



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**

[75] Inventors: **Ira Pastan**, Potomac; **Itai Benhar**, Rockville; **Eduardo A. Padlan**, Kensington; **Sun-Hee Jung**, Rockville; **Byungkook Lee**, Potomac, all of Md.

[73] Assignee: **The United States of America as represented by the Department of Health and Human Services**, Washington, D.C.

[*] 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

[56] References Cited

U.S. PATENT DOCUMENTS

4,545,985 10/1985 Pastan et al. .
4,867,962 9/1989 Abrams .
5,242,813 9/1993 Pastan et al. .
5,258,498 11/1993 Houston et al. 530/350

FOREIGN PATENT DOCUMENTS

93 07286 4/1993 WIPO .

OTHER PUBLICATIONS

Bast et al., *J. Clin. Invest.*, 68:1331-1337 (1981).
Batra et al., *J. Biol. Chem.* 265: 15198-15202 (1990).
Batra et al., *Proc. Natl. Acad. Sci. USA* 86: 8545-8549 (1989).
Better et al., *Science*, 240: 1041-1043 (1988).
Bird et al., *Science*, 242: 423-26 (1988).
Brinkmann et al., *Proc. Nat. Acad. Sci. USA* 89: 3075-3079 (1992).
Chaudhary et al., *Nature*, 339: 394-97 (1989).
Chaudhary et al., *Proc. Natl. Acad. Sci. USA* 87:9491-94 (1990).
Chaudhary et al., *Proc. Natl. Acad. Sci. USA* 87: 1066-1070 (1990).

Hartman et al., *E.M.B.O. J.* 3: 3023-3030 (1984).
Hellstrom et al., *Cancer Res.*, 50: 2183-2190 (1990).
Hoess et al., *Gene*, 128: 43-49 (1993).
Huston et al., *Proc. Natl. Acad. Sci. USA*, 85:5879-83 (1988).
Jones et al., *Nature*, 321: 522-525 (1986).
Jones et al., *Nature*, 321: 522-525(1986).
Kondo et al., *J. Biol. Chem.*, 263: 9470-75 (1988).
Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81: 6851-6855 (1984).
Pai et al., *Proc. Natl. Acad. Sci. USA*, 88: 3358-62 (1991).
Pastan et al., *Cancer Res.*, 51: 3781-3787 (1991).
Wawrzynczak et al., *Mol. Immunol.*, 29: 213-220 (1992).
Willingham et al., *Proc. Natl. Acad. Sci. USA*, 84: 2474-2478 (1987).
Brinkmann et al., *Bochem. Biophys Acta.*, 1198:27-45, 1994.
Proceedings of the National Academy of Sciences of the USA, vol. 88, No. 19, 1 Oct. 1991 Washington, DC, USA, pp. 8616-8620, U. Brinkmann et al. 'B3 (Fv)-PE38KDEL, a single-chain immunotoxin that causes complete regression of a human carcinoma in mice' cited in the application see abstract see FIGS. 1,2.

Bioconjugate Chemistry, vol. 5, No. 4, Jul. 1994 Washington, DC, USA, pp. 321-326, XP 000564453 I. Benhar et al. 'Mutations of two lysine residues in the CDR loops of a recombinant immunotoxin that reduce its sensitivity to chemical derivatization.' cited in the application see abstract see FIG. 1.

Cancer Research, vol. 53, No. 2, 15 Jan. 1993 Philadelphia, PA, USA, pp. 334-339, P. Friedman et al. 'BR96 sFv-PE40, a potent single-chain immunotoxin that selectively kills carcinoma cells.' see abstract see discussion.

The Journal of Immunology, vol. 152, No. 5, 1 Mar. 1994 Baltimore, MD, USA, pp. 2377-2384, C. Siegall et al., 'In vitro and in vivo characterization of BR96 sFv-PE40.' see abstract.

Primary Examiner—Frank C. Eisenschenk
Attorney, Agent, or Firm—Townsend and Townsend and Crew LLP

[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

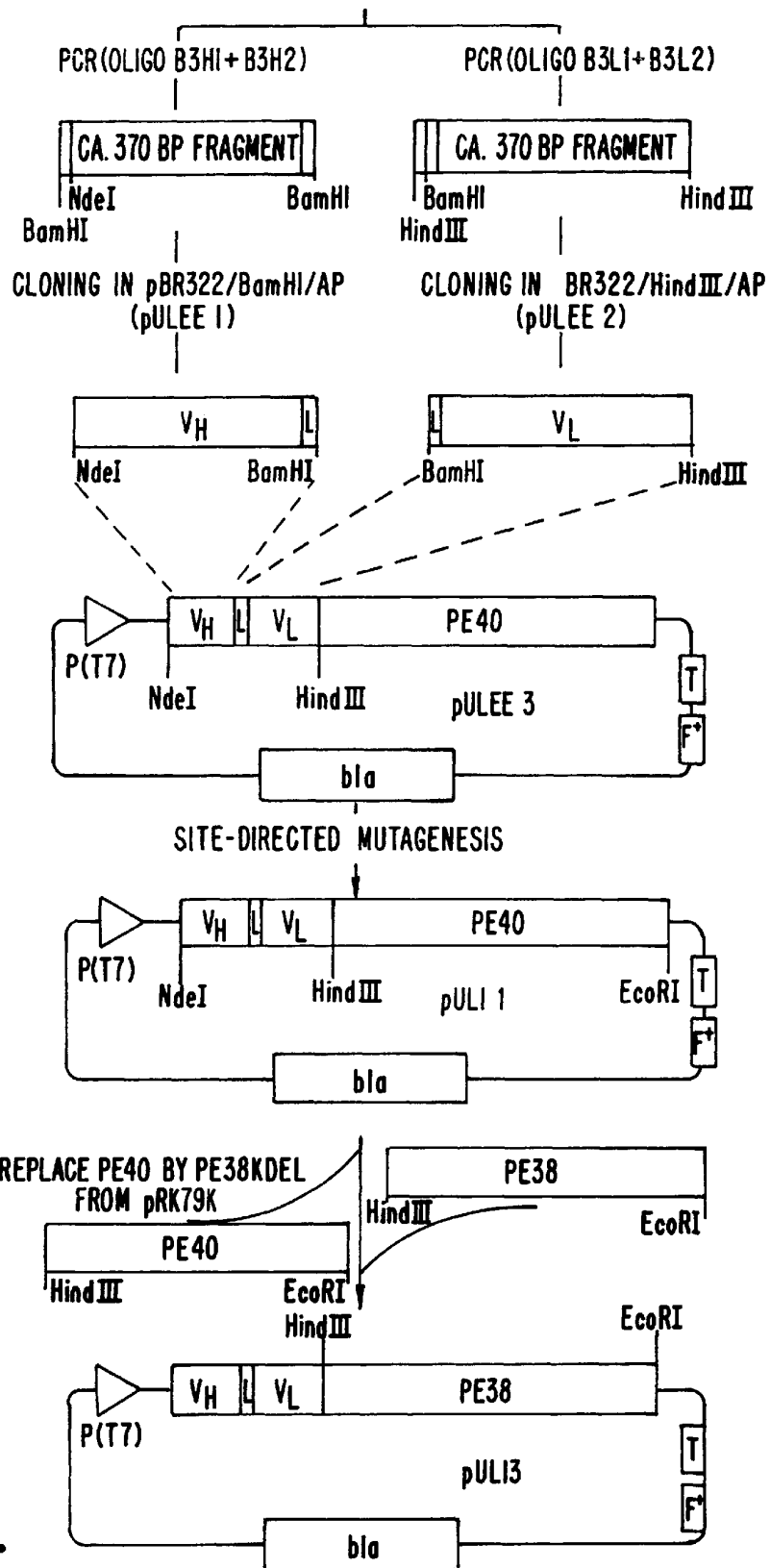


FIG. 1A.

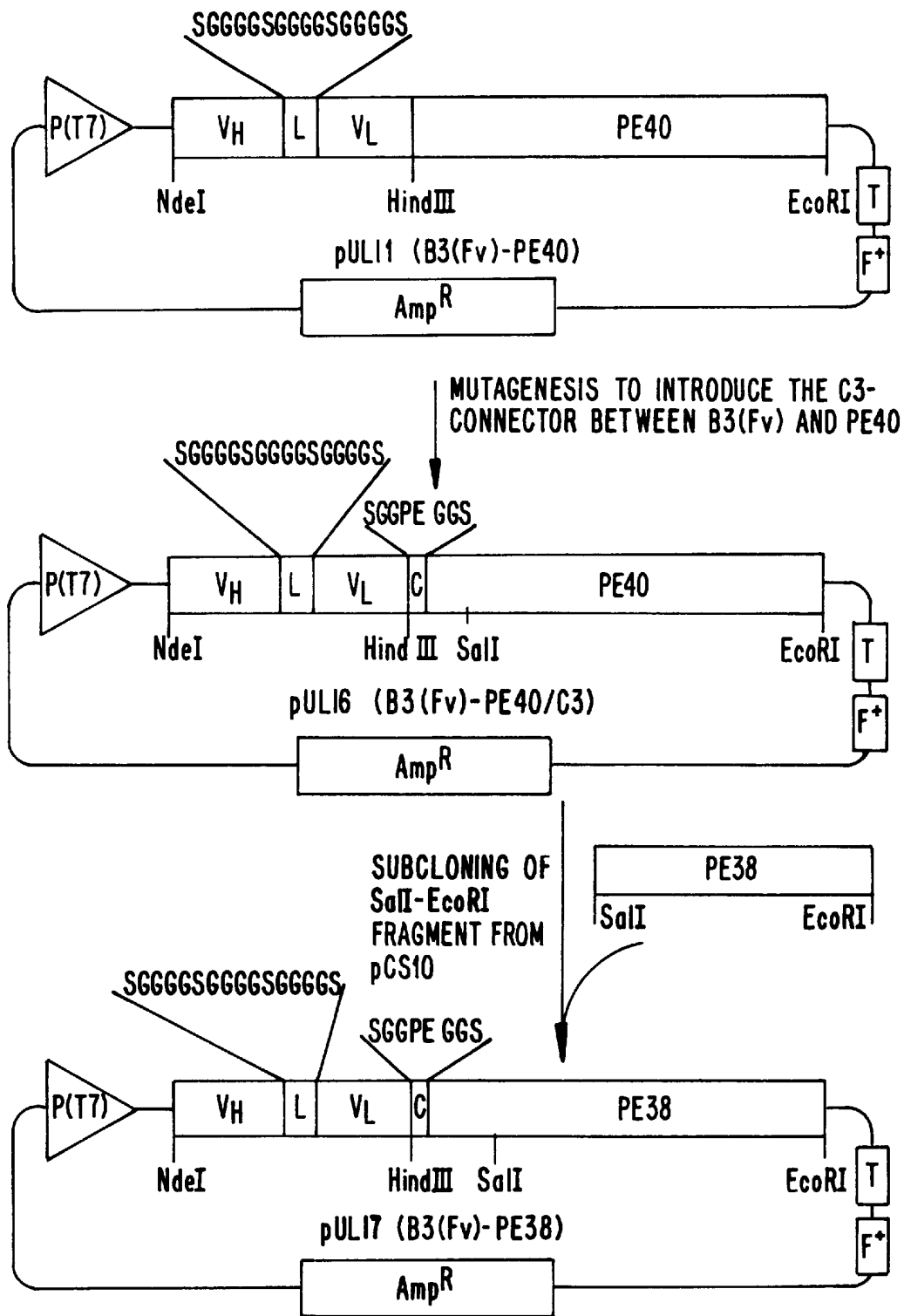


FIG. 1B.

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.

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.