

Exhibit C



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

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

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(52) **U.S. Cl.**
USPC **536/24.5**

(58) **Field of Classification Search**
USPC 536/24.5
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	Epanand et al.
5,320,906 A	6/1994	Eley et al.
5,334,761 A	8/1994	Gebeyehu et al.
5,545,412 A	8/1996	Eppstein et al.
5,578,475 A	11/1996	Jessee et al.

5,656,743 A	8/1997	Busch et al.
5,674,908 A	10/1997	Haces et al.
5,703,055 A	12/1997	Felgner et al.
5,705,385 A	1/1998	Bally et al.
5,736,392 A	4/1998	Hawley-Nelson et al.
5,820,873 A	10/1998	Choi et al.
5,877,220 A	3/1999	Schwartz et al.
5,885,613 A	3/1999	Holland et al.
5,958,901 A	9/1999	Dwyer et al.
5,976,567 A	11/1999	Wheeler et al.
5,981,501 A	11/1999	Wheeler et al.
6,020,202 A	2/2000	Jessee
6,020,526 A	2/2000	Schwartz et al.
6,034,135 A	3/2000	Schwartz et al.
6,051,429 A	4/2000	Hawley-Nelson et al.
6,075,012 A	6/2000	Gebeyehu et al.
6,165,501 A	12/2000	Tirosh et al.
6,172,049 B1	1/2001	Dwyer et al.
6,251,939 B1	6/2001	Schwartz et al.
6,284,267 B1	9/2001	Aneja
6,287,591 B1	9/2001	Semple et al.
6,339,173 B1	1/2002	Schwartz et al.
6,376,248 B1	4/2002	Hawley-Nelson et al.
6,534,484 B1	3/2003	Wheeler et al.
6,586,410 B1	7/2003	Wheeler et al.
6,638,529 B2	10/2003	Schwartz et al.

(Continued)

FOREIGN PATENT DOCUMENTS

CA	2309727 A1	4/1999
CA	2271582 A1	11/1999

(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.
Barinaga, M., "Step Taken Toward Improved Vectors for Gene Transfer," *Science*, 1994, vol. 266, p. 1326.
Bass, "The Short Answer," *Nature*, 2001, 411: 428-9.

(Continued)

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(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.

(56)

References Cited

U.S. PATENT DOCUMENTS

6,649,780 B1 11/2003 Eibl et al.
 6,671,393 B2 12/2003 Hays et al.
 6,696,424 B1 2/2004 Wheeler
 6,815,432 B2 11/2004 Wheeler et al.
 6,858,224 B2 2/2005 Wheeler et al.
 7,166,745 B1 1/2007 Chu et al.
 7,422,902 B1 9/2008 Wheeler et al.
 7,479,573 B2 1/2009 Chu et al.
 7,601,872 B2 10/2009 Chu et al.
 7,687,070 B2 3/2010 Gebeyehu et al.
 7,745,651 B2 6/2010 Heyes et al.
 7,799,565 B2* 9/2010 MacLachlan et al. 435/458
 7,803,397 B2 9/2010 Heyes et al.
 7,807,815 B2* 10/2010 MacLachlan et al. 536/24.5
 7,838,658 B2* 11/2010 MacLachlan et al. 536/24.5
 7,901,708 B2 3/2011 MacLachlan et al.
 7,915,450 B2 3/2011 Chu et al.
 7,982,027 B2* 7/2011 MacLachlan et al. 536/24.5
 8,058,068 B2 11/2011 Hawley-Nelson et al.
 8,058,069 B2* 11/2011 Yaworski et al. 435/458
 8,101,741 B2* 1/2012 MacLachlan et al. 536/24.5
 8,158,827 B2 4/2012 Chu et al.
 8,188,263 B2* 5/2012 MacLachlan et al. 536/24.5
 8,227,443 B2* 7/2012 MacLachlan et al. 514/44 A
 8,236,943 B2* 8/2012 Lee et al. 536/24.5
 8,283,333 B2* 10/2012 Yaworski et al. 514/44 A
 8,455,455 B1* 6/2013 Robbins et al. 514/44 A
 8,492,359 B2* 7/2013 Yaworski et al. 514/44 A
 8,513,403 B2* 8/2013 MacLachlan et al. 536/24.5
 8,569,256 B2* 10/2013 Heyes et al. 514/44 A
 8,598,333 B2* 12/2013 MacLachlan et al. 536/24.5
 2001/0048940 A1 12/2001 Tousignant et al.
 2003/0069173 A1 4/2003 Hawley-Nelson et al.
 2003/0072794 A1 4/2003 Boulikas
 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.
 2005/0260757 A1 11/2005 Gebeyehu et al.
 2006/0008910 A1 1/2006 MacLachlan et al.
 2006/0147514 A1 7/2006 Gebeyehu et al.
 2006/0228406 A1 10/2006 Chiou et al.
 2007/0042031 A1 2/2007 MacLachlan et al.
 2007/0202598 A1 8/2007 Chu et al.
 2007/0202600 A1 8/2007 Chu et al.
 2009/0143583 A1 6/2009 Chu et al.
 2009/0291131 A1 11/2009 MacLachlan et al.
 2010/0159593 A1 6/2010 Chu et al.
 2012/0136073 A1 5/2012 Yang et al.
 2012/0238747 A1 9/2012 Chu et al.

FOREIGN PATENT DOCUMENTS

CA 2330741 A1 11/1999
 CA 2397016 A1 7/2001
 CA 2513623 8/2004
 JP 03-126211 5/1991
 JP 05-202085 8/1993
 JP 06-080560 3/1994
 WO 91/16024 A1 10/1991
 WO 93/05162 A1 3/1993
 WO 93/12240 A1 6/1993
 WO 93/12756 A2 7/1993
 WO 93/24640 A2 12/1993
 WO 93/25673 A1 12/1993
 WO 95/02698 A1 1/1995
 WO 95/18863 A1 7/1995
 WO 95/35301 A1 12/1995

WO 96/40964 A2 12/1996
 WO 96/41873 A1 12/1996
 WO 98/51285 A2 11/1998
 WO 00/03683 A2 1/2000
 WO 00/15820 A1 3/2000
 WO 00/62813 A2 10/2000
 WO 01/05374 A1 1/2001
 WO 01/05873 A1 1/2001
 WO 01/93836 12/2001
 WO 02/34236 A2 5/2002
 WO 02/087541 A1 11/2002
 WO 03/097805 A2 11/2003
 WO 2004/065546 A2 8/2004
 WO 2004/110499 A1 12/2004
 WO 2005/007196 A2 1/2005
 WO 2005/026372 A1 3/2005
 WO 2005/035764 A1 4/2005
 WO 2005/120152 A2 12/2005
 WO 2006/002538 A1 1/2006
 WO 2006/053430 A1 5/2006
 WO 2007/056861 A1 5/2007
 WO 2009/086558 A1 7/2009
 WO 2009/111658 A2 9/2009
 WO 2010/042877 A1 4/2010
 WO 2010/048228 A2 4/2010
 WO 2010/088537 A2 8/2010
 WO 2010/105209 A1 9/2010

OTHER PUBLICATIONS

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.
 Chonn et al., "Recent advances in liposomal drug-delivery systems," *Current Opinion in Biotechnology*, 1995, vol. 6, pp. 698-708.
 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.
 Elbashir et al., "Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells," *Nature*, May 2001, pp. 494-498, vol. 411.
 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

(56)

References Cited

OTHER PUBLICATIONS

- 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.
- Global Newswire, retrieved from <http://globalnewswire.com> on Feb. 27, 2013, Tekmira sues Alnylam Pharmaceuticals for repeated misuse of tradesecrets and confidential information, Mar. 16, 2011, pp. 1-3.
- 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.
- Heyes et al., "Cationic lipid saturation influences intracellular delivery of encapsulated nucleic acids," *Journal of Controlled Release*, 2005, vol. 107, pp. 276-287.
- Heyes et al., "Synthesis of novel cationic lipids: effect of structural modification on the efficiency of gene transfer," *J. Med. Chem.*, 2002, vol. 45, pp. 99-114.
- 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.
- JP06080560—English abstract from CAplus 2 pages, 1994.
- 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.
- Krichevsky, A. et al., "RNAi functions in cultured mammalian neurons," *PNAS*, 99(18):11926-29, 2002.
- Lawrence et al., "The formation, characterization and stability of non-ionic surfactant vesicles," *S.T.P. Pharma Sciences*, 1996, vol. 6, No. 1, pp. 49-60.
- Lawrence et al., "Synthesis and aggregation properties of dialkyl polyoxyethylene glycerol ethers," *Chemistry and Physics of Lipids*, 1996, 82(2):89-100.
- 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.
- Murahashi et al., "Synthesis and evaluation of neoglycolipid for liposome modification," *Biol. Pharm. Bull.*, 1997, 20(6):704-707.
- Orkin, S. et al., NIH Report, Report and Recommendations of the Panel to Assess the NIH Investment in Research on Gene Therapy, 1995, pp. 1-41.
- Parr et al., Factors influencing the retention and chemical stability of poly(ethylene glycol)-lipid conjugates incorporated into large unilamellar vesicles, *Biochimica et Biophysica Acta*, 1994, 1195:21-30.
- 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.
- Sawada et al., "Microemulsions in supercritical CO₂ utilizing the polyethyleneglycol dialkylglycerol and their use for the solubilization of hydrophiles," *Dyes and Pigments*, 2005, pp. 64-74, vol. 65.
- Shin et al. "Acid-triggered release via dePEGylation of DOPE liposomes containing acid-labile vinyl ether PEG-lipids," *Journal of Controlled Release*, 2003, vol. 91, pp. 187-200.
- Song et al., "Characterization of the inhibitory effect of PEG-lipid conjugates on the intracellular delivery of plasmid and antisense DNA mediated by cationic lipid liposomes," *Biochimica et Biophysica Acta*, 2002, 1558:1-13.
- Sorensen et al., "Gene Silencing by Systemic Delivery of Synthetic siRNAs in Adult Mice", *J. Biol. Chem.*, 2003, V. 327, 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.

* cited by examiner

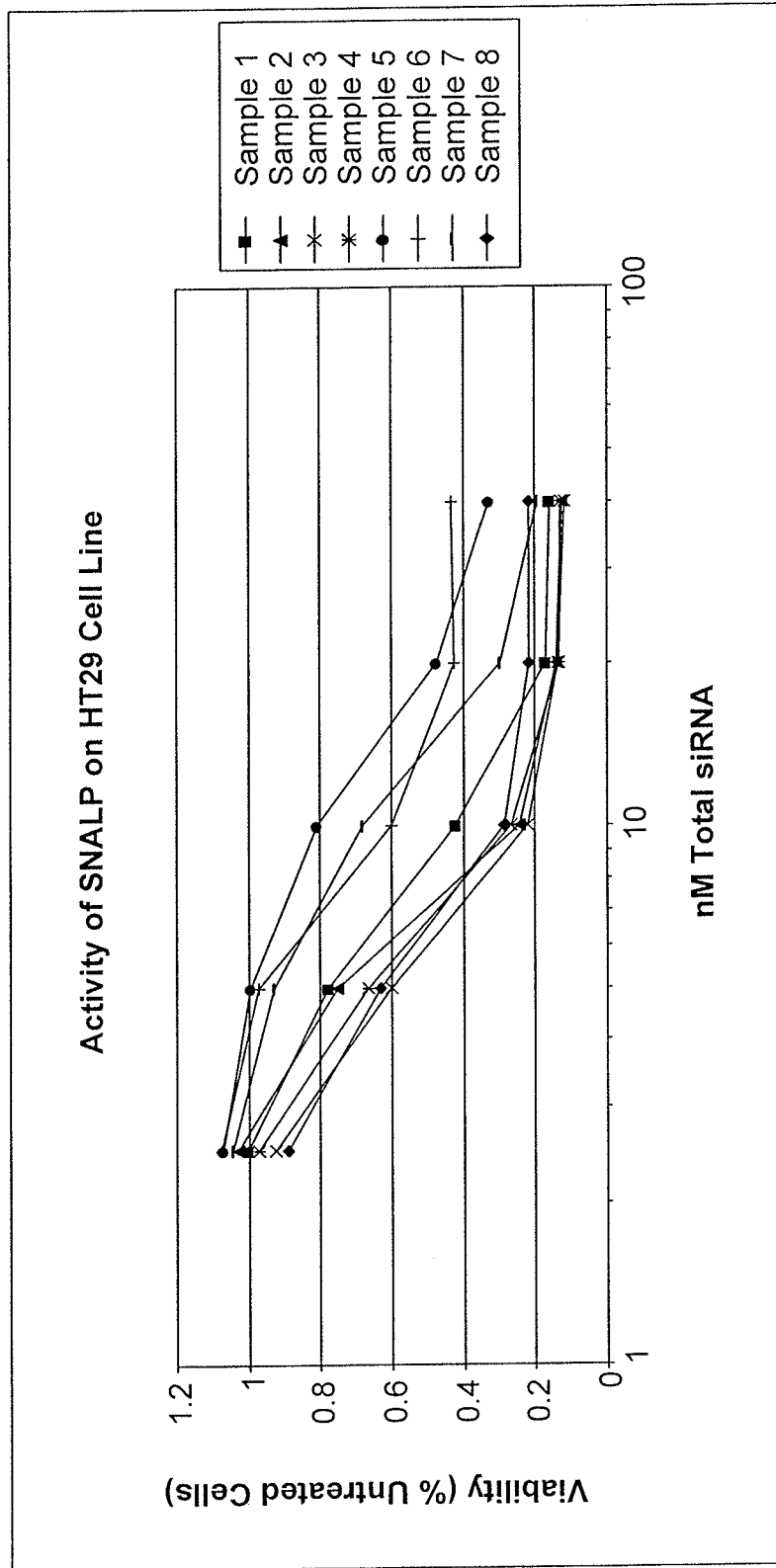


FIG. 1A

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