

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 07-052069- -00004-0000

Fecha: 2008-06-23 15:57:26 Dep. 2020 NUECREACIONE
Tra. 11 PATENTEIYII Eve: 379 FASENACIONALII
Act. 538 PETICION Folios: 3

Clarke, Modet & C^o

COLOMBIA

SEÑORES
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES
E.S.D.

REF: EXPEDIENTE No.07-052.069
SOLICITANTE: CENTRO DE INMUNOLOGÍA MOLECULAR

ASUNTO: SOLICITUD EXAMEN DE PATENTABILIDAD

DILIA RODRÍGUEZ D'ALEMAN identificada como aparece al pie de mi firma, domiciliada en Bogotá, en la Carrera 11 No. 86-53, piso 6, obrando como apoderada de la sociedad solicitante, pido a usted que se proceda a examinar si la invención, cuya patente se tramita en el expediente de la referencia, es patentable.

Adjunto al presente memorial los recibos Nrs. 08-43,943 y 08-43,988 de 4 de junio de 2008, por la suma de \$211.000.00, con lo cual queda cubierta la tasa oficial correspondiente a dicho estudio.

Señor Superintendente,

DILIA RODRIGUEZ D'ALEMAN
C.C. 41.654.882 de Bogotá
T.P. 20824 del C.S.J.

P-941

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE DATA : 08 - 43.943
FECHA : JUNIO 4 DE 2008

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***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	Vr. PAGO
CLARKE ROSET Y CA. COLOMB	CONSIGNACION	BANCO DE BOGOTA	062754387	3418225	03/06/2008	39,390,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01	SOLICITUDES	
		B2 INVOCACION DE UNA PRIORIDAD EN NU	140,000.00
		TOTAL :	140,000.00

SON: CIENTO CUARENTA MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176099-2

RECIBO OFICIAL DE CAJA : 08 - 43,982
FECHA : JUNIO 4 DE 2008

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	Nº. PAGO	FECHA PGO	Vr. PAGO
CLARKE MODEY Y CO. COLOMBIA	CONSIGNACION	BANCO DE BOGOTA	062754387	3418225	03.06.2008	79,090,000.00

***** CONCEPTO *****

CANT.	RENTISTAS	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	83 INVOCACION DE UNA PRIORIDAD EN SI	71,000.00
		TOTAL :	71,000.00

SON: SETENTA Y UNO MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

P-941

DIVISIÓN DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No. 07-52069

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCION**

FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No **587** DE

FECHA **18 DE ENERO DE 2008** EL TÉRMINO HÁBIL SEÑALADO POR LA LEY

EXPIRA EL **16 DE ABRIL DE 2008**

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI _____

NO X

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30 November 2001 (30.11.2001) IT

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(81) Designated States (*national*): AE, AG, AI, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SI, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: FUSION PROTEINS CONTAINING TLP PEPTIDES

(57) Abstract: Disclosed are fusion proteins obtained from a combination of TLP peptides and interleukin-2 (IL-2), and their use in cancer immunotherapy.

WO 03/045997 A2

FUSION PROTEINS CONTAINING TLP PEPTIDES

This invention relates to fusion proteins obtained from a combination of TLP peptides and interleukin-2 (IL-2), and their use in cancer immune therapy.

The abbreviation TLP ("Tumour Liberated Particles") identifies protein
5 complexes isolated from tumour tissues in accordance with the procedure initially described in EP 283433. Various peptides and proteins forming the TLP complex have been characterised. A 214 kDa TLP protein mainly expressed by lung cancer is described in Oncology, 1983 (40:248-253). Epitopes of the said protein and antibodies against them are described in
10 WO98/01462. EP649433 describes a 100 kDa TLP protein isolated from lung cancer, and immunogenic peptides derived from it. These peptides have been used to generate monoclonal and polyclonal antibodies which are employed to characterise the TLP expression pattern in tissues of various origins and to validate TLP proteins as tumour antigens (WO94/01458 and WO98/15282).

15 Patent application MI2001A001380 describes a method for the preparation of TLP which allows its use in early tumour diagnosis.

Published patent WO01/62786 describes the isolation of cDNA that codes for a peptide of the TLP 100kDa complex and its use in tumour immune therapy.

20 It has now been found that the immune response induced by the TLP peptides can be increased by their combination with an active fragment of interleukin 2 (IL-2). In particular it has been found that fusion proteins combining the amino acid sequences of TLP peptides and IL-2, elicit a cytotoxic response against tumour cells superior to that observed with TLP
25 peptides alone.

The present invention therefore relates to a fusion protein or

polypeptide obtained by combining the amino acid sequence of a TLP peptide with the amino acid sequence of an active fragment of IL-2.

The TLP peptides which can be used in accordance with the invention are described in WO 98/01462, WO 98/1458, WO 98/15282, EP 649433 and
5 WO 01/62786, herein incorporated by reference in their entirety, and have the following amino acid sequences (the same peptide reference number will be used throughout the description):

1. GPPEVQNAN
2. RTKNEASI
- 10 3. NQRNRD
4. GPPEVQNAN
5. TNKEASICPSVYLSTLPSIHSFTNSSIYYPCIHLSISLSVHPPLI
HPSIYPSYSSSIHSSSHHPCTHLSTHSVINPVKNFEHLLPIRPCTWTLG.

Derivatives of the preceding peptides obtained by deletion, addition or
15 substitution of one or more amino acids which retain the same immunogenic activity as the starting peptides are also included in the scope of the invention.

"Active fragment of IL-2" means a region of the molecule comprising the first 90, preferably the first 100 amino acids starting from the amino
20 terminal residue.

IL-2 may be of human or animal origin, and may be natural or recombinant. The human form whose sequence is deposited at GenBank accession number NP_000577 is preferred. Moreover, it is possible to use variants of the IL-2 sequence (Cytokine (1997) 7:488-498) which retain its
25 activity, e.g. which induce the growth of T-lymphocytes, regulate the activity of T-helper lymphocytes and stimulate cytotoxic T CD8 cells and NK cells (Theze et al., 1996, Immunol. Today, 17:481-486).

The term "fusion protein or polypeptide" in accordance with the

invention means a (poly)peptide molecule constituted partly by the amino acid sequence of an active fragment of IL-2, the remainder being constituted by a TLP peptide sequence as specified above. Although the two sequences can be combined with any mutual orientation, it is preferable for the amino and carboxy-terminal domains to be occupied by IL-2 and the TLP peptide respectively. A linker with a sequence length which allows correct folding of the two sub-units can also be inserted between the two sequences. The linker will preferably comprise 10 to 30 neutral amino acids without hindering side chains, more preferably Gly and Ser residues.

10 In accordance with a preferred embodiment, the fusion protein will contain a TLP peptide selected from 2 and 5 fused with an active fragment of IL-2. Even more preferred is the fusion protein having SEQ ID No. 1 (Fig. 1), wherein sequence 1-100 of IL-2 is fused with TLP peptide 2.

The two peptidic portions can be fused by chemical methods or recombinant DNA techniques. In this case, the cDNA that codes for IL-2 is fused "in frame" to the cDNA coding for the TLP peptide; after insertion of the recombinant cDNA in a suitable vector, the fusion protein is expressed in a eukaryote or prokaryote expression system. Alternatively, the cDNA that codes for the fusion product can be prepared with PCR using suitable amplification primers.

The fusion protein can also be prepared by synthesis in solution or solid phase (Merrifield, 1986, Science 232:341-347 and Barany and Merrifield, The Peptides, Gross and Meiehnhofer, eds N.Y. Academic Press, 1989, pp. 1-284), or with an automatic synthesiser (Stewart and Young, Solid Phase Peptide Synthesis, 2nd edition, Rockford Ill., Pierce Chemical Co., 1984).

The activity of the fusion proteins in accordance with the invention has been evaluated in cytotoxicity assays. Specifically, the activity of cytotoxic

T-lymphocytes (CTL) against a syngenic tumour cell line naturally expressing human TLP and, respectively, the reactivity of an anti-human TLP immune rabbit serum against various human tumour lines have been tested. The details of the experiments are set out in the examples. The results clearly
5 show that IL-2/TLP fusion proteins are more effective than single TLP peptides in inducing an antigen-specific cytotoxic immune response against tumour cells.

In accordance with another aspect, the invention therefore relates to the use of IL-2/TLP fusion polypeptides be the preparation a pharmaceutical
10 composition useful in the prevention or treatment of tumours. The pharmaceutical compositions in accordance with the invention will contain an effective amount of fusion protein together with pharmaceutically acceptable vehicles and excipients. "Effective amount" means a quantity sufficient to activate the lymphocytes and trigger a cytotoxic response to the tumour. In
15 accordance with a preferred embodiment, the said compositions are indicated for preventive vaccination of persons at risk of developing tumours or therapeutic vaccination of tumour patients. The techniques for preparation and use of vaccines are known to those skilled in the art, and are described, for example, in Paul, Fundamental Immunology, Raven Press, New York
20 (1989) or Cryz, S.J., Immunotherapy and Vaccines, VCH Verlagsgesellschaft (1991). The vaccines are usually in the form of an injectable preparation, suspension or solution, but can also be made in the form of a solid or liposome-based preparation. The active constituents can be mixed with excipients such as emulsifying, buffering and adjuvant agents, thus increasing
25 the efficacy of the vaccine.

The pharmaceutical compositions in accordance with the invention are particularly indicated for the prevention and treatment of tumours of the lung, colon/rectum, kidneys, bladder, testicles, ovaries, prostate, breast and cervix.