

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

(71) Applicant: **Protiva Biotherapeutics, Inc.**, Burnaby (CA)

(72) 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, BC (CA)

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(58) **Field of Classification Search**

CPC . C12N 15/113; C12N 2310/14; A61K 9/1271
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 Deussen

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.

5,334,761 A 8/1994 Gebeyehu et al.

5,545,412 A 8/1996 Eppstein et al.

5,578,475 A 11/1996 Jessee

5,627,159 A 5/1997 Shih et al.

5,641,662 A 6/1997 Debs 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

(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.

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.

(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.

(56)

References Cited

FOREIGN PATENT DOCUMENTS

U.S. PATENT DOCUMENTS

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.
 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 et al.
 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.
 7,803,397 B2 9/2010 Heyes et al.
 7,807,815 B2 10/2010 MacLachlan et al.
 7,838,658 B2 11/2010 MacLachlan et al.
 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.
 8,058,068 B2 11/2011 Hawley-Nelson et al.
 8,058,069 B2 11/2011 Yaworski et al.
 8,101,741 B2 1/2012 MacLachlan et al.
 8,158,827 B2 4/2012 Chu et al.
 8,188,263 B2 5/2012 MacLachlan et al.
 8,227,443 B2 7/2012 MacLachlan et al.
 8,236,943 B2* 8/2012 Lee et al. 536/24.5
 8,283,333 B2 10/2012 Yaworski et al.
 8,455,455 B1 6/2013 Robbins et al.
 8,492,359 B2* 7/2013 Yaworski et al. 514/44 A
 8,513,403 B2 8/2013 MacLachlan et al.
 8,569,256 B2 10/2013 Heyes et al.
 8,598,333 B2 12/2013 MacLachlan et al.
 8,822,668 B2* 9/2014 Yaworski et al. 536/24.5
 9,006,417 B2* 4/2015 Yaworski 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 Bouliskas
 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 Tuschi 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.
 2006/0240093 A1 10/2006 MacLachlan 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.
 2010/0130588 A1 5/2010 Yaworski et al.
 2010/0159593 A1 6/2010 Chu et al.
 2012/0058188 A1 3/2012 MacLachlan et al.
 2012/0136073 A1 5/2012 Yang et al.
 2012/0183581 A1 7/2012 Yaworski et al.
 2012/0228747 A1 9/2012 Chu et al.

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
 JP 2002-525063 8/2002
 JP 2003-505401 2/2003
 JP 2007-524349 8/2007
 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 96/02655 A1 2/1996
 WO 96/10390 A1 4/1996
 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 01/75164 A2 10/2001
 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

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.
 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,

(56)

References Cited

OTHER PUBLICATIONS

- 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 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.
- Global Newswire, retrieved from <http://globalnewswire.com> on Feb. 27, 2013, Tekmira sues Alnylam Pharmaceuticals for repeated misuse of tradeseecrets 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.
- 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.
- 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.
- 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

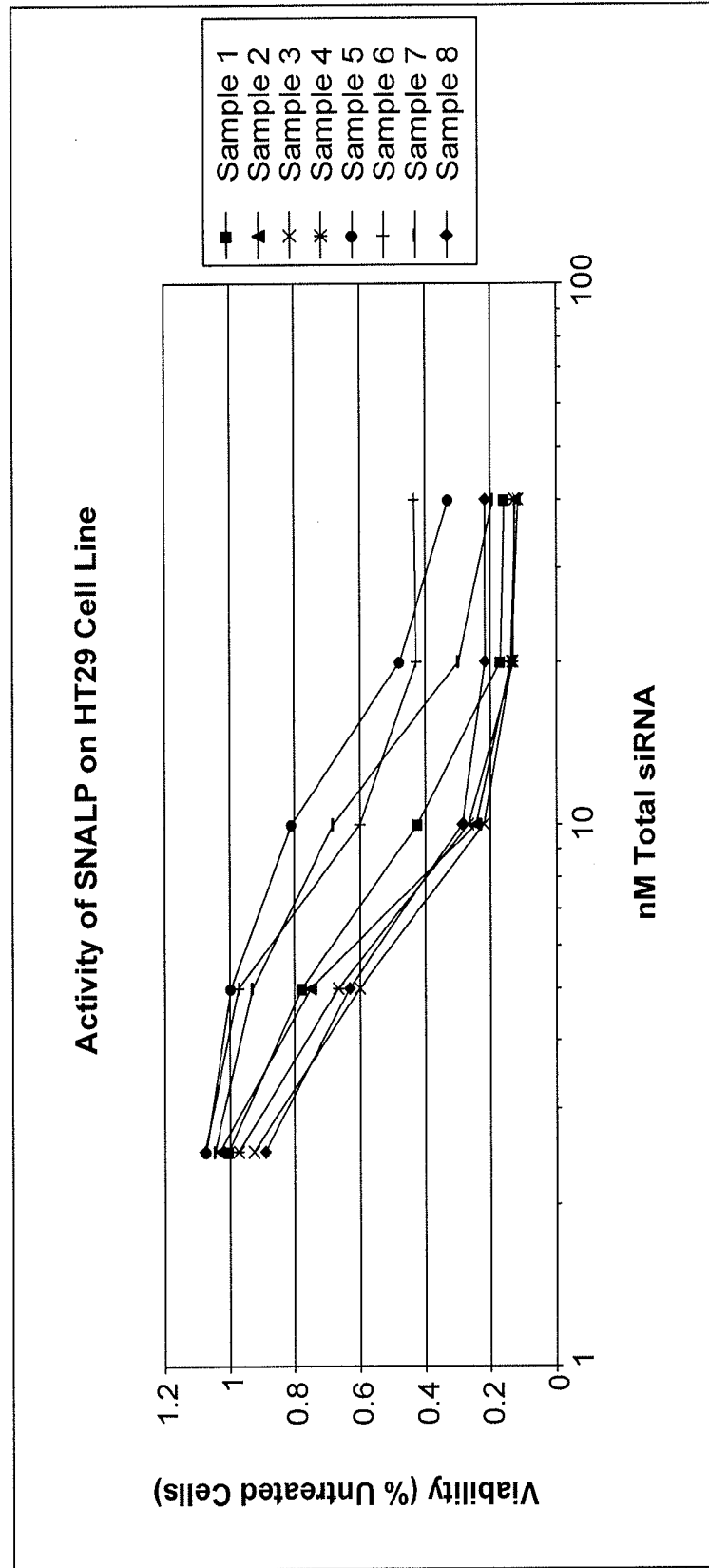


FIG. 1A

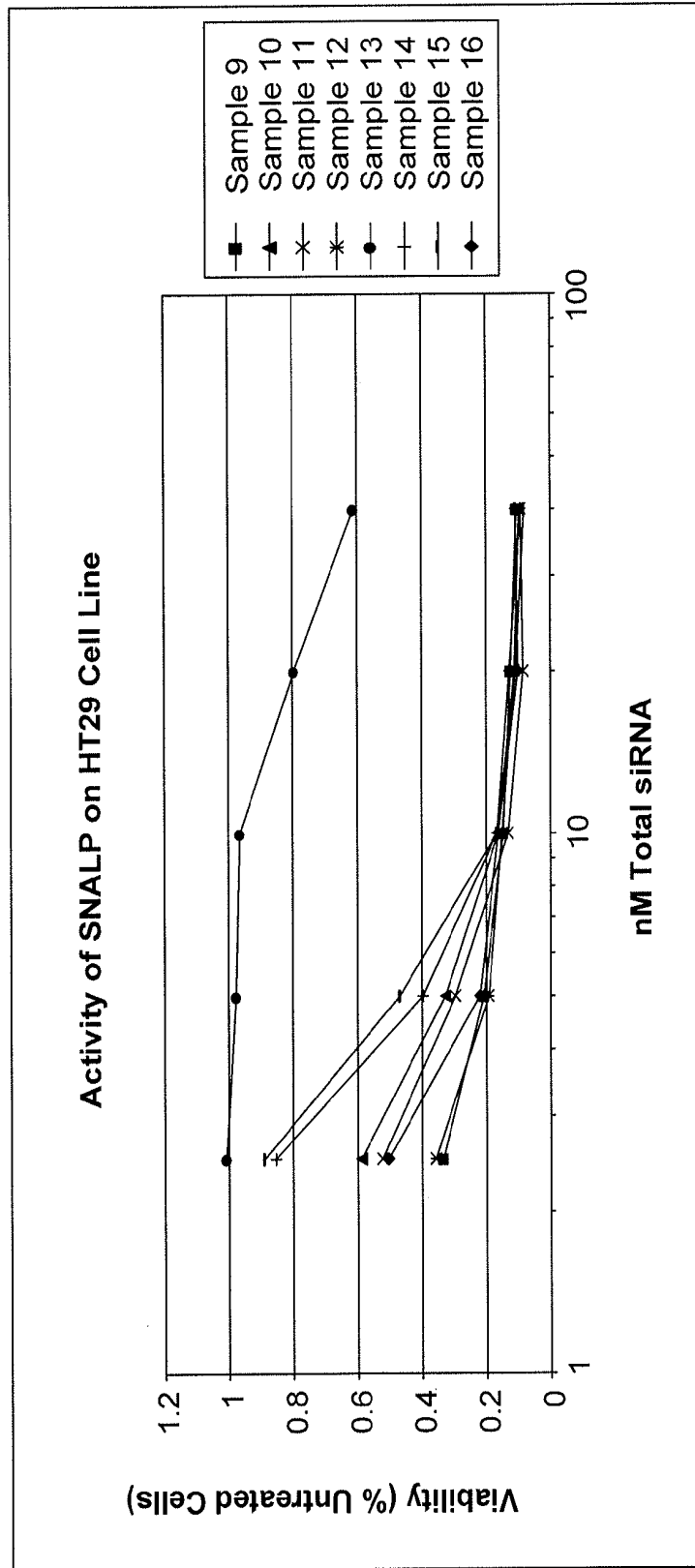


FIG. 1B

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