



US005889157A

United States Patent [19]

[11] Patent Number: **5,889,157**

Pastan et al.

[45] Date of Patent: ***Mar. 30, 1999**

[54] **HUMANIZED B3 ANTIBODY FRAGMENTS, FUSION PROTEINS, AND USES THEREOF**

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[*] Notice: The term of this patent shall not extend beyond the expiration date of Pat. No. 5,242,813.

[21] Appl. No.: **331,396**

[22] Filed: **Oct. 28, 1994**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 767,331, Sep. 30, 1991, abandoned, which is a continuation-in-part of Ser. No. 596,289, Oct. 12, 1990, Pat. No. 5,242,813.

[51] Int. Cl.⁶ **C07K 16/00**; A61K 39/395

[52] U.S. Cl. **530/387.1**; 530/387.3; 530/387.5; 530/387.7; 530/388.1; 530/388.8; 424/133.1; 435/328; 435/7.1; 536/23.53

[58] Field of Search 530/387.1, 387.3, 530/387.5, 387.7, 388.1, 388.8, 390.5, 866, 867; 435/69.1, 69.7, 91.1, 7.1, 328; 536/23.53; 424/133.1

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[57] ABSTRACT

This invention provides for recombinant single chain antibodies capable of specifically binding to a Lewis^Y-related carbohydrate antigen and fusion proteins comprising these antibodies. More particularly, the invention provides for humanized chain Fv regions of the monoclonal antibodies B1, B3 and B5 and fusion proteins incorporating these humanized antibodies. The antibodies may comprise humanized variable heavy (V_H) chains, humanized variable light (V_L) chains, or both. The invention also provides for DNA sequences encoding the various humanized antibodies. In addition, the invention provides for methods of detecting cells bearing a Lewis^Y antigen in a patient and for methods of killing or inhibiting the growth of cells bearing a Lewis^Y antigen in a patient.

21 Claims, 17 Drawing Sheets

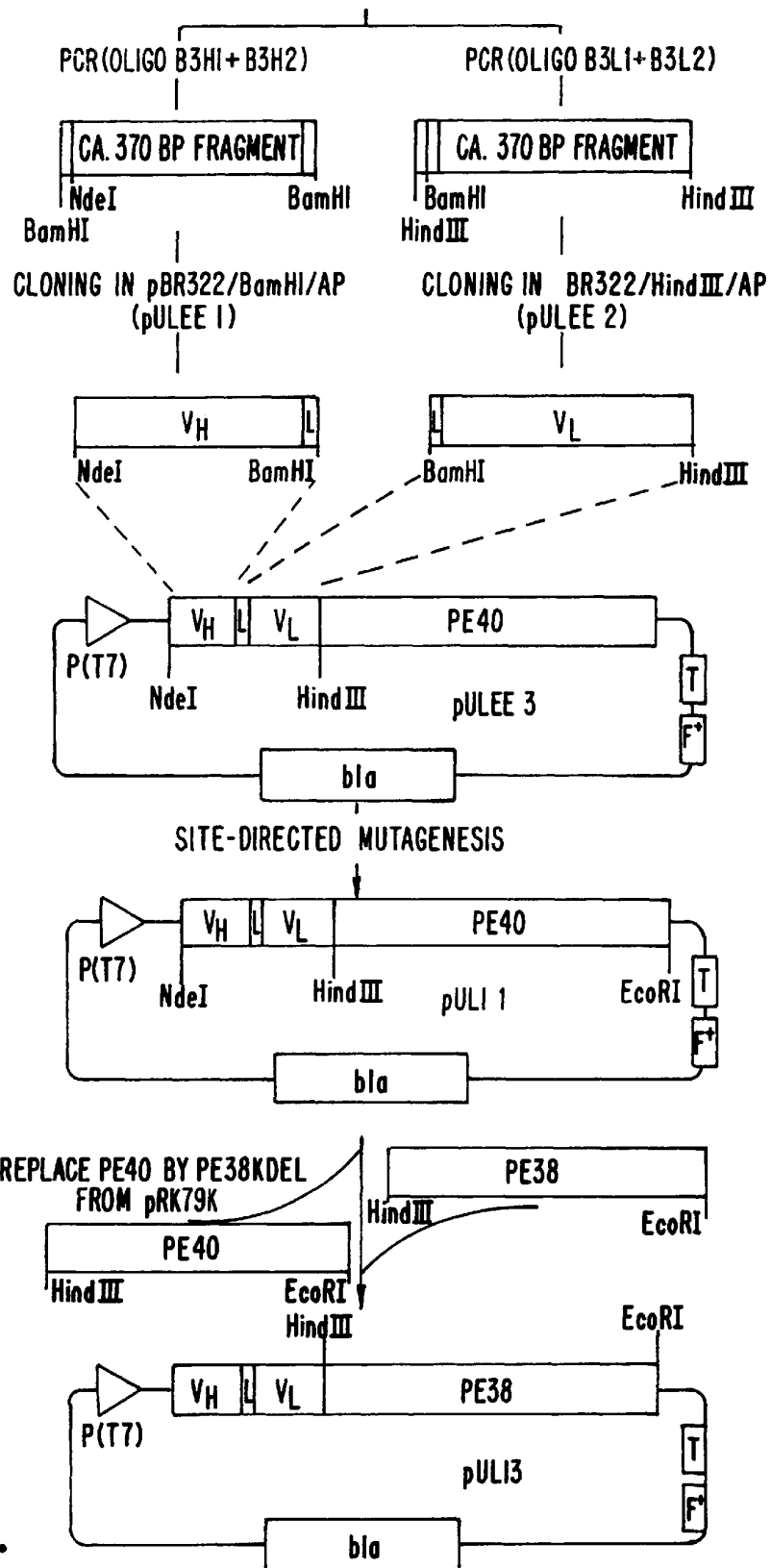


FIG. 1A.

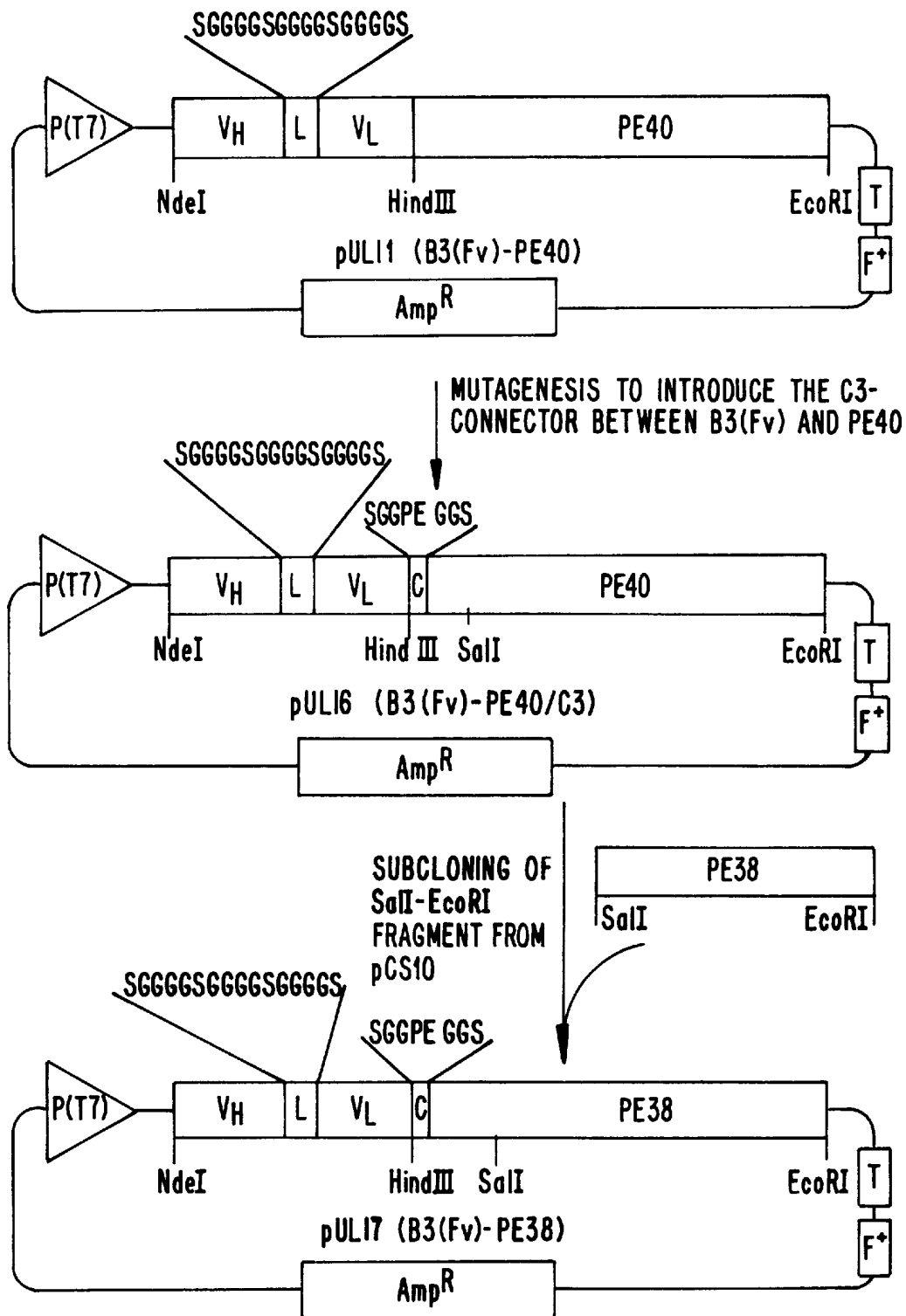


FIG. 1B.

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                                NdeI |----Fv HEAVY CHAIN--
1  TTTAACTTTAAGAAGGAGATATACATATGGATGTGAAGCTGGTGGAGTCT  50
                                SD          M D V K L V E S
                                           E V K L V E S
----->
51  GGGGGAGGCTTAGTGCAGCCTGGAGGGTCCCTGAAACTCTCCTGTGCAAC  100
    G G G L V Q P G G S L K L S C A T
    G G G L V Q P G G S L
101 CTCTGGATTCACTTTCAGTGACTATTACATGTATTGGGTTCCGCCAGACTC  150
    S G F T F S D Y Y M Y W V R Q T P
151 CAGAGAAGAGGCTGGAGTGGGTGCGATACATTAGTAATGATGATAGTTCC  200
    E K R L E W V A Y I S N D D S S
201 GCCGCTTATTCAGACACTGTAAAGGGCCGGTTCACCATCTCCAGAGACAA  250
    A A Y S D T V K G R F T I S R D N
251 TGCCAGGAACACCCTCTACCTGCAAATGAGCCGTCTGAAGTCTGAGGACA  300
    A R N T L Y L Q M S R L K S E D T
301 CAGCCATATATTCCTGTGCAAGAGGACTGGCCTGGGGAGCCTGGTTTGCT  350
    A I Y S C A R G L A W G A W F A
                                BamHI
351 TACTGGGGCCAAGGGACTCTGGTCACTGTCTCCTCAGGCGGAGGCGGATC  400
    Y W G Q G T L V T V S S G G G G S
    <----Fv HEAVY CHAIN--|-----LINKER-
-----LINKER-----|--Fv LIGHT CHAIN---
401 CGGTGGTGGCGGATCTGGAGGTGGCGGAAGCGATGTGCTGATGACCCAGT  450
    G G G G S G G G S D V L M T Q S
                                D V L M T Q S
-----Fv LIGHT CHAIN----->
451 CTCCATTGAGTTTACCTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTGC  500
    P L S L P V S L G D Q A S I S C
    P L S L P V S L G ? Q
501 AGATCTAGTCAGATCATTGTACATAGTAATGGAAACACCTATTTAGAATG  550
    R S S Q I I V M S N G N T Y L E W
551 GTACCTGCAGAAACCAGGCCAGTCTCAAAGCTCCTGATCTACAAAGTTT  600
    Y L Q K P G Q S P K L L I Y K V S

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FIG. 2A-1.

601 CCAACCGATTTTCTGGGGTCCCAGACAGGTTTCAGTGGCAGTGGATCAGGG 650
N R F S G V P D R F S G S G S G

651 ACAGATTTCACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGT 700
T D F T L K I S R V E A E D L G V

701 TTATTACTGCTTTCAAGGTTACATGTTCCATTCACGTTTCGGCTCGGGGA 750
Y Y C F Q G S N V P F T F G S G T

HindIII

751 CAAAGCTGGAAATTAAGCTTT..... 772
K L E I K A F -> PE40

FIG. 2A-2.

721 CACATGTTCCATTCACGTTTCGGCTCGGGGACAAAGCTGGAAATTAATAA 770
H V P F T F G S G T K L E I K *

EcoRI

771 TGAATTCC.. 779
* -> TERM

FIG. 2B.

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