours at either room temperature or 37°C . degrees and then detection of bound proteins is accomplished by adding a secondary alkaline phosphatase-conjugated anti-human Fc

antibody to the wells. Finally, alkaline phosphatase substrate is added to the wells and optical density is measured. FIG. 2 shows the results of this assay. Like the irrelevant control protein rTie2-Fc, acetylated Flt1(1-3)-Fc does not exhibit any binding to the Matrigel coated plate, whereas the non-acetylated Flt1(1-3)-Fc protein exhibits significant binding. This result indicates that acetylation of basic amino acid residues is an effective way to interfere with the charge interactions that exist between positively charged proteins and the negatively charged extracellular matrix components they are exposed to in vivo.

Example 5

Pegylation of Flt1(1-3)-Fc Protein

Although pegylation (polyethylene glycol—PEG) of proteins has been shown to increase their in vivo potency by enhancing stability and bioavailability while minimizing immunogenicity (see references cited supra), it is counter-intuitive that pegylating molecules that are too large to be filtered by the kidney glomeruli would improve their pharmacokinetic properties. Without being bound by theory, Applicants postulated that pegylation of the Flt1(1-3)-Fc molecules could improve the pharmacokinetic properties, possibly not by altering the positive charge or by decreasing the pI of Flt1(1-3)-Fc, but rather by physically shielding the positive charges from interacting with the extracellular matrix. Applicants decided to attempt to improve the pharmacokinetic properties of Flt1(1-3)-Fc molecules by attaching strands of 20K PEGs as described infra.

Materials and Methods

Purified Flt1(1-3)-Fc derived from CHO cells (see supra) was used in the following pegylation experiments. Functionalized PEGs were obtained from Shearwater Polymers, Huntsville, Ala.; Bicine from Sigma, St Louis, Mo.; Superose 6 column from Pharmacia, Piscataway, N.J.; PBS as a 10 \times concentrate from Life Technologies, Gaithersburg, Md.; Glycerol from J. T. Baker, Phillipsburg, N.J.; and Bis-Tris precast gels from Novex, Calif.

20K PEG strands functionalized with amine-specific terminal moieties were used in small-scale reaction studies that were set-up to evaluate different reaction conditions in which the PEG:protein stoichiometry was varied. Based on these reactions and the analyses of samples on standard SDS-PAGE, Flt1(1-3)-Fc at a concentration of 1.5 mg/mL was reacted at pH 8.1 with 20K SPA-PEG (PEG succinimidyl propionate) molecules at a PEG-to-Flt1(1-3)-Fc monomer molar ratio of 1:6. The reaction was allowed to proceed at 8°C . overnight. For initial purification, the reaction products were applied to a 10 mm \times 30 cm Superose 6 column equilibrated with PBS containing 5% Glycerol. The column appeared to separate pegylated Flt1(1-3)-Fc molecules based on the extent of pegylation. Fractions corresponding to what appeared to be primarily mono-pegylated and di-pegylated dimeric Flt1(1-3)-Fc, as judged by banding patterns on reducing and non-reducing SDS-PAGE gels were pooled. The protein concentration was determined by measuring absorbance at 280 nm. The pegylated Flt1(1-3)-Fc protein was sterile filtered, aliquoted and stored at -40°C .

19

Example 6

Binding of Unmodified, Acetylated, and Pegylated Flt1(1-3)-Fc in a BIACORE™-Based Assay

Unmodified, acetylated, and pegylated Flt1(1-3)-Fc proteins were tested in a BIACORE™-based assay to evaluate their ability to bind to the Flt1 ligand, VEGF. In this assay, unmodified Flt1(1-3)-Fc protein was immobilized on the surface of a BIACORE™ chip (see BIACORE™ Instruction Manual, Pharmacia, Inc., Piscataway, N.J., for standard procedures) and a sample containing 0.2 µg/ml VEGF and either unmodified Flt1(1-3)-Fc, acetylated Flt1(1-3)-Fc or pegylated Flt1(1-3)-Fc (each at 25 µg/ml) was passed over the Flt1(1-3)-Fc-coated chip. To minimize the effects of non-specific binding, the bound samples were washed with a 0.5M NaCl wash. In one sample, unmodified Flt1(1-3)-Fc was mixed with heparin. Heparin is a negatively charged molecule and the Flt1(1-3)-Fc protein is a positively charged molecule, so when the two molecules are mixed together, they should interact through their respective charges. This essentially neutralizes Flt1(1-3)-Fc's inherent positive charge making the molecule behave as if it has been chemically or genetically modified so as to reduce its charge and its tendency to bind via charge interactions. As shown in FIG. 3, acetylated (columns 13-16), pegylated (columns 17-20), and heparin-treated Flt1(1-3)-Fc (columns 21-24) are each able to completely compete with the BIACORE™ chip-bound Flt1(1-3)-Fc for VEGF binding as compared to control (columns 1-4) and irrelevant protein (columns 5-8). Unmodified Flt1(1-3)-Fc (columns 5-6) appeared to only partially compete with BIACORE™ chip-bound Flt1(1-3)-Fc for VEGF binding. However, washing the bound samples with 0.5M NaCl (columns 7-8) resulted in a binding profile similar to the modified forms of Flt1(1-3)-Fc, indicating that the unmodified protein was exhibiting non-specific binding to the chip that could be eliminated by the salt wash.

Example 7

Binding of Unmodified, Acetylated, and Pegylated Flt1(1-3)-Fc in an ELISA-Based Assay

Unmodified, acetylated, and pegylated Flt1(1-3)-Fc proteins were tested in a standard ELISA-based assay to evaluate their ability to bind the Flt1 receptor ligand VEGF. As shown in FIG. 4, both pegylated and acetylated Flt1(1-3)-Fc proteins are capable of binding to VEGF, demonstrating that modifying the protein either by pegylation or acetylation does not destroy its ability to bind its ligand.

Example 8

Pharmacokinetic Analysis of Unmodified Flt1(1-3)-Fc, Acetylated Flt1(1-3)-Fc, and Pegylated Flt1(1-3)-Fc

In vivo experiments were designed to assess the pharmacokinetic profiles of unmodified Flt1(1-3)-Fc, acetylated Flt1(1-3)-Fc, and pegylated Flt1(1-3)-Fc protein. Balb/c mice (23-28 g; 3 mice/group) were injected subcutaneously with 4 mg/kg of unmodified, acetylated, or pegylated Flt1(1-3)-Fc. The mice were tail bled at 1, 2, 4, 6, 24 hours, 2 days, and 3 days after injection of protein. The sera were assayed in a standard ELISA-based assay designed to detect Flt1(1-3)-Fc protein. Briefly, the assay involves coating an ELISA plate with VEGF, binding the unmodified, acety-

20

lated, or pegylated Flt1(1-3)-Fc-containing sera, and reporting with an anti-Fc antibody linked to alkaline phosphatase. As shown in FIG. 5, the T_{max} for all of the Flt1(1-3)-Fc proteins was between the 6 hour and 24 hour time points. The C_{max} for the different proteins was as follows: Unmodified: 0.06 µg/ml-0.15 µg/ml; acetylated: 1.5 µg/ml-4.0 µg/ml; and pegylated: approximately 5 µg/ml.

Example 9

Step-Acetylation of Flt1(1-3)-Fc

To determine what minimal amount of acetylation is necessary to eliminate binding to extracellular matrix components, an experiment was designed that acetylated the Flt1(1-3)-Fc protein in a step-wise fashion by using increasing amounts of molar excess of acetylation reagent in the acetylation reaction mixture. The range of molar excess was as follows: 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 moles of acetylation reagent per 1 mole of Flt1(1-3)-Fc monomer. The reactions were performed as detailed in the instruction manual provided with the sulfo-NHS-Acetate modification kit (Pierce Chemical Co., Rockford, Ill, Cat.#26777).

Example 10

Characterization of Step-Acetylated Flt1(1-3)-Fc

(a) IEF analysis Unmodified Flt1(1-3)-Fc and step-acetylated Flt1(1-3)-Fc proteins were analyzed by standard IEF analysis. As shown in FIG. 6A-6B, unmodified Flt1(1-3)-Fc protein was not able to migrate into the gel due to its extremely high pI (greater than 9.3). However, most of the step-acetylated Flt1(1-3)-Fc samples (30-100 fold molar excess samples) were able to migrate into the gel and equilibrate at pIs ranging between 4.55-8.43, depending on the degree of acetylation of the protein. This result demonstrates that acetylation can change the positive charge of the protein in a dose-dependent manner and that reduction of the pI can be controlled by controlling the degree of acetylation.

(b.) Binding of Step-Acetylated Flt1(1-3)-Fc to Extracellular Matrix Components

To test for binding to extracellular matrix components, Flt1(1-3)-Fc and step-acetylated Flt1(1-3)-Fc were tested in the above-described assay designed to mimic the interaction with extracellular matrix components. Varying concentrations of either unmodified Flt1(1-3)-Fc, step-acetylated Flt1(1-3)-Fc (10, 20, and 30 fold molar excess samples), or rTie2-Fc (an irrelevant control) protein were added to the wells. The plates were incubated for 1-2 hours at room temperature or 37° C. and then detection of bound proteins was accomplished by adding a secondary alkaline phosphatase-conjugated anti-human Fc antibody to the wells. Alkaline phosphatase substrate was subsequently added to the wells and optical density measured. FIG. 7 shows the results of this assay. Like the irrelevant control protein rTie2-Fc, step-acetylated Flt1(1-3)-Fc (20 and 30 fold molar excess samples) did not exhibit any significant binding to the Matrigel coated plate, whereas the non-acetylated Flt1(1-3)-Fc protein exhibited significant binding. The binding is saturable, indicating that the Flt1(1-3)-Fc protein may be binding to specific sites, rather than a more general charge-mediated interaction that might not be saturable. The 10 fold molar excess sample showed reduced binding, but the degree of acetylation was not enough to

completely block binding to extracellular matrix components. The 20 fold molar excess and higher samples displayed no detectable binding, despite the fact that by IEF analysis (FIGS. 6A and 6B) the lower molar excess samples still had a large net positive charge. This result demonstrates that it is not necessary to completely acetylate all available basic amino acids in order to eliminate binding to extracellular matrix components.

(c.) Binding of Step-Acetylated Flt1(1-3)-Fc in a BIACORE™-Based Assay

Unmodified and step-acetylated Flt1(1-3)-Fc proteins were tested in a BIACORE™-based assay to evaluate their ability to bind to the Flt1 ligand, VEGF. In this assay, unmodified Flt1(1-3)-Fc protein (0.5, 1.0, or 5.0 µg/ml) was immobilized on the surface of a BIACORE™ chip (see BIACORE™ Instruction Manual, Pharmacia, Inc., Piscataway, N.J., for standard procedures) and a solution containing 0.2 µg/ml VEGF and either unmodified Flt1(1-3)-Fc (at either 0.5, 1.0, or 5.0 µg/ml) or 10 different step-acetylated Flt1(1-3)-Fc samples (at 0.5, 1.0, or 5.0 µg/ml each) were passed over the Flt1(1-3)-Fc-coated chip. As shown in FIG. 8, at a sub-stoichiometric ratio (0.5 µg/ml of either unmodified Flt1(1-3) or step-acetylated Flt1(1-3)-Fc vs. 0.2 µg/ml VEGF), there is not enough Flt1(1-3)-Fc (either unmodified or step-acetylated) in the solution to completely bind the VEGF. At 1.0 µg/ml, which approximates a 1:1 stoichiometric ratio, both unmodified and step-acetylated Flt1(1-3)-Fc are better able to compete for VEGF binding, but there is still insufficient Flt1(1-3)-Fc protein (either unmodified or step-acetylated) to completely bind the available VEGF. However, at 5.0 µg/ml, which is several times greater than a 1:1 stoichiometric ratio, both the Flt1(1-3)-Fc and the step-acetylated Flt1(1-3)-Fc proteins are able to bind the VEGF, regardless of the degree of acetylation. This clearly demonstrates that acetylation does not alter Flt1(1-3)-Fc's ability to bind VEGF.

(d.) Pharmacokinetic Analysis of Step-Acetylated Flt1(1-3)-Fc

In vivo experiments were designed to assess the pharmacokinetic profiles of unmodified Flt1(1-3)-Fc and step-acetylated Flt1(1-3)-Fc protein. Balb/c mice (23-28 g) were injected subcutaneously with 4 mg/kg of unmodified or 10, 20, 40, 60 and 100 fold molar excess samples of step-acetylated Flt1(1-3)-Fc (3 mice for unmodified, 10, 20 and 40 fold molar excess samples and 2 mice for 60 and 100 fold molar excess samples). The mice were tail bled at 1, 2, 4, 6, 24 hours, 2 days and 3 days after injection. The sera were assayed in an ELISA-based assay designed to detect Flt1(1-3)-Fc (described supra). FIG. 9 details the results of this study. The T_{max} for all of the Flt1(1-3)-Fc proteins tested was at the 6 hour time point but the C_{max} was as follows: Unmodified Flt1(1-3)-Fc: 0.06 µg/ml; 10 fold molar excess sample:—0.71 µg/ml, 20 fold molar excess sample—2 µg/ml, 40 fold molar excess sample—4 µg/ml, 60 fold molar excess sample—2 µg/ml, 100 fold molar excess sample—1 µg/ml. This results demonstrates that acetylation or pegylation of Flt1(1-3)-Fc significantly improves its pharmacokinetic profile.

Example 11

Construction of Flt1(1-3)-Fc Basic Region Deletion Mutant Designated Mut1: Flt1(1-3_{ΔB})-Fc

Based on the observation that acetylated Flt1(1-3)-Fc, which has a pI below 6, has much better pharmacokinetics

than the highly positive unmodified Flt1(1-3)-Fc (pI>9.3), it was asked whether the difference in pharmacokinetics could be attributed to the net charge of the protein, which made it stick to negatively charged extracellular matrix components, or whether there were perhaps specific locations on the surface of the Flt1(1-3)-Fc protein that constituted specific binding sites for extracellular matrix components. For example, many proteins are known to have heparin binding sites, often consisting of a cluster of basic residues. Sometimes these residues are found in a cluster on the primary sequence of the protein; some of the literature has identified "consensus sequences" for such heparin binding sites (see for example Hileman, et al., 1998, *Bioessays* 20(2):156-67). In other cases, the known crystal structure of a protein reveals a cluster of positively charged residues on the surface of a protein, but the residues come from different regions of the primary sequence and are only brought together when the protein folds into its tertiary structure. Thus it is difficult to deduce whether an isolated amino acid residue forms part of a cluster of basic residues on the surface of the protein. However, if there is a cluster of positively charged amino acid residues in the primary sequence, it is not unreasonable to surmise that the residues are spatially close to one another and might therefore be part of an extracellular matrix component binding site. Flt1 receptor has been studied extensively and various domains have been described (see for example Tanaka et al., 1997, *Jpn. J. Cancer Res* 88:867-876). Referring to the nucleic acid and amino acid sequence set forth in FIG. 10A-10D of this application, one can identify the signal sequence for secretion which is located at the beginning of the sequence and extends to the glycine coded for by nucleotides 76-78. The mature protein begins with Ser-Lys-Leu-Lys, starting at nucleotide 79 of the nucleic acid sequence. Flt1 Ig domain 1 extends from nucleotide 79 to 393, ending with the amino acids Ser-Asp-Thr. Flt1 Ig domain 2 extends from nucleotide 394 to 687 (encoding Gly-Arg-Pro to Asn-Thr-Ile), and Flt1 Ig domain 3 extends from nucleotides 688 to 996 (encoding Ile-Asp-Val to Asp-Lys-Ala). There is a bridging amino acid sequence, Gly-Pro-Gly, encoded by nucleotides 997-1005, followed by the nucleotide sequence encoding human Fc (nucleotides 1006-1701 or amino acids Glu-Pro-Lys to Pro-Gly-Lys-stop).

A more detailed analysis of the Flt1 amino acid sequence reveals that there is a cluster, namely, amino acid residues 272-281 (KNKRASVRR) of FIG. 10A-10D, in which 6 out of 10 amino acid residues are basic. This sequence is located in Flt1 Ig domain 3 of the receptor (see FIG. 11), which is not itself essential for binding of VEGF ligand, but which confers a higher affinity binding to ligand. An alignment of the sequence of Ig domain 3 with that of Ig domain 2 reveals that in this region, there is very poor alignment between the two Ig domains, and that there are about 10 additional amino acids in Ig domain 3. An analysis of the hydrophilicity profiles (MaeVaster MACVECTOR™ computer software) of these two domains clearly indicates the presence of a hydrophilic region in the protein (FIG. 12A-12B). These observations raised the possibility that the actual three dimensional conformation of Flt1 Ig domain 3 allowed for some type of protrusion that is not in Flt1 Ig domain 2. To test this hypothesis, the 10 additional amino acids were deleted and the resulting protein was tested to see whether the deletion would affect the pharmacokinetics favorably without seriously compromising the affinity of the receptor for VEGF. This DNA construct, which was constructed using standard molecular biology techniques (see e.g., *Molecular Cloning, A Laboratory Manual* (Sambrook, F et

23

al., Cold Spring Harbor Laboratory), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, N.Y.) in the mammalian expression vector pMT21, (Genetics Institute, Inc., Cambridge, Mass.), is referred to as Mut1: Flt1(1-3_{ΔB})-Fc. The Mut1: Flt1(1-3_{ΔB})-Fc construct was derived from Flt1(1-3)-Fc by deletion of nucleotides 814-843 (set forth in FIG. 10A-10D), which deletes the highly basic 10-amino acid residue sequence Lys-Asn-Lys-Arg-Ala-Ser-Val-Arg-Arg-Arg from Flt1 Ig domain 3.

The final DNA construct was sequence-verified using an ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, Calif.). The sequence of Mut1: Flt1(1-3_{ΔB})-Fc is set forth in FIG. 13A-13D.

Example 12

Construction of Flt1(1-3)-Fc Basic region Deletion Mutant Designated Mut2: Flt1(2-3_{ΔB})-Fc

A second deletion mutant construct, designated Mut2: Flt1(2-3_{ΔB})-Fc, was derived from the Mut1: Flt1(1-3_{ΔB})-Fc construct by deletion of Flt1 Ig domain 1 encoded by nucleotides 79-393 (see FIG. 10A-10D); for convenience, nucleotides 73-78 (TCA GGT) were changed to TCC GGA. This introduced a restriction site (BspE1) without altering the associated amino acid sequence, Ser-Gly. This DNA construct, which was constructed using standard molecular biology techniques (see e.g., Molecular Cloning, A Laboratory Manual (Sambrook, et al., Cold Spring Harbor Laboratory), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY) in the mammalian expression vector pMT21 (Genetics Institute, Inc., Cambridge, Mass.), was also sequence-verified using an ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, Calif.). The sequence of Mut2: Flt1(2-3_{ΔB})-Fc is set forth in FIG. 14A-14C.

Example 13

Construction of Flt1(1-3)-Fc Deletion Mutant Designated Mut3: Flt1(2-3)-Fc

A third deletion mutant construct, designated Mut3: Flt1(2-3)-Fc, was constructed the same way as the Mut2: Flt1(2-3_{ΔB})-Fc construct, except that Flt1 Ig domain 3 was left intact (the basic region amino acids were not deleted). The construct was constructed using standard molecular biology techniques and the final construct was sequence-verified as described supra. The sequence of Mut3: Flt1(2-3)-Fc is set forth in FIG. 15A-15C.

Example 14

Construction of Flt1(1-3)-Fc Basic Region N-Glycosylation Mutant Designated Mut4: Flt1(1-3_{R→N})-Fc

A final construct was made in which a N-glycosylation site was introduced into the middle of the basic region of Flt1 Ig domain 3. This construct was designated Mut4: Flt1(1-3_{R→N})-Fc and was made by changing nucleotides 824-825 from GA to AC, consequently changing the coded Arg residue (AGA) into an Asn residue (AAC) (see FIG. 10A-10). The resulting amino acid sequence is therefore changed from Arg-Ala-Ser to Asn-Ala-Ser, which matches

24

the canonical signal (Asn-Xxx-Ser/Thr) for the addition of a N-glycosylation site at the Asn residue. The sequence of Mut4: Flt1(1-3_{R→N})-Fc is set forth in FIG. 16A-16D.

Example 15

Characterization of Acetylated Flt1(1-3)-Fc, Mut1: Flt1(2-3)-Fc, and Mut4: Flt1(1-3_{R→N})-Fc Mutants

(a.) Binding to Extracellular Matrix Components

To determine whether the three modified proteins were more or less likely to have improved pharmacokinetic properties, Matrigel coated 96-well dishes (as described supra) were incubated with varying concentrations of the mutant proteins and detected with anti-human Fc/alkaline-phosphatase conjugated antibodies. As shown in FIG. 18, this experiment showed that while the unmodified Flt1(1-3)-Fc protein could bind avidly to these wells, the Mut3: Flt1(2-3)-Fc protein bound somewhat more weakly, the Mut1: Flt1(1-3_{ΔB})-Fc protein bound more weakly still, and the Mut2: Flt1(2-3_{ΔB})-Fc protein showed the best profile, binding more weakly than any of the other mutant proteins. The Mut4: Flt1(1-3_{R→N})-Fc glycosylation mutant protein showed only marginal benefit on the Matrigel assay. These results confirm the hypothesis that a linear sequence of positive amino acids can be deleted from the primary sequence resulting in a decrease in charge interaction with extracellular matrix components.

(b.) Binding of Mut1: Flt1(1-3_{ΔB})-Fc and Mut4: Flt1(1-3_{R→N})-Fc in a BIACORE™-Based Assay.

Unmodified and acetylated Flt1(1-3)-Fc and genetically modified Mut1: Flt1(1-3_{ΔB})-Fc and Mut4: Flt1(1-3_{R→N})-Fc proteins were tested in a BIACORE™-based assay to evaluate their ability to bind to the Flt1 ligand, VEGF. In this assay, unmodified Flt1(1-3)-Fc protein (0.25, 0.5, or 1.0 μg/ml) was immobilized on the surface of a BIACORE™ chip (see BIACORE™ Instruction Manual, Pharmacia, Inc., Piscataway, N.J., for standard procedures) and a solution containing 0.1 μg/ml VEGF and either purified or COS cell supernatant containing unmodified Flt1(1-3)-Fc (at approximately 0.25, 0.5, or 1.0 μg/ml), purified acetylated Flt1(1-3)-Fc (at 0.25, 0.5, or 1.0 μg/ml), COS cell supernatant containing Mut1: Flt1(1-3_{ΔB})-Fc (at approximately 0.25, 0.5, or 1.0 μg/ml), or COS cell supernatant containing Mut4: Flt1(1-3_{R→N})-Fc (at approximately 0.25, 0.5, or 1.0 μg/ml) were passed over the Flt1(1-3)-Fc-coated chip. As shown in FIG. 17, at the sub-stoichiometric ratio (0.25 μg/ml Flt1(1-3)-Fc of unmodified, acetylated or genetically modified samples vs. 0.1 μg/ml VEGF), there is insufficient Flt1(1-3)-Fc protein to block binding of VEGF to the Flt1(1-3)-Fc immobilized on the BIACORE™ chip. At 0.5 μg/ml of unmodified acetylated or genetically modified Flt1(1-3)-Fc proteins, the stoichiometric ratio approximates 1:1 and there is an increased ability to block VEGF binding to the BIACORE™ chip. At 1.0 μg/ml of unmodified, acetylated or genetically modified Flt1(1-3)-Fc proteins, which is approximately a 10:1 stoichiometric ratio, the Flt1(1-3)-Fc proteins are able to block binding of VEGF to the BIACORE™ chip, but they are not equivalent. Unmodified, acetylated, and Mut1: Flt1(1-3_{ΔB})-Fc are essentially equal in their ability to block VEGF binding, whereas Mut4: Flt1(1-3_{R→N})-Fc is somewhat less efficient at blocking binding. These results confirm the hypothesis that it is possible to reduce the non-specific binding of a positively charged

molecule by genetically removing a linear sequence of predominantly negatively charged amino acids.

(c.) Binding of Mut1: Flt1(1-3_{ΔB})-Fc, Mut2: Flt1(2-3_{ΔB})-Fc, Mut3: Flt1(2-3)-Fc, and in an ELISA-Based Assay.

To determine whether the three mutant proteins could bind the Flt1 ligand VEGF, binding experiments were done in which 96-well plates coated with VEGF were incubated with varying concentrations of the respective mutant protein, and after washing, the amount bound was detected by incubating with an alkaline phosphatase conjugated anti-human Fc antibody and quantitated colorimetrically by the addition of an appropriate alkaline phosphatase substrate. As shown in FIG. 19, this experiment showed that all the mutant proteins could bind VEGF similarly, at the concentrations tested.

Example 16

Pharmacokinetic Analysis of Acetylated Flt1(1-3)-Fc, Mut1: Flt1(1-3_{ΔB})-Fc, and Unmodified Flt1(1-3)-Fc

In vivo experiments were designed to assess the pharmacokinetic profiles of unmodified Flt1(1-3)-Fc, Mut1: Flt1(1-3_{ΔB})-Fc, and 40 fold molar excess acetylated Flt1(1-3)-Fc protein. Balb/c mice (25-30 g) were injected subcutaneously with 4 mg/kg of unmodified Flt1(1-3)-Fc, 40 fold molar excess acetylated Flt1(1-3)-Fc, and Mut1: Flt1(1-3_{ΔB})-Fc proteins (4 mice each). These mice were tail bled at 1, 2, 4, 6, 24 hours, 2 days, 3 days, and 5 days after injection. The sera were assayed in an ELISA designed to detect Flt1(1-3)-Fc protein which involves coating an ELISA plate with VEGF, binding the Flt1(1-3)-Fc and reporting with an anti-Fc antibody linked to alkaline phosphatase. As shown in FIG. 20, the C_{max} for these reagents was as follows: Unmodified Flt1(1-3)-Fc—0.15 μg/ml; 40 fold molar excess acetylated Flt1(1-3)-Fc—1.5 μg/ml; and Mut1: Flt1(1-3_{ΔB})-Fc—0.7 μg/ml.

Example 17

Modified Flt1 Receptor Vector Construction

The rationale for constructing modified versions of the Flt1 receptor (also known as VEGFR1) was based on the observation that the protein sequence of Flt1 was highly basic, and was therefore likely to stick to extracellular matrix (ECM). The highly basic nature of Flt1 probably explains why unmodified Flt1(1-3)-Fc (described supra) has poor pharmacokinetics that make it difficult to use as a therapeutic agent. As described supra, the chemically modified form of 40 fold molar excess acetylated Flt1(1-3)-Fc, hereinafter termed A40, exhibited a greatly improved pharmacokinetic (PK) profile over the non-acetylated Flt1(1-3)-Fc. Therefore, attempts were made to engineer DNA molecules that could be used to recombinantly express modified forms of a Flt1 receptor molecule that would possess the improved PK profile exhibited by A40 and still maintain the ability to bind tightly to VEGF.

It is known in the literature that the first Ig domain of Flt1 (which has a net charge of +5 at neutral pH) is not essential for tight binding to VEGF, so this domain was deleted. The third Ig domain (having a net charge of +11) is not essential for binding, but confers higher affinity for VEGF than the second Ig domain, so instead of deleting it entirely, it was replaced with the equivalent domains of the Flt1 receptor

relatives Flk1 (also known as VEGFR2) and Flt4 (also known as VEGFR3). These chimeric molecules (denoted R1R2 (Flt1.D2.Flk1D3.FcΔC1(a)) and VEGFR1R2-FcΔC1(a)) and R1R3 (Flt1D2.VEGFR3D3-FcΔC1(a)) and VEGFR₁R3-FcΔC1(a) respectively, wherein R1 and Flt1D2=Ig domain 2 of Flt1 (VEGFR1); R2 and Flk1D3=Ig domain 3 of Flk1 (VEGFR2); and R3 and VEGFR3D3=Ig domain 3 of Flt4 (VEGFR3)) were much less sticky to ECM, as judged by an in vitro ECM binding assay as described infra, had greatly improved PK as described infra. In addition, these molecules were able to bind VEGF tightly as described infra and block phosphorylation of the native Flk1 receptor expressed in endothelial cells as described infra.

(a) Construction of the Expression Plasmid pFlt1D2.Flk1D3.FcΔC1(a)

Expression plasmids pMT21.Flt1(1-3).Fc (6519 bp) and pMT21.Flk-1(1-3).Fc (5230 bp) are plasmids that encode ampicillin resistance and Fc-tagged versions of Ig domains 1-3 of human Flt1 and human Flk1, respectively. These plasmids were used to construct a DNA fragment consisting of a fusion of Ig domain 2 of Flt1 with Ig domain 3 of Flk1, using PCR amplification of the respective Ig domains followed by further rounds of PCR to achieve fusion of the two domains into a single fragment. For Ig domain 2 of Flt1, the 5' and 3' amplification primers were as follows:

```
5': bsp/flt1D2
(5'-GACTAGCAGTCCGGAGGTAGACCTTTCGTAGAGATG-3')
3': Flt1D2-Flk1D3.as
(5'-CGGACTCAGAACACATCTATGATTGGT-3')
```

The 5' amplification primer encodes a BspE1 restriction enzyme site upstream of Ig domain 2 of Flt1, defined by the amino acid sequence GRPFVEM (corresponding to amino acids 27-33 of FIG. 21A-21C). The 3' primer encodes the reverse complement of the 3' end of Flt1 Ig domain 2 fused directly to the 5' beginning of Flk1 Ig domain 3, with the fusion point defined as TIID of Flt1 (corresponding to amino acids 123-126 of FIG. 21A-21C) and continuing into VVLS (corresponding to amino acids 127-130 of FIG. 21A-21C) of Flk1.

For Ig domain 3 of Flk1, the 5' and 3' amplification primers were as follows:

```
5': Flt1D2-Flk1D3.s
(5'-ACAATCATAGATGTGGTTCTGAGTCCGTCTCATGG-3')
3': Flk1D3/apa/srf.as
(5'-GATAATGCCCGGCCCTTTTCATGGACCCTGACAAATG-3')
```

The 5' amplification primer encodes the end of Flt1 Ig domain 2 fused directly to the beginning of Flk1 Ig domain 3, as described above. The 3' amplification primer encodes the end of Flk1 Ig domain 3, defined by the amino acids VRVHEK (corresponding to amino acids 223-228 of FIG. 21A-21C), followed by a bridging sequence that includes a recognition sequence for the restriction enzyme SrfI, and encodes the amino acids GPG. The bridging sequence corresponds to amino acids 229-231 of FIG. 21A-21C.

After a round of PCR amplification to produce the individual domains, the products were combined in a tube and subjected to a further round of PCR with the primers bsp/flt1D2 and Flk1D3/apa/srf.as (described supra) to produce the fusion product. This PCR product was subsequently digested with the restriction enzymes BspEI and SmaI and the resulting 614 bp fragment was subcloned into the BspEI

to SrfI restriction sites of the vector pMT21/ΔB2.Fc, to create the plasmid pMT21/Flt1D2.Flk1D3.Fc. The nucleotide sequence of the Flt1D2-Flk1D3 gene fusion insert was verified by standard sequence analysis. This plasmid was then digested with the restriction enzymes EcoRI and SrfI and the resulting 702 bp fragment was transferred into the EcoRI to SrfI restriction sites of the plasmid pFlt1(1-3)B2-FcΔC1(a) to produce the plasmid pFlt1D2.Flk1D3.FcΔC1(a). The complete DNA and deduced amino acid sequences of the Flt1D2.Flk1D3.FcΔC1(a) chimeric molecule is set forth in FIG. 21A-21C.

(b) Construction of the Expression Plasmid pFlt1D2VEGFR3D3FcΔC1(a)

The expression plasmid pMT21.Flt1(1-3).Fc (6519 bp) encodes ampicillin resistance and an Fc-tagged version of Ig domains 1-3 of human Flt1 receptor. This plasmid was used to produce a DNA fragment containing Ig domain 2 of Flt1 by PCR. RNA from the cell line HEL921.7 was used to produce Ig domain 3 of Flk1, using standard RT-PCR methodology. A further round of PCR amplification was used to achieve fusion of the two Ig domains into a single fused fragment. For Ig domain 2 of Flt1, the 5' and 3' amplification primers were as follows:

5': bsp/flt1D2
(5'-GACTAGCAGTCCGGAGGTAGACCTTTCGTAGAGATG-3')

3': Flt1D2.VEGFR3D3.as
(TTCCGGGCAACAGCTGGATATCTATGATTGTATTGGT)

The 5' amplification primer encodes a BspEI restriction site upstream of Ig domain 2 of Flt1, defined by the amino acid sequence GRPFVEM (corresponding to amino acids 27-33 of FIG. 22A-22C). The 3' amplification primer encodes the reverse complement of the end of Flt1 Ig domain 2 fused directly to the beginning of VEGFR3 Ig domain 3, with the fusion point defined as THD of Flt1 (corresponding to amino acids 123-126 of FIG. 22A-22C) and continuing into IQLL of VEGFR3 (corresponding to amino acids 127-130 of FIG. 22A-22C).

For Ig domain 3 of VEGFR3, the 5' and 3' primers used for RT-PCR were as follows:

5': R3D3.s
(ATCCAGCTGTTGCCAGGAAGTCGCTGGAGCTGCTGGTA)

3': R3D3.as
(ATTTTCATGCACAATGACCTCGGTGCTCTCCCGAAATCG)

Both the 5' and 3' amplification primers match the sequence of VEGFR3. The 296 bp amplification product of this RT-PCR reaction was isolated by standard techniques and subjected to a second round of PCR to add suitable sequences to allow for fusion of the Flt1D2 with the Flk1D3 domains and fusion of the Flk1D3 and Fc domains via a GPG bridge (see below). The amplification primers were as follows:

5': Flt1D2.VEGFR3D3.s
(TCATAGATATCCAGCTGTTGCCAGGAAGTCGCTGGAG)

3': VEGFR3D3/srf.as
(GATAATGCCCGGCCATTTTCATGCACAATGACCTCGGT)

The 5' amplification primer encodes the 3' end of Flt1 Ig domain 2 fused directly to the beginning (5' end) of VEGFR3 Ig domain 3, as described above. The 3' amplifi-

cation primer encodes the 3' end of VEGFR3 Ig domain 3, defined by the amino acids VIVHEN (corresponding to amino acids 221-226 of FIG. 22A-22C), followed by a bridging sequence that includes a recognition sequence for SrfI, and encodes the amino acids GPG. The bridging sequence corresponds to amino acids 227-229 of FIG. 22A-22C.

After one round (for Flt1 Ig domain 2) or two rounds (for Flt4 Ig domain 3) of PCR to produce the individual Ig domains, the PCR products were combined in a tube and subjected to a further round of PCR amplification with the amplification primers bsp/flt1D2 and VEGFR3D3/srf.as described supra, to produce the fusion product. This PCR product was subsequently digested with the restriction enzymes BspEI and SmaI and the resulting 625 bp fragment was subcloned into the BspEI to SrfI restriction sites of the vector pMT21/Flt1ΔB2.Fc (described supra), to create the plasmid pMT21/Flt1D2.VEGFR3D3.Fc. The sequence of the Flt1D2-VEGFR3D3 gene fusion insert was verified by standard sequence analysis. This plasmid was then digested with the restriction enzymes EcoRI and SrfI and the resulting 693 bp fragment was subcloned into the EcoRI to SrfI restriction sites of the plasmid pFlt1(1-3)ΔB2-FcΔC1(a) to produce the plasmid designated pFlt1D2.VEGFR3D3.FcΔC1(a). The complete DNA deduced amino acid sequence of the Flt1D2.VEGFR3D3.FcΔC1(a) chimeric molecule is set forth in FIG. 22A-22C.

Example 18

Extracellular Matrix Binding (ECM) Binding Assay

ECM-coated plates (Becton Dickinson catalog #35-4607) were rehydrated with warm DME supplemented with glutamine (2 mM), 100 U penicillin, 100 U streptomycin, and 10% BCS for at least 1 hr. before adding samples. The plates were then incubated for 1 hr. at room temperature with varying concentrations of Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a) starting at 10 nM with subsequent 2-fold dilutions in PBS plus 10% BCS. The plates were then washed 3 times with PBS plus 0.1% Triton-X and incubated with alkaline phosphatase-conjugated anti-human Fc antibody (Promega, 1:4000 in PBS plus 10% BCS) for 1 hr. at room temperature. The plates were then washed 4 times with PBS 0.1% Triton-X and alkaline phosphatase buffer/pNPP solution (Sigma) was added for color development. Plates were read at λ=405-570 nm. The results of this experiment are shown in FIG. 23 and demonstrate that the Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a) proteins are considerably less sticky to the ECM as compared to the Flt1(1-3)-Fc protein.

Example 19

Transient Expression of pFlt1D2.Flk1D3.FcΔC1(a) in CHO-K1 (E1A) Cells

A large scale (2 L) culture of *E. coli* DH1B cells carrying the pFlt1D2.Flk1D3.FcΔC1(a) plasmid described supra in Example 17(a) was grown overnight in Terrific Broth (TB) plus 100 μg/ml ampicillin. The next day, the plasmid DNA was extracted using a QIAgen ENDOPREEM™ Megaprep kit following the manufacturer's protocol. The concentration of the purified plasmid DNA was determined by standard techniques using a UV spectrophotometer and fluorometer. The plasmid DNA was verified by standard restriction

enzyme digestion of aliquots using the restriction enzymes EcoRI plus NotI and AseI. All restriction enzyme digest fragments corresponded to the predicted sizes when analyzed on a 1% agarose gel.

Forty 15 cm petri plates were seeded with CHO-K1/E1A cells at a density of 4×10^6 cells/plate. Plating media was Gibco Ham's F-12 supplemented with 10% HYCLONE™ Fetal Bovine Serum (EBS), 100 U penicillin/100 U streptomycin and glutamine (2 mM). The following day each plate of cells was transfected with 6 µg of the pFlt1D2.Flk1D3.FcΔC1(a) plasmid DNA using Gibco Optimem and Gibco Lipofectamine in 12 ml volume, following the manufacturer's protocol. Four hours after adding the transfection mix to the cells, 12 ml/plate of Optimem supplemented with 10% FBS was added. Plates were incubated at 37° C. in a 5% CO₂ incubator overnight. The following day the media was removed from each plate and 25 ml expression media (Gibco CHO-S-SFM II supplemented with glutamine (2 mM) and 1 mM sodium butyrate) was added. The plates were incubated at 37° C. for 3 days. After 3 days of incubation, the media was aspirated from each plate and centrifuged at 400 rpm in a swinging bucket rotor to pellet cells. The supernatant was decanted into sterile 1L bottles and purification of the expressed protein was performed as described infra.

Example 20

Construction pVEGFR1R2-FcΔC1(a) Expression Vector

The pVEGFR1R2.FcΔC1(a) expression plasmid was constructed by insertion of DNA encoding amino acids SDT (corresponding to amino acids 27-29 of FIG. 24A-24C) between Flt1d2-Flk1d3-FcΔC1(a) amino acids 26 and 27 of FIG. 21A-21C (GG) and removal of DNA encoding amino acids GPG corresponding to amino acids 229-231 of Figure. The SDT amino acid sequence is native to the Flt1 receptor and was added back in to decrease the likelihood of heterogeneous N-terminal processing. The GPG (bridging sequence) was removed so that the Flt1 and Flk1 Ig domains were fused directly to one another. The complete DNA and deduced amino acid sequences of the pVEGFR1R2.FcΔC1(a) chimeric molecule is set forth in FIG. 24A-24C.

Example 21

Cell Culture Process Used to Produce Modified Flt1 Receptors

(a) Cell Culture Process Used to Produce Flt1D2.Flk1D3.FcΔC1(a)

The process for production of Flt1D2.Flk1D3.FcΔC1(a) protein using the expression plasmid pFlt1D2.Flk1D3.FcΔC1(a) described supra in Example 1 involves suspension culture of recombinant Chinese hamster ovary (CHO K1/E1A) cells which constitutively express the protein product. The cells are grown in bioreactors and the protein product is isolated and purified by affinity and size exclusion chromatography. The process is provided in greater detail below.

Cell Expansion

Two confluent T-225 cm² flasks containing the Flt1D2.Flk1D3.FcΔC1(a) expressing cell line were expanded by passaging cells into eight T-225 cm² flasks in medium (GMEM+10% serum, GIBCO) and incubated at

37° C. and 5% CO₂. When the flasks approached confluence (approximately 3 to 4 days) the cells were detached using trypsin. Fresh medium was added to protect the cells from further exposure to the trypsin. The cells were centrifuged and resuspended in fresh medium then transferred to eight 850 cm² roller bottles and incubated at 37° C. and 5% CO₂ until confluent.

Suspension Culture in Bioreactors

Cells grown in roller bottles were trypsinized to detach them from the surface and washed with suspension culture medium. The cells are aseptically transferred to a 5L bioreactor (New Brunswick Celligen Plus) where the cells are grown in 3.5L of suspension culture. The suspension culture medium was a glutamine-free low glucose modification of IS-CHO (Irvine Scientific) to which 5% fetal bovine serum (Hyclone), GS supplement (Life Technologies) and 25 µM methionine sulfoximine (Sigma) was added. The pH was controlled at 7.2 by addition of carbon dioxide to the inlet gas or by addition of a liquid solution of sodium carbonate to the bioreactor. Dissolved oxygen level was maintained at 30% of saturation by addition of oxygen or nitrogen to the inlet gas and temperature controlled at 37° C. When a density of 4×10^6 cells/mL was reached the cells were transferred to a 40L bioreactor containing the same medium and setpoints for controlling the bioreactor. The temperature setpoint was reduced to 34° C. to slow cell growth and increase the relative rate of protein expression.

(b) Cell Culture Process Used to Produce Flt1D2.VEGFR3D3.FcΔC1(a)

The same methodologies as described supra for Flt1D2.Flk1D3.FcΔC1(a) were used to produce Flt1D2.VEGFR3D3.FcΔC1(a).

Example 22

Harvest and Purification of Modified Flt1 Receptors

(a) Harvest and Purification of Flt1D2.Flk1D3.FcΔC1(a)

The product protein was aseptically harvested from the bioreactor while retaining cells using Millipore ProstaK tangential-flow filtration modules and a low-shear mechanical pump (Fristam). Fresh medium was added to the bioreactor to replace that removed during the harvest filtration. Approximately 40L of harvest filtrate was then loaded onto a 400 mL column containing Protein A SEPHAROSE™ resin (Amersham Pharmacia). After loading the resin was washed with buffer containing 10 mM sodium phosphate, 500 mM sodium chloride, pH 7.2 to remove any unbound contaminating proteins. Flt1 D2.Flk1D3.FcΔC1(a) protein was eluted with a pH 3.0 citrate buffer. The eluted protein was neutralized by addition of Tris base and frozen at -20° C.

Several frozen lots of Flt1D2.Flk1D3.FcΔC1(a) protein from the Protein A step above were thawed, pooled and concentrated using a Millipore 30 kD nominal molecular weight cutoff (NMWCO) tangential flow filtration membrane. The protein was transferred to a stirred cell concentrator (Millipore) and further concentrated to 30 mg/mL using a 30 kD NMWCO membrane. The concentrated protein was loaded onto a size exclusion column packed with Superdex 200 resin (Amersham Pharmacia) that was equilibrated with phosphate buffered saline plus 5% glycerol. The same buffer was used to run the column. The fractions corresponding to Flt1D2.Flk1D3.FcΔC1(a) dimer were pooled, sterile filtered through a 0.22 micron filter, aliquoted and frozen.

31

(b) Harvest and Purification of Flt1D2.VEGFR3D3.FcΔC1(a)

(a) The same methodologies as described supra for Flt1D2.Flk1D3.FcΔC1(a) were used to harvest and purify Flt1D2.VEGFR3D3.FcΔC1(a).

Example 23

Phosphorylation Assay for Transiently Expressed VEGFR2

Primary human umbilical vein endothelial cells (HU-VECs), passage 4-6, were starved for 2 hrs in serum-free DME high glucose media. Samples containing 40 ng/ml (1 nM) human VEGF165, which is a ligand for the VEGF receptors Flt1, Flk1 and Flt4(VEGFR3) were prepared and were preincubated for 1 hr. at room temperature with varying amounts of the modified Flt1 receptors Flt1(1-3)-Fc, Flt1(1-3)-Fc (A40), Flt1D2Flk1D3.FcΔC1(a) and Flt1D2VEGFR3D3.FcΔC1(a) in serum-free DME-high glucose media containing 0.1% BSA. Cells were challenged for minutes with the samples prepared above ±VEGF165, followed by whole cell lysis using complete lysis buffer. Cell lysates were immunoprecipitated with an antibody directed against the C-terminus of VEGFR2 receptor. The immunoprecipitated lysates were loaded onto 4-12% SDS-PAGE Novex gel and then transferred to PVDF membrane using standard transfer methodologies. Detection of phosphorylated VEGFR2 was done by immunoblotting with the anti-phospho Tyrosine mAb called 4G10 (UBI) and developed using ECL-reagent (Amersham).

FIGS. 25A-25C and 26A-26B show the results of this experiment. FIG. 25A-25C reveals that detection by Western blot of tyrosine phosphorylated VEGFR2(Flk1) by VEGF165 ligand stimulation shows that cell-surface receptors are phosphorylated to varying levels depending on which modified Flt1 receptor is used during the preincubations with VEGF. As is seen in FIG. 25A, at a 1.5 molar excess of either Flt1(1-3)-Fc, Flt1(1-3)-Fc (A40) or transient Flt1D2Flk1D3.FcΔC1(a) there is complete blockage of receptor stimulation by these three modified Flt1 receptors as compared to control media challenge. In contrast, transient Flt1D2VEGFR3D3.FcΔC1(a) does not show significant blockage at this molar excess, as compared with VEGF positive control challenge. Similar results are seen in FIG. 25B, where the modified Flt receptors are in a 3-fold molar excess to VEGF165 ligand. In FIG. 25C, where the modified Flt1 receptors are in a 6-fold molar excess to VEGF165 ligand, transient Flt1D2VEGFR3D3.FcΔC1(a) can now be shown to be partially blocking VEGF165-induced stimulation of cell-surface receptors.

In FIG. 26A-26B, detection by Western blot of tyrosine phosphorylated VEGFR2(Flk1) by VEGF165 ligand stimulation shows that cell-surface receptors are not phosphorylated by challenge samples which have VEGF165 preincubated with 1 and 2 fold molar excess (FIG. 26A) or 3 and 4 fold molar excess (FIG. 26B) of either transient Flt1D2Flk1D3.FcΔC1(a), stable Flt1D2Flk1D3.FcΔC1(a), or transient VEGFR1R2-FcΔC1(a). At all modified Flt1 receptor concentrations tested there is complete binding of VEGF165 ligand during the preincubation, resulting in no detectable stimulation of cell surface receptors by unbound VEGF1-65 as compared to control media challenge.

32

Example 24

Cell Proliferation Bioassay

The test cell population is MG87 cells that have been stably transfected with a expression plasmid that contains a DNA insert encoding the VEGFR2(Flk1) extracellular domain fused to the TrkB intracellular kinase domain, thus producing a chimeric molecule. The reason the TrkB intracellular kinase domain was used rather than the native VEGFR2(Flk1) intracellular kinase domain is that the intracellular kinase domain of VEGFR2(Flk1) does not cause a strong proliferative response when stimulated by VEGF165 in these cells. It is known that MG87 cells containing full length TrkB receptor give a robust proliferative response when stimulated with BDNF, so the TrkB intracellular kinase domain was engineered to replace the intracellular kinase domain of VEGFR2(Flk1) to take advantage of this proliferative response capability.

5 × 10³ cells/well were plated in a 96 well plate and allowed to settle for 2 hrs at 37° C. The following modified Flt receptors Flt1(1-3)-Fc, Flt1D2.Flk1D3.FcΔC1(a) and Flt1 D2.VEGFR3D3.FcΔC1(a), plus an irrelevant receptor termed Tie2-Fc as a negative control, were titrated from 40 nM to 20 pM and incubated on the cells for 1 hr at 37° C. Human recombinant VEGF165 in defined media was then added to all the wells at a concentration of 1.56 nM. The plates were incubated for 72 hrs at 37° C. and then MTS (Owen's reagent, Promega) added and the plates were incubated for an additional for 4 hrs. Finally, the plates were read on a spectrophotometer at 450/570 nm. The results of this experiment are shown in FIG. 27. The control receptor Tie2-Fc does not block VEGF165-induced cell proliferation at any concentration whereas Flt1D2.Flk1D3.FcΔC1(a) blocks 1.56nM VEGF165 with a half maximal dose of 0.8 nM. Flt1(1-3)-Fc and Flt1D2.VEGFR3D3.FcΔC1(a) are less effective in blocking VEGF165 in this assay with a half maximal dose of ~2nM. VEGF165 alone gives a reading of 1.2 absorbance units and the background is 0.38 absorbance units.

Example 25

Binding Stoichiometry of Modified Flt Receptors to VEGF165

(a) BIACORE™ Analysis

The stoichiometry of Flt1D2Flk1D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a) interaction with human VEGF165 was determined by measuring either the level of VEGF saturation binding to the Flt1D2Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a) surfaces or measuring concentration of VEGF165 needed to completely prevent binding of Flt1D2Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a) to VEGF BIACORE™ chip surface.

Modified Flt receptors Flt1D2Flk1D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a), were captured with an anti-Fc specific antibody that was first immobilized on a BIACORE™ chip using amine-coupling chemistry. A blank antibody surface was used as a negative control. VEGF165 was injected at a concentration of 1 nM, 10 nM, and 50 nM over the Flt1D2Flk1D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a) surfaces at 10 μl/min for one hour. A real-time binding signal was recorded and saturation binding was achieved at the end of each injection. Binding stoichiometry was calculated as a molar ratio of bound VEGF165 to the immobilized

FIt1D2F1k1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a), using the conversion factor of 1000 RU equivalent to 1 ng/ml. The results indicated binding stoichiometry of one VEGF165 dimeric molecule per one FIt1 D2F1k1 D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a) molecule (FIG. 28).

In solution, FIt1D2F1k1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a) at a concentration of 1 nM (estimated to be 1000 times higher than the KD of the FIt1 D2F1k1 D3.FcΔC1(a) or VEGFR1 R2-FcΔC1(a)NEGF165 interaction) were mixed with varied concentrations of VEGF165. After one hour incubation, concentrations of the free FIt1D2F1k1D3.FcΔC1(a) in solution were measured as a binding signal to an amine-coupled VEGF165 surface. A calibration curve was used to convert the FIt1D2F1k1D3.FcΔC1(a) BIACORE™ binding signal to its molar concentration. The data showed that the addition of 1 nM VEGF165 into the FIt1D2F1k1D3.FcΔC1(a) solution completely blocked FIt1D2F1k1D3.FcΔC1(a) binding to the VEGF165 surface. This result suggested the binding stoichiometry of one VEGF165 molecule per one FIt1D2F1k1D3.FcΔC1(a) molecule (FIG. 29 and FIG. 30). When the concentration of FIt1D2F1k1D3.FcΔC1(a) was plotted as a function of added concentration of VEGF165, the slope of the linear portion was 1.06 for FIt1D2F1k1D3.FcΔC1(a) and -1.07 for VEGFR1R2-FcΔC1(a). The magnitude of the slope, very close to negative one, was indicative that one molecule of VEGF165 bound to one molecule of either FIt1D2F1k1D3.FcΔC1(a) or VEGFR1 R2-FcΔC1(a).

(b) Size Exclusion Chromatography

FIt1D2F1k1D3.FcΔC1(a) was mixed with a 3-fold excess of VEGF165 and the receptor-ligand complex was purified using a Pharmacia SUPEROSE™ 6 size exclusion chromatography column. The receptor-ligand complex was then incubated in a buffer containing 6M guanidine hydrochloride in order to dissociate it into its component proteins. FIt1D2F1k1D3.FcΔC1(a) was separated from VEGF165 using SUPEROSE™ 6 size exclusion chromatography column run in 6M guanidium chloride. In order to determine complex stoichiometry, several injections of FIt1D2F1k1D3.FcΔC1(a) and VEGF165 were made and peak height or peak integrated intensity was plotted as a function of the concentration of injected protein. The calibration was done under condition identical to one used in separating components of FIt1D2F1k1D3.FcΔC1(a)/VEGF complex. Quantification of the FIt1D2F1k1D3.FcΔC1(a)/VEGF complex composition was based on the calibration curves. The results of this experiment are set forth in FIG. 28, which shows the ratio of VEGF165 to FIt1D2F1k1D3.FcΔC1(a) in a complex to be 1:1.

Example 26

Determination of the Binding Stoichiometry of FIt1D2F1k1D3.FcΔC1(a)/VEGF165 Complex by Size Exclusion Chromatography

FIt1D2F1k1D3.FcΔC1(a)/VEGF165 Complex Preparation

VEGF165 (concentration=3.61 mg/ml) was mixed with CHO cell transiently expressed FIt1D2.F1k1D3.FcΔC1(a) (concentration=0.9 mg/ml) in molar ratio of 3:1 (VEGF165:FIt1D2.F1k1D3.FcΔC1(a)) and incubated overnight at 4° C.

(a) Size Exclusion Chromatography (SEC) Under Native Conditions

To separate the complex from excess of unbound VEGF165, 50 μl of the complex was loaded on a Pharmacia

SUPEROSE™ 12 PC 3.2/30 which was equilibrated in PBS buffer. The sample was eluted with the same buffer at flow rate 40 μl/min. at room temperature. The results of this SEC are shown in FIG. 31. Peak #1 represents the complex and peak #2 represents unbound VEGF165. Fractions eluted between 1.1 and 1.2 ml were combined and guanidinium hydrochloride (GuHCl) was added to a final concentration 4.5M to dissociate the complex.

(b) Size Exclusion Chromatography (SEC) Under Dissociative Conditions

To separate the components of the receptor-ligand complex and to determine their molar ratio, 50 μl of dissociated complex as described supra was loaded onto a SUPEROSE™ 12 PC 3.2/30 equilibrated in 6M GuHCl and eluted with the same solution at a flow rate 40 μl/min. at room temperature. The results of this SEC are shown in FIG. 32.

(c) Calculation of FIt1D2F1k1D3.FcΔC1(a):VEGF165 Complex Stoichiometry

The stoichiometry of the receptorligand complex was determined from the peak area or the peak height of the components. Concentrations of VEGF165 and FIt1D2F1k1D3.FcΔC1(a) corresponding to the peak height or peak area, respectively, were obtained from the standard curves for VEGF165 and FIt1D2F1k1D3.FcΔC1(a). To obtain a standard curve, four different concentrations (0.04 mg/ml-0.3 mg/ml) of either component were injected onto a Pharmacia SEPHAROSE™ 12 PC 3.2/30 column equilibrated in 6M guanidinium chloride and eluted with the same solution at flow rate 40 μl/min. at room temperature. The standard curve was obtained by plotting peak area or peak height vs protein concentration. The molar ratio of VEGF165:FIt1D2F1k1D3.FcΔC1(a) determined from the peak area of the components was 1.16. The molar ratio of VEGF165:FIt1D2F1k1D3.FcΔC1(a) determined from the peak height of the components was 1.10.

Example 27

Determination of the Stoichiometry of the FIt1D2F1k1D3.FcΔC1(a)/VEGF165 Complex by Size Exclusion Chromatography with On-Line Light Scattering

Complex Preparation

VEGF165 was mixed with CHO transiently expressed FIt1D2.F1k1D3.FcΔC1(a) protein in molar ratio of 3:1 (VEGF165:FIt1D2F1k1D3.FcΔC1(a)) and incubated overnight at 4° C.

(a) Size Exclusion Chromatography (SEC) with On-Line Light Scattering

Size exclusion chromatography column with a MiniDawn on-line light scattering detector (Wyatt Technology, Santa Barbara, Calif.) and refractive index (RI) detectors (Shimadzu, Kyoto, Japan) was used to determine the molecular weight (MW) of the receptor-ligand complex. Samples were injected onto a SUPEROSE™ 12 HR 10/30 column (Pharmacia) equilibrated in PBS buffer and eluted with the same buffer at flow rate 0.5 ml/min. at room temperature. As shown in FIG. 33, the elution profile shows two peaks. Peak #1 represents the receptor-ligand complex and peak #2 represents the unbound VEGF165. MW was calculated from LS and RI signals. The same procedure was used to determine MW of the individual components of the receptor-ligand complex. The results of these determinations are as follows: MW of the FIt1D2F1k1D3.FcΔC1(a)/VEGF-165

35

complex at the peak position is 157 300 (FIG. 33), the MW of VEGF165 at the peak position is 44 390 (FIG. 34) and the MW of R1R2 at the peak is 113 300 (FIG. 35).

These data indicated that the stoichiometry of the Flt1D2.Flk1D3.FcΔC1(a)/VEGF complex is 1:1 as its corresponds to the sum of molecular weights for Flt1D2.Flk1D3.FcΔC1(a) and VEGF165. Importantly, this method conclusively proved that the Flt1D2.Flk1D3.FcΔC1(a)/VEGF165 complex was indeed composed of only one molecule of VEGF165 ligand and only one molecule of the Flt1D2.Flk1D3.FcΔC1(a).

Example 28

Peptide Mapping of Flt1D2.Flk1D3.FcΔC1(a)

The disulfide structures and glycosylation sites in Flt1D2.Flk1D3.FcΔC1(a) were determined by a peptide mapping method. In this method, the protein was first cleaved with trypsin. Tryptic fragments were analyzed and identified by HPLC coupled with mass spectrometry, in addition to an N-terminal sequencing technique. Reduction of the tryptic digest was employed to help identify disulfide-bond-containing fragments. Treatment of the tryptic digest with PNGase F (Glyko, Novato, Calif.) was employed to help identify fragments with N-linked glycosylation sites. The results are summarized in the accompanying FIG. 36.

There are a total of ten cysteines in Flt1D2.Flk1D3.FcΔC1(a); six of them belong to the Fc region. Cys127 has been confirmed to be disulfide bonded to Cys76. Cys121 is confirmed to be disulfide bonded to Cys182. The first two cysteines in the Fc region (Cys211 and Cys214) form an intermolecular disulfide bond with the same two cysteines in another Fc chain. However, because these two cysteines can not be separated enzymatically from each other, it can not be determined whether disulfide bonding is occurring between same cysteines (Cys211 to Cys211, for example) or between Cys211 and Cys214. Cys216 is confirmed to be disulfide bonded to Cys306. Cys352 is confirmed to be disulfide bonded to Cys410.

There are five possible N-linked glycosylation sites in Flt1D2.Flk1D3.FcΔC1(a). All five of them are found to be glycosylated to varying degrees. Complete glycosylation was observed at Asn33 (amino acid sequence NIT), Asn193 (amino acid sequence NST), and Asn282 (amino acid sequence NST). In addition, partial glycosylation is observed on Asn65 and Asn120. Sites of glycosylation are highlighted by underline in the FIG. 36.

Example 29

Pharmacokinetic Analysis of Modified Flt Receptors

(a) Pharmacokinetic Analysis of Flt1(1-3)-Fc (A40), Flt1D2.Flk1D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a)

Balb/c mice (25-30 g) were injected subcutaneously with 4 mg/kg of Flt1(1-3)-Fc (A40), CHO transiently expressed Flt1D2.Flk1D3.FcΔC1(a), CHO stably expressed Flt1D2.Flk1D3.FcΔC1(a), and CHO transiently expressed VEGFR1R2-FcΔC1(a). The mice were tail bled at 1, 2, 4, 6, 24 hrs, 2 days, 3 days and 6 days after injection. The sera were assayed in an ELISA designed to detect Flt1(1-3)-Fc (A40), Flt1D2.Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a). The ELISA involves coating an ELISA plate with VEGF165, binding the detect Flt1(1-3)-Fc (A40),

36

Flt1D2.Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a) and reporting with an anti-Fc antibody linked to horse radish peroxidase. The results of this experiments are shown in FIG. 37. The T_{max} for Flt1(1-3)-Fc (A40) was at 6 hrs while the T_{max} for the transient and stable Flt1D2.Flk1D3.FcΔC1(a) and the transient VEGFR1R2-FcΔC1(a) was 24 hrs. The C_{max} for Flt1(1-3)-Fc (A40) was 8 μg/ml. For both transients (Flt1D2.Flk1D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a)) the C_{max} was 18 μg/ml and the C_{max} for the stable VEGFR1R2-FcΔC1(a) was 30 μg/ml.

(b) Pharmacokinetic Analysis of Flt1(1-3)-Fc (A40), Flt1D2.Flk1D3.FcΔC1(a) and Flt1 D2.VEGFR3D3.FcΔC1(a)

Balb/c mice (25-30 g) were injected subcutaneously with 4 mg/kg of Flt1(1-3)-Fc (A40), CHO transiently expressed Flt1D2.Flk1D3.FcΔC1(a) and CHO transiently expressed Flt1D2.VEGFR3D3.FcΔC1(a). The mice were tail bled at 1, 2, 5, 6, 7, 8, 12, 15 and 20 days after injection. The sera were assayed in an ELISA designed to detect Flt1(1-3)-Fc, Flt1D2.Flk1D3.FcΔC1(a) and Flt1 D2.VEGFR3D3.FcΔC1(a). The ELISA involves coating an ELISA plate with 165, binding the Flt1(1-3)-Fc, Flt1D2.Flk1D3.FcΔC1(a) or Flt1D2.VEGFR3D3.FcΔC1(a) and reporting with an anti-Fc antibody linked to horse radish peroxidase. Flt1(1-3)-Fc (A40) could no longer be detected in the serum after day 5 whereas Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a) were detectable for 15 days or more. The results of this experiment are shown in FIG. 38.

Example 30

Evaluation of the Ability of Flt1D2.Flk1D3.FcΔC1(a) to Inhibit Tumor Growth In Vivo

To evaluate the ability of Flt1D2.Flk1D3.FcΔC1(a) to inhibit tumor growth in vivo a model in which tumor cell suspensions are implanted subcutaneously on the right flank of male severe combined immunodeficiency (SCID) mice was employed. Two cell lines, the human HT-1080 fibrosarcoma cell line (ATCC accession no. CCL-121) and the rat C6 glioma cell line (ATCC accession no. CCL-107), each of which exhibit distinctly different morphologies and growth characteristics, were used in the assay. The first dose of Flt1D2.Flk1D3.FcΔC1(a) (at 25 mg/Kg or as indicated in FIGS. 39 and 40) was given on the day of tumor implantation. Animals subsequently received subcutaneous injections of Flt1(1-3)-Fc (A40), Flt1D2.Flk1D3.FcΔC1(a) or vehicle either every other day (EOD) or two times per week (2x/wk) for a period of 2 weeks. After 2 weeks, animals were perfused with fixative, tumors were removed and samples were blinded. Tumor volume was determined by measuring the length and width of visible subcutaneous tumors. Both of Flt1(1-3)-Fc (A40) and Flt1D2.Flk1D3.FcΔC1(a) significantly reduced the growth of tumors formed by HT-1080 and C6 cells. The results of these experiments are shown in FIG. 39 and FIG. 40.

Example 31

The Effect of VEGF165 and Modified Flt Receptors in Female Reproductive System

The stereotypic pattern of vascular remodeling which occur in the uterus and ovary over the course of the reproductive cycle has been well characterized, making

these tissues particularly well suited to the study of mechanisms which regulate angiogenesis, vascular remodeling and vascular regression. Indeed, in situ hybridization studies in the reproductive tissues provided the first clear evidence that VEGF acts as a mediator of physiological angiogenesis in mature rodents, as well as humans and non-human primates (Phillips et al, 1990; Ravindranath et al, 1992; Shweiki et al, 1993; Kamat et al, 1995). As cyclic angiogenesis and vascular remodeling are prominent features of the normal ovary and uterus, it is not surprising that abnormal blood vessel growth and/or vascular dysfunction have been found to characterize many pathological conditions which affect these organs. Furthermore, these pathogenic vascular abnormalities are thought to be caused or perpetuated by the dysregulated expression of one or more angiogenic or anti-angiogenic factors, most prominently VEGF.

For example, abnormal angiogenesis is characteristic of polycystic ovary disease, endometriosis and endometrial carcinoma, and in each case VEGF is over expressed in the affected tissue (Kamat et al, 1995; Shifren et al, 1996; Guidi et al, 1996; Donnez et al, 1998). Overexpression of VEGF is also thought to play a pathogenic role in the establishment of systemic vascular hyperpermeability in ovarian hyperstimulation syndrome (McClure et al, 1994; Levin, et al, 1998) and preeclampsia (Baker et al, 1995; Sharkey et al, 1996). In addition, VEGF has been implicated as the permeability factor responsible for the production of ascites associated with ovarian carcinoma and other tumors (Senger et al, 1983; Boockook et al, 1995). Agents which effectively neutralize the biological actions of VEGF can reasonably be anticipated to be of therapeutic benefit in the above and related conditions.

Angiogenesis and vascular remodeling are also hallmarks of blastocyst implantation and placental development (Findlay, 1986). VEGF is strongly expressed both in the maternal decidua and in embryonic trophoblasts, where it is thought to first stimulate expansion and hyperpermeability of the uterine vasculature during the peri-implantation period and subsequently mediate formation of both the maternal and embryonic components of the placental vasculature (Shweiki et al, 1993; Cullinan-Bove and Koos, 1993; Chakraborty et al, 1995; Das et al, 1997). VEGF is also required for luteal angiogenesis and associated progesterone secretion necessary to prepare the uterus for implantation (Ferrara et al, 1998). Thus, agents which inhibit the biological actions of VEGF may prove to be useful as contraceptive agents (by preventing implantation), or as abortifacients in the early stages of gestation. The latter application might find particular use as a non-surgical intervention for the termination of ectopic pregnancies.

While the expression of VEGF receptors is largely confined to the vascular endothelium in normal reproductive tissues, Flt1 is also expressed by trophoblasts in the placenta in both humans and animals (Clark et al, 1996; He et al, 1999) where it has been proposed to play a role in trophoblast invasion. Interestingly, both Flt1 and KDR Flk1 are expressed by choriocarcinoma cell line BeWo (Charnock-Jones et al, 1994), and VEGF has been shown to promote DNA synthesis and tyrosine phosphorylation of MAP kinase in these cells. Furthermore, primary and metastatic ovarian carcinomas not only to express high levels of VEGF, but—in addition to the vascular endothelium—the tumor cells themselves express KDR and/or Flt1 (Boockook et al, 1995). These findings suggest that VEGF may not only be critically involved in the generation and maintenance of tumor vasculature, but that at least in some tumors of reproductive origin VEGF may subserve an autocrine role, directly sup-

porting the survival and proliferation of the tumor cells. Thus agents which block the actions of VEGF may have particularly beneficial applications to the treatment of tumors of reproductive origin.

5 Methods and Results

(a) Assessment of VEGF-Induced Uterine Hyperpermeability

Pregnant mare's serum gonadotrophin (PMSG) was injected subcutaneously (5 IU) to induce ovulation in prepubertal female rats. This results in a surge of estradiol after 2 days which in turn causes an induction of VEGF in the uterus. It is reported that this induction results in hyperpermeability of the uterus and an increase in uterine wet weight 6 hrs. later and, therefore, could potentially be blocked by the modified Flt receptors Flt1(1-3)-Fc (A40), Flt1D2.Flk1D3.FcΔC1(a) and Flt1D2.VEGFR3D3.FcΔC1(a). In this in vivo model, the normal weight of the rat uterus is about 50 mg and this can be induced to 300-350 mg by PMSG. Desiccation of the tissue reveals that this is all water weight. Subcutaneous injection of Flt1(1-3)-Fc (A40), Flt1D2.Flk1D3.FcΔC1(a) and Flt1 D2.VEGFR3D3.FcΔC1(a) at 25 mg/kg at 1 hr. after PMSG injection results in about a 50% inhibition of the increase in uterine wet weight. Increasing the dose of modified Flt receptor does not further reduce the increase in wet weight suggesting that there is a VEGF-independent component to this model. The results of this experiment are shown in FIG. 41.

30 (a) Assessment of Corpus Luteum Angiogenesis Using Progesterone as a Readout

Pregnant mare's serum gonadotrophin (PMSG) is injected subcutaneously (5 IU) to induce ovulation in prepubertal female rats. This results in a fully functioning corpus luteum containing a dense network of blood vessels after 4 days that allows for the secretion of progesterone into the blood stream in order to prepare the uterus for implantation. The induction of angiogenesis in the corpus luteum requires VEGF; therefore, blocking VEGF would result in a lack of new blood vessels and thus a lack of progesterone secreted into the blood stream. In this in vivo model, resting levels of progesterone are about 5 ng/ml and this can be induced to a level of 25-40 ng/ml after PMSG. Subcutaneous injection of Flt1(1-3)-Fc (A40) or Flt1D2.Flk1D3.FcΔC1(a) at 25 mg/kg or 5 mg/kg at 1 hr. after PMSG injection results in a complete inhibition of the progesterone induction on day 4. The results of this experiment are shown in FIG. 42A-42B.

Example 33

50 Pharmacokinetic Analysis of Flt1(1-3)-Fc (A40) and Pegylated Flt1(1-3)-Fc

Flt1(1-3)-Fc was PEGylated with either 10 kD PEG or 20 kD PEG and tested in balb/c mice for their pharmacokinetic profile. Both PEGylated forms of Flt1(1-3)-Fc were found to have much better PK profiles than Flt1(1-3)-Fc (A40), with the T_{max} occurring at 24 hrs. for the PEGylated molecules as opposed to 6 hrs. for Flt1(1-3)-Fc (A40).

Example 34

VEGF165 ELISA to Test Affinity of Modified Flt1 Receptor Variants

10 pM of VEGF165 was incubated overnight at room temperature with modified Flt1 receptor variants ranging

from 160 pM to 0.1 pM. The modified Flt1 receptor variants used in this experiment were Flt1(1-3)-Fc, Flt1(1-3)-Fc (A40), transiently expressed Flt1D2Fk1D3.FcΔC1(a), transiently expressed Flt1D2VEFGFR3D3-FcΔC1(a), Flt1(1-³_{NAS})-Fc, Flt1(1-3_{R→C})-Fc and Tie2-Fc. Flt1(1-3_{NAS})-Fc is a modified version of Flt1(1-3)-Fc in which the highly basic amino acid sequence KNKRASVRRR is replaced by NASVNGSR, resulting in the incorporation of two new glycosylation sites and a net reduction of five positive charges, both with the purpose of reducing the unfavorable effects of this sequence on PK. Flt1(1-3_{R→C})-Fc is a modification in which a single arginine (R) residue within the same basic amino acid sequence is changed to a

cysteine (C) (KNKRASVRRR→KNKCASVRRR) to allow for pegylation at that residue, which could then shield the basic region from exerting its unfavorable effects on PK. After incubation the solution was transferred to a plate containing a capture antibody for VEGF165 (R&D). The amount of free VEGF165 was then determined using an antibody to report free VEGF165. This showed that the modified Flt1 receptor variant with the highest affinity for VEGF165 (determined as the lowest amount of free VEGF165) was Flt1D2Fk1D3.FcΔC1(a), followed by Flt1(1-3)-Fc and Flt1(1-3)-Fc (A40) and then by Flt1(1-3_{R→C})-Fc, Flt1(1-3_{NAS})-Fc and Flt1D2VEFGFR3D3-FcΔC1(a). Tie2Fc has no affinity for VEGF165.

SEQUENCE LISTING

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<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1) ... (1701)

<400> SEQUENCE: 1

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  1                    5                10                15

tgt ctg ctt ctc aca gga tct agt tca ggt tca aaa tta aaa gat cct      96
Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro
  20                25                30

gaa ctg agt tta aaa ggc acc cag cac atc atg caa gca ggc cag aca     144
Glu Leu Ser Leu Lys Gly Thr Gln His Ile Met Gln Ala Gly Gln Thr
  35                40                45

ctg cat ctc caa tgc agg ggg gaa gca gcc cat aaa tgg tct ttg cct     192
Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys Trp Ser Leu Pro
  50                55                60

gaa atg gtg agt aag gaa agc gaa agg ctg agc ata act aaa tct gcc     240
Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala
  65                70                75                80

tgt gga aga aat ggc aaa caa ttc tgc agt act tta acc ttg aac aca     288
Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr
  85                90                95

gct caa gca aac cac act ggc ttc tac agc tgc aaa tat cta gct gta     336
Ala Gln Ala Asn His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val
  100               105               110

cct act tca aag aag aag gaa aca gaa tct gca atc tat ata ttt att     384
Pro Thr Ser Lys Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile
  115               120               125

agt gat aca ggt aga cct ttc gta gag atg tac agt gaa atc ccc gaa     432
Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu
  130               135               140

att ata cac atg act gaa gga agg gag ctc gtc att ccc tgc cgg gtt     480
Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val
  145               150               155               160

acg tca cct aac atc act gtt act tta aaa aag ttt cca ctt gac act     528
Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
  165               170               175

ttg atc cct gat gga aaa cgc ata atc tgg gac agt aga aag ggc ttc     576
Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe
  180               185               190

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atc	ata	tca	aat	gca	acg	tac	aaa	gaa	ata	ggg	ctt	ctg	acc	tgt	gaa	624
Ile	Ile	Ser	Asn	Ala	Thr	Tyr	Lys	Glu	Ile	Gly	Leu	Leu	Thr	Cys	Glu	
		195					200					205				
gca	aca	gtc	aat	ggg	cat	ttg	tat	aag	aca	aac	tat	ctc	aca	cat	cga	672
Ala	Thr	Val	Asn	Gly	His	Leu	Tyr	Lys	Thr	Asn	Tyr	Leu	Thr	His	Arg	
		210				215					220					
caa	acc	aat	aca	atc	ata	gat	gtc	caa	ata	agc	aca	cca	cgc	cca	gtc	720
Gln	Thr	Asn	Thr	Ile	Ile	Asp	Val	Gln	Ile	Ser	Thr	Pro	Arg	Pro	Val	
		225			230					235					240	
aaa	tta	ctt	aga	ggc	cat	act	ctt	gtc	ctc	aat	tgt	act	gct	acc	act	768
Lys	Leu	Leu	Arg	Gly	His	Thr	Leu	Val	Leu	Asn	Cys	Thr	Ala	Thr	Thr	
				245					250					255		
ccc	ttg	aac	acg	aga	gtt	caa	atg	acc	tgg	agt	tac	cct	gat	gaa	aaa	816
Pro	Leu	Asn	Thr	Arg	Val	Gln	Met	Thr	Trp	Ser	Tyr	Pro	Asp	Glu	Lys	
			260					265					270			
aat	aag	aga	gct	tcc	gta	agg	cga	cga	att	gac	caa	agc	aat	tcc	cat	864
Asn	Lys	Arg	Ala	Ser	Val	Arg	Arg	Arg	Ile	Asp	Gln	Ser	Asn	Ser	His	
		275				280						285				
gcc	aac	ata	ttc	tac	agt	gtt	ctt	act	att	gac	aaa	atg	cag	aac	aaa	912
Ala	Asn	Ile	Phe	Tyr	Ser	Val	Leu	Thr	Ile	Asp	Lys	Met	Gln	Asn	Lys	
		290				295					300					
gac	aaa	gga	ctt	tat	act	tgt	cgt	gta	agg	agt	gga	cca	tca	ttc	aaa	960
Asp	Lys	Gly	Leu	Tyr	Thr	Cys	Arg	Val	Arg	Ser	Gly	Pro	Ser	Phe	Lys	
		305			310					315					320	
tct	gtt	aac	acc	tca	gtg	cat	ata	tat	gat	aaa	gca	ggc	ccg	ggc	gag	1008
Ser	Val	Asn	Thr	Ser	Val	His	Ile	Tyr	Asp	Lys	Ala	Gly	Pro	Gly	Glu	
				325					330					335		
ccc	aaa	tct	tgt	gac	aaa	act	cac	aca	tgc	cca	ccg	tgc	cca	gca	cct	1056
Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	
			340					345					350			
gaa	ctc	ctg	ggg	gga	ccg	tca	gtc	ttc	ctc	ttc	ccc	cca	aaa	ccc	aag	1104
Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	
			355				360						365			
gac	acc	ctc	atg	atc	tcc	cgg	acc	cct	gag	gtc	aca	tgc	gtg	gtg	gtg	1152
Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	
		370				375						380				
gac	gtg	agc	cac	gaa	gac	cct	gag	gtc	aag	ttc	aac	tgg	tac	gtg	gac	1200
Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	
		385			390					395					400	
ggc	gtg	gag	gtg	cat	aat	gcc	aag	aca	aag	ccg	cgg	gag	gag	cag	tac	1248
Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	
				405					410					415		
aac	agc	acg	tac	cgt	gtg	gtc	agc	gtc	ctc	acc	gtc	ctg	cac	cag	gac	1296
Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	
			420					425					430			
tgg	ctg	aat	ggc	aag	gag	tac	aag	tgc	aag	gtc	tcc	aac	aaa	gcc	ctc	1344
Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	
		435				440							445			
cca	gcc	ccc	atc	gag	aaa	acc	atc	tcc	aaa	gcc	aaa	ggg	cag	ccc	cga	1392
Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	
		450				455						460				
gaa	cca	cag	gtg	tac	acc	ctg	ccc	cca	tcc	cgg	gat	gag	ctg	acc	aag	1440
Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	
					470					475					480	
aac	cag	gtc	agc	ctg	acc	tgc	ctg	gtc	aaa	ggc	ttc	tat	ccc	agc	gac	1488
Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	
				485					490					495		
atc	gcc	gtg	gag	tgg	gag	agc	aat	ggg	cag	ccg	gag	aac	aac	tac	aag	1536
Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	

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500		505		510												
acc	acg	cct	ccc	gtg	ctg	gac	tcc	gac	ggc	tcc	ttc	ttc	ctc	tac	agc	1584
Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	
	515						520					525				
aag	ctc	acc	gtg	gac	aag	agc	agg	tgg	cag	cag	ggg	aac	gtc	ttc	tca	1632
Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	
	530				535						540					
tgc	tcc	gtg	atg	cat	gag	gct	ctg	cac	aac	cac	tac	acg	cag	aag	agc	1680
Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	
	545				550					555					560	
ctc	tcc	ctg	tct	ccg	ggc	aaa	tga									1704
Leu	Ser	Leu	Ser	Pro	Gly	Lys										
			565													
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<211> LENGTH: 567																
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Cys	Leu	Leu	Leu	Thr	Gly	Ser	Ser	Ser	Gly	Ser	Lys	Leu	Lys	Asp	Pro	
	20						25					30				
Glu	Leu	Ser	Leu	Lys	Gly	Thr	Gln	His	Ile	Met	Gln	Ala	Gly	Gln	Thr	
	35						40					45				
Leu	His	Leu	Gln	Cys	Arg	Gly	Glu	Ala	Ala	His	Lys	Trp	Ser	Leu	Pro	
	50					55					60					
Glu	Met	Val	Ser	Lys	Glu	Ser	Glu	Arg	Leu	Ser	Ile	Thr	Lys	Ser	Ala	
	65				70					75					80	
Cys	Gly	Arg	Asn	Gly	Lys	Gln	Phe	Cys	Ser	Thr	Leu	Thr	Leu	Asn	Thr	
			85						90					95		
Ala	Gln	Ala	Asn	His	Thr	Gly	Phe	Tyr	Ser	Cys	Lys	Tyr	Leu	Ala	Val	
	100							105					110			
Pro	Thr	Ser	Lys	Lys	Lys	Glu	Thr	Glu	Ser	Ala	Ile	Tyr	Ile	Phe	Ile	
	115					120						125				
Ser	Asp	Thr	Gly	Arg	Pro	Phe	Val	Glu	Met	Tyr	Ser	Glu	Ile	Pro	Glu	
	130				135					140						
Ile	Ile	His	Met	Thr	Glu	Gly	Arg	Glu	Leu	Val	Ile	Pro	Cys	Arg	Val	
	145			150						155				160		
Thr	Ser	Pro	Asn	Ile	Thr	Val	Thr	Leu	Lys	Lys	Phe	Pro	Leu	Asp	Thr	
			165						170					175		
Leu	Ile	Pro	Asp	Gly	Lys	Arg	Ile	Ile	Trp	Asp	Ser	Arg	Lys	Gly	Phe	
	180							185					190			
Ile	Ile	Ser	Asn	Ala	Thr	Tyr	Lys	Glu	Ile	Gly	Leu	Leu	Thr	Cys	Glu	
	195						200					205				
Ala	Thr	Val	Asn	Gly	His	Leu	Tyr	Lys	Thr	Asn	Tyr	Leu	Thr	His	Arg	
	210					215					220					
Gln	Thr	Asn	Thr	Ile	Ile	Asp	Val	Gln	Ile	Ser	Thr	Pro	Arg	Pro	Val	
	225				230					235					240	
Lys	Leu	Leu	Arg	Gly	His	Thr	Leu	Val	Leu	Asn	Cys	Thr	Ala	Thr	Thr	
			245						250					255		
Pro	Leu	Asn	Thr	Arg	Val	Gln	Met	Thr	Trp	Ser	Tyr	Pro	Asp	Glu	Lys	
	260							265					270			
Asn	Lys	Arg	Ala	Ser	Val	Arg	Arg	Arg	Ile	Asp	Gln	Ser	Asn	Ser	His	
	275						280					285				

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Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys
 290 295 300

Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys
 305 310 315 320

Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Gly Pro Gly Glu
 325 330 335

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
 340 345 350

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
 355 360 365

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
 370 375 380

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
 385 390 395 400

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
 405 410 415

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
 420 425 430

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
 435 440 445

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
 450 455 460

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
 465 470 475 480

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
 485 490 495

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
 500 505 510

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
 515 520 525

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
 530 535 540

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
 545 550 555 560

Leu Ser Leu Ser Pro Gly Lys
 565

<210> SEQ ID NO 3
 <211> LENGTH: 1674
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)...(1671)

<400> SEQUENCE: 3

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1 5 10 15	
tgt ctg ctt ctc aca gga tct agt tca ggt tca aaa tta aaa gat cct	96
Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro	
20 25 30	
gaa ctg agt tta aaa ggc acc cag cac atc atg caa gca ggc cag aca	144
Glu Leu Ser Leu Lys Gly Thr Gln His Ile Met Gln Ala Gly Gln Thr	
35 40 45	
ctg cat ctc caa tgc agg ggg gaa gca gcc cat aaa tgg tct ttg cct	192

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Leu	His	Leu	Gln	Cys	Arg	Gly	Glu	Ala	Ala	His	Lys	Trp	Ser	Leu	Pro	
	50					55					60					
gaa	atg	gtg	agt	aag	gaa	agc	gaa	agg	ctg	agc	ata	act	aaa	tct	gcc	240
Glu	Met	Val	Ser	Lys	Glu	Ser	Glu	Arg	Leu	Ser	Ile	Thr	Lys	Ser	Ala	
65				70					75						80	
tgt	gga	aga	aat	ggc	aaa	caa	ttc	tgc	agt	act	tta	acc	ttg	aac	aca	288
Cys	Gly	Arg	Asn	Gly	Lys	Gln	Phe	Cys	Ser	Thr	Leu	Thr	Leu	Asn	Thr	
				85					90						95	
gct	caa	gca	aac	cac	act	ggc	ttc	tac	agc	tgc	aaa	tat	cta	gct	gta	336
Ala	Gln	Ala	Asn	His	Thr	Gly	Phe	Tyr	Ser	Cys	Lys	Tyr	Leu	Ala	Val	
			100					105					110			
cct	act	tca	aag	aag	aag	gaa	aca	gaa	tct	gca	atc	tat	ata	ttt	att	384
Pro	Thr	Ser	Lys	Lys	Lys	Glu	Thr	Glu	Ser	Ala	Ile	Tyr	Ile	Phe	Ile	
			115			120							125			
agt	gat	aca	ggt	aga	cct	ttc	gta	gag	atg	tac	agt	gaa	atc	ccc	gaa	432
Ser	Asp	Thr	Gly	Arg	Pro	Phe	Val	Glu	Met	Tyr	Ser	Glu	Ile	Pro	Glu	
130						135					140					
att	ata	cac	atg	act	gaa	gga	agg	gag	ctc	gtc	att	ccc	tgc	cgg	gtt	480
Ile	Ile	His	Met	Thr	Glu	Gly	Arg	Glu	Leu	Val	Ile	Pro	Cys	Arg	Val	
145					150					155					160	
acg	tca	cct	aac	atc	act	ggt	act	tta	aaa	aag	ttt	cca	ctt	gac	act	528
Thr	Ser	Pro	Asn	Ile	Thr	Val	Thr	Leu	Lys	Lys	Phe	Pro	Leu	Asp	Thr	
					165				170					175		
ttg	atc	cct	gat	gga	aaa	cgc	ata	atc	tgg	gac	agt	aga	aag	ggc	ttc	576
Leu	Ile	Pro	Asp	Gly	Lys	Arg	Ile	Ile	Trp	Asp	Ser	Arg	Lys	Gly	Phe	
			180						185				190			
atc	ata	tca	aat	gca	acg	tac	aaa	gaa	ata	ggg	ctt	ctg	acc	tgt	gaa	624
Ile	Ile	Ser	Asn	Ala	Thr	Tyr	Lys	Glu	Ile	Gly	Leu	Leu	Thr	Cys	Glu	
		195					200						205			
gca	aca	gtc	aat	ggg	cat	ttg	tat	aag	aca	aac	tat	ctc	aca	cat	cga	672
Ala	Thr	Val	Asn	Gly	His	Leu	Tyr	Lys	Thr	Asn	Tyr	Leu	Thr	His	Arg	
		210				215					220					
caa	acc	aat	aca	atc	ata	gat	gtc	caa	ata	agc	aca	cca	cgc	cca	gtc	720
Gln	Thr	Asn	Thr	Ile	Ile	Asp	Val	Gln	Ile	Ser	Thr	Pro	Arg	Pro	Val	
225				230						235					240	
aaa	tta	ctt	aga	ggc	cat	act	ctt	gtc	ctc	aat	tgt	act	gct	acc	act	768
Lys	Leu	Leu	Arg	Gly	His	Thr	Leu	Val	Leu	Asn	Cys	Thr	Ala	Thr	Thr	
				245					250					255		
ccc	ttg	aac	acg	aga	ggt	caa	atg	acc	tgg	agt	tac	cct	gat	gaa	att	816
Pro	Leu	Asn	Thr	Arg	Val	Gln	Met	Thr	Trp	Ser	Tyr	Pro	Asp	Glu	Ile	
			260					265					270			
gac	caa	agc	aat	tcc	cat	gcc	aac	ata	ttc	tac	agt	ggt	ctt	act	att	864
Asp	Gln	Ser	Asn	Ser	His	Ala	Asn	Ile	Phe	Tyr	Ser	Val	Leu	Thr	Ile	
		275				280							285			
gac	aaa	atg	cag	aac	aaa	gac	aaa	gga	ctt	tat	act	tgt	cgt	gta	agg	912
Asp	Lys	Met	Gln	Asn	Lys	Asp	Lys	Gly	Leu	Tyr	Thr	Cys	Arg	Val	Arg	
		290			295						300					
agt	gga	cca	tca	ttc	aaa	tct	ggt	aac	acc	tca	gtg	cat	ata	tat	gat	960
Ser	Gly	Pro	Ser	Phe	Lys	Ser	Val	Asn	Thr	Ser	Val	His	Ile	Tyr	Asp	
305				310						315					320	
aaa	gca	ggc	ccg	ggc	gag	ccc	aaa	tct	tgt	gac	aaa	act	cac	aca	tgc	1008
Lys	Ala	Gly	Pro	Gly	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	
				325					330					335		
cca	ccg	tgc	cca	gca	cct	gaa	ctc	ctg	ggg	gga	ccg	tca	gtc	ttc	ctc	1056
Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	
			340					345					350			
ttc	ccc	cca	aaa	ccc	aag	gac	acc	ctc	atg	atc	tcc	cgg	acc	cct	gag	1104
Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	
		355				360						365				

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gtc	aca	tgc	gtg	gtg	gtg	gac	gtg	agc	cac	gaa	gac	cct	gag	gtc	aag	1152
Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	
	370					375					380					
ttc	aac	tgg	tac	gtg	gac	ggc	gtg	gag	gtg	cat	aat	gcc	aag	aca	aag	1200
Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	
	385				390					395					400	
cgg	egg	gag	gag	cag	tac	aac	agc	acg	tac	cgt	gtg	gtc	agc	gtc	ctc	1248
Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	
				405					410					415		
acc	gtc	ctg	cac	cag	gac	tgg	ctg	aat	ggc	aag	gag	tac	aag	tgc	aag	1296
Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	
			420					425					430			
gtc	tcc	aac	aaa	gcc	ctc	cca	gcc	ccc	atc	gag	aaa	acc	atc	tcc	aaa	1344
Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	
		435					440					445				
gcc	aaa	ggg	cag	ccc	cga	gaa	cca	cag	gtg	tac	acc	ctg	ccc	cca	tcc	1392
Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	
	450					455					460					
cgg	gat	gag	ctg	acc	aag	aac	cag	gtc	agc	ctg	acc	tgc	ctg	gtc	aaa	1440
Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	
	465				470					475					480	
ggc	ttc	tat	ccc	agc	gac	atc	gcc	gtg	gag	tgg	gag	agc	aat	ggg	cag	1488
Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	
				485					490					495		
cgg	gag	aac	aac	tac	aag	acc	acg	cct	ccc	gtg	ctg	gac	tcc	gac	ggc	1536
Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	
			500					505					510			
tcc	ttc	ttc	ctc	tac	agc	aag	ctc	acc	gtg	gac	aag	agc	agg	tgg	cag	1584
Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	
		515					520					525				
cag	ggg	aac	gtc	ttc	tca	tgc	tcc	gtg	atg	cat	gag	gct	ctg	cac	aac	1632
Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	
	530					535					540					
cac	tac	acg	cag	aag	agc	ctc	tcc	ctg	tct	ccg	ggt	aaa	tga			1674
His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys				
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<210> SEQ ID NO 4
 <211> LENGTH: 557
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

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			20					25					30		
Glu	Leu	Ser	Leu	Lys	Gly	Thr	Gln	His	Ile	Met	Gln	Ala	Gly	Gln	Thr
			35				40					45			
Leu	His	Leu	Gln	Cys	Arg	Gly	Glu	Ala	Ala	His	Lys	Trp	Ser	Leu	Pro
			50			55					60				
Glu	Met	Val	Ser	Lys	Glu	Ser	Glu	Arg	Leu	Ser	Ile	Thr	Lys	Ser	Ala
65				70						75				80	
Cys	Gly	Arg	Asn	Gly	Lys	Gln	Phe	Cys	Ser	Thr	Leu	Thr	Leu	Asn	Thr
				85					90					95	
Ala	Gln	Ala	Asn	His	Thr	Gly	Phe	Tyr	Ser	Cys	Lys	Tyr	Leu	Ala	Val
			100					105					110		
Pro	Thr	Ser	Lys	Lys	Lys	Glu	Thr	Glu	Ser	Ala	Ile	Tyr	Ile	Phe	Ile
		115					120					125			

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Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu
 130 135 140

Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val
 145 150 155 160

Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
 165 170 175

Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe
 180 185 190

Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
 195 200 205

Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg
 210 215 220

Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val
 225 230 235 240

Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr
 245 250 255

Pro Leu Asn Thr Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Ile
 260 265 270

Asp Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile
 275 280 285

Asp Lys Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg
 290 295 300

Ser Gly Pro Ser Phe Lys Ser Val Asn Thr Ser Val His Ile Tyr Asp
 305 310 315 320

Lys Ala Gly Pro Gly Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
 325 330 335

Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
 340 345 350

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
 355 360 365

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
 370 375 380

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
 385 390 395 400

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
 405 410 415

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
 420 425 430

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
 435 440 445

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
 450 455 460

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
 465 470 475 480

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
 485 490 495

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
 500 505 510

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
 515 520 525

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
 530 535 540

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His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 545 550 555

<210> SEQ ID NO 5
 <211> LENGTH: 1359
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)...(1356)

<400> SEQUENCE: 5

atg gtc agc tac tgg gac acc ggg gtc ctg ctg tgc gcg ctg ctc agc	48
Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser	
1 5 10 15	
tgt ctg ctt ctc aca gga tct agt tcc gga ggt aga cct ttc gta gag	96
Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu	
20 25 30	
atg tac agt gaa atc ccc gaa att ata cac atg act gaa gga agg gag	144
Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu	
35 40 45	
ctc gtc att ccc tgc cgg gtt acg tca cct aac atc act gtt act tta	192
Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu	
50 55 60	
aaa aag ttt cca ctt gac act ttg atc cct gat gga aaa cgc ata atc	240
Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile	
65 70 75 80	
tgg gac agt aga aag ggc ttc atc ata tca aat gca acg tac aaa gaa	288
Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu	
85 90 95	
ata ggg ctt ctg acc tgt gaa gca aca gtc aat ggg cat ttg tat aag	336
Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys	
100 105 110	
aca aac tat ctc aca cat cga caa acc aat aca atc ata gat gtc caa	384
Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln	
115 120 125	
ata agc aca cca cgc cca gtc aaa tta ctt aga ggc cat act ctt gtc	432
Ile Ser Thr Pro Arg Pro Val Lys Leu Leu Arg Gly His Thr Leu Val	
130 135 140	
ctc aat tgt act gct acc act ccc ttg aac acg aga gtt caa atg acc	480
Leu Asn Cys Thr Ala Thr Thr Pro Leu Asn Thr Arg Val Gln Met Thr	
145 150 155 160	
tgg agt tac cct gat gaa att gac caa agc aat tcc cat gcc aac ata	528
Trp Ser Tyr Pro Asp Glu Ile Asp Gln Ser Asn Ser His Ala Asn Ile	
165 170 175	
ttc tac agt gtt ctt act att gac aaa atg cag aac aaa gac aaa gga	576
Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys Asp Lys Gly	
180 185 190	
ctt tat act tgt cgt gta agg agt gga cca tca ttc aaa tct gtt aac	624
Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys Ser Val Asn	
195 200 205	
acc tca gtg cat ata tat gat aaa gca ggc ccg ggc gag ccc aaa tct	672
Thr Ser Val His Ile Tyr Asp Lys Ala Gly Pro Gly Glu Pro Lys Ser	
210 215 220	
tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg	720
Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu	
225 230 235 240	
ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc	768
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu	
245 250 255	
atg atc tcc ccg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc	816

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Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser		
			260					265					270				
cac	gaa	gac	cct	gag	gtc	aag	ttc	aac	tgg	tac	gtg	gac	ggc	gtg	gag		864
His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu		
		275					280					285					
gtg	cat	aat	gcc	aag	aca	aag	ccg	cgg	gag	gag	cag	tac	aac	agc	acg		912
Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr		
		290				295					300						
tac	cgt	gtg	gtc	agc	gtc	ctc	acc	gtc	ctg	cac	cag	gac	tgg	ctg	aat		960
Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn		
305					310					315					320		
ggc	aag	gag	tac	aag	tgc	aag	gtc	tcc	aac	aaa	gcc	ctc	cca	gcc	ccc		1008
Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro		
				325					330					335			
atc	gag	aaa	acc	atc	tcc	aaa	gcc	aaa	ggg	cag	ccc	cga	gaa	cca	cag		1056
Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln		
			340					345						350			
gtg	tac	acc	ctg	ccc	cca	tcc	cgg	gat	gag	ctg	acc	aag	aac	cag	gtc		1104
Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val		
		355				360						365					
agc	ctg	acc	tgc	ctg	gtc	aaa	ggc	ttc	tat	ccc	agc	gac	atc	gcc	gtg		1152
Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val		
		370				375					380						
gag	tgg	gag	agc	aat	ggg	cag	ccg	gag	aac	aac	tac	aag	acc	acg	cct		1200
Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro		
385					390				395						400		
ccc	gtg	ctg	gac	tcc	gac	ggc	tcc	ttc	ttc	ctc	tac	agc	aag	ctc	acc		1248
Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr		
				405					410					415			
gtg	gac	aag	agc	agg	tgg	cag	cag	ggg	aac	gtc	ttc	tca	tgc	tcc	gtg		1296
Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val		
			420					425					430				
atg	cat	gag	gct	ctg	cac	aac	cac	tac	acg	cag	aag	agc	ctc	tcc	ctg		1344
Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu		
			435			440							445				
tct	ccg	ggt	aaa	tga													1359
Ser	Pro	Gly	Lys														
			450														

<210> SEQ ID NO 6
 <211> LENGTH: 452
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

Met	Val	Ser	Tyr	Trp	Asp	Thr	Gly	Val	Leu	Leu	Cys	Ala	Leu	Leu	Ser		
1				5					10					15			
Cys	Leu	Leu	Leu	Thr	Gly	Ser	Ser	Ser	Gly	Gly	Arg	Pro	Phe	Val	Glu		
			20					25					30				
Met	Tyr	Ser	Glu	Ile	Pro	Glu	Ile	Ile	His	Met	Thr	Glu	Gly	Arg	Glu		
			35				40						45				
Leu	Val	Ile	Pro	Cys	Arg	Val	Thr	Ser	Pro	Asn	Ile	Thr	Val	Thr	Leu		
			50			55					60						
Lys	Lys	Phe	Pro	Leu	Asp	Thr	Leu	Ile	Pro	Asp	Gly	Lys	Arg	Ile	Ile		
65				70						75				80			
Trp	Asp	Ser	Arg	Lys	Gly	Phe	Ile	Ile	Ser	Asn	Ala	Thr	Tyr	Lys	Glu		
				85					90					95			
Ile	Gly	Leu	Leu	Thr	Cys	Glu	Ala	Thr	Val	Asn	Gly	His	Leu	Tyr	Lys		
			100					105						110			

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Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln
 115 120 125
 Ile Ser Thr Pro Arg Pro Val Lys Leu Leu Arg Gly His Thr Leu Val
 130 135 140
 Leu Asn Cys Thr Ala Thr Thr Pro Leu Asn Thr Arg Val Gln Met Thr
 145 150 155 160
 Trp Ser Tyr Pro Asp Glu Ile Asp Gln Ser Asn Ser His Ala Asn Ile
 165 170 175
 Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys Asp Lys Gly
 180 185 190
 Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys Ser Val Asn
 195 200 205
 Thr Ser Val His Ile Tyr Asp Lys Ala Gly Pro Gly Glu Pro Lys Ser
 210 215 220
 Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 225 230 235 240
 Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 245 250 255
 Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 260 265 270
 His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 275 280 285
 Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
 290 295 300
 Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 305 310 315 320
 Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
 325 330 335
 Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
 340 345 350
 Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
 355 360 365
 Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 370 375 380
 Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 385 390 395 400
 Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 405 410 415
 Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
 420 425 430
 Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
 435 440 445
 Ser Pro Gly Lys
 450

<210> SEQ ID NO 7
 <211> LENGTH: 1389
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1) ... (1386)

<400> SEQUENCE: 7

atg gtc agc tac tgg gac acc ggg gtc ctg ctg tgc gcg ctg etc agc

48

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Met	Val	Ser	Tyr	Trp	Asp	Thr	Gly	Val	Leu	Leu	Cys	Ala	Leu	Leu	Ser				
1				5					10						15				
tgt	ctg	ctt	ctc	aca	gga	tct	agt	tcc	gga	ggt	aga	cct	ttc	gta	gag				96
Cys	Leu	Leu		Thr	Gly	Ser	Ser	Ser	Gly	Gly	Arg	Pro	Phe	Val	Glu				
			20					25					30						
atg	tac	agt	gaa	atc	ccc	gaa	att	ata	cac	atg	act	gaa	gga	agg	gag				144
Met	Tyr	Ser	Glu	Ile	Pro	Glu	Ile	Ile	His	Met	Thr	Glu	Gly	Arg	Glu				
		35					40					45							
ctc	gtc	att	ccc	tgc	egg	gtt	acg	tca	cct	aac	atc	act	gtt	act	tta				192
Leu	Val	Ile	Pro	Cys	Arg	Val	Thr	Ser	Pro	Asn	Ile	Thr	Val	Thr	Leu				
	50						55				60								
aaa	aag	ttt	cca	ctt	gac	act	ttg	atc	cct	gat	gga	aaa	cgc	ata	atc				240
Lys	Lys	Phe	Pro	Leu	Asp	Thr	Leu	Ile	Pro	Asp	Gly	Lys	Arg	Ile	Ile				
	65				70					75				80					
tgg	gac	agt	aga	aag	ggc	ttc	atc	ata	tca	aat	gca	acg	tac	aaa	gaa				288
Trp	Asp	Ser	Arg	Lys	Gly	Phe	Ile	Ile	Ser	Asn	Ala	Thr	Tyr	Lys	Glu				
				85					90					95					
ata	ggg	ctt	ctg	acc	tgt	gaa	gca	aca	gtc	aat	ggg	cat	ttg	tat	aag				336
Ile	Gly	Leu	Leu	Thr	Cys	Glu	Ala	Thr	Val	Asn	Gly	His	Leu	Tyr	Lys				
			100					105					110						
aca	aac	tat	ctc	aca	cat	cga	caa	acc	aat	aca	atc	ata	gat	gtc	caa				384
Thr	Asn	Tyr	Leu	Thr	His	Arg	Gln	Thr	Asn	Thr	Ile	Ile	Asp	Val	Gln				
		115					120					125							
ata	agc	aca	cca	cgc	cca	gtc	aaa	tta	ctt	aga	ggc	cat	act	ctt	gtc				432
Ile	Ser	Thr	Pro	Arg	Pro	Val	Lys	Leu	Leu	Arg	Gly	His	Thr	Leu	Val				
	130					135					140								
ctc	aat	tgt	act	gct	acc	act	ccc	ttg	aac	acg	aga	ggt	caa	atg	acc				480
Leu	Asn	Cys	Thr	Ala	Thr	Thr	Pro	Leu	Asn	Thr	Arg	Val	Gln	Met	Thr				
	145				150					155				160					
tgg	agt	tac	cct	gat	gaa	aaa	aat	aag	aga	gct	tcc	gta	agg	cga	cga				528
Trp	Ser	Tyr	Pro	Asp	Glu	Lys	Asn	Lys	Arg	Ala	Ser	Val	Arg	Arg	Arg				
				165				170						175					
att	gac	caa	agc	aat	tcc	cat	gcc	aac	ata	ttc	tac	agt	ggt	ctt	act				576
Ile	Asp	Gln	Ser	Asn	Ser	His	Ala	Asn	Ile	Phe	Tyr	Ser	Val	Leu	Thr				
		180						185					190						
att	gac	aaa	atg	cag	aac	aaa	gac	aaa	gga	ctt	tat	act	tgt	cgt	gta				624
Ile	Asp	Lys	Met	Gln	Asn	Lys	Asp	Lys	Gly	Leu	Tyr	Thr	Cys	Arg	Val				
		195					200					205							
agg	agt	gga	cca	tca	ttc	aaa	tct	gtt	aac	acc	tca	gtg	cat	ata	tat				672
Arg	Ser	Gly	Pro	Ser	Phe	Lys	Ser	Val	Asn	Thr	Ser	Val	His	Ile	Tyr				
		210				215					220								
gat	aaa	gca	ggc	ccg	ggc	gag	ccc	aaa	tct	tgt	gac	aaa	act	cac	aca				720
Asp	Lys	Ala	Gly	Pro	Gly	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr				
	225				230					235					240				
tgc	cca	ccg	tgc	cca	gca	cct	gaa	ctc	ctg	ggg	gga	ccg	tca	gtc	ttc				768
Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe				
				245						250				255					
ctc	ttc	ccc	cca	aaa	ccc	aag	gac	acc	ctc	atg	atc	tcc	cgg	acc	cct				816
Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro				
			260				265						270						
gag	gtc	aca	tgc	gtg	gtg	gtg	gac	gtg	agc	cac	gaa	gac	cct	gag	gtc				864
Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val				
		275					280					285							
aag	ttc	aac	tgg	tac	gtg	gac	ggc	gtg	gag	gtg	cat	aat	gcc	aag	aca				912
Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr				
		290				295					300								
aag	ccg	cgg	gag	gag	cag	tac	aac	agc	acg	tac	cgt	gtg	gtc	agc	gtc				960
Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val				
	305				310					315					320				

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ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc	1008
Leu Thr Val Leu His Gln Asp Trp Leu Leu Gly Lys Glu Tyr Lys Cys	
325 330 335	
aag gtc tcc aac aaa gcc ctg cca gcc ccc atc gag aaa acc atc tcc	1056
Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser	
340 345 350	
aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca	1104
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro	
355 360 365	
tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc	1152
Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val	
370 375 380	
aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg	1200
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly	
385 390 395 400	
cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac	1248
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp	
405 410 415	
ggc tcc ttc ttc ctg tac agc aag ctg acc gtg gac aag agc agg tgg	1296
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp	
420 425 430	
cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac	1344
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His	
435 440 445	
aac cac tac acg cag aag agc ctg tcc ctg tct ccg ggt aaa	1386
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys	
450 455 460	
tga	1389

<210> SEQ ID NO 8

<211> LENGTH: 462

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser	
1 5 10 15	
Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu	
20 25 30	
Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu	
35 40 45	
Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu	
50 55 60	
Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile	
65 70 75 80	
Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu	
85 90 95	
Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys	
100 105 110	
Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln	
115 120 125	
Ile Ser Thr Pro Arg Pro Val Lys Leu Leu Arg Gly His Thr Leu Val	
130 135 140	
Leu Asn Cys Thr Ala Thr Thr Pro Leu Asn Thr Arg Val Gln Met Thr	
145 150 155 160	
Trp Ser Tyr Pro Asp Glu Lys Asn Lys Arg Ala Ser Val Arg Arg Arg	
165 170 175	

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Ile Asp Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu Thr
 180 185 190

Ile Asp Lys Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr Cys Arg Val
 195 200 205

Arg Ser Gly Pro Ser Phe Lys Ser Val Asn Thr Ser Val His Ile Tyr
 210 215 220

Asp Lys Ala Gly Pro Gly Glu Pro Lys Ser Cys Asp Lys Thr His Thr
 225 230 235 240

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
 245 250 255

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
 260 265 270

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
 275 280 285

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
 290 295 300

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
 305 310 315 320

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
 325 330 335

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
 340 345 350

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
 355 360 365

Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
 370 375 380

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
 385 390 395 400

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
 405 410 415

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
 420 425 430

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
 435 440 445

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 450 455 460

<210> SEQ ID NO 9
 <211> LENGTH: 1704
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)...(1701)

<400> SEQUENCE: 9

atg gtc agc tac tgg gac acc ggg gtc ctg ctg tgc gcg ctg ctc agc	48
Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser	
1 5 10 15	
tgt ctg ctt ctc aca gga tct agt tca ggt tca aaa tta aaa gat cct	96
Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro	
20 25 30	
gaa ctg agt tta aaa ggc acc cag cac atc atg caa gca ggc cag aca	144
Glu Leu Ser Leu Lys Gly Thr Gln His Ile Met Gln Ala Gly Gln Thr	
35 40 45	
ctg cat ctc caa tgc agg ggg gaa gca gcc cat aaa tgg tct ttg cct	192
Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys Trp Ser Leu Pro	

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50	55	60	
gaa atg gtg agt aag gaa agc gaa agg ctg agc ata act aaa tct gcc Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala 65 70 75 80			240
tgt gga aga aat ggc aaa caa ttc tgc agt act tta acc ttg aac aca Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr 85 90 95			288
gct caa gca aac cac act ggc ttc tac agc tgc aaa tat cta gct gta Ala Gln Ala Asn His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val 100 105 110			336
cct act tca aag aag aag gaa aca gaa tct gca atc tat ata ttt att Pro Thr Ser Lys Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile 115 120 125			384
agt gat aca ggt aga cct ttc gta gag atg tac agt gaa atc ccc gaa Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu 130 135 140			432
att ata cac atg act gaa gga agg gag ctc gtc att ccc tgc cgg gtt Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val 145 150 155 160			480
acg tca cct aac atc act gtt act tta aaa aag ttt cca ctt gac act Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr 165 170 175			528
ttg atc cct gat gga aaa cgc ata atc tgg gac agt aga aag ggc ttc Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe 180 185 190			576
atc ata tca aat gca acg tac aaa gaa ata ggg ctt ctg acc tgt gaa Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu 195 200 205			624
gca aca gtc aat ggg cat ttg tat aag aca aac tat ctc aca cat cga Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg 210 215 220			672
caa acc aat aca atc ata gat gtc caa ata agc aca cca cgc cca gtc Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val 225 230 235 240			720
aaa tta ctt aga ggc cat act ctt gtc ctc aat tgt act gct acc act Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr 245 250 255			768
ccc ttg aac acg aga gtt caa atg acc tgg agt tac cct gat gaa aaa Pro Leu Asn Thr Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys 260 265 270			816
aat aag aac gct tcc gta agg cga cga att gac caa agc aat tcc cat Asn Lys Asn Ala Ser Val Arg Arg Arg Ile Asp Gln Ser Asn Ser His 275 280 285			864
gcc aac ata ttc tac agt gtt ctt act att gac aaa atg cag aac aaa Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys 290 295 300			912
gac aaa gga ctt tat act tgt cgt gta agg agt gga cca tca ttc aaa Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys 305 310 315 320			960
tct gtt aac acc tca gtg cat ata tat gat aaa gca ggc ccg ggc gag Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Gly Pro Gly Glu 325 330 335			1008
ccc aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro 340 345 350			1056
gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys 355 360 365			1104
gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg			1152

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Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
 370                               375                               380

gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac    1200
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
385                               390                               395                               400

ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac    1248
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
405                               410                               415

aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac    1296
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
420                               425                               430

tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc    1344
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
435                               440                               445

cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga    1392
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
450                               455                               460

gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag    1440
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
465                               470                               475                               480

aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac    1488
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
485                               490                               495

atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag    1536
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
500                               505                               510

acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc    1584
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
515                               520                               525

aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca    1632
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
530                               535                               540

tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc    1680
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
545                               550                               555                               560

ctc tcc ctg tct ccg ggt aaa tga
Leu Ser Leu Ser Pro Gly Lys
565
    
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<210> SEQ ID NO 10
<211> LENGTH: 567
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
    
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<400> SEQUENCE: 10

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Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
 1                               5                               10                               15

Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro
20                               25                               30

Glu Leu Ser Leu Lys Gly Thr Gln His Ile Met Gln Ala Gly Gln Thr
35                               40                               45

Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys Trp Ser Leu Pro
50                               55                               60

Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala
65                               70                               75                               80

Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr
85                               90                               95

Ala Gln Ala Asn His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val
100                              105                              110
    
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Pro Thr Ser Lys Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile
 115 120 125
 Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu
 130 135 140
 Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val
 145 150 155 160
 Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
 165 170 175
 Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe
 180 185 190
 Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
 195 200 205
 Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg
 210 215 220
 Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val
 225 230 235 240
 Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr
 245 250 255
 Pro Leu Asn Thr Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys
 260 265 270
 Asn Lys Asn Ala Ser Val Arg Arg Arg Ile Asp Gln Ser Asn Ser His
 275 280 285
 Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys
 290 295 300
 Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys
 305 310 315 320
 Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Gly Pro Gly Glu
 325 330 335
 Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
 340 345 350
 Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
 355 360 365
 Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
 370 375 380
 Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
 385 390 395 400
 Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
 405 410 415
 Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
 420 425 430
 Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
 435 440 445
 Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
 450 455 460
 Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
 465 470 475 480
 Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
 485 490 495
 Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
 500 505 510
 Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
 515 520 525

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Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
 530 535 540

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
 545 550 555 560

Leu Ser Leu Ser Pro Gly Lys
 565

<210> SEQ ID NO 11
 <211> LENGTH: 1453
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (69)...(1442)

<400> SEQUENCE: 11

aagcttgggc tgcaggtcga tcgactctag aggatcgatc cccgggagag ctcgaaattcg 60

caaccacc atg gtc agc tac tgg gac acc ggg gtc ctg ctg tgc gcg ctg 110
 Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu
 1 5 10

ctc agc tgt ctg ctt ctc aca gga tct agt tcc gga ggt aga cct ttc 158
 Leu Ser Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe
 15 20 25 30

gta gag atg tac agt gaa atc ccc gaa att ata cac atg act gaa gga 206
 Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly
 35 40 45

agg gag ctc gtc att ccc tgc cgg gtt acg tca cct aac atc act gtt 254
 Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val
 50 55 60

act tta aaa aag ttt cca ctt gac act ttg atc cct gat gga aaa cgc 302
 Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg
 65 70 75

ata atc tgg gac agt aga aag ggc ttc atc ata tca aat gca acg tac 350
 Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr
 80 85 90

aaa gaa ata ggg ctt ctg acc tgt gaa gca aca gtc aat ggg cat ttg 398
 Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu
 95 100 105 110

tat aag aca aac tat ctc aca cat cga caa acc aat aca atc ata gat 446
 Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp
 115 120 125

gtg gtt ctg agt ccg tct cat gga att gaa cta tct gtt gga gaa aag 494
 Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu Lys
 130 135 140

ctt gtc tta aat tgt aca gca aga act gaa cta aat gtg ggg att gac 542
 Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp
 145 150 155

ttc aac tgg gaa tac cct tct tgc aag cat cag cat aag aaa ctt gta 590
 Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu Val
 160 165 170

aac cga gac cta aaa acc cag tct ggg agt gag atg aag aaa ttt ttg 638
 Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe Leu
 175 180 185 190

agc acc tta act ata gat ggt gta acc cgg agt gac caa gga ttg tac 686
 Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu Tyr
 195 200 205

acc tgt gca gca tcc agt ggg ctg atg acc aag aag aac agc aca ttt 734
 Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr Phe
 210 215 220

gtc agg gtc cat gaa aag ggc ccg ggc gac aaa act cac aca tgc cca 782

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Val	Arg	Val	His	Glu	Lys	Gly	Pro	Gly	Asp	Lys	Thr	His	Thr	Cys	Pro		
		225					230					235					
cgc	tgc	cca	gca	cct	gaa	ctc	ctg	ggg	gga	cgc	tca	gtc	ttc	ctc	ttc	830	
Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe		
		240				245					250						
ccc	cca	aaa	ccc	aag	gac	acc	ctc	atg	atc	tcc	egg	acc	cct	gag	gtc	878	
Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val		
255				260						265				270			
aca	tgc	gtg	gtg	gtg	gac	gtg	agc	cac	gaa	gac	cct	gag	gtc	aag	ttc	926	
Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe		
				275					280					285			
aac	tgg	tac	gtg	gac	ggc	gtg	gag	gtg	cat	aat	gcc	aag	aca	aag	cgc	974	
Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro		
			290					295					300				
cgg	gag	gag	cag	tac	aac	agc	acg	tac	cgt	gtg	gtc	agc	gtc	ctc	acc	1022	
Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr		
		305					310					315					
gtc	ctg	cac	cag	gac	tgg	ctg	aat	ggc	aag	gag	tac	aag	tgc	aag	gtc	1070	
Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val		
		320			325						330						
tcc	aac	aaa	gcc	ctc	cca	gcc	ccc	atc	gag	aaa	acc	atc	tcc	aaa	gcc	1118	
Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala		
335				340						345				350			
aaa	ggg	cag	ccc	cga	gaa	cca	cag	gtg	tac	acc	ctg	ccc	cca	tcc	cgg	1166	
Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg		
				355				360						365			
gat	gag	ctg	acc	aag	aac	cag	gtc	agc	ctg	acc	tgc	ctg	gtc	aaa	ggc	1214	
Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly		
			370				375						380				
ttc	tat	ccc	agc	gac	atc	gcc	gtg	gag	tgg	gag	agc	aat	ggg	cag	cgc	1262	
Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro		
		385				390						395					
gag	aac	aac	tac	aag	acc	acg	cct	ccc	gtg	ctg	gac	tcc	gac	ggc	tcc	1310	
Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser		
		400				405					410						
ttc	ttc	ctc	tat	agc	aag	ctc	acc	gtg	gac	aag	agc	agg	tgg	cag	cag	1358	
Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln		
		415			420					425				430			
ggg	aac	gtc	ttc	tca	tgc	tcc	gtg	atg	cat	gag	gct	ctg	cac	aac	cac	1406	
Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His		
				435				440						445			
tac	acg	cag	aag	agc	ctc	tcc	ctg	tct	ccg	ggt	aaa	tgagcggccg				1452	
Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys						
			450					455									
c																	1453

<210> SEQ ID NO 12
 <211> LENGTH: 458
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
 1 5 10 15
 Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu
 20 25 30
 Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu
 35 40 45
 Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu

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50				55				60							
Lys 65	Lys	Phe	Pro	Leu	Asp 70	Thr	Leu	Ile	Pro	Asp 75	Gly	Lys	Arg	Ile	Ile 80
Trp	Asp	Ser	Arg	Lys 85	Gly	Phe	Ile	Ile	Ser 90	Asn	Ala	Thr	Tyr	Lys 95	Glu
Ile	Gly	Leu	Leu	Thr 100	Cys	Glu	Ala	Thr 105	Val	Asn	Gly	His	Leu	Tyr 110	Lys
Thr	Asn	Tyr 115	Leu	Thr	His	Arg	Gln	Thr 120	Asn	Thr	Ile	Ile	Asp 125	Val	Val
Leu	Ser	Pro	Ser	His	Gly 135	Ile	Glu	Leu	Ser	Val	Gly 140	Glu	Lys	Leu	Val
Leu	Asn	Cys	Thr	Ala	Arg 150	Thr	Glu	Leu	Asn	Val 155	Gly	Ile	Asp	Phe	Asn 160
Trp	Glu	Tyr	Pro	Ser 165	Ser	Lys	His	Gln	His 170	Lys	Lys	Leu	Val	Asn 175	Arg
Asp	Leu	Lys	Thr	Gln	Ser	Gly	Ser	Glu	Met 185	Lys	Lys	Phe	Leu	Ser 190	Thr
Leu	Thr	Ile	Asp	Gly	Val	Thr	Arg	Ser 200	Asp	Gln	Gly	Leu	Tyr	Thr 205	Cys
Ala	Ala	Ser	Ser	Gly	Leu	Met 215	Thr	Lys	Lys 220	Asn	Ser	Thr	Phe	Val	Arg
Val	His	Glu	Lys	Gly	Pro	Gly 230	Asp	Lys	Thr 235	His	Thr	Cys	Pro	Pro	Cys 240
Pro	Ala	Pro	Glu	Leu	Leu	Gly 245	Gly	Pro	Ser 250	Val	Phe	Leu	Phe	Pro 255	Pro
Lys	Pro	Lys	Asp 260	Thr	Leu	Met	Ile	Ser 265	Arg	Thr	Pro	Glu	Val	Thr 270	Cys
Val	Val	Val	Asp 275	Val	Ser	His	Glu	Asp 280	Pro	Glu	Val	Lys	Phe	Asn 285	Trp
Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn 295	Ala	Lys	Thr	Lys	Pro	Arg 300	Glu
Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val 310	Val	Ser 315	Val	Leu	Thr	Val 320	Leu
His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu 325	Tyr	Lys 330	Cys	Lys	Val	Ser 335	Asn
Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys 345	Thr	Ile	Ser	Lys	Ala	Lys 350	Gly
Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr 360	Leu	Pro	Pro	Ser	Arg	Asp 365	Glu
Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr 375	Cys	Leu	Val	Lys	Gly	Phe 380	Tyr
Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu 385	Ser	Asn 395	Gly	Gln	Pro	Glu 400	Asn
Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu 405	Asp	Ser 410	Asp	Gly	Ser	Phe 415	Phe
Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys 420	Ser	Arg	Trp	Gln	Gln	Gly 430	Asn
Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu 435	Ala	Leu	His	Asn	His	Tyr 445	Thr
Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly 450	Lys						

<210> SEQ ID NO 13

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<211> LENGTH: 1444
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (69)...(1433)

<400> SEQUENCE: 13

aagcttgggc tgcaggtcga tcgactctag aggatcgatc cccgggagag ctccaattcg      60
caaccacc atg gtc agc tac tgg gac acc ggg gtc ctg ctg tgc gcg ctg      110
      Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu
      1          5          10
ctc agc tgt ctg ctt ctc aca gga tct agt tcc gga ggt aga cct ttc      158
Leu Ser Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe
      15          20          25          30
gta gag atg tac agt gaa atc ccc gaa att ata cac atg act gaa gga      206
Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly
      35          40          45
agg gag ctc gtc att ccc tgc cgg gtt acg tca cct aac atc act gtt      254
Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val
      50          55          60
act tta aaa aag ttt cca ctt gac act ttg atc cct gat gga aaa cgc      302
Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg
      65          70          75
ata atc tgg gac agt aga aag ggc ttc atc ata tca aat gca acg tac      350
Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr
      80          85          90
aaa gaa ata ggg ctt ctg acc tgt gaa gca aca gtc aat ggg cat ttg      398
Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu
      95          100          105          110
tat aag aca aac tat ctc aca cat cga caa acc aat aca atc ata gat      446
Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp
      115          120          125
atc cag ctg ttg ccc agg aag tcg ctg gag ctg ctg gta ggg gag aag      494
Ile Gln Leu Leu Pro Arg Lys Ser Leu Glu Leu Leu Val Gly Glu Lys
      130          135          140
ctg gtc ctc aac tgc acc gtg tgg gct gag ttt aac tca ggt gtc acc      542
Leu Val Leu Asn Cys Thr Val Trp Ala Glu Phe Asn Ser Gly Val Thr
      145          150          155
ttt gac tgg gac tac cca ggg aag cag gca gag cgg ggt aag tgg gtg      590
Phe Asp Trp Asp Tyr Pro Gly Lys Gln Ala Glu Arg Gly Lys Trp Val
      160          165          170
ccc gag cga cgc tcc caa cag acc cac aca gaa ctc tcc agc atc ctg      638
Pro Glu Arg Arg Ser Gln Thr His Thr Glu Leu Ser Ser Ile Leu
      175          180          185          190
acc atc cac aac gtc agc cag cac gac ctg ggc tgg tat gtg tgc aag      686
Thr Ile His Asn Val Ser Gln His Asp Leu Gly Ser Tyr Val Cys Lys
      195          200          205
gcc aac aac ggc atc cag cga ttt cgg gag agc acc gag gtc att gtg      734
Ala Asn Asn Gly Ile Gln Arg Phe Arg Glu Ser Thr Glu Val Ile Val
      210          215          220
cat gaa aat ggc ccg ggc gac aaa act cac aca tgc cca ccg tgc cca      782
His Glu Asn Gly Pro Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro
      225          230          235
gca cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa      830
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
      240          245          250
ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg      878
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
      255          260          265          270

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gtg gac gtc agc cac gaa gac cct gag gtc aag ttc aac tgg tac	926
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr	
275 280 285	
gtg gac ggc gtc gag gtc cat aat gcc aag aca aag ccg cgg gag gag	974
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu	
290 295 300	
cag tac aac agc acg tac cgt gtc gtc agc gtc ctc acc gtc ctg cac	1022
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His	
305 310 315	
cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa	1070
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys	
320 325 330	
gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag	1118
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln	
335 340 345 350	
ccc cga gaa cca cag gtc tac acc ctg ccc cca tcc cgg gat gag ctg	1166
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu	
355 360 365	
acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc	1214
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro	
370 375 380	
agc gac atc gcc gtc gag tgg gag agc aat ggg cag ccg gag aac aac	1262
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn	
385 390 395	
tac aag acc acg cct ccc gtc ctg gac tcc gac ggc tcc ttc ttc ctc	1310
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu	
400 405 410	
tat agc aag ctc acc gtc gac aag agc agg tgg cag cag ggg aac gtc	1358
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val	
415 420 425 430	
ttc tca tgc tcc gtc atg cat gag gct ctg cac aac cac tac acg cag	1406
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln	
435 440 445	
aag agc ctc tcc ctg tct cgg ggt aaa tgagcggccg c	1444
Lys Ser Leu Ser Leu Ser Pro Gly Lys	
450 455	

<210> SEQ ID NO 14
 <211> LENGTH: 455
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 14

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser	
1 5 10 15	
Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu	
20 25 30	
Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu	
35 40 45	
Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu	
50 55 60	
Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile	
65 70 75 80	
Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu	
85 90 95	
Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys	
100 105 110	
Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Ile Gln	
115 120 125	

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Leu Leu Pro Arg Lys Ser Leu Glu Leu Leu Val Gly Glu Lys Leu Val
 130 135 140

Leu Asn Cys Thr Val Trp Ala Glu Phe Asn Ser Gly Val Thr Phe Asp
 145 150 155 160

Trp Asp Tyr Pro Gly Lys Gln Ala Glu Arg Gly Lys Trp Val Pro Glu
 165 170 175

Arg Arg Ser Gln Gln Thr His Thr Glu Leu Ser Ser Ile Leu Thr Ile
 180 185 190

His Asn Val Ser Gln His Asp Leu Gly Ser Tyr Val Cys Lys Ala Asn
 195 200 205

Asn Gly Ile Gln Arg Phe Arg Glu Ser Thr Glu Val Ile Val His Glu
 210 215 220

Asn Gly Pro Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
 225 230 235 240

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
 245 250 255

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
 260 265 270

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
 275 280 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
 290 295 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
 305 310 315 320

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
 325 330 335

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
 340 345 350

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
 355 360 365

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
 370 375 380

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
 385 390 395 400

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
 405 410 415

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
 420 425 430

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
 435 440 445

Leu Ser Leu Ser Pro Gly Lys
 450 455

<210> SEQ ID NO 15
 <211> LENGTH: 1377
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1) ... (1374)

<400> SEQUENCE: 15

atg gtc agc tac tgg gac acc ggg gtc ctg ctg tgc gcg ctg ctc agc
 Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
 1 5 10 15

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tgt ctg ctt ctc aca gga tct agt tcc gga agt gat acc ggt aga cct	96
Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Ser Asp Thr Gly Arg Pro	
20 25 30	
ttc gta gag atg tac agt gaa atc ccc gaa att ata cac atg act gaa	144
Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu	
35 40 45	
gga agg gag ctc gtc att ccc tgc cgg gtt acg tca cct aac atc act	192
Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr	
50 55 60	
gtt act tta aaa aag ttt cca ctt gac act ttg atc cct gat gga aaa	240
Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys	
65 70 75 80	
cgc ata atc tgg gac agt aga aag ggc ttc atc ata tca aat gca acg	288
Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr	
85 90 95	
tac aaa gaa ata ggg ctt ctg acc tgt gaa gca aca gtc aat ggg cat	336
Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His	
100 105 110	
ttg tat aag aca aac tat ctc aca cat cga caa acc aat aca atc ata	384
Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile	
115 120 125	
gat gtg gtt ctg agt ccg tct cat gga att gaa cta tct gtt gga gaa	432
Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu	
130 135 140	
aag ctt gtc tta aat tgt aca gca aga act gaa cta aat gtg ggg att	480
Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile	
145 150 155 160	
gac ttc aac tgg gaa tac cct tct tcg aag cat cag cat aag aaa ctt	528
Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu	
165 170 175	
gta aac cga gac cta aaa acc cag tct ggg agt gag atg aag aaa ttt	576
Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe	
180 185 190	
ttg agc acc tta act ata gat ggt gta acc cgg agt gac caa gga ttg	624
Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu	
195 200 205	
tac acc tgt gca gca tcc agt ggg ctg atg acc aag aag aac agc aca	672
Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr	
210 215 220	
ttt gtc agg gtc cat gaa aag gac aaa act cac aca tgc cca ccg tgc	720
Phe Val Arg Val His Glu Lys Asp Lys Thr His Thr Cys Pro Pro Cys	
225 230 235 240	
cca gca cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca	768
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro	
245 250 255	
aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc	816
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys	
260 265 270	
gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg	864
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp	
275 280 285	
tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag	912
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu	
290 295 300	
gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg	960
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu	
305 310 315 320	
cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac	1008
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn	
325 330 335	

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aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg 1056
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
      340                      345                      350

cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag 1104
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
      355                      360                      365

ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat 1152
Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
      370                      375                      380

ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac 1200
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
      385                      390                      395

aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc 1248
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
      405                      410                      415

ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac 1296
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
      420                      425                      430

gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg 1344
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
      435                      440                      445

cag aag agc ctc tcc ctg tct cgg ggt aaa tga 1377
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
      450                      455
    
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<210> SEQ ID NO 16
<211> LENGTH: 458
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
    
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<400> SEQUENCE: 16

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Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
 1      5      10      15

Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Ser Asp Thr Gly Arg Pro
 20      25      30

Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu
 35      40      45

Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr
 50      55      60

Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys
 65      70      75      80

Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr
 85      90      95

Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His
100     105     110

Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile
115     120     125

Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu
130     135     140

Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile
145     150     155     160

Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
165     170     175

Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
180     185     190

Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
195     200     205
    
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Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
 210 215
 Phe Val Arg Val His Glu Lys Asp Lys Thr His Thr Cys Pro Pro Cys
 225 230 235 240
 Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
 245 250 255
 Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
 260 265 270
 Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
 275 280 285
 Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
 290 295 300
 Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
 305 310 315 320
 His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
 325 330 335
 Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
 340 345 350
 Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
 355 360 365
 Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
 370 375 380
 Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
 385 390 395 400
 Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
 405 410 415
 Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
 420 425 430
 Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
 435 440 445
 Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 450 455

<210> SEQ ID NO 17
 <211> LENGTH: 430
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His
 1 5 10 15
 Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro
 20 25 30
 Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro
 35 40 45
 Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser
 50 55 60
 Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val
 65 70 75 80
 Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn
 85 90 95
 Thr Ile Ile Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser
 100 105 110
 Val Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn

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115			120			125									
Val	Gly	Ile	Asp	Phe	Asn	Trp	Glu	Tyr	Pro	Ser	Ser	Lys	His	Gln	His
130						135						140			
Lys	Lys	Leu	Val	Asn	Arg	Asp	Leu	Lys	Thr	Gln	Ser	Gly	Ser	Glu	Met
145				150						155					160
Lys	Lys	Phe	Leu	Ser	Thr	Leu	Thr	Ile	Asp	Gly	Val	Thr	Arg	Ser	Asp
			165						170					175	
Gln	Gly	Leu	Tyr	Thr	Cys	Ala	Ala	Ser	Ser	Gly	Leu	Met	Thr	Lys	Lys
			180						185					190	
Asn	Ser	Thr	Phe	Val	Arg	Val	His	Glu	Lys	Gly	Pro	Gly	Asp	Lys	Thr
			195					200				205			
His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser
						215						220			
Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg
225					230						235				240
Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro
					245					250				255	
Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala
			260					265						270	
Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val
			275				280					285			
Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr
			290			295					300				
Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr
305					310					315					320
Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu
				325						330				335	
Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys
			340					345					350		
Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser
			355				360					365			
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp
			370			375					380				
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser
385					390					395					400
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala
				405					410					415	
Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Pro	Gly	Lys		
			420					425					430		

<210> SEQ ID NO 18
 <211> LENGTH: 36
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: primer

 <400> SEQUENCE: 18
 gactagcagt ccggaggtag acctttcgta gagatg

<210> SEQ ID NO 19
 <211> LENGTH: 33
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: primer

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<400> SEQUENCE: 19
 cggactcaga accacatcta tgattgtatt ggt 33

<210> SEQ ID NO 20
 <211> LENGTH: 7
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20
 Gly Arg Pro Phe Val Glu Met
 1 5

<210> SEQ ID NO 21
 <211> LENGTH: 35
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: primer

<400> SEQUENCE: 21
 acaatcatag atgtggttct gactcctct catgg 35

<210> SEQ ID NO 22
 <211> LENGTH: 38
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: primer

<400> SEQUENCE: 22
 gataatgccc gggccctttt catggaccct gacaaatg 38

<210> SEQ ID NO 23
 <211> LENGTH: 6
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 23
 Val Arg Val His Glu Lys
 1 5

<210> SEQ ID NO 24
 <211> LENGTH: 36
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: primer

<400> SEQUENCE: 24
 gactagcagt ccggaggtag acctttcgta gagatg 36

<210> SEQ ID NO 25
 <211> LENGTH: 38
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: primer

<400> SEQUENCE: 25
 ttctctggca acagctggat atctatgatt gtattggt 38

<210> SEQ ID NO 26
 <211> LENGTH: 4
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 26

Ile Gln Leu Leu
1<210> SEQ ID NO 27
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 27

atccagctgt tgcccaggaa gtcgctggag ctgctggta 39

<210> SEQ ID NO 28
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 28

attttcatgc acaatgaact cgggtctctc ccgaaatcg 39

<210> SEQ ID NO 29
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 29

tcatagatat ccagctgttg cccaggaagt cgctggag 38

<210> SEQ ID NO 30
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 30

gataatgcc gggccatttt catgcacaat gacctcggg 39

<210> SEQ ID NO 31
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 31

Val Ile Val His Glu Asn
1 5<210> SEQ ID NO 32
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: modified Flt1 receptor

<400> SEQUENCE: 32

Lys Asn Lys Arg Ala Ser Val Arg Arg Arg
1 5 10

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<210> SEQ ID NO 33
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: modified Flt1 receptor

<400> SEQUENCE: 33

Asn Ala Ser Val Asn Gly Ser Arg
1 5

<210> SEQ ID NO 34
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: modified Flt1 receptor

<400> SEQUENCE: 34

Lys Asn Lys Cys Ala Ser Val Arg Arg Arg
1 5 10

<210> SEQ ID NO 35
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 35

Ser Lys Leu Lys
1

<210> SEQ ID NO 36
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 36

Lys Asn Lys Arg Ala Ser Val Arg Arg
1 5

<210> SEQ ID NO 37
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 37

Thr Ile Ile Asp
1

<210> SEQ ID NO 38
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 38

Val Val Leu Ser
1

97

We claim:

1. 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 human Flt1 and Ig domain 3 of a second VEGF receptor human Flk1; and
 - (b) a multimerizing component, wherein the fusion protein binds VEGF.
2. The fusion protein of claim 1, wherein the first VEGF receptor component is upstream of the second VEGF receptor component.
3. The fusion protein of claim 1, wherein the first VEGF receptor component is downstream of the second VEGF receptor component.
4. The fusion protein of claim 1, wherein the multimerizing component comprises an immunoglobulin domain.

98

5. The fusion protein of claim 4, wherein the immunoglobulin domain is selected from the group consisting of the Fc domain of IgG, and the heavy chain of IgG.
6. A fusion protein encoded by a nucleic acid sequence selected from:
 - (a) SEQ ID NO:15; and
 - (b) nucleic acid sequences which, as a result of the degeneracy of the genetic code, differ from the nucleic acid sequence of SEQ ID NO:15.
7. A fusion protein consisting of immunoglobulin-like (Ig) domain 2 of a first vascular endothelial growth factor (VEGF) receptor upstream of Ig domain 3 of a second VEGF receptor and a multimerizing component, wherein the fusion protein comprises the amino acid sequence SEQ ID NO:16.

* * * * *