

SEQUENCE LISTING

<110> National Cancer Center Japan
Rin Institute Inc.

<120> Fusion protein suitable for use in dissolving fibrin clot and
pharmaceutical composition comprising the same

<130> PR15-9006WO

<150> JP2020-062316

<151> 2020-03-31

<160> 19

<170> PatentIn version 3.5

<210> 1

<211> 411

<212> PRT

<213> Homo sapiens

<400> 1

Ser Asn Glu Leu His Gln Val Pro Ser Asn Cys Asp Cys Leu Asn Gly
1 5 10 15

Gly Thr Cys Val Ser Asn Lys Tyr Phe Ser Asn Ile His Trp Cys Asn
20 25 30

Cys Pro Lys Lys Phe Gly Gly Gln His Cys Glu Ile Asp Lys Ser Lys
35 40 45

Thr Cys Tyr Glu Gly Asn Gly His Phe Tyr Arg Gly Lys Ala Ser Thr
50 55 60

Asp Thr Met Gly Arg Pro Cys Leu Pro Trp Asn Ser Ala Thr Val Leu
65 70 75 80

Gln Gln Thr Tyr His Ala His Arg Ser Asp Ala Leu Gln Leu Gly Leu

85

90

95

Gly Lys His Asn Tyr Cys Arg Asn Pro Asp Asn Arg Arg Arg Pro Trp
 100 105 110

Cys Tyr Val Gln Val Gly Leu Lys Pro Leu Val Gln Glu Cys Met Val
 115 120 125

His Asp Cys Ala Asp Gly Lys Lys Pro Ser Ser Pro Pro Glu Glu Leu
 130 135 140

Lys Phe Gln Cys Gly Gln Lys Thr Leu Arg Pro Arg Phe Lys Ile Ile
 145 150 155 160

Gly Gly Glu Phe Thr Thr Ile Glu Asn Gln Pro Trp Phe Ala Ala Ile
 165 170 175

Tyr Arg Arg His Arg Gly Gly Ser Val Thr Tyr Val Cys Gly Gly Ser
 180 185 190

Leu Ile Ser Pro Cys Trp Val Ile Ser Ala Thr His Cys Phe Ile Asp
 195 200 205

Tyr Pro Lys Lys Glu Asp Tyr Ile Val Tyr Leu Gly Arg Ser Arg Leu
 210 215 220

Asn Ser Asn Thr Gln Gly Glu Met Lys Phe Glu Val Glu Asn Leu Ile
 225 230 235 240

Leu His Lys Asp Tyr Ser Ala Asp Thr Leu Ala His His Asn Asp Ile
 245 250 255

Ala Leu Leu Lys Ile Arg Ser Lys Glu Gly Arg Cys Ala Gln Pro Ser
 260 265 270

Arg Thr Ile Gln Thr Ile Cys Leu Pro Ser Met Tyr Asn Asp Pro Gln
275 280 285

Phe Gly Thr Ser Cys Glu Ile Thr Gly Phe Gly Lys Glu Asn Ser Thr
290 295 300

Asp Tyr Leu Tyr Pro Glu Gln Leu Lys Met Thr Val Val Lys Leu Ile
305 310 315 320

Ser His Arg Glu Cys Gln Gln Pro His Tyr Tyr Gly Ser Glu Val Thr
325 330 335

Thr Lys Met Leu Cys Ala Ala Asp Pro Gln Trp Lys Thr Asp Ser Cys
340 345 350

Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Ser Leu Gln Gly Arg Met
355 360 365

Thr Leu Thr Gly Ile Val Ser Trp Gly Arg Gly Cys Ala Leu Lys Asp
370 375 380

Lys Pro Gly Val Tyr Thr Arg Val Ser His Phe Leu Pro Trp Ile Arg
385 390 395 400

Ser His Thr Lys Glu Glu Asn Gly Leu Ala Leu
405 410

<210> 2

<211> 411

<212> PRT

<213> Artificial Sequence

<220>

<223> Human pro-urokinase mutant K135G, K136G

<400> 2

Ser Asn Glu Leu His Gln Val Pro Ser Asn Cys Asp Cys Leu Asn Gly
1 5 10 15

Gly Thr Cys Val Ser Asn Lys Tyr Phe Ser Asn Ile His Trp Cys Asn
20 25 30

Cys Pro Lys Lys Phe Gly Gly Gln His Cys Glu Ile Asp Lys Ser Lys
35 40 45

Thr Cys Tyr Glu Gly Asn Gly His Phe Tyr Arg Gly Lys Ala Ser Thr
50 55 60

Asp Thr Met Gly Arg Pro Cys Leu Pro Trp Asn Ser Ala Thr Val Leu
65 70 75 80

Gln Gln Thr Tyr His Ala His Arg Ser Asp Ala Leu Gln Leu Gly Leu
85 90 95

Gly Lys His Asn Tyr Cys Arg Asn Pro Asp Asn Arg Arg Arg Pro Trp
100 105 110

Cys Tyr Val Gln Val Gly Leu Lys Pro Leu Val Gln Glu Cys Met Val
115 120 125

His Asp Cys Ala Asp Gly Gly Gly Pro Ser Ser Pro Pro Glu Glu Leu
130 135 140

Lys Phe Gln Cys Gly Gln Lys Thr Leu Arg Pro Arg Phe Lys Ile Ile
145 150 155 160

Gly Gly Glu Phe Thr Thr Ile Glu Asn Gln Pro Trp Phe Ala Ala Ile
165 170 175

Tyr Arg Arg His Arg Gly Gly Ser Val Thr Tyr Val Cys Gly Gly Ser
180 185 190

Leu Ile Ser Pro Cys Trp Val Ile Ser Ala Thr His Cys Phe Ile Asp
195 200 205

Tyr Pro Lys Lys Glu Asp Tyr Ile Val Tyr Leu Gly Arg Ser Arg Leu
210 215 220

Asn Ser Asn Thr Gln Gly Glu Met Lys Phe Glu Val Glu Asn Leu Ile
225 230 235 240

Leu His Lys Asp Tyr Ser Ala Asp Thr Leu Ala His His Asn Asp Ile
245 250 255

Ala Leu Leu Lys Ile Arg Ser Lys Glu Gly Arg Cys Ala Gln Pro Ser
260 265 270

Arg Thr Ile Gln Thr Ile Cys Leu Pro Ser Met Tyr Asn Asp Pro Gln
275 280 285

Phe Gly Thr Ser Cys Glu Ile Thr Gly Phe Gly Lys Glu Asn Ser Thr
290 295 300

Asp Tyr Leu Tyr Pro Glu Gln Leu Lys Met Thr Val Val Lys Leu Ile
305 310 315 320

Ser His Arg Glu Cys Gln Gln Pro His Tyr Tyr Gly Ser Glu Val Thr
325 330 335

Thr Lys Met Leu Cys Ala Ala Asp Pro Gln Trp Lys Thr Asp Ser Cys
340 345 350

Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Ser Leu Gln Gly Arg Met
355 360 365

Thr Leu Thr Gly Ile Val Ser Trp Gly Arg Gly Cys Ala Leu Lys Asp
370 375 380

Lys Pro Gly Val Tyr Thr Arg Val Ser His Phe Leu Pro Trp Ile Arg
385 390 395 400

Ser His Thr Lys Glu Glu Asn Gly Leu Ala Leu
405 410

<210> 3

<211> 675

<212> PRT

<213> Artificial Sequence

<220>

<223> Fusion protein of pro-urokinase mutant and Fab region of heavy
chain of anti-fibrin antibody 1101 clone

<400> 3

Met Gly Ser Thr Ala Ile Leu Ala Leu Leu Ala Val Leu Gln Gly
1 5 10 15

Val Cys Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
20 25 30

Pro Gly Glu Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe
35 40 45

Thr Ser Tyr Trp Met His Trp Val Arg Gln Met Pro Gly Lys Gly Leu
50 55 60

Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Ser Asp Thr Arg Tyr Ser

65 70 75 80

Pro Ser Phe Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser
 85 90 95

Thr Ala Tyr Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met
 100 105 110

Tyr Tyr Cys Thr Arg Lys Ala His Tyr Gly Asn Tyr Gly Phe Ala Tyr
 115 120 125

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
 130 135 140

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
145 150 155 160

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
 165 170 175

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
 180 185 190

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
 195 200 205

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
 210 215 220

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
225 230 235 240

Ser Cys Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
 245 250 255

Ser Ser Ser Asn Glu Leu His Gln Val Pro Ser Asn Cys Asp Cys Leu
260 265 270

Asn Gly Gly Thr Cys Val Ser Asn Lys Tyr Phe Ser Asn Ile His Trp
275 280 285

Cys Asn Cys Pro Lys Lys Phe Gly Gly Gln His Cys Glu Ile Asp Lys
290 295 300

Ser Lys Thr Cys Tyr Glu Gly Asn Gly His Phe Tyr Arg Gly Lys Ala
305 310 315 320

Ser Thr Asp Thr Met Gly Arg Pro Cys Leu Pro Trp Asn Ser Ala Thr
325 330 335

Val Leu Gln Gln Thr Tyr His Ala His Arg Ser Asp Ala Leu Gln Leu
340 345 350

Gly Leu Gly Lys His Asn Tyr Cys Arg Asn Pro Asp Asn Arg Arg Arg
355 360 365

Pro Trp Cys Tyr Val Gln Val Gly Leu Lys Pro Leu Val Gln Glu Cys
370 375 380

Met Val His Asp Cys Ala Asp Gly Gly Gly Pro Ser Ser Pro Pro Glu
385 390 395 400

Glu Leu Lys Phe Gln Cys Gly Gln Lys Thr Leu Arg Pro Arg Phe Lys
405 410 415

Ile Ile Gly Gly Glu Phe Thr Thr Ile Glu Asn Gln Pro Trp Phe Ala
420 425 430

Ala Ile Tyr Arg Arg His Arg Gly Gly Ser Val Thr Tyr Val Cys Gly
435 440 445

Gly Ser Leu Ile Ser Pro Cys Trp Val Ile Ser Ala Thr His Cys Phe
450 455 460

Ile Asp Tyr Pro Lys Lys Glu Asp Tyr Ile Val Tyr Leu Gly Arg Ser
465 470 475 480

Arg Leu Asn Ser Asn Thr Gln Gly Glu Met Lys Phe Glu Val Glu Asn
485 490 495

Leu Ile Leu His Lys Asp Tyr Ser Ala Asp Thr Leu Ala His His Asn
500 505 510

Asp Ile Ala Leu Leu Lys Ile Arg Ser Lys Glu Gly Arg Cys Ala Gln
515 520 525

Pro Ser Arg Thr Ile Gln Thr Ile Cys Leu Pro Ser Met Tyr Asn Asp
530 535 540

Pro Gln Phe Gly Thr Ser Cys Glu Ile Thr Gly Phe Gly Lys Glu Asn
545 550 555 560

Ser Thr Asp Tyr Leu Tyr Pro Glu Gln Leu Lys Met Thr Val Val Lys
565 570 575

Leu Ile Ser His Arg Glu Cys Gln Gln Pro His Tyr Tyr Gly Ser Glu
580 585 590

Val Thr Thr Lys Met Leu Cys Ala Ala Asp Pro Gln Trp Lys Thr Asp
595 600 605

Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Ser Leu Gln Gly
610 615 620

Arg Met Thr Leu Thr Gly Ile Val Ser Trp Gly Arg Gly Cys Ala Leu
625 630 635 640

Lys Asp Lys Pro Gly Val Tyr Thr Arg Val Ser His Phe Leu Pro Trp
645 650 655

Ile Arg Ser His Thr Lys Glu Glu Asn Gly Leu Ala Leu Ser Ala Gly
660 665 670

Gly Ala Ser
675

<210> 4

<211> 236

<212> PRT

<213> Artificial Sequence

<220>

<223> Light chain of humanized 1101 clone

<400> 4

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
20 25 30

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
35 40 45

Gln His Ile Asn Asn Trp Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys
50 55 60

Ala Pro Lys Leu Leu Ile Ser Gly Ala Thr Ser Leu Gln Ser Gly Val
65 70 75 80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
 85 90 95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
 100 105 110

Tyr Trp Ser Thr Pro Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
 115 120 125

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
 130 135 140

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145 150 155 160

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
 165 170 175

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
 180 185 190

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
 195 200 205

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
 210 215 220

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 5
<211> 242
<212> PRT
<213> Artificial Sequence

<220>
<223> Fab region of heavy chain of humanized 1101 clone

<400> 5

Met Gly Ser Thr Ala Ile Leu Ala Leu Leu Ala Val Leu Gln Gly
1 5 10 15

Val Cys Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
20 25 30

Pro Gly Glu Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe
35 40 45

Thr Ser Tyr Trp Met His Trp Val Arg Gln Met Pro Gly Lys Gly Leu
50 55 60

Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Ser Asp Thr Arg Tyr Ser
65 70 75 80

Pro Ser Phe Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser
85 90 95

Thr Ala Tyr Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met
100 105 110

Tyr Tyr Cys Thr Arg Lys Ala His Tyr Gly Asn Tyr Gly Phe Ala Tyr
115 120 125

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
130 135 140

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
145 150 155 160

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
165 170 175

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
180 185 190

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
195 200 205

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
210 215 220

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
225 230 235 240

Ser Cys

<210> 6

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer 1

<400> 6

acttgaattc caccatgggc tctacagcaa tcctcgcttt g 41

<210> 7

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer 2

<400> 7

ataaggatcc ttaccgga gacagggaga ggctcttc

38

<210> 8

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer 3

<400> 8

gaggctcgag tcttaagttt caatgcggcc ag

32

<210> 9

<211> 78

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer 4

<400> 9

ataagcggcc gctcaatggt ggtggtggtg atgatggtga cttgctccgc ctgcggaaag 60

cgccagacca ttttcttc

78

<210> 10

<211> 102

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer 5

<400> 10

gaagagcctc tccctgtctc cggttaaagg atccggtgga ggtggcagtg gtgggggagg 60

ctcaggaggc tcgagtctta agtttcaatg cggccagaaa ac

102

<210> 11

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> HCDR1 of humanized 1101 clone

<400> 11

Ser Tyr Trp Met His

1 5

<210> 12

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> HCDR2 of humanized 1101 clone

<400> 12

Ala Ile Tyr Pro Gly Asn Ser Asp Thr Arg Tyr Ser Pro Ser Phe Gln

1 5 10 15

Gly

<210> 13

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> HCDR3 of humanized 1101 clone

<400> 13

Lys Ala His Tyr Gly Asn Tyr Gly Phe Ala Tyr
1 5 10

<210> 14

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> LCDR1 of humanized 1101 clone

<400> 14

Arg Ala Ser Gln His Ile Asn Asn Trp Leu Ala
1 5 10

<210> 15

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> LCDR2 of humanized 1101 clone

<400> 15

Gly Ala Thr Ser Leu Gln Ser
1 5

<210> 16

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> LCDR3 of humanized 1101 clone

<400> 16

Gln Gln Tyr Trp Ser Thr Pro Leu Thr
1 5

<210> 17
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Fibrinogen Bbeta 231-246

<400> 17

Cys Asn Ile Pro Val Val Ser Gly Lys Glu Cys Glu Glu Ile Ile Arg
1 5 10 15

<210> 18
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Fibrinogen gamma 232-246

<400> 18

Lys Asn Trp Ile Gln Tyr Lys Glu Gly Phe Gly His Leu Ser Pro
1 5 10 15

<210> 19
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> linker used in Examples

<400> 19

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Ser Ser
1 5 10 15