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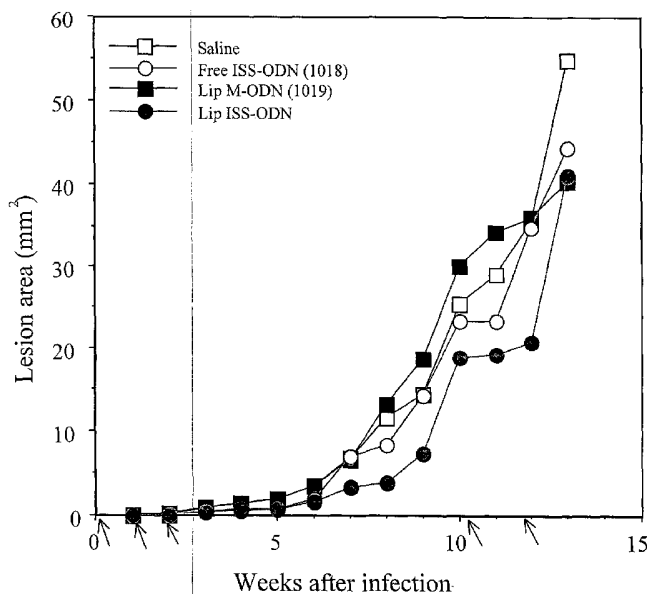
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[Continued on next page]

(54) Title: METHOD FOR PREPARATION OF VESICLES LOADED WITH IMMUNOSTIMULATORY OLIGODEOXYNUCLEOTIDES



(57) Abstract: The present invention is based on the finding of a simple and fast method for an effective entrapment of active ISS-ODN in liposomes, with more than 60% loading). The method is based on drying a suspension of amphipathic material and then hydrating it with an aqueous solution containing the ISS-ODN thereby entrapping it in liposomes formed from the lipid. Thus, the present invention concerns an efficient method for loading, and retaining, ISS-ODN in liposomal vesicles. Animals treated with liposomal ISS-ODN of the invention are shown to demonstrate a slower tempo of disease development as compared to free ISS-ODN or to liposomes loaded with mutant ISS-ODN (lip M-ODN).

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European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/IL 02/00507

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 7 A61K9/127 A61K39/39

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 7 A61K

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)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, EMBASE, CHEM ABS Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>LUDEWIG B ET AL: "In vivo antigen loading and activation of dendritic cells via a liposomal peptide vaccine mediates protective antiviral and anti-tumour immunity." VACCINE, vol. 19, no. 1, 15 August 2000 (2000-08-15), pages 23-32, XP004228807 ISSN: 0264-410X abstract paragraphs '02.3!', '02.4! --- -/--</p>	<p>1,3-5, 11,12, 18,19, 21-25, 31-44</p>

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)
- \*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

- \*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.
- \*&\* document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

28 January 2003

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## INTERNATIONAL SEARCH REPORT

 Internati  
 Application No  
 PCT/IL 02/00507

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 172 007 A (SYNTEX INC) 19 February 1986 (1986-02-19) page 1, line 24 -page 4, line 9 page 9, line 30 -page 10, line 8 page 18, line 28 -page 19, line 5 examples claims ---	1-44
Y	GB 2 164 624 A (SHIONOGI & CO) 26 March 1986 (1986-03-26) page 1, line 34 - line 52 page 2, line 13 - line 17 page 2, line 18 - line 19 examples claims ---	1-44
X	WO 99 22772 A (ISIS PHARMACEUTICALS) 14 May 1999 (1999-05-14)  example 3 ---	1,3-5,9, 12,13, 15,18, 19, 21-25, 29,31, 33-35, 38,39
X	SONEHARA K ET AL: "Hexamer palindromic oligonucleotides with 5'-CG-3' motif(s) induce production of interferon." JOURNAL OF INTERFERON AND CYTOKINE RESEARCH, vol. 16, no. 10, 1996, pages 799-803, XP000863569 ISSN: 1079-9907 abstract "Materials and Methods" ---	34,35, 38-41
X	WO 01 15726 A (INEX PHARMACEUTICALS CORP) 8 March 2001 (2001-03-08) page 2, line 10 -page 3, line 6 examples claims ---	34,35, 38-41
X	GÜRSEL M ET AL: "Immunoadjuvant action of plasmid DNA in liposomes." VACCINE, vol. 17, no. 11-12, March 1999 (1999-03), pages 1376-1383, XP004158265 ISSN: 0264-410X abstract paragraph '02.1! - paragraph '02.4! --- -/--	34-44

## INTERNATIONAL SEARCH REPORT

Internatio Application No

PCT/IL 02/00507

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 45849 A (GENZYME CORP; STRESSGEN BIOTECHNOLOGIES CORP) 10 August 2000 (2000-08-10) page 4, paragraph 2 examples claims ---	34, 35, 38-41
P, X	LI WM ET AL: "Enhanced immune response to T-independent antigen by using CpG oligodeoxynucleotides encapsulated in liposomes." VACCINE, vol. 20, no. 1-2, 12 October 2001 (2001-10-12), pages 148-157, XP004305127 ISSN: 0264-410X abstract paragraph '02.1! - paragraph '02.3!; table 1 paragraph '03.5! ---	1, 3-5, 9, 11-13, 15, 18, 19, 21-25, 29, 31-44
P, X	GURSEL I ET AL: "Sterically stabilized cationic liposomes improve the uptake and immunostimulatory activity of CpG oligonucleotides." JOURNAL OF IMMUNOLOGY, vol. 167, no. 6, 15 September 2001 (2001-09-15), pages 3324-3328, XP002228991 ISSN: 0022-1767 abstract "Materials and Methods" ---	34-44
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A	MCCLUSKIE MJ ET AL.: "The potential of CpG oligodeoxynucleotides as mucosal adjuvants" CRITICAL REVIEWS IN IMMUNOLOGY, vol. 21, no. 1-3, 2001, pages 103-120, XP008012997 ISSN: 1040-8401 the whole document -----	

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/IL 02/00507

## 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:  

Although claims 39-45 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 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:

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.:

### Remark on Protest

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

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IL 02/00507

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