

EXHIBIT A



US008058069B2

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

(10) **Patent No.:** **US 8,058,069 B2**
(45) **Date of Patent:** **Nov. 15, 2011**

(54) **LIPID FORMULATIONS FOR NUCLEIC ACID DELIVERY**

(75) Inventors: **Edward Yaworski**, Maple Ridge (CA);
Kieu Lam, Surrey (CA); **Lloyd Jeffs**,
Delta (CA); **Lorne Palmer**, Vancouver
(CA); **Ian MacLachlan**, Mission (CA)

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

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **12/424,367**

(22) Filed: **Apr. 15, 2009**

(65) **Prior Publication Data**

US 2010/0130588 A1 May 27, 2010

Related U.S. Application Data

(60) Provisional application No. 61/045,228, filed on Apr. 15, 2008.

(51) **Int. Cl.**
C07H 21/04 (2006.01)
C12N 15/88 (2006.01)

(52) **U.S. Cl.** **435/458; 536/24.5**

(58) **Field of Classification Search** **536/24.5; 435/458**

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 Epand et al.
5,320,906 A 6/1994 Eley et al.
5,545,412 A 8/1996 Eppstein 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.
6,815,432 B2 11/2004 Wheeler et al.
6,858,224 B2 2/2005 Wheeler et al.
7,422,902 B1 9/2008 Wheeler et al.
7,799,565 B2* 9/2010 MacLachlan et al. 435/458

2001/0048940 A1 12/2001 Tousignant et al.
2003/0077829 A1 4/2003 MacLachlan
2003/0143732 A1 7/2003 Fosnaugh et al.
2004/0063654 A1 4/2004 Davis et al.
2004/0142892 A1 7/2004 Finn et al.
2004/0253723 A1 12/2004 Tachas et al.
2004/0259247 A1 12/2004 Tuschl et al.
2005/0064595 A1 3/2005 MacLachlan et al.
2005/0118253 A1 6/2005 MacLachlan et al.
2006/0008910 A1 1/2006 MacLachlan et al.
2007/0042031 A1 2/2007 MacLachlan et al.
2009/0291131 A1* 11/2009 MacLachlan et al. 424/450

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 96/40964 A2 12/1996
WO WO 96/41873 A1 12/1996
WO WO 01/05374 A1 1/2001
WO WO 02/034236 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

(Continued)

OTHER PUBLICATIONS

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, Inc.), 1999, vol. 26, pp. 759-760.
Arpicco, S., et al., "Synthesis, characterization and transfection activity of new saturated and unsaturated cationic lipids," IL Farmaco, 2004, vol. 59, pp. 869-878.
Ballas, N., et al., "Liposomes bearing a quaternary ammonium detergent as an efficient vehicle for functional transfer of TMV-RNA into plant protoplasts," Biochimica et Biophysica Acta, 1988, vol. 939, pp. 8-18.

(Continued)

Primary Examiner — Brian Whiteman
(74) *Attorney, Agent, or Firm* — Kilpatrick Townsend & Stockton LLP

(57) **ABSTRACT**

The present invention provides novel, stable lipid particles comprising one or more active agents or therapeutic agents, methods of making the lipid particles, and methods of delivering and/or administering the lipid particles. More particularly, the present invention provides stable nucleic acid-lipid particles (SNALP) comprising a nucleic acid (such as one or more interfering RNA), methods of making the SNALP, and methods of delivering and/or administering the SNALP.

FOREIGN PATENT DOCUMENTS

WO	WO 2005/007196	A2	1/2005
WO	WO 2005/026372	A1	3/2005
WO	WO 2005/120152	A2	12/2005
WO	WO 2009/086558	A1	7/2009
WO	WO 2009/111658	A2	9/2009
WO	WO 2010/042877	A1	4/2010
WO	WO 2010/048228	A2	4/2010
WO	WO 2010/088537	A2	8/2010
WO	WO 2010/105209	A1	9/2010

OTHER PUBLICATIONS

Barinaga, M., "Step Taken Toward Improved Vectors for Gene Transfer," *Science*, 1994, vol. 266, p. 1326.

Bass, *Nature*, 2001, 411: 428-9.

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.

Behr, J.-P., "Synthetic Gene-Transfer Vectors," *Acc. Chem. Res.*, 1993, vol. 26, pp. 274-278.

Brigham, K., et al., "Rapid Communication: In vivo Transfection of Murine Lungs with a Functioning Prokaryotic Gene Using a Liposome Vehicle," *The American Journal of the Medical Sciences*, vol. 298, No. 4, pp. 278-281, 1989.

Brummelkamp, et al., "A System for Stable Expression of Short Interfering RNAs in Mammalian Cells," *Science*, 2002, V. 296, pp. 550-553.

Cevc, G., "How Membrane Chain-Melting Phase-Transition Temperature is Affected by the Lipid Chain Asymmetry and Degree of Unsaturation: An Effective Chain-Length Model," *Biochemistry*, 1991, vol. 30, pp. 7186-7193.

Cortesi, R., et al., "Effect of cationic liposome composition on in vitro cytotoxicity and protective effect on carried DNA," *International Journal of Pharmaceutics*, 1996, vol. 139, pp. 69-78.

Crystal, R., "Transfer of Genes to Humans: Early Lessons and Obstacles to Success," *Science*, 1995, vol. 270, pp. 404-410.

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, vol. 11, pp. 195-286.

Dwarki, V.J., et al., "Cationic Liposome-Mediated RNA Transfection," *Methods in Enzymology*, 1993, vol. 217, pp. 644-654.

Enoch, H., et al., "Formation and properties of 1000-Å-diameter, single-bilayer phospholipid vesicles," *Proc. Natl. Acad. Sci. USA*, 1979, vol. 76, No. 1, pp. 145-149.

Felgner, J., et al., "Cationic Lipid-Mediated Transfection in Mammalian Cells: 'Lipofection,'" *J. Tiss. Cult. Meth.*, 1993, vol. 15, pp. 63-68.

Felgner, J., et al., "Enhanced Gene Delivery and Mechanism Studies with a Novel Series of Cationic Lipid Formulations," *The Journal of Biological Chemistry*, 1994, vol. 269, No. 4, pp. 2550-2561.

Felgner, P., et al., "Lipofection: A highly efficient, lipid-mediated DNA-transfection procedure," *Proc. Natl. Acad. Sci. USA*, 1987, vol. 84, pp. 7413-7417.

Felgner, P.L., et al., "Cationic Liposome Mediated Transfection," *Proc. West. Pharmacol. Soc.*, 1989, vol. 32, pp. 115-121.

Gao, X., et al., "A Novel Cationic Liposome Reagent for Efficient Transfection of Mammalian Cells," *Biochem. Biophys. Res. Comm.*, 1991, vol. 179, No. 1, pp. 280-285.

Gershon, H., et al., "Mode of Formation and Structural Feature of DNA-Cationic Liposome Complexes Used for Transfection," *Biochemistry*, 1993, vol. 32, pp. 7143-7151.

Guy-Caffey, J., et al., "Novel Polyaminolipids Enhance the Cellular Uptake of Oligonucleotides," *The Journal of Biological Chemistry*, 1995, vol. 270, No. 52, pp. 31391-31396.

Hawley-Nelson, P., et al., "LipofectAmine™ Reagent: A New, Higher Efficiency Polycationic Liposome Transfection Reagent," *Focus*, 1993, vol. 15, No. 3, pp. 73-80.

Hyde, S., et al., "Correction of the ion transport defect in cystic fibrosis transgenic mice by gene therapy," *Nature*, 1993, vol. 362, pp. 250-255.

Jiang, L., et al., "Comparison of protein precipitation methods for sample preparation prior to proteomic analysis," *Journal of Chromatography A*, 2004, vol. 1023, pp. 317-320.

Juliano, R., et al., "The Effect of Particle Size and Charge on the Clearance Rates of Liposomes and Liposome Encapsulated Drugs," *Biochem. Biophys. Res. Commun.*, 1975, vol. 63, No. 3, pp. 651-658.

Keough, K., "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.

Legendre, J.-Y., et al., "Delivery of Plasmid DNA into Mammalian Cell Lines Using pH-Sensitive Liposomes: Comparison with Cationic Liposomes," *Pharm. Res.*, 1992, vol. 9, No. 10, pp. 1235-1242.

Leventis, R., et al., "Interactions of mammalian cells with lipid dispersions containing novel metabolizable cationic amphiphiles," *Biochem. Biophys. Acta*, 1990, vol. 1023, pp. 124-132.

Liu, et al., "Cationic Liposome-mediated Intravenous Gene Delivery," *J. Biol. Chem.*, 1995, V. 270, pp. 24864-24870.

Marshall, E., "Gene Therapy's Growing Pains," *Science*, 1995, vol. 269, pp. 1050-1055.

Orkin, S., et al., NIH Report, Report and Recommendations of the Panel to Assess the NIH Investment in Research on Gene Therapy, 1995.

Paul, C., et al., "Effective expression of small interfering RNA in human cells," *Nature Biotech.*, 2002, 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, vol. 228, pp. 697-703.

Sorensen, et al., "Gene Silencing by Systemic Delivery of Synthetic siRNAs in Adult Mice," *J. Biol. Chem.*, 2003, V. 278, pp. 761-766.

Spagnou, S., et al., "Lipidic Carriers of siRNA: Differences in the Formulation, Cellular Uptake, and Delivery with Plasmid DNA," *Biochemistry*, 2004, vol. 43, pp. 13348-13356.

Stamatatos, L., et al., "Interactions of Cationic Lipid Vesicles with Negatively Charged Phospholipid Vesicles and Biological Membranes," *Biochemistry*, 1988, vol. 27, pp. 3917-3925.

Szoka, F., et al., "Comparative Properties and Methods of Preparation of Lipid Vesicles (Liposomes)," *Ann. Rev. Biophys. Bioeng.*, 1980, vol. 9, pp. 467-508.

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, vol. 75, No. 9, pp. 4194-4198.

Templeton, "Cationic Liposome-mediated Gene Delivery In vivo", *Bioscience Reports*, 2002, vol. 22, No. 2, pp. 283-295.

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, vol. 1240, pp. 34-40.

Wheeler, et al., "Stabilized Plasmid-lipid Particles: Constructions and Characterization," *Gene Therapy*, V. 6, pp. 271-281, 1999.

Wilson, R., et al., "Counterion-Induced Condensation of Deoxyribonucleic Acid," *A Light-Scattering Study*, *Biochemistry*, 1979, vol. 18, No. 11, pp. 2192-2196.

Woodle, M.C., et al., "Versatility in lipid compositions showing prolonged circulation with sterically stabilized liposomes," *Biochimica et Biophysica Acta*, 1992, vol. 1105, pp. 193-200.

Zhu, N., et al., "Systemic Gene Expression After Intravenous DNA Delivery into Adult Mice," *Science*, 1993, vol. 261, pp. 209-211.

Elbashir, et al., Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells; *Nature*; May 2001; pp. 494-498; vol. 411.

* cited by examiner

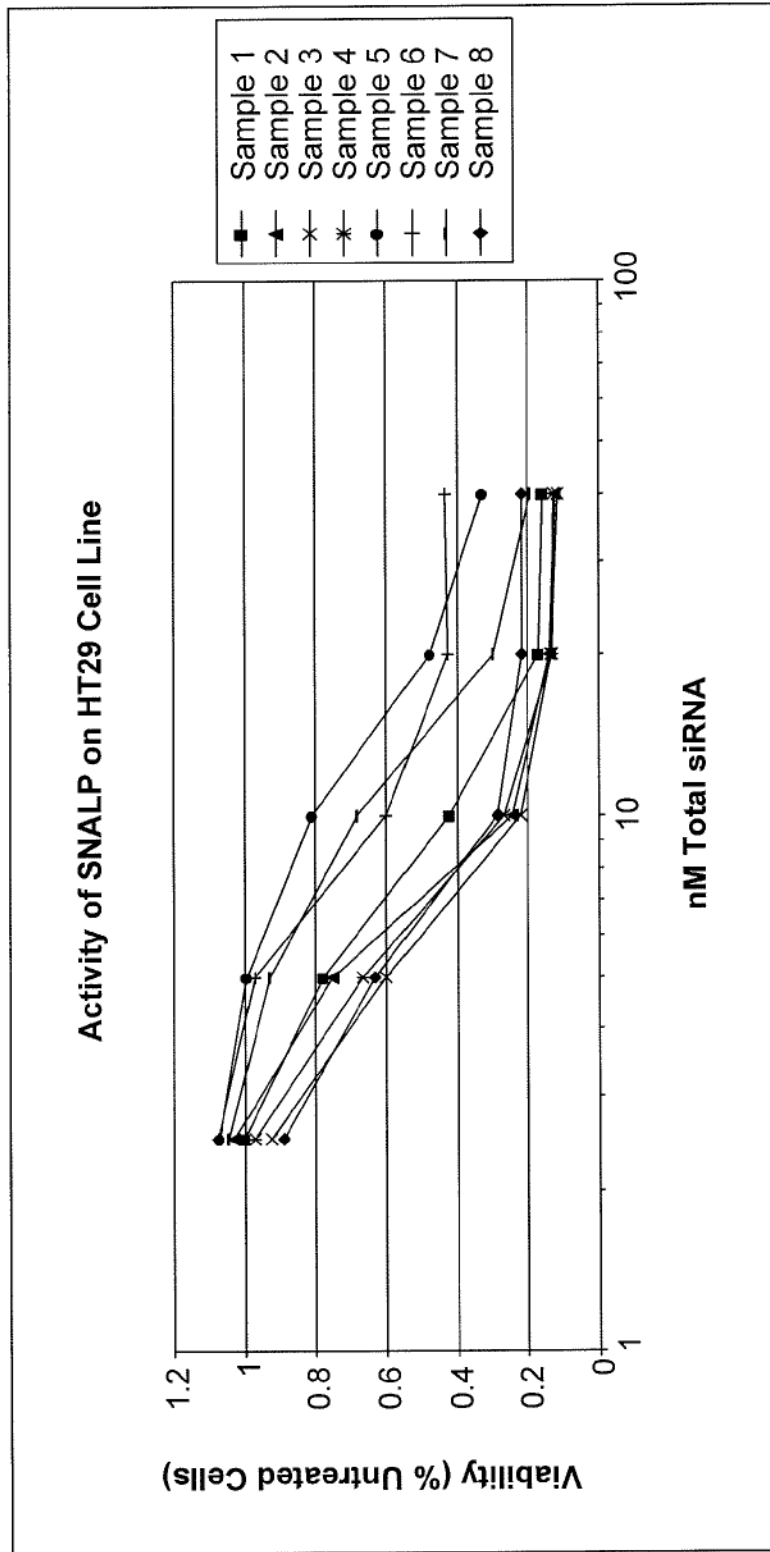


FIG. 1A

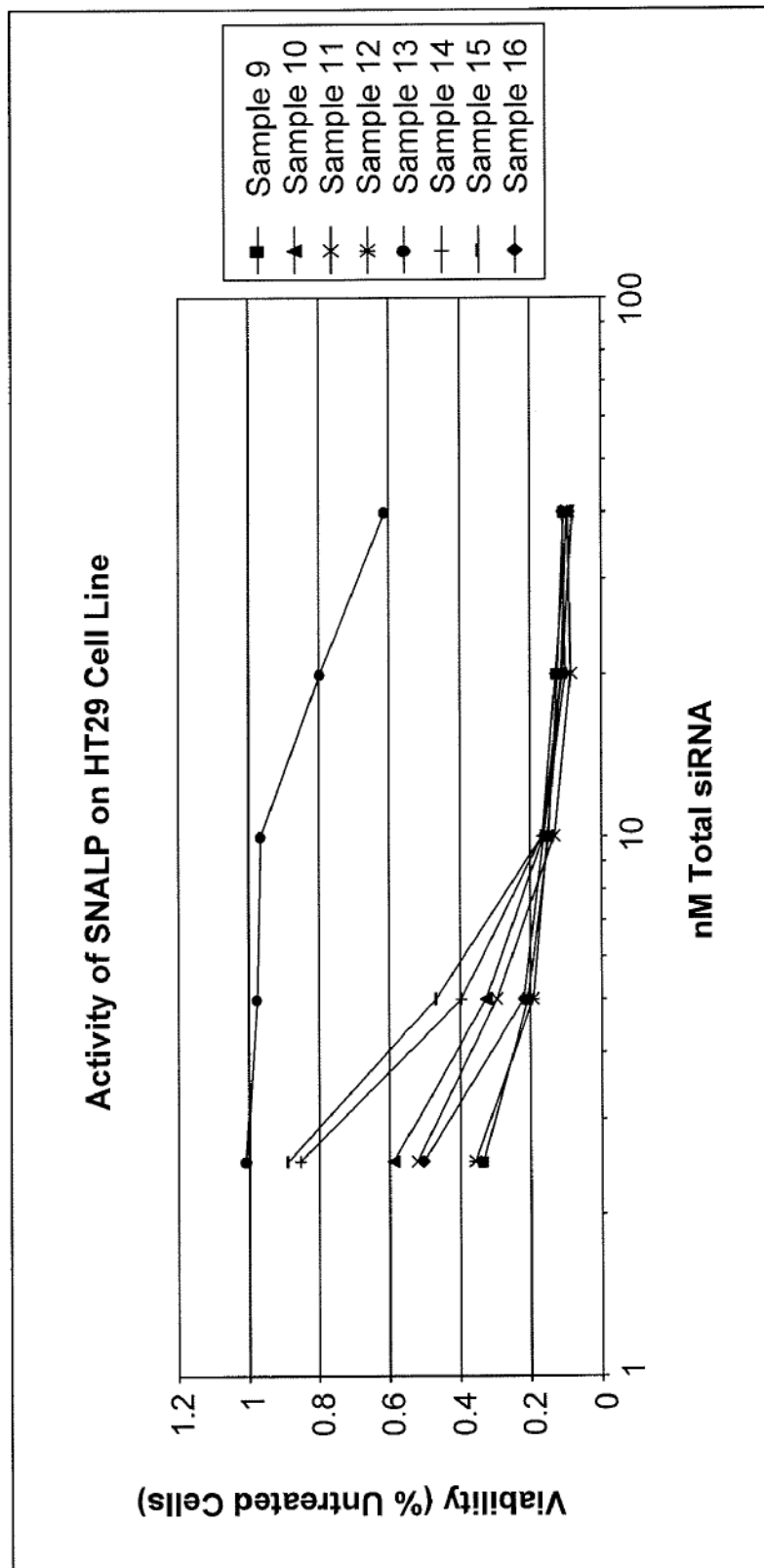


FIG. 1B

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.