



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification⁵ : A61K 37/02, 37/24</p>	<p>A1</p>	<p>(11) International Publication Number: WO 92/20364 (43) International Publication Date: 26 November 1992 (26.11.92)</p>
<p>(21) International Application Number: PCT/US92/04093 (22) International Filing Date: 15 May 1992 (15.05.92) (30) Priority data: 701,932 17 May 1991 (17.05.91) US (71) Applicant: SERAGEN, INC. [US/US]; 97 South Street, Hopkinton, MA 01748 (US). (72) Inventors: WATERS, Cory, Ann ; 2 Clark Road, Bedford, MA 01730 (US). POISSON, Louis, R. ; 14 Portland Street, Manville, RI 02838 (US). (74) Agent: CLARK, Paul, T.; Fish & Richardson, 225 Franklin Street, Boston, MA 02110 (US).</p>	<p>(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), MC (European patent), NL (European patent), SE (European patent).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>	
<p>(54) Title: CYTOKINE RECEPTOR TARGETED MOLECULES FOR TREATMENT OF NEOPLASTIC CELL GROWTH</p>		
<p>(57) Abstract</p> <p>The invention features a method for treating meoplastic cell growth in a patient, wherein the meoplastic cell is of non-lymphoid and non-monocytic origin. The method includes administering to the patient a molecule which is capable of specifically binding to a cytokine receptor expressed on the meoplastic cell, which molecule is capable of decreasing the viability of the meoplastic cell. The cytokine receptor is a receptor normally expressed on cells of lymphoid or monocytic origin.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FI	Finland	MI	Mali
AU	Australia	FR	France	MN	Mongolia
BB	Barbados	GA	Gabon	MR	Mauritania
BE	Belgium	GB	United Kingdom	MW	Malawi
BF	Burkina Faso	GN	Guinea	NL	Netherlands
BG	Bulgaria	GR	Greece	NO	Norway
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IE	Ireland	RO	Romania
CA	Canada	IT	Italy	RU	Russian Federation
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland	KR	Republic of Korea	SN	Senegal
CI	Côte d'Ivoire	LI	Liechtenstein	SU	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
DE	Germany	MC	Monaco	US	United States of America
DK	Denmark	MG	Madagascar		
ES	Spain				

- 1 -

CYTOKINE RECEPTOR TARGETED MOLECULES FOR
TREATMENT NEOPLASTIC CELL GROWTH

Background of the Invention

5 This invention relates to the treatment of
neoplastic cell growth, e.g., cancer.

 There have been many attempts to limit neoplastic
cell growth using drugs targeted to neoplastic cells.
Both monoclonal antibodies and receptor ligands have been
10 suggested as targeting agents.

 Murphy (United States Patent 4,675,382) suggests
that a cytotoxin/interleukin-2 hybrid protein could be
used for treatment of cancers involving the immune
system.

15 LeMaistre et al. (*Lancet* 337:1124, 1991) describe
the treatment of a patient with chronic lymphocytic
leukemia using a diphtheria toxin/interleukin-2 hybrid
molecule.

Summary of the Invention

20 In general, the invention features a method for
treating neoplastic cell growth in a patient, wherein the
neoplastic cell is of non-lymphoid and non-monocytic
origin. The method includes administering to the patient
a molecule which is capable of specifically binding to a
25 cytokine receptor expressed on the neoplastic cell, which
molecule is capable of decreasing the viability of the
neoplastic cell. The cytokine receptor is a receptor
normally expressed on cells of lymphoid or monocytic
origin.

30 In various preferred embodiments, the neoplastic
cell is not of hematopoietic stem cell origin; the
neoplastic cell is a sarcoma; the neoplastic cell is a

- 2 -

osteosarcoma; the neoplastic cell is a fibrosarcoma; the
neoplastic cell is a leiomyosarcoma; the neoplastic cell
is a carcinoma; and the neoplastic cell is a
rhabdomyosarcoma. In more preferred embodiments, the
5 carcinoma is a lung carcinoma; and the carcinoma is
hepatocellular carcinoma.

In a preferred embodiment, the cytokine receptor
is an interleukin receptor. In more preferred
embodiments, the interleukin receptor is an interleukin-
10 2 receptor; the interleukin receptor is an interleukin-4
receptor; and the interleukin receptor is an interleukin-
6 receptor. In an even more preferred embodiment, the
interleukin-2 receptor is a high affinity interleukin-2
receptor. In another preferred embodiment, the cytokine
15 receptor is not expressed on non-neoplastic cells of non-
lyphoid and non-monocytic origin.

In still other preferred embodiments, the molecule
kills cells bearing the cytokine receptor; the molecule
is a hybrid molecule includes a first and a second
20 portion joined together covalently, the first portion
includes a molecule capable of decreasing cell viability
and the second portion includes a molecule capable of
specifically binding to the cytokine receptor. In more
preferred embodiments, the second portion includes all or
25 a binding portion of an antibody specific for the
cytokine receptor; the second portion includes all or a
binding portion of a ligand for the cytokine receptor;
and the first portion includes a cytotoxin; and the
antibody is a complement activating antibody. In an even
30 more preferred embodiment, the cytokine receptor is an
interleukin receptor. In a yet more preferred
embodiment, the ligand is an interleukin.

In another preferred embodiment, the cytotoxin is
a fragment of a peptide toxin which is enzymatically
35 active but which does not possess generalized eukaryotic

- 3 -

receptor binding activity. In a more preferred embodiment, the fragment of a peptide toxin includes fragment A of diphtheria toxin and enough of fragment B of diphtheria toxin to facilitate translocation into the
5 cytosol.

In other preferred embodiments, the molecule is DAB₃₈₉IL-2; the molecule is DAB₃₈₉IL-4; the molecule is DAB₃₈₉IL-6; the molecule is DAB₄₈₆IL-2; the molecule is DAB₄₈₆IL-4; and the molecule is DAB₄₈₆IL-6.

10 By "non-lymphoid and non-monocytic origin" is meant all cells that are not descended from lymphoid stem cells or monocytes, e.g., cells other than T lymphocytes, B lymphocytes, and macrophages. By "lymphoid and monocytic origin" is meant cells descended from lymphoid
15 stem cells or monocytes, e.g., T lymphocytes, B lymphocytes, plasma cells, and macrophages. By "specifically binding" is meant that the molecule does not substantially bind to other cell surface receptors. By "not of hematopoietic stem cell origin" is meant all
20 cell are not descended from pluripotent stem cells.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Detailed Description

25 DAB₃₈₉IL-4 and DAB₄₈₆IL-2 Reduce the Viability of Several Cancer Cell Types

Our *in vitro* toxicity experiments have demonstrated that certain cancer cells are sensitive to interleukin-4 or interleukin-2 receptor targeted
30 cytotoxins. Accordingly these interleukin receptor-targeted cytotoxins, described in detail below, can provide a means by which to decrease the viability of cancer cells.

- 4 -

In the experiments described below various cancer cell lines are shown to be sensitive to a fusion protein (DAB₃₈₉IL-4) in which the receptor binding domain of diphtheria toxin (a single molecule of which, when present intracellularly, can block protein synthesis) has been replaced by a portion of human interleukin-4.

Various cell lines were exposed to DAB₃₈₉IL-4 and the level of protein synthesis was measured as described below. The results of this analysis are presented in Table 1 where the IC₅₀ is defined as the concentration of drug required to cause a 50% decrease in protein synthesis. Cancer cell lines from striated muscle, smooth muscle, liver, bone, and lung are sensitive to DAB₃₈₉IL-4; the IC₅₀ for these cell lines was found to be 10⁻¹⁰M to 10⁻⁸M.

Table 1: Sensitivity of Cancer Cells to DAB₃₈₉IL-4

Cell Line	*ATCC No.	Classification	** IC ₅₀ (M)
Hep G2	HB 8065	Human Hepatocellular Carcinoma, Hepatitis B ⁻	2.7 x 10 ⁻¹⁰
HT-1080	CCL 121	Human Fibrosarcoma	3.9 x 10 ⁻⁹
A673	CRL 1598	Human Rhabdomyosarcoma	2.7 x 10 ⁻⁹
A549	CCL 185	Human Lung Carcinoma	2.1 x 10 ⁻⁸
U-2 OS	HTB 96	Human Osteogenic Sarcoma, bone primary	2.6 x 10 ⁻⁸
SK-ES-1	HTB 86	Human Anaplastic Osteosarcoma, bone primary	2.2 x 10 ⁻⁸
SK-LMS-1	HTB 88	Human Leiomyosarcoma, vulva	1.5 x 10 ⁻⁸

* American Type Culture Collection (Bethesda, MD) accession number.

** The concentration of DAB₃₈₉IL-4 which leads to a 50% decrease in protein synthesis.

The sensitivity of these cancer cell lines is comparable to that of cells, such as HTLV-1 transformed T-lymphocytes and phytohemagglutinin activated T-lymphocytes, which are expected to express high numbers of interleukin-4 receptors (Table 2). In contrast, activated rat T-lymphocytes, which express an interleukin-4 receptor that is not recognized by human

- 5 -

interleukin-4, are not nearly as sensitive to DAB₃₈₉IL-4, even though they are sensitive to diphtheria toxin (Table 2). This result demonstrates that DAB₃₈₉IL-4 selectively intoxicates interleukin-4 receptor expressing cells.

5 **Table 2: DAB₃₈₉IL-4 Sensitivity of Normal and Neoplastic Cells Derived from Lymphocytes and Monocytes**

10	Cell or Cell Line	Classification	IC ₅₀ (M)
	T cell origin HUT 102/6TG C91/PL	Human, CTCL, HTLV-I ⁺ Human, HTLV-I ⁺ , transformed	2.9 X 10 ⁻¹¹ 6.3 X 10 ⁻¹¹
15	B cell origin Raji	Human, Burkitt's lymphoma EBV ⁺	7.2 X 10 ⁻¹⁰
	Myelomononuclear cell U937	Human, histiocytic lymphoma	2.0 X 10 ⁻⁹
20	Normal PBMC PHA activated T cells	Human	1.6 X 10 ⁻¹⁰
	Non-primate Con A-activated normal splenic T.cells	Rat	>10 ⁻⁷

25 * This is the concentration of drug which leads to a 50% decrease in protein synthesis.

A similar set of experiments demonstrated that human rhabdomyosarcoma cell line A673 (ATCC Accession No. CRL 1598) is sensitive to a fusion protein (DAB₄₈₆IL-2) in which the receptor binding domain of diphtheria toxin has
30 been replaced by a portion of human interleukin-2. DAB₄₈₆IL-2 at 7.6 x 10⁻⁸M decreased protein synthesis by 50%.

Assay for Sensitivity of Neoplastic Cells to Interleukin Receptor Targeted Cytotoxins

35 Cytotoxicity was evaluated by measuring protein synthesis. [¹⁴C]leucine incorporation in the presence and absence of DAB₃₈₉IL-4 (or DAB₄₈₆IL-2) as follows. Cells were plated 96 well microtiter plates in growth medium appropriate to the cell type. DAB₃₈₉IL-4 ((or DAB₄₈₆IL-2)

- 6 -

was added at various concentrations and the cultures were incubated for 20 hours prior to pulse labelling with [¹⁴C]leucine. Cells were then trypsinized, harvested onto glass fiber filter mats and counted. The IC₅₀ is the concentration of cytotoxin which leads to a 50% decrease in [¹⁴C]leucine incorporation.

The sensitivity of other cell types, including cells isolated from a tissue sample (e.g., a biopsy) obtained from a patient, can be evaluated as described above. Viability can also be measured using any standard viability assay appropriate to the cell type being studied. For example, viability can be determined using a trypan blue dye exclusion assay (Kruse et al., eds. *Tissue Culture: Methods and Applications*, Academic Press, 1989).

Molecules Useful in the Method of the Invention

The molecules useful in the method of the invention are targeted to a cytokine receptor. In general, there are three ways in which the molecules useful in the invention can act: (1) the molecule can kill a cell because the molecule has a cytotoxic domain; (2) the molecule (an antibody) can cause cell lysis by inducing complement fixation; and (3) the molecule can block binding or uptake of receptor's ligand. In all three cases the molecule must be targeted to receptor bearing cells; this is accomplished by including the receptor's ligand (or a portion or derivative thereof) or an anti-receptor antibody as part of the molecule.

Interleukin-2 (IL-2) receptor targeted molecules provide examples of each of these three approaches. A fusion molecule which includes the IL-2 receptor binding portion of IL-2 and a cytotoxin can be used to kill neoplastic cells bearing the interleukin-2 receptor. Likewise, the second type of molecule described above, a complement fixing antibody, in this instance directed

- 7 -

against the IL-2 receptor, can eliminate IL-2 receptor-bearing cells. In this example, the third type of molecule could be a molecule that blocks binding of IL-2 to its receptor. This molecule would prevent neoplastic
5 cells that bear the interleukin-2 receptor from receiving a proliferation signal from IL-2.

Molecules useful for treating neoplastic cell growth can take a number of forms. When IL-2 itself is the targeting agent, the molecule can be a cytotoxic
10 hybrid molecule in which IL-2 is fused to a toxin molecule, preferably a polypeptide toxin. Derivatives of IL-2 which bind to IL-2R, lack IL-2 activity and block binding and/or uptake of bona fide IL-2 are useful in the method of the invention because they will prevent IL-2-
15 induced proliferation of IL-2R bearing cells. When an anti-IL-2R antibody is the targeting agent, a cytotoxic hybrid molecule can be formed by fusing all or part of the antibody to a cytotoxin. The effectiveness of such an antibody/toxin hybrid, like that of an IL-2/toxin
20 hybrid, depends on the hybrid molecule being taken up by cells to which it binds. Anti-IL-2R antibodies which block binding and/or uptake of IL-2 are also useful in the method of the invention. Lytic anti-IL-2R antibodies are useful in the invention because they can cause
25 complement-mediated lysis of IL-2R-bearing cells.

Some of the molecules can be hybrid molecules formed by the fusion of all or part of two or more molecules. The hybrid molecule can be a hybrid protein encoded by a recombinant DNA molecule, in which case the
30 two domains are joined (directly or through an intermediary domain) by a peptide bond. Alternatively, two domains can be produced separately and joined by a covalent bond in a separate chemical linkage step. In some cases, the cytotoxic domain of a hybrid molecule may
35 itself be derived from two separate molecules.

- 8 -

Interleukin-2 as a Targeting Agent

Interleukin-2 (IL-2) or any IL-2 receptor binding derivative thereof can be used as a targeting agent for a cytotoxin. The DNA and amino acid sequences of IL-2 are known (Tadatsugu et al., *Nature* 302:305, 1983), and its structure has been predicted by x-ray crystallography (Brandhuber et al., *Science* 238:1707, 1987). Analysis of genetically engineered variants of IL-2 has provided some information concerning which residues are important for IL-2R binding (Collins et al., *Proc. Natl. Acad. Sci. USA* 85:7709, 1988) and bioactivity (Cohen et al. *Science* 234:349, 1989; Collins et al., *supra*). Variants of IL-2 which are useful in the invention include deletion mutants (Genbauffe et al., USSN 388,557, hereby incorporated by reference) which lack one or more amino acid residues in the region between residue 74 and residue 79 (numbering according to Williams et al., *Nucl. Acids Res.* 16:1045, 1988). These mutants effectively target toxins to IL-2R-bearing cells (Genbauffe et al., *supra*). Generally, IL-2 variants useful for targeting a cytotoxin must efficiently bind IL-2R and be endocytosed. The ability of various derivatives to bind to the IL-2 receptor can be tested with an IL-2R binding assay described below.

In designing molecules targeted to cells bearing the IL-2 receptor it must be recognized that the IL-2 receptor, like other receptors, has several forms; and it may be desirable to target cells bearing one form and not another. The human interleukin-2 receptor has a high-, an intermediate-, and a low-affinity form. The high affinity receptor has an apparent K_d of $\sim 10^{-10}$ M and is composed of two subunits, p55 and p75 (also called p70). When expressed on the cell surface, both the p75 and p55 subunits are capable of binding IL-2. The p75 subunit corresponds to the intermediate affinity receptor ($K_d \sim$

- 9 -

8.2 x 10⁻¹⁰M), and p55 subunit corresponds to the low affinity receptor (K_d ~ 1-3 x 10⁻⁸M). The p75 subunit is expressed on the surface of resting T cells, natural killer cells, monocytes/macrophages, and lymphokine-
5 activated killer (LAK) cell precursors, while the high affinity receptor is expressed on activated T- and B-cells.

In the method of the invention it may be desirable to target only cells bearing the high affinity receptor.
10 In these circumstances useful molecules will eliminate or neutralize cells bearing the high affinity IL-2 receptor at a concentration which leaves cells bearing the intermediate or low affinity receptor largely unaffected. When the molecule, like IL-2 itself, has affinity for all
15 three classes of IL-2 receptor, selectivity can be accomplished by administering the molecule at a concentration which does not permit significant binding to cells bearing lower affinity receptors. A hybrid molecule may have altered receptor affinities compared to
20 IL-2. Such hybrid molecules may be more or less selective for cells bearing the high affinity IL-2 receptor. For example, cells bearing the high-affinity receptor are 500-1000 times more sensitive to DAB₄₈₆IL-2, a fusion protein consisting of part of diphtheria toxin
25 and part of IL-2, than are cells bearing the intermediate- affinity receptor (Waters et al., *Eur. J. Immunol.* 20:785, 1990).

A cytotoxin can be attached to an IL-2 derivative in a number of ways. Preferably, an IL-2/toxin hybrid is
30 a hybrid protein produced by the expression of a fused gene. Alternatively, the cytotoxin and the IL-2 derivative can be produced separately and later coupled by means of a non-peptide covalent bond. Linkage methods are described below.

- 10 -

Useful cytotoxins are preferably significantly cytotoxic only when present intracellularly and are substantially excluded from any given cell in the absence of a targeting domain. Peptide toxins fulfill both of these criteria and are readily incorporated into hybrid molecules. A mixed cytotoxin, a cytotoxin composed of all or part of two or more toxins, can also be used. Several useful toxins are described in more detail below.

Interleukin-4 and Interleukin-6 as a Targeting Agents

10 Interleukin-4 (IL-4) is a cytokine which acts on a variety of cell types. Its receptor is expressed on a number of cell types, including CD4+ T cells and monocytes. IL-4 can act as a T cell growth factor and it is thought to have an influence on IL-2 induced lymphocyte proliferation.

15 A cytotoxin directed against IL-4 receptor-bearing cells or IL-6 receptor-bearing cells may enhance the effectiveness of molecules directed against IL-2R-bearing cells. The protein and DNA sequence of IL-4 and IL-6 are known (Lee et al., *J. Biol. Chem.* 263:10817, 1988; Hirano et al., *Nature* 324:73, 1986). These lymphokines can be used to create hybrid lymphokine/toxin molecules similar to IL-2/toxin hybrid molecules.

Monoclonal Antibodies as Targeting Agents

25 Monoclonal antibodies directed against the lymphokine receptor of choice can be used to direct toxins to cells bearing that receptor. These antibodies or antibody fragments can be fused to a cytotoxin either by virtue of the toxin and the antibody being encoded by a fused gene which encodes a hybrid protein molecule, or by means of a non-peptide covalent bond which is used to join separately produced ligand and toxin molecules. Several useful toxins are described below.

35 Antibody/toxin hybrids can be tested for their ability to kill receptor bearing cells using a toxicity

- 11 -

assay similar to that which is described below for IL-2R bearing cells.

Monoclonal antibodies useful in the method of the invention can be made by immunizing mice with human IL-2R or cultured T-lymphocytes, fusing the murine splenocytes with appropriate myeloma cells, and screening the antibodies produced by the resultant hybridoma lines for the requisite IL-2R binding properties by means of an ELISA assay. Antibody production and screening can be performed according to Uchiyama et al. (*J. Immunol.* 126:1393, 1981). Alternatively, useful antibodies may be isolated from a combinatorial library produced by the method of Huse et al. (*Science* 246:1275, 1989).

The invention can employ not only intact monoclonal or polyclonal antibodies, but also an immunologically-active antibody fragment, for example, a Fab or (Fab)₂ fragment; an antibody heavy chain, an antibody light chain; a genetically engineered single-chain Fv molecule (Ladner et al., U.S. Patent No. 4,946,778); or a chimeric antibody, for example, a "humanized" antibody which contains the binding specificity of a murine antibody, but in which most or all of the remaining portions are of human origin (Reichman et al., *Nature* 332:323, 1988).

25 Toxins

The toxin molecules useful in the method of the invention are preferably toxins, such as peptide toxins, which are significantly cytotoxic only when present intracellularly. Of course, under these circumstances the molecule must be able to enter a cell bearing the targeted receptor. This ability depends on the nature of the molecule and the nature of the cell receptor. For example, cell receptors which naturally allow uptake of a ligand are likely to provide a means for a molecule which includes a toxin to enter a cell bearing that receptor.

- 12 -

Preferably, a peptide toxin is fused to an IL-2R binding domain by producing a recombinant DNA molecule which encodes a hybrid protein molecule. Such an approach ensures consistency of composition.

5 Many peptide toxins have a generalized eukaryotic receptor binding domain; in these instances the toxin must be modified to prevent intoxication of non-receptor bearing cells. Any such modifications must be made in a manner which preserves the cytotoxic functions of the
10 molecule (see U.S. Department of Health and Human Services, U.S. Serial No. 401,412). Potentially useful toxins include, but are not limited to: cholera toxin, ricin, 0-Shiga-like toxin (SLT-I, SLT-II, SLT II_v), LT toxin, C3 toxin, Shiga toxin, pertussis toxin, tetanus
15 toxin, *Pseudomonas* exotoxin, alorin, saporin, modeccin, and gelanin.

Diphtheria Toxin-based Molecules

Diphtheria toxin can be used to produce molecules useful in the method of the invention. Diphtheria toxin,
20 whose sequence is known, is described in detail in Murphy U.S. Patent 4,675,382, hereby incorporated by reference. The natural diphtheria toxin molecule secreted by *Corynebacterium diphtheriae* consists of several functional domains which can be characterized, starting
25 at the amino terminal end of the molecule, as enzymatically-active Fragment A (amino acids Gly₁ - Arg₁₉₃) and Fragment B (amino acids Ser₁₉₄ - Ser₅₃₅), which includes a translocation domain and a generalized cell binding domain (amino acid residues 475 through 535).

30 The process by which diphtheria toxin intoxicates sensitive eukaryotic cells involves at least the following steps: (i) the binding domain of diphtheria toxin binds to specific receptors on the surface of a sensitive cell; (ii) while bound to its receptor, the
35 toxin molecule is internalized into an endocytic vesicle;

- 13 -

(iii) either prior to internalization, or within the endocytic vesicle, the toxin molecule undergoes a proteolytic cleavage between fragments A and B; (iv) as the pH of the endocytic vesicle decreases to below 6, the toxin crosses the endosomal membrane, facilitating the delivery of Fragment A into the cytosol; (v) the catalytic activity of Fragment A (i.e., the nicotinamide adenine dinucleotide - dependent adenosine diphosphate (ADP) ribosylation of the eukaryotic protein synthesis factor termed "Elongation Factor 2") causes the death of the intoxicated cell. It is apparent that a single molecule of Fragment A introduced into the cytosol is sufficient to block down the cell's protein synthesis machinery and kill the cell. The mechanism of cell killing by *Pseudomonas* exotoxin A, and possibly by certain other naturally-occurring toxins, is very similar.

DAB₄₈₆IL-2, a fusion protein in which the receptor binding domain of diphtheria toxin has been replaced by a portion of human IL-2 (Williams et al., *J. Biol. Chem.* 35:20673, 1990; see also Williams et al., *Protein Eng.* 1:493, 1987), is an example of a molecule useful in the method of the invention. This molecule selectively kills IL-2R-expressing tumor cells and lymphocytes (Waters et al., *Eur. J. Immunol.* 20:785, 1990; Kiyokawa et al., *Cancer Res.* 49:4042, 1989). Because of its ability to kill activated lymphocytes, DAB₄₈₆IL-2 has been used to control graft rejection (Pankewycz et al., *Transplantation* 47:318, 1989; Kickman et al., *Transplantation* 47:327, 1989) and to treat certain autoimmune disorders (Forte et al., *2nd International Symposium on Immunotoxins*, 1990).

DAB₄₈₆IL-2 is a chimeric molecule consisting of Met followed by amino acid residues 1 through 485 of the mature diphtheria toxin fused to amino acid residues 2

- 14 -

through 133 of IL-2. Thus, DAB₄₈₆IL-2 includes all of diphtheria toxin fragment A, which encodes the enzymatically active portion of the molecule, and a portion of fragment B. The portion of fragment B present
5 in DAB₄₈₆IL-2 does not include the generalized receptor binding domain but does include the translocation domain which facilitates delivery of the enzymatically active portion into the cytosol.

Preparation of DAB₄₈₆IL-2 and DAB₃₈₉IL-2

10 DAB₄₈₆IL-2 was produced in *E. coli* harboring the DAB₄₈₆IL-2 encoding plasmid, pDW24 (Williams et al., J. Biol. Chem. 265:20673, 1990, except amp^r is replaced by kan^r). The protein was purified by immunoaffinity chromatography and high pressure liquid chromatography
15 (Williams et al., *supra*). DAB₃₈₉IL-2 can be prepared as described below for DAB₃₈₉IL-4 by substituting IL-2 for IL-4.

Preparation of DAB₃₈₉IL-4 and DAB₄₈₆IL-2

A synthetic gene encoding human interleukin-4 was
20 synthesized (Milligen/Bioscience 7500 DNA synthesizer). The IL-4 sequence (Yodota et al., *Proc Nat'l Acad Sci USA*, 83:58994, 1986) was modified to incorporate *E. coli*-preferred codon usage (deBoer et al., in *Maximizing Gene Expression*, Reznikioff et al., eds., 1986, Butterworths,
25 Boston), and restriction endonuclease cleavage sites were added to facilitate subsequent cloning steps. IL-4 coding sequence (His¹ to Ser¹²⁹) was inserted into pDW27 plasmid. pDW27 is derived from pDW24 (Williams et al., J. Biol. Chem. 265:11885, 1990) by deleting DNA
30 corresponding to amino acids 388 to 485 of native diphtheria toxin. DAB₄₈₆IL-4 can be prepared as described above for DAB₄₈₆IL-2 by substituting IL-4 for IL-2.

Cytotoxicity of DAB₃₈₉IL-4

- 15 -

The ability of DAB₃₈₉IL-4 to reduce viability of various cell types was measured using an inhibition of protein synthesis assay; the results of this assay are presented in Table 3. IC₅₀ (M) is the concentration of DAB₃₈₉IL-4 required for a 50% decrease in protein synthesis. The rat, Con A-activated, normal splenic lymphocytes were far less sensitive to DAB₃₈₉IL-4 than any of the other cells or cell lines. Since the rat interleukin-4 receptor does not bind human interleukin-4, this result demonstrates the specificity of DAB₃₈₉IL-4. These rat cells are sensitive to a diphtheria toxin/rat interleukin-2 hybrid molecule.

Preparation of DAB₃₈₉IL-6 and DAB₄₈₆IL-6

A synthetic gene encoding human interleukin-6 was synthesized (Milligen/Biosearch 7500 DNA synthesizer). The IL-6 sequence (Revel et al., EPA 8611404.9) was modified to incorporate *E. Coli* preferred codon usage (deBoer et al., *supra*), and restriction endonuclease cleavage sites were added to facilitate subsequent cloning steps. The entire IL-6 coding sequence was inserted into pDW27 plasmid as described above for DAB₃₈₉IL-4. DAB₄₈₆IL-6 can be produced as described above for DAB₄₈₆IL-2 by substituting IL-6 for IL-2.

Mixed Toxins

The cytotoxic portion of some molecules useful in the invention can be provided by a mixed toxin molecule. A mixed toxin molecule is a molecule derived from two different polypeptide toxins. Generally, as discussed above in connection with diphtheria toxin, polypeptide toxins have, in addition to the domain responsible for generalized eukaryotic cell binding, an enzymatically active domain and a translocation domain. The binding and translocation domains are required for cell recognition and toxin entry respectively. The

- 16 -

enzymatically active domain is the domain responsible for cytotoxic activity once the molecule is inside a cell.

Naturally-occurring proteins which are known to have a translocation domain include diphtheria toxin, 5 *Pseudomonas* exotoxin A, and possibly other peptide toxins. The translocation domains of diphtheria toxin and *Pseudomonas* exotoxin A are well characterized (see, e.g., Hoch et al., *Proc. Natl. Acad. Sci. USA* 82:1692, 1985; Colombatti et al., *J. Biol. Chem.* 261:3030, 1986; 10 and Deleers et al., *FEBS Lett.* 160:82, 1983), and the existence and location of such a domain in other molecules may be determined by methods such as those employed by Hwang et al., *Cell* 48:129, 1987; and Gray et al., *Proc. Natl. Acad. Sci. USA* 81:2645, 1984).

15 One useful IL-2/mixed toxin hybrid molecule is formed by fusing the enzymatically active A subunit of *E. coli* Shiga-like toxin (Calderwood et al., *Proc. Natl. Acad. Sci. USA* 84:4364, 1987) to the translocation domain (amino acid residues 202 through 460) of diphtheria 20 toxin, and to IL-2. This three-part hybrid molecule, SLT-A/DTB'/IL-2, is useful in the method of the invention in the same way as DAB₄₈₆IL-2 described above. The IL-2 portion of the three-part hybrid causes the molecule to attach specifically to IL-2R-bearing cells, and the 25 diphtheria toxin translocation portion acts to insert the enzymatically active A subunit of the Shiga-like toxin into the targeted cell. The enzymatically active portion of Shiga-like toxin, like diphtheria toxin, acts on the protein synthesis machinery of the cell to prevent 30 protein synthesis, thus killing the cell. The difference between these two types of hybrid toxins is the nature of their enzymatic activities: the enzymatic portion of DAB₄₈₆IL-2 catalyzes the ADP-ribosylation by nicotinamide adenine dinucleotide of Elongation Factor 2, thereby 35 inactivating this factor which is necessary for protein

- 17 -

synthesis, while the enzymatic portion of SLT-A/DTB'/IL-2 is a ribonuclease capable of cleaving ribosomal RNA at a critical site, thereby inactivating the ribosome. SLT-A/DTB'/IL-2 hybrid would therefore be useful as a
5 treatment for the same indications as DAB₄₈₆IL-2, and could be substituted or used in conjunction with it.

Linkage of Toxins to Binding Ligands

The binding ligand and the cytotoxin of useful hybrid molecules can be linked in several ways. If the
10 hybrid molecule is produced by expression of a fused gene, a peptide bond serves as the link between the cytotoxin and the binding ligand. Alternatively, the toxin and the binding ligand can be produced separately and later coupled by means of a non-peptide covalent
15 bond.

For example, the covalent linkage may take the form of a disulfide bond. In this case, if the IL-2R binding ligand is a protein, e.g., IL-2, the DNA encoding IL-2 can be engineered to contain an extra cysteine codon
20 as described in Murphy et al. U.S. Serial No. 313,599, hereby incorporated by reference. The cysteine must be positioned so as to not interfere with the IL-2R binding activity of the molecule. For example, the cysteine codon can be inserted just upstream of the DNA encoding
25 Pro² of the mature form of IL-2. The toxin molecule must be derivatized with a sulfhydryl group reactive with the cysteine the modified IL-2. In the case of a peptide toxin this can be accomplished by inserting a cysteine codon into the DNA sequence encoding the toxin.
30 Alternatively, a sulfhydryl group, either by itself or as part of a cysteine residue, can be introduced using solid phase polypeptide techniques. For example, the introduction of sulfhydryl groups into peptides is described in Hiskey (*Peptides* 3:137, 1981).
35 Derivatization can also be carried out according to the

- 18 -

method described for the derivatization of a peptide hormone in Bacha et al. U.S. Patent No. 4,468,382, hereby incorporated by reference. Similarly, proteins can be derivatized at the DNA or protein chemistry level. The
5 introduction of sulfhydryl groups into proteins is described in Maasen et al. (*Eur. J. Biochem.* 134:32, 1983). The cytotoxin and the IL-2R binding ligand are then produced and purified, and the disulfide bond
10 between the purified molecules formed by reducing both sulfur groups, mixing toxin and ligand, in a ratio of about 1:5 to 1:20, and allowing disulfide bond formation to proceed to completion (generally 20 to 30 minutes) at room temperature. The mixture is then dialyzed against phosphate buffered saline to remove unreacted ligand and
15 toxin molecules. Sephadex chromatography or the like is then carried out to separate on the basis of size the desired toxin-ligand conjugates from toxin-toxin and ligand-ligand conjugates.

Assays for IL-2 Receptor Binding and IL-4 Receptor

20 Binding

The IL-2R binding ability of various molecules can be measured using an IL-2R assay for high affinity (Ju et al., *J. Biol. Chem.* 262:5723, 1987) or intermediate affinity receptors (Rob et al., *Proc. Natl. Acad. Sci.*
25 *USA* 84:2002, 1987). The IL-4R binding activity of various molecules can be measured using the assay described by Park et al. (*J. Exp. Med.* 166:176, 1984) or the assay described by Foxwell et al. (*Eur. J. Immunol.* 19:1637, 1989).

30 Assays for Toxicity

Molecules of the invention (both antibodies and hybrid molecules) can be screened for the ability to decrease viability of cells bearing the targeted receptor by means of assays such as those described below.

- 19 -

Toxicity towards IL-2R bearing cells can be tested as follows. Cultured HUT 102/6TG (Tsuda et al., *Proc. Natl. Acad. Sci. USA* 83:9694, 1986) or YT2C2 (Teshigawari et al., *J. Exp. Med.* 165:223, 1987) cells are maintained
5 in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 25 mM HEPES (pH 7.4), 2mM 1-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 10% fetal calf serum (Hazelton, Lenexa, KS). Cells are seeded in 96-well V-bottomed plates (Linbro-Flow
10 Laboratories, McLean, VA) at a concentration of 1×10^5 per well in complete medium. Putative toxins are added to varying concentrations (10^{-12} M to 10^{-6} M) and the cultures are incubated for 18 hrs. at 37°C in a 5% CO₂ atmosphere. Following incubation, the plates are
15 centrifuged for 5 min. at 170 x g, and the medium removed and replaced with 100 µl leucine-free medium (MEM, Gibco) containing 8 µCi/ml (³H-leucine; New England Nuclear, Boston, MA). After an additional 90 min. at 37°C, the plates are centrifuged for 5 min. at 170 x g, the medium
20 is removed, and the cells are collected on glass fiber filters using a cell harvester (Skatron, Sterling, VA). Filters are washed, dried, and counted according to standard methods. Cells cultured with medium alone serve as the control.

25 Toxicity towards cells bearing IL-4R may be tested by an assay similar to that described above for IL-2R bearing cells, but utilizing MLA144 cells (Rabin et al. *J. Immunol.* 127:1852, 1981) or HUT 102/6TG cells, seeded at 1×10^5 cells per well and incubated for 40 hours.

30 Therapy

Generally, the molecules of the invention will be administered by intravenous infusion. They may also be administered subcutaneously. Dosages of molecules useful in the methods of the invention will vary, depending on
35 factors such as whether the substance is a cytotoxin, a

- 20 -

lytic antibody, or an IL-2R blocking molecule. In the case of toxic molecules that act intracellularly, the extent of cell uptake is an important factor; less permeable molecules must be administered at a higher
5 dose.

More than 60 patients have received DAB₄₈₆IL-2 in Phase I/II clinical protocols. The maximum tolerated dose (MTD) established by transient asymptomatic hepatic transaminase elevations in about 30% of patients treated
10 at the MTD. Anti-tumor effects have been seen in approximately 40% of patients; responses were noted in B-cell leukemias and lymphomas, cutaneous T-cell lymphoma and Hodgkin's disease (LeMaistre et al., *Blood*
360a:abstract 1429, 1990; Woodworth et al., *Fourth*
15 *International Conference on Human Retrovirology*, 1991). Serum concentrations of 10^{-8} M DAB₄₈₆IL-2 have been achieved in patients with IL-2 receptor expressing malignancies. Anti-tumor effects have been observed in
20 highly refractory leukemia/lymphoma patients and these effects have occurred despite the presence of elevated soluble IL-2R levels in all patients. This observation is consistent with data which suggest that soluble IL-2R does not interfere with binding of IL-2 to the high
25 affinity interleukin-2 receptor. Animal and human studies have demonstrated that DAB₄₈₆IL-2 has no general immunosuppressive effect (LeMaistre et al., *supra*; Woodworth et al., *supra*).

Experiments indicate that binding and internalization of DAB₄₈₆IL-2 by cells bearing the high
30 affinity IL-2 receptor occurs within 30 minutes of exposure, resulting in maximal inhibition of protein synthesis within several hours. Therefore, the molecule should be effective even if the serum half-life is rather short.

- 21 -

Generally, drugs targeted to IL-2 receptor will be administered immediately after (e.g., within several minutes or less) vascular injury. Preferably, treatment begins before the accumulation of platelets and
5 leukocytes. Animal models of denuding balloon catheter injury have been used to show that platelet aggregation and thrombus formation occurs immediately after injury and that leukocyte adhesion begins within several hours. Autopsy of a patient who died 5 days after angioplasty
10 revealed that proliferating smooth muscle cells were invading the dilated region (Austin et al., *J. Amer. Coll. Cardiol.* 6:369, 1985).

Once initiated, endothelial regeneration is complete within one to two weeks. Since re-
15 endothelialization of the vessel wall appears to inhibit smooth muscle cell proliferation (Ip et al., *J. Amer. Coll. Cardiol.* 15:1667, 1990), treatment may need to last for only a few weeks. Accordingly, it is desirable to administer the compounds of the invention periodically
20 over a period adequate to allow regeneration of the endothelium.

The hybrid molecule can be administered as an unmodified molecule or in the form of a pharmaceutically acceptable salt, admixed with a therapeutically
25 acceptable carrier, e.g., saline. Examples of preferred salts are therapeutically acceptable organic acids, e.g., acetic, lactic, maleic, citric, or salicylic. For example, the hybrid molecule may be purified and sterile filtered using 2 micron filters and suspended in sterile
30 phosphated buffer saline (0.15M NaCl; 0.02M phosphate buffer, pH 7.2).

Other Embodiments

Derivatives of IL-2 which block utilization of endogenous IL-2 are useful for preventing proliferation

- 22 -

of IL-2R bearing cells. Activated cells deprived of IL-2 fail to proliferate and, in the absence of the essential anabolic stimulus provided by IL-2, will eventually die. The ability of a given IL-2 derivative to interfere with IL-2 function can be tested in an IL-2 bioactivity assay such as the one described by Ju et al. (*J. Biol. Chem.* 262:5723, 1987). IL-2R/toxin hybrids in which the toxin has been rendered inactive can be also used to block IL-2 receptors. A non-toxic mutant diphtheria toxin molecule has been described (Uchida et al. *J. Biol. Chem.* 248:3838, 1973), and this molecule can be used to produce a non-toxic IL-2/diphtheria toxin hybrid. See Svrluga et al. U.S. Serial No. 590,113, hereby incorporated by reference, for an example of such a hybrid molecule.

Monoclonal antibodies which interfere with the binding and/or uptake of IL-2 are useful in the method of the invention because IL-2R bearing cells deprived of IL-2 fail to proliferate. Blocking monoclonal antibodies can be tested for their ability to interfere with IL-2 bioactivity using the method of Ju et al., (*supra*).

Monoclonal antibodies which induce complement can be used to destroy IL-2R-bearing cells. Complement inducing antibodies are generally those of the IgG1, IgG2, IgG3, and IgM isotypes. Monoclonal anti-IL-2R antibodies can be screened for those able to induce complement using a complement-dependent cytotoxicity test, as follows.

Human T-lymphocytes and EBV transformed B-lymphocytes are labeled with ^{51}Cr sodium chromate and used as target cells; these cells are incubated with hybridoma culture supernatants and with complement, and then the supernatants are collected and counted with a gamma counter. Those supernatants exhibiting toxicity against activated T-lymphocytes, but not resting T- or B-

- 23 -

lymphocytes, are selected, and then subjected to a further screening step to select those supernatants containing antibody which precipitates (i.e., is specifically reactive with) the 50 kd glycoprotein IL-2
5 receptor (described in detail in Leonard et al. (*Proc. Natl. Acad. Sci. USA* 80:6957, 1983). The desired anti-IL-2 receptor antibody is purified from the supernatants using conventional methods.

- 24 -

Claims

1 1. A method of making a medicament for treating a
2 sarcoma characterized by growth of neoplastic cells of
3 non-lymphoid and non-monocytic origin, said method
4 comprising admixing a pharmaceutically acceptable carrier
5 and a molecule which is capable of specifically binding
6 to a cytokine receptor expressed on said neoplastic cell,
7 wherein said cytokine receptor is normally expressed on
8 cells of lymphoid or monocytic origin, said molecule
9 being capable of decreasing the viability of said
10 neoplastic cell.

1 2. The method of claim 1 wherein said neoplastic
2 cell is not of hematopoietic stem cell origin.

1 3. The method of claim 1 wherein said sarcoma is
2 an osteosarcoma.

1 4. The method of claim 1 wherein said sarcoma is
2 a fibrosarcoma.

1 5. The method of claim 1 wherein said sarcoma is
2 a leiomyosarcoma.

1 6. The method of claim 1 wherein said sarcoma is
2 a rhabdomyosarcoma.

1 7. A method of making a medicament for treating a
2 lung carcinoma characterized by growth of neoplastic
3 cells of non-lymphoid and non-monocytic origin, said
4 method comprising admixing a pharmaceutically acceptable
5 carrier and a molecule which is capable of specifically
6 binding to a cytokine receptor expressed on said
7 neoplastic cell, wherein said cytokine receptor is

- 25 -

8 normally expressed on cells of lymphoid or monocytic
9 origin, said molecule being capable of decreasing the
10 viability of said neoplastic cell.

1 8. The method of claim 1 wherein said cytokine
2 receptor is an interleukin receptor.

1 9. The method of claim 8 wherein said interleukin
2 receptor is an interleukin-2 receptor.

1 10. The method of claim 9 wherein said
2 interleukin-2 receptor is a high affinity interleukin-2
3 receptor.

1 11. The method of claim 8 wherein said
2 interleukin receptor is an interleukin-4 receptor.

1 12. The method of claim 8 wherein said
2 interleukin receptor is an interleukin-6 receptor.

1 13. The method of claim 1 wherein said molecule
2 kills cells bearing said cytokine receptor.

1 14. The method of claim 1 wherein said molecule
2 is a hybrid molecule comprising a first and a second
3 portion joined together covalently, said first portion
4 comprising a molecule capable of decreasing cell
5 viability and said second portion comprising a molecule
6 capable of specifically binding to said cytokine
7 receptor.

1 15. The method of claim 14 wherein said second
2 portion comprises all or a binding portion of an antibody
3 specific for said cytokine receptor.

- 26 -

1 16. The method of claim 14 wherein said second
2 portion comprises all or a binding portion of a ligand
3 for said cytokine receptor.

1 17. The method of claim 16 wherein said cytokine
2 receptor is an interleukin receptor.

1 18. The method of claim 16 wherein said ligand is
2 an interleukin.

1 19. The method of claim 14 wherein said first
2 portion comprises a cytotoxin.

1 20. The method of claim 19 wherein said cytotoxin
2 is a fragment of a peptide toxin which is enzymatically
3 active but which does not possess generalized eukaryotic
4 receptor binding activity.

1 21. The method of claim 20 wherein said fragment
2 of a peptide toxin comprises fragment A of diphtheria
3 toxin and enough of fragment B of diphtheria toxin to
4 facilitate translocation into the cytosol.

1 22. The method of claim 21 wherein said molecule
2 is DAB₃₈₉IL-2.

1 23. The method of claim 21 wherein said molecule
2 is DAB₃₈₉IL-4.

1 24. The method of claim 21 wherein said molecule
2 is DAB₃₈₉IL-6.

1 25. The method of claim 21 wherein said molecule
2 is DAB₄₈₆IL-2.

- 27 -

1 26. The method of claim 21 wherein said molecule
2 is DAB₄₈₆IL-4.

1 27. The method of claim 21 wherein said molecule
2 is DAB₄₈₆IL-6.

1 28. The method of claim 15 wherein said antibody
2 is a complement activating antibody.

1 29. The method of claim 1 wherein said cytokine
2 receptor is not expressed on non-neoplastic cells of non-
3 lymphoid and non-monocytic origin.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/04093

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/02, 37/24

US CL :424/85.1, 85.2, 85.8, 85.91; 514/2, 8, 12, 21

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)

U.S. : 424/85.1, 85.2, 85.8, 85.91; 514/2, 8, 12, 21
435/69.5, 69.52, 69.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Please See Extra Sheet.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	The Journal Immunol., Vol. 145, No. 8, issued 15 October 1990, Kozak et al, "IL-2-PE40 Prevents The Development of Tumors in Mice Injected with IL-2 Receptor Expressing EL4 Transfectant Tumor Cells", pages 2766-2771, see entire document.	1-10,13-22,25,28-29 1-10,13-22,25,28-29
X Y	Proc. Natl. Acad. Sci., Vol. 85, issued December 1988, Siegall et al, "Cytotoxic Activity of an Interleukin 6-Pseudomonas Exotoxin Fusion Protein on Human Myeloma Cells", pages 9738-9742, see entire document.	1-8,12,13-21,24,27-29 1-8,12,13-221,24,27-29
X Y	Prog. Leukocyte Biol. Vol 10B, issued 1990, Siegall et al, "Tumor Specific Cytotoxicity of The Chimeric Toxins TGF α -PE40 and IL-6-PE40", pages 401-406, see entire document.	1-8,12,13-21,24,27-29 1-8,12,13-21,24,27-29
X,P Y	Cancer Research , Vol. 51, issued 1 June 1991, Puri et al, "Expression of High-Affinity Interleukin 4 Receptors on Murine Sarcoma Cells and Receptor-mediated Cytotoxicity of Tumor Cells to Chimeric Protein between Interleukin 4 and Pseudomonas Exotoxin", pages 3011-3017, see entire document	1-8,11,13-21,23,26,27-29 1-8,11,13-21,23,26,27-29

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be part of particular relevance	"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
"E" earlier document published on or after the international filing date	"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
"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)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
01 SEPTEMBER 1992

Date of mailing of the international search report
15 SEP 1992

Name and mailing address of the ISA/
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Authorized officer *Garnette D. Draper*
GARNETTE D. DRAPER, PRIM EXM

Facsimile No. NOT APPLICABLE

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/04093

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P Y	US, A, 5,084,556 (Brown) 28 January 1992, see col 5 and the claims	1-7,13-16, 19-21,28-29 1-7,13-16,19-21,28-29
Y,P	US, A, 5,082,927 (Pastan) 21 January 1992, see entire document.	1-8,11,13-21,23,26,28-29

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/04093

B. FIELDS SEARCHED

Documentation other than minimum documentation that are included in the fields searched:

Lymphokines, vols 1-15; Immunophysiology: The Role of Cells and Cytokines in Immunity and Inflammation, ed. Oppenheim, 1990; Adoptive Cellular Immunology of Cancer, ed. Stevenson, 1989; and Biological Response Modifiers and Cancer Therapy, ed. Chiao, 1988

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

CAS, DIALOG, MEDLINE

FOR: treat/inhibit/prevent/suppress, tumor/cancer/neoplasma, hybrid/chimeric/fuse/conjugate, toxin/cytotoxin, interleukin