



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 19/00, A61K 47/48, 31/365, 31/16, 31/57, 35/28 // (A61K 47/48, 31:365) (A61K 47/48, 31:16) (A61K 47/48, 31:57) (A61K 47/48, 35:28)	A3	(11) International Publication Number: WO 98/39363 (43) International Publication Date: 11 September 1998 (11.09.98)
(21) International Application Number: PCT/US98/04303 (22) International Filing Date: 5 March 1998 (05.03.98) (30) Priority Data: 60/039,987 5 March 1997 (05.03.97) US (71) Applicants (for all designated States except US): THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; National Institutes of Health, Office of Technology Transfer, Suite 325, 6011 Executive Boulevard, Rockville, MD 20852-3804 (US). UNIVERSITY OF ALABAMA RESEARCH FOUNDATION [US/US]; Suite 1120-G, 701 South 20th Street, Birmingham, AL 35294-0111 (US). WISCONSIN ALUMNI RESEARCH FOUNDATION [US/US]; 614 Walnut Street, Madison, WI 53705 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): NEVILLE, David, M. [US/US]; 9624 Parkwood Drive, Bethesda, MD 20814 (US). SCHARFF, Joshua, E. [US/US]; 4600 Harlan Street, Rockville, MD 20853 (US). KNECHTLE, Stuart [US/US];		2268 Caine Road, Oregon, WI 53575 (US). THOMAS, Judith, M. [US/US]; 2117 Brook Highland Ridge, Birmingham, AL 35242 (US). THOMPSON, Jerry, T. [US/US]; 103 Winchester Circle, Goose Creek, SC 29445 (US). HU, Huaizhong [CN/SG]; Blk D #05-08, 113 Clementi Road, Singapore 129793 (SG). MA, Shenglin [CN/US]; 911 16th Place South, Birmingham, AL 35205 (US). (74) Agents: SPRATT, Gwendolyn, D. et al.; Needle & Rosenberg, P.C., 127 Peachtree Street, N.E., Atlanta, GA 30303 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 1 April 1999 (01.04.99)
(54) Title: IMMUNOTOXINS AND METHODS OF INDUCING IMMUNE TOLERANCE		
(57) Abstract Provided are novel DT- and ETA-based immunotoxins and a method of treating an immune system disorder not involving T cell proliferation, comprising administering to the animal an immunotoxin comprising a mutant diphtheria toxin moiety linked to an antibody moiety which routes by the anti-CD3 pathway, or derivatives thereof under conditions such that the disorder is treated. Thus, the present method can treat graft-versus-host disease. Also provided is a method of inhibiting a rejection response by inducing immune tolerance in a recipient to a foreign mammalian donor tissue or cells, comprising the steps of: a) exposing the recipient to an immunotoxin so as to reduce the recipients' peripheral blood T-cell lymphocyte population by at least 80%, wherein the immunotoxin is anti-CD3 antibody linked to a diphtheria protein toxin, wherein the protein has a binding site mutation; and b) transplanting the donor cells into the recipient, whereby a rejection response by the recipient to the donor organ cell is inhibited, and the host is tolerized to the donor cell.		

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INTERNATIONAL SEARCH REPORT

International Application No

PCT, JS 98/04303

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K19/00 A61K47/48 A61K31/365 A61K31/16 A61K31/57
 A61K35/28 //(A61K47/48,31:365), (A61K47/48,31:16),
 (A61K47/48,31:57), (A61K47/48,35:28)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 32137 A (KNECHTLE STUART J ;THOMAS JUDITH (US); US HEALTH (US); NEVILLE DAV) 17 October 1996 see page 10, line 8 - page 27, line 12 see page 69 - page 85; examples 10,11 ---	1,3,5,7, 9,11,13, 15,17, 19, 21-23, 25-28, 30-42
X	MA ET AL: "EXPRESSION AND CHARACTERIZATION OF A DIVALENT CHIMERIC ANTI-HUMAN CD3 SINGLE CHAIN ANTIBODY" SCANDINAVIAN JOURNAL OF IMMUNOLOGY, vol. 43, 1996, pages 134-139, XP002083387 cited in the application see the whole document ---	1,3,5,7, 9,11,13, 15,17, 19, 21-23, 25-28, 30-42

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Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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Date of the actual completion of the international search

5 November 1998

Date of mailing of the international search report

16. 02. 1999

Name and mailing address of the ISA

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SITCH, D

INTERNATIONAL SEARCH REPORT

International Application No

PC1/US 98/04303

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ANAND ET AL: "BACTERIAL EXPRESSION AND SECRETION OF VARIOUS SINGLE-CHAIN FV GENES ENCODING PROTEINS SPECIFIC FOR A SALMONELLA SEROTYPE B O-ANTIGEN" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 266, no. 32, 15 November 1991, pages 21874-2879, XP002083388 cited in the application see page 21874 see abstract	21,22, 25,26
A	--- HOSAKA M ET AL: "ARG-X-LYS/ARG-ARG MOTIF AS A SIGNAL FOR PRECURSOR CLEAVAGE CATALYZED BY FURIN WITHIN THE CONSTITUTIVE SECRETORY PATHWAY" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 266, no. 19, 5 July 1991, pages 12127-12130, XP000672721 see page 12127 see abstract	9
A	--- THOMPSON ET AL: "AN ANTI-CD3 SINGLE-CHAIN IMMUNOTOXIN WITH A TRUNCATED DIPHTHERIA TOXIN AVOIDS INHIBITION BY PRE-EXISTING ANTIBODIES IN HUMAN BLOOD" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, 1995, pages 28037-28041, XP002083389 cited in the application see page 28037 see abstract	
A	--- NEVILLE ET AL: "IN VIVO T-CELL ABLATION BY A HOLO-IMMUNOTOXIN DIRECTED AT HUMAN CD3" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, USA, vol. 89, 1992, pages 2585-2589, XP002083390 cited in the application see page 2585 see abstract -----	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/04303

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 27, 28, 30-42 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see extra sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see extra sheet, subject 1.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 23,
28 (all completely), 21, 22, 25-27,
30-42 (all partially)

A divalent anti-T cell immunotoxin targeting moiety comprising at least the structure constituted by two monovalent antibody chains joined by a disulphide bond at C337 of residues 228-340 within the natural IgM domain in muCH2; a divalent anti-T cell immunotoxin comprising the above targeting moiety and a toxin moiety; a method of inhibiting a rejection response by inducing tolerance in a recipient to foreign mammalian donor cells involving the use of the above immunotoxin.

2. Claims: 2,4,6, 8, 10, 12, 14, 16, 18, 20, 24,
29 (all completely), 21, 22, 25, 26, 27,
30-42 (all partially)

A divalent anti-T cell immunotoxin targeting moiety comprising at least the structure constituted by two monovalent antibody chains joined by a disulphide bond at C227 or C229, or C227 and C229, of residues 216-238 of the gamma IgG hinge region, and wherein C220 is changed to P; a divalent anti-T cell immunotoxin comprising the above targeting moiety and a toxin moiety; a method of inhibiting a rejection response by inducing tolerance in a recipient to foreign mammalian donor cells involving the use of the above immunotoxin.

3. Claims: 21, 22, 25-27, 30-42 (all partially)

An immunotoxin being a fusion protein and having a sequence of domains from the amino terminus from left to right of: toxin moiety, VL, L, VH, L, VL, L, VH; a method of inhibiting a rejection response by inducing tolerance in a recipient to foreign mammalian donor cells involving the use of the above immunotoxin.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/04303

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9632137 A	17-10-1996	AU 5663796 A	30-10-1996
		CA 2218166 A	17-10-1996
		EP 0830146 A	25-03-1998
		US 5762927 A	09-06-1998
