



US007799565B2

(12) **United States Patent**
MacLachlan et al.

(10) **Patent No.:** **US 7,799,565 B2**
(45) **Date of Patent:** ***Sep. 21, 2010**

(54) **LIPID ENCAPSULATED INTERFERING RNA**

(75) Inventors: **Ian MacLachlan**, Vancouver (CA);
Lorne R. Palmer, Vancouver (CA);
James Heyes, Vancouver (CA)

(73) Assignee: **Protiva Biotherapeutics, Inc.**, Burnaby,
BC (CA)

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 135 days.

This patent is subject to a terminal dis-
claimer.

(21) Appl. No.: **11/148,152**

(22) Filed: **Jun. 7, 2005**

(65) **Prior Publication Data**

US 2006/0008910 A1 Jan. 12, 2006

Related U.S. Application Data

(60) Provisional application No. 60/679,427, filed on May
9, 2005, provisional application No. 60/610,746, filed
on Sep. 17, 2004, provisional application No. 60/578,
075, filed on Jun. 7, 2004, provisional application No.
60/577,961, filed on Jun. 7, 2004.

(51) **Int. Cl.**

C12N 15/88 (2006.01)
C12N 15/00 (2006.01)
C12N 15/87 (2006.01)
A61K 9/127 (2006.01)
A61N 43/04 (2006.01)
C07C 53/00 (2006.01)
A01N 43/04 (2006.01)
A61K 31/70 (2006.01)
A61K 9/48 (2006.01)
A61B 8/00 (2006.01)

(52) **U.S. Cl.** **435/458**; 424/9.2; 424/93.2;
424/450; 435/6; 435/455; 435/466; 514/44;
554/1; 554/103; 554/107

(58) **Field of Classification Search** None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,394,448 A 7/1983 Szoka, Jr. et al.
4,438,052 A 3/1984 Weder et al.
4,515,736 A 5/1985 Deamer
4,598,051 A 7/1986 Papahadjopoulos et al.
4,897,355 A 1/1990 Eppstein et al.
5,013,556 A 5/1991 Woodle et al.
5,171,678 A 12/1992 Behr et al.
5,208,036 A 5/1993 Eppstein et al.
5,225,212 A 7/1993 Martin et al.
5,264,618 A 11/1993 Felgner et al.
5,279,833 A 1/1994 Rose
5,283,185 A 2/1994 Epan et al.

5,578,475 A 11/1996 Jessee et al.
5,641,662 A 6/1997 Debs et al.
5,656,743 A 8/1997 Busch et al.
5,703,055 A 12/1997 Felgner et al.
5,705,385 A 1/1998 Bally et al.
5,820,873 A 10/1998 Choi et al.
5,976,567 A 11/1999 Wheeler et al.
5,981,501 A 11/1999 Wheeler et al.
6,534,484 B1 3/2003 Wheeler et al.
6,586,410 B1 7/2003 Wheeler et al.
6,649,780 B1* 11/2003 Eibl et al. 554/110
6,815,432 B2 11/2004 Wheeler et al.
6,858,224 B2 2/2005 Wheeler et al.
2003/0077829 A1 4/2003 MacLachlan
2003/0125263 A1 7/2003 Gold et al.
2003/0143732 A1 7/2003 Fosnaugh et al.
2004/0063654 A1* 4/2004 Davis et al. 514/44
2004/0142892 A1 7/2004 Finn et al.
2004/0253723 A1 12/2004 Tachas et al.
2004/0259247 A1 12/2004 Tuschl et al.
2006/0105976 A1 5/2006 Soutschek et al.

FOREIGN PATENT DOCUMENTS

WO WO 91/16024 A1 10/1991
WO WO 93/05162 A1 3/1993
WO WO 93/12240 A1 6/1993
WO WO 93/12756 A2 7/1993
WO WO 93/24640 A2 12/1993
WO WO 93/25673 A1 12/1993
WO WO 95/02698 A1 1/1995
WO WO 95/18863 A1 7/1995
WO WO 95/35301 A1 12/1995
WO WO 96/02655 A1 2/1996
WO WO 96/10390 A1 4/1996
WO WO 96/41873 A1 12/1996
WO WO 01/05374 A1* 1/2001
WO WO 02/34236 A2 5/2002
WO WO 02/087541 A1 11/2002
WO WO 03/097805 A2 11/2003
WO WO 2004/065546 A2 8/2004
WO WO 2004/110499 A1 12/2004
WO WO 2005/007196 A2 1/2005
WO WO 2005/026372 A1 3/2005
WO WO 2005/120152 A2 12/2005

OTHER PUBLICATIONS

Ballas, N. et al., "Liposomes bearing a quarternary ammonium deter-
gent as an efficient vehicle for functional transfer of TMV-RNA into
plant protoplasts," *Biochim. Biophys. Acta*, 1998, pp. 8-18, vol. 939.
Barinaga, M., "Step Taken Toward Improved Vectors for Gene Trans-
fer," *Science*, 1994, p. 1326, vol. 266.

(Continued)

Primary Examiner—Robert M Kelly
Assistant Examiner—Kelaginamane Hiriyanna
(74) *Attorney, Agent, or Firm*—Townsend and Townsend and
Crew LLP

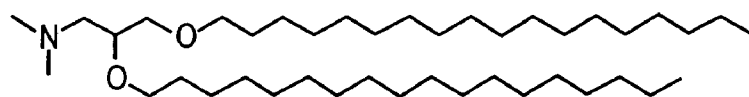
(57) **ABSTRACT**

The present invention provides lipid-based formulations for
delivering, e.g., introducing, nucleic acid-lipid particles com-
prising an interference RNA molecule to a cell, and assays for
optimizing the delivery efficiency of such lipid-based formu-
lations.

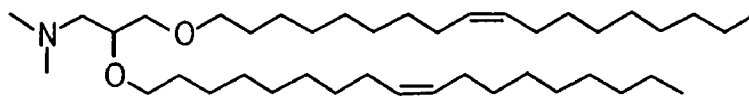
OTHER PUBLICATIONS

- Behr, J.-P., "Synthetic Gene-Transfer Vectors," *Acc. Chem. Res.* 1993, pp. 274-278, vol. 26.
- Brigham, K. et al., "Rapid Communication: In vivo Transfection of Murine Lungs with a Functioning Prokaryotic Gene Using a Liposome Vehicle," *Am. J. Med. Sci.*, 1989, pp. 278-281, vol. 298.
- Cortesi, R., et al., "Effect of cationic liposome composition on in vitro cytotoxicity and protective effect on carried DNA," *International Journal of Pharmaceutics*, 1996, pp. 69-78, vol. 139.
- Crystal, R., "Transfer of Genes to Humans: Early Lessons and Obstacles to Success," *Science*, 1995, pp. 404-410, vol. 270.
- Culver K., "The First Human Gene Therapy Experiment," *Gene Therapy: A Handbook for Physicians*, 1994, pp. 33-40.
- Duzgunes, N., "Membrane Fusion," *Subcellular Biochemistry*, 1985, pp. 195-286, vol. 11.
- Dwarki, V.J., et al., "Cationic Liposome-Mediated RNA Transfection," *Methods in Enzymology*, 1993, pp. 644-654, vol. 217.
- Enoch, H. et al., "Formation and properties of 1000-Å-diameter, single-bilayer phospholipid vesicles," *Proc. Natl. Acad. Sci. USA*, 1979, pp. 145-149, vol. 76, No. 1.
- Felgner, P. et al., "Lipofection: A highly efficient, lipid-mediated DNA-transfection procedure," *Proc. Natl. Acad. Sci. USA*, 1987, pp. 7413-7417, vol. 84.
- Felgner, J.H., et al., "Enhanced Gene Delivery and Mechanism Studies with a Novel Series of Cationic Lipid Formulations," *The Journal of Biological Chemistry*, Jan. 1994, pp. 2550-2561, vol. 269, No. 4.
- Felgner, J., et al., "Cationic Lipid-Mediated Transfection in Mammalian Cells: "Lipofection," *J. Tiss. Cult. Meth.*, 1993, pp. 63-68, vol. 15.
- Felgner, P.L., et al., "Cationic Liposome Mediated Transfection," *Proc. West. Pharmacol. Soc.*, 1989, pp. 115-121, vol. 32.
- Gao, X. et al., "A Novel Cationic Liposome Reagent for Efficient Transfection of Mammalian Cells," *Biochem. Biophys. Res. Comm.*, 1991, pp. 280-285, vol. 179.
- Gershon, H. et al., "Mode of Formation and Structural Feature of DNA-Cationic Liposome Complexes Used for Transfection," *Biochemistry*, 1993, pp. 7413-7151, vol. 32.
- Guy-Caffey, J., et al., "Novel Polyaminolipids Enhance the Cellular Uptake of Oligonucleotides," *The Journal of Biological Chemistry*, Dec. 1995, pp. 31391-31396, vol. 270, No. 52.
- Hawley-Nelson, et al., "LipofectAmine™ Reagent: A New, Higher Efficiency Polycationic Liposome Transfection Reagent," *Focus*, 1993, p. 73-80, vol. 15, No. 3.
- Hyde, S., et al., "Correction of the ion transport defect in cystic fibrosis transgenic mice by gene therapy," *Nature*, 1993, pp. 250-256, vol. 362.
- Jiang, Lei et al.; "Comparison of protein precipitation methods for sample preparation prior to proteomic analysis"; 2004, *Journal of Chromatography*, vol. 1023, No. 2, pp. 317-320.
- Juliano R., and Stamp, D., "The Effect of Particle Size and Charge on the Clearance Rates of Liposomes and Liposome Encapsulated Drugs," *Biochem. Biophys. Res. Commun.*, 1975, pp. 651-658, vol. 63.
- Legendre, J.Y. and Szoka, F., "Delivery of Plasmid DNA into Mammalian Cell Lines Using pH-Sensitive Liposomes: Comparison with Cationic Liposomes," *Pharm. Res.*, 1992, pp. 1235-1242, vol. 9, No. 10.
- Leventis, R., et al., "Interactions of mammalian cells with lipid dispersions containing novel metabolizable cationic amphiphiles," *Biochem. Biophys. Acta*, 1990, p. 124, vol. 1023.
- Marshall, E., "Gene Therapy's Growing Pains," *Science*, 1995, pp. 1050-1055, vol. 269.
- Orkin, et al., *NIH Report, Report and Recommendations of the Panel to Assess the NIH Investment in Research on Gene Therapy*, 1995.
- Paul, Cynthia P. et al.; "Effective expression of small interfering RNA in human cells"; 2002, *Nature Biotechnology*, vol. 20, pp. 505-508.
- Puyal, C., et al., "A new cationic liposome encapsulating genetic material: A potential delivery system for polynucleotides," *Eur. J. Biochem.*, 1995, pp. 697-703, vol. 228.
- Spagnou, Sebastien et al.; "Lipidic Carriers of siRNA: Differences in the Formulation, Cellular Uptake, and Delivery with Plasmid DNA"; 2004, *Biochemistry*, vol. 43, pp. 13348-13356.
- Stamatatos, L., et al., "Interactions of Cationic Lipid Vesicles with Negatively Charged Phospholipid Vesicles and Biological Membranes," *Biochemistry*, 1988, pp. 3917-3925, vol. 27.
- Szoka, F. et al., "Comparative Properties and Methods of Preparation of Lipid Vesicles (Liposomes)," *Ann. Rev. Biophys. Bioeng.*, 1980, pp. 467-508, vol. 9.
- Szoka, F. et al., "Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation," *Proc. Natl. Acad. Sci. USA*, 1978, pp. 4194-4198, vol. 75, No. 9.
- Van Der Woude, I., et al., "Parameters influencing the introduction of plasmid DNA into cells by the use of synthetic amphiphiles as a carrier system," *Biochimica et Biophysica Acta*, 1995, pp. 34-40, vol. 1240.
- Wilson, R. et al., "Counterion-Induced Condensation of Deoxyribonucleic Acid. A Light-Scattering Study," *Biochemistry*, 1979, pp. 2192-2196, vol. 18.
- Woodle, M.C. et al., "Versatility in lipid compositions showing prolonged circulation with sterically stabilized liposomes," *Biochim. Biophys. Acta*, 1992, pp. 193-200, vol. 1105.
- Zhu, N., et al., "Systemic Gene Expression After Intravenous DNA Delivery into Adult Mice," *Science*, 1993, pp. 209-211, vol. 261.
- Arpicco S. et al., "Preparation and Characterization of Novel Cationic Lipids Developed for Gene Transfection." *Proceed. Int'l. Symp. Control Rel. Bioact. Mater. (Controlled Release Society, Torchilin, V.P.; Beronese Francesco m., Eds)*, 1999, vol. 26, pp. 759-760. (ISSN 1022-0178).
- Arpicco S. et al., "Synthesis, Characterization and Transfection Activity of New Saturated and Unsaturated Cationic Lipids." *II Farmaco*, Nov. 2004, vol. 59, No. 11, pp. 869-878, (ISSN 0014-827X) See Compound 13 (Scheme 1, p. 871 and section 2.2.2., p. 872), p. 876, right column, first full paragraph, Figure 3.
- Cevc, G., "How Membrane Chain-Melting Phase-Transition Temperature is Affected by Lipid Chain Asymmetry and Degree of Unsaturation: An Effective Chain Length Model." *Biochemistry*, Jul. 1991, vol. 30, No. 29, pp. 7186-7193. (ISSN 0006-2960).
- Keough, K.M.W., "Influence of Chain Unsaturation and Chain Position on Thermotropism and Intermolecular Interactions in Membranes." *Biochem. Soc. Transactions*, 1990, vol. 18, No. 5, pp. 835-837. (ISSN 0300-5127).
- Heyes, James et al., "Cationic lipid saturation influences intracellular delivery of encapsulated nucleic acids," *Journal of Controlled Release*, 2005, vol. 107, No. 2, pp. 276-287.
- Heyes, James et al., "Synthesis of Novel Cationic Lipids: Effect of Structural Modification on the Efficiency of Gene Transfer," *J. Med. Chem.*, 2002, vol. 45, No. 1, pp. 99-114.
- Beale, G., et al. "Gene Silencing Nucleic Acids Designed by Scanning Arrays: Anti-EGFR Activity of siRNA, Ribozyme and DNA Enzymes Targeting a Single Hybridization-accessible Region using the Same Delivery System," *Journal of Drug Targeting*, 2003, vol. 11, No. 7, pp. 449-456.
- Brummelkamp, et al. "A System for Stable Expression of Short Interfering RNAs in Mammalian Cells," *Science*, 2002, vol. 296, pp. 550-553.
- Mashek et al. "Short Communication: Net Uptake of Nonesterified Long Chain Fatty Acids by the Perfused Caudate Lobe of the Caprine Liver," *J. Dairy Sci.*, 2003, 86:1218-1220.
- Vigh et al. "Does the membrane's physical state control the expression of heat shock and other genes?" *TIBS*, 1998, 23:369-374.

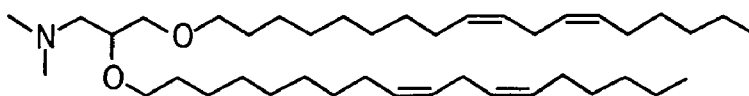
* cited by examiner



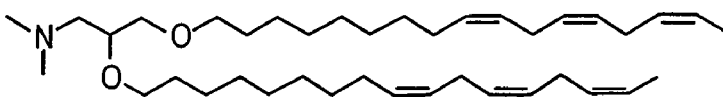
DSDMA



DODMA

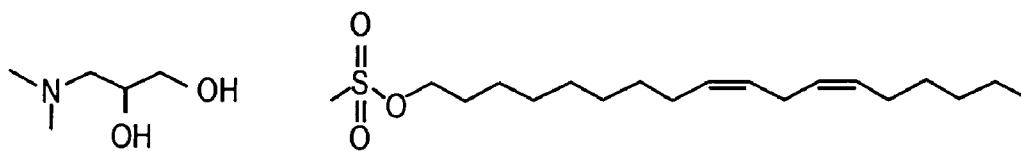


DLinDMA

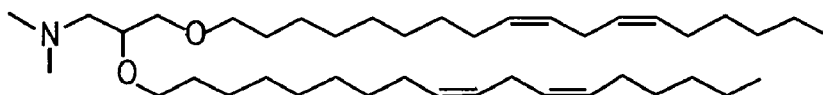


DLenDMA

FIG. 1



NaH, benzene
Reflux 3 hours



DLinDMA.

FIG. 2

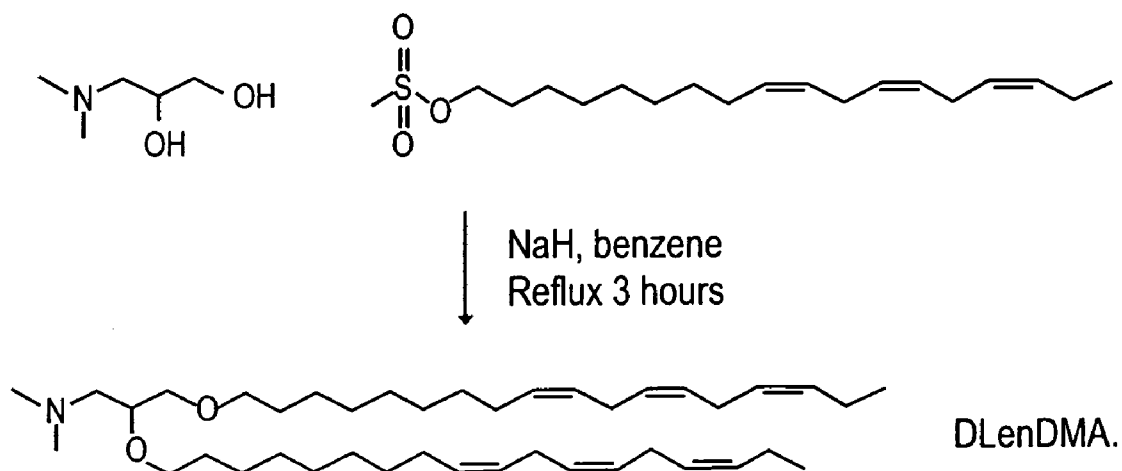


FIG. 3

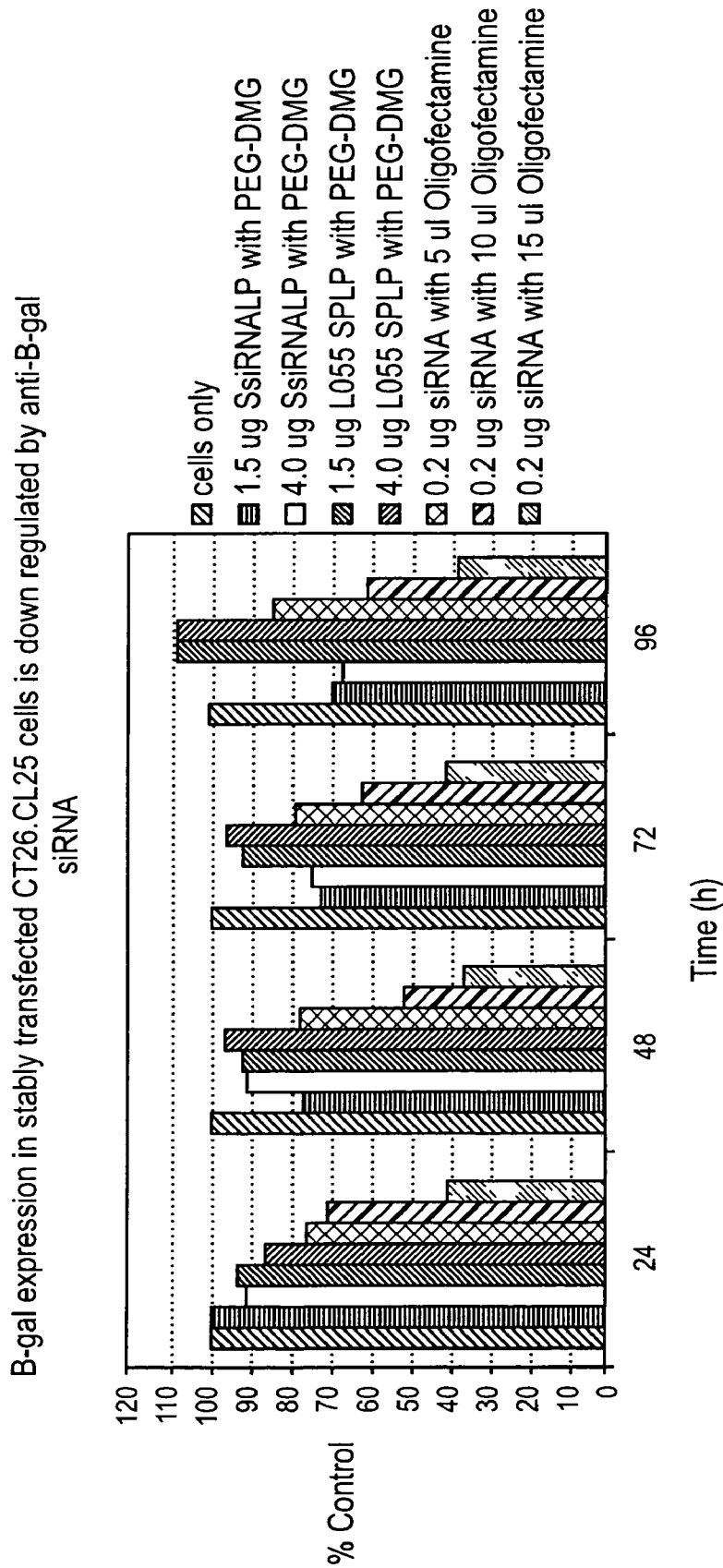


FIG. 4

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.