AUSTRALIA

Patents Act 1990

IN THE MATTER OF Patent Acceptance 2020202755 in the Name of **Shire Human Genetic Therapies**

- and -

IN THE MATTER OF Opposition thereto by **JLTF Holdings Pty Ltd**

Declaration of: Leaf Huang

Address: University of North Carolina, 1315 Kerr Hall, CB# 7571, Chapel Hill, NC, 27599

Occupation: Professor in the Eshelman School of Pharmacy at the University of North Carolina

Date: 5th September 2023

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4.	**Exhibit LH-4** which is a copy of the journal article *Gene Transfer In Vivo with DNA-Liposome Complexes*: *Safety and Acute Toxicity in Mice*, Stewart, M. et al., *Human Gene Therapy* 3:267-275 (1992)
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	Multilamellar Vesicle Structures, Semple, S., *et al.,* Biochimica et Biophysica Acta, 1510, 152-166 (2001)
11.	**Exhibit LH-11** which is a copy of the journal article *Stabilized Plasmid-Lipid Particles: Pharmacokinetics and Plasmid Delivery to Distal Tumors following Intravenous Injection,* Monck, M.A., et al. Journal of Drug Targeting, 7(6), 439-452 (2000)
12.	**Exhibit LH-12**, a copy of WO2005/120152A2, *Cationic Lipids and Methods of Use* (**D1**)
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19.	**Exhibit LH-19** which is a copy of Martinon et al., "Induction of virus-specific cytotoxic T lymphocytes in vivo by liposome- entrapped mRNA". *Eur. J. Immunol.* 1993. 23: 1719-1722 (**D14**)
20.	**Exhibit LH-20** being a copy of Pascolo, S. (2008). "Vaccination with Messenger RNA (mRNA)". In: Bauer, S., Hartmann, G. (eds) Toll-Like Receptors (TLRs) and Innate Immunity. Handbook of Experimental Pharmacology, vol 183. Springer, Berlin, Heidelberg (**D15**)
21.	**Exhibit LH-21** which is a copy of Hess et al., "Vaccination with mRNAs encoding tumor-associated antigens and granulocyte-macrophage colony-stimulating factor efficiently primes CTL responses, but is insufficient to overcome tolerance to a model tumor/self antigen". *Cancer Immunol. Immunother.* (2006) 55: 672-683 (**D16**)
22.	**Exhibit LH-22** which is a copy of Fenske DB et al. "Liposomal Nanomedicines: An emerging field". *Toxicol. Pathol.* 2008 Jan; 36(1):21-9 (**D17**)
23.	**Exhibit LH-23** being a copy of Malone RW et al. "Cationic liposome-mediated RNA transfection". *Proc Natl Acad Sci U S A.* 1989 Aug; 86(16):6077-81 (**D18**)
24.	**Exhibit LH-24** being a copy of WO1990/011092A1, *Expression of Exogenous Polynucleotide Sequences in a Vertebrate* (**D19**)

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25.	**Exhibit LH-25** which is a copy of the Opposed Australian Patent Application No. 2020202755
26.	**Exhibit LH-26** being a copy of Gómez-Aguado, I., et al. "Nanomedicines to Deliver mRNA: State of the Art and Future Perspectives". *Nanomaterials*. Feb 2020; 10: 364
27.	**Exhibit LH-27** being a copy of Buschmann, M.D., et al. "Nanomaterial Delivery Systems for mRNA Vaccines". *Vaccines*. Jan 2021; 9: 65
28.	**Exhibit LH-28** which is a copy of WO2012/170930A1, *Lipid Nanoparticle Compositions and Methods for mRNA Delivery*

I, **LEAF HUANG**, Professor in the Eshelman School of Pharmacy at the University of North Carolina, 1315 Kerr Hall, CB# 7571, Chapel Hill, NC, 27599, do declare as follows:-

A. INTRODUCTION

1. I have been informed that my evidence will be used in a dispute about a patent application. I was told that the patent application being opposed ("**the Opposed Application**") was filed in Australia in the name of Shire Human Genetic Therapies ("**Shire**"). The Opposed Application was not identified to me, initially, and I was asked not to look for it. I have been requested to act as an independent expert witness in respect of this matter.

2. I have been provided with a copy of the Federal Court of Australia Expert Evidence Practice Note (GPN-EXPT) dated 25 October 2016 (Practice Note), a copy of which is exhibited hereto and marked **Exhibit LH-1**. I have read and understood the Practice Note, and have complied with the Practice Note in the course of preparing this Declaration.

3. Although, prior to giving my comments in relation to the matters set out in sections A to E of this Declaration I was not told the identity of the Opposed Application, or anything that could identify the patent application which is the subject of this dispute, I was told that the relevant patent application has a claimed priority date of 01 December 2009 ("**the Priority Date**" or "**relevant date**"). I was also told that the priority date is a matter at issue in this opposition and that it may be later, up until 30 November 2010. I was told that I should assume that the relevant date for considering what was the common general knowledge of the skilled addressee, and for assessing whether the claims of the opposed application are novel and inventive, is 01 December 2009. I was also told that if I considered that there were material changes in what was commonly known between the 01 December 2009 and 30 November 2010 dates, I should indicate that.

4. While, in some parts of my Declaration below I have used the present tense and in others the past tense, each of my comments in those sections reflects my opinion as to what was known and understood by me as at the Priority Date, unless the context suggests otherwise.

5. My Declaration is organised as follows:

a) Section A contains some introductory comments;

b) Section B outlines my experience and qualifications;

c) Section C identifies the sources of information I consulted at the Priority Date;

d) Section D provides some background as to my state of knowledge in lipid delivery vehicles formulated with nucleic acids ("**the Field**") at the Priority Date;

e) Section E contains my initial comments on documents which I understand were published before the Priority Date or the valid priority date ("t**he Prior Art**");

f) Section F sets out my comments on the Opposed Application, which was provided to me after I had given my opinion in relation to the matters the subject of sections A to E; and

g) Section G sets out my opinion as to whether the Prior Art discloses the features of claims of the Opposed Application.

B. MY EXPERIENCE AND QUALIFICATIONS

6. Exhibited hereto and marked **Exhibit LH-2** is a copy of my CV, which includes details of my academic qualifications, professional experience, and memberships to industry bodies.

7. I am the Fred Eshelman Distinguished Professor in the Eshelman School of Pharmacy at the University of North Carolina at Chapel Hill. My research has been in the area of gene therapy and targeted drug delivery. I pioneered the liposome non-viral vector and designed and manufactured the cationic lipid vector for the first non-viral clinical trial in 1992. I was also the first to publish the activity of polyethylene glycol (PEG) in prolonging the circulation time of liposomes.

8. My research has been in the area of gene therapy and targeted drug delivery. I pioneered the liposome non-viral vector and designed and manufactured the cationic lipid vector for the first non-viral clinical trial in 1992. I was also the first to publish the activity of polyethylene glycol (PEG) in prolonging the circulation time of liposomes.

9. In 2004, I received the Alec D. Bangham MD FRS Achievement Award, which is the highest honour in liposome research. I was the recipient of the 2013 Distinguished Pharmaceutical Scientist Award

which is the highest scientific recognition of the American Association of Pharmaceutical Scientists. I was named a Highly Cited Researcher in “Pharmacology & Toxicology” and then in “Cross Field” since 2016. I have also co-founded 7 biotech start-up companies in the past, including Lipogen, Rx Therapeutics, Lipella and PDS Biotechnology.

10. In addition to my role as the Fred Eshelman Distinguished Professor within the Division of Molecular Pharmaceutics (now Division of Pharmacoengineering and Molecular Pharmaceutics) of the Eshelman School of Pharmacy at the University of North Carolina, I am also an Honorary Professor at the Sichuan University in China (2009), a Visiting Chair Professor at Chung-Yuan Christian University in Taiwan (2010-2017), a Visiting Professor at the Fourth Military Medical University in China (2012), an Advanced Visiting Scholar at Fudan University in China (2013-2016), a Visiting Professor at Shenyang Pharmaceutical University in China (2016-2017), and an Adjunct Professor at Shanghai University of Traditional Chinese Medicine. I also spend some of my time at the University of North Carolina lecturing, and over the years have lectured a number of subjects including Advances in Drug Delivery and Nanomedicine, and Pharmaceutical Analysis. I have been responsible for the supervision of a number of graduate and post-graduate students, for example, to date I have supervised over 20 graduate students and over 20 postdoctoral fellows on various projects in the field of liposome technology and cellular delivery.

11. I have a PhD in biophysics at Michigan State University, and a Bachelor of Science with a major in physics. Before carrying out my post-doctoral research fellowship in the field of biophysics at Carnegie Institute of Washington, I worked for four years as a research assistant within the department of biophysics at Michigan State University. It was during my post-doctoral research that I became involved with liposome research and went on to be a pioneer of liposomal delivery of genes and other nucleic acid-based therapies as well as other small molecular weight drugs.

12. During the period from 1976 to the present, I have worked in the field of biophysics with a focus on gene therapy and targeted drug delivery employing lipid-related technologies.

13. From 1976 to 1991, at the University of Tennessee, I had a number of roles within the Department of Biochemistry. From 1976 to 1980, I was an Assistant Professor, followed by an Associate Professor from 1980-1985, and then a Professor from 1985-1991. In my time at the University, I taught/group taught various subjects including Biochemistry, Advanced Cell Biology, and Liposome Technology, to graduate students and pre-med/pre-vet students. I was also responsible for the supervision of over 25 graduate students and over 10 postdoctoral fellows on various projects in the field of liposome technology and cellular delivery.

14. From 1991 to 2005, I was at the University of Pittsburgh. I was appointed Professor within the Department of Pharmacology at the University of Pittsburgh and was also Head of the Laboratory of Drug Targeting until 1999. During my time at the University of Pittsburgh I was appointed to a number of various roles, such as the Director of Liposome Vector Core within Pittsburgh Centre for Human Gene Therapy in 1993, Director of the Molecular Pharmacology Training Program from 1997-1999, Associate Dean for Research within the School of Pharmacy also in 1999, and Director within the Centre for Pharmacogenetics from 1999-2005 whilst also sharing my time as a Professor within the Department of Pharmacology (1999-2005) and Department of Bioengineering (2001-2005). In my time at the University, I also lectured various subjects including Gene Therapy Vectors, Drug Delivery Systems, Cancer Biology and Therapeutics, Advanced Topics in Drug Delivery and Targeting, Molecular Pharmacology, and Receptors and Signal Transduction. I was also responsible for the supervision of over 15 graduate students and over 30 postdoctoral fellows on various projects in the field of liposome technology and cellular delivery.

15. The research and development that I have conducted during this period, provided me with knowledge and experience in gene therapy and targeted drug delivery, such as in the development of ligand targeted delivery systems for cDNA, mRNA, siRNA, proteins, and peptides for tumour growth inhibition and for vaccines in treating cancer and infected diseases.

16. In addition, I have been or am a member of the following professional associations:
    - Society of Chinese Bio-scientists in America;
    - Controlled Release Society;
    - American Association of Pharmaceutical Scientists;
    - American Society of Gene and Cell Therapy; and
    - American Association of Colleges of Pharmacy.

17. These organizations have members and contributors from all over the world, including Australia. During meetings of these associations, global researchers would regularly attend and indeed, present their work.

18. During my over 40 years of experience in the area of gene therapy and targeted drug delivery, I have attended and participated in numerous international conferences, symposiums and meetings. I have served as a committee chair, committee member, and session chair for a number of committees, conferences, symposium, courses and seminar series. For these organizations, I have been actively engaged in the development and execution of sessions, workshops and national meeting programs, all relating to drug delivery systems. My role as both a leader of and participant in these sessions, as well as my teaching and research responsibilities, required me to keep abreast of the latest research

and developments in gene therapy and targeted drug delivery field on an ongoing basis.

19. I have authored or co-authored more than 600 papers with an H-index of 146, principally related to lipid-based approaches to drug delivery. I have published in many of the top scientific journals including *Nature, Nature Medicine, Nature Nanotechnology, Nature Communications* and *Proceedings of the National Academy of Sciences*. I have authored or co-authored over 120 invited reviews/book chapters, and have co-edited two books, some of which include, for example, a book chapter within "*Nanoparticulate Drug Delivery Systems: Strategies, Technologies, and Applications*", first published in early 2013, that I co-authored and having a chapter title "*Lipid Nanoparticles for the Delivery of Nucleic Acids*"; and co-edited the books *Nonviral Vectors for Gene Therapy: Advances in Genetics*, Part I and Part II, and *Nonviral Vectors for Gene Therapy: Lipid and Polymer Based Gene Transfer*. These books and book chapters discuss some of the challenges in gene delivery systems, and contain in-depth discussions of different non-viral approaches, including cationic lipid nanoparticles and polymers, naked DNA and various physical methods of delivery, as well as a comprehensive coverage of the nucleic acid payloads. I am also the inventor or co-inventor of about 49 US and foreign patents in lipid-delivery technology and general drug delivery.

20. I am the Editor Emeritus of *Journal of Liposome Research.* I served on the editorial board of this Journal from 1986-1991, and was the Editor-in-Chief from 1991-1997. I was also an Associate Editor of *Gene Therapy* in 1999 and *Molecular Therapy* from 1999-2004. These leading journals cover both the research and clinical applications of novel therapeutic techniques based on a genetic component, gene transfer, vector development and design, stem cell manipulation, development of gene-, peptide-, protein-, oligonucleotide-, and cell-based therapeutics to correct genetic and acquired diseases, vaccine development, pre-clinical target validation, safety/efficacy studies, and clinical trials.

21. I have also served on the editorial board of the following:
    - *Journal of Drug Targeting* (1992-1999),
    - *Gene Therapy* (1993-1999, 2000),
    - *Advanced Drug Delivery Reviews* (1995-2019),
    - *Journal of Gene Medicine* (1999-2006),
    - *Journal of Controlled Release* (2000-2015),
    - *Molecular Pharmaceutics* (2003-2010),
    - *Biological and Pharmaceutical Bulletin* (2004-present),
    - *BBA – Biomembranes* (2010-2015),
    - *Acta Pharmaceutica Sinica B* (2011-2019), *and*

- *Molecular Therapy: Nucleic Acids* (2011-present).

C. SOURCES OF INFORMATION

22. I have been asked to describe any steps I took to keep up-to-date with developments in the Field before the Priority Date.

23. It was and remains important for me to keep abreast of key developments in the Field. At the Priority Date, I acquired relevant knowledge and information through:

    a) my interactions with colleagues all over the world;

    b) the editing, authoring and preparation of publications as outlined above;

    c) my membership of various industry and professional organisations as outlined above;

    d) keeping up to date with the literature;

    e) attending and speaking at conferences in the Field.

D. BACKGROUND KNOWLEDGE

24. I was asked what I consider the skillset and knowledge base to be, of a skilled person or team given the task of providing compositions having a nucleic acid formulated within a liposome, working immediately before the priority date of the Opposed Application.

25. In my view, providing compositions having a nucleic acid formulated within a liposome would require a team including a person with knowledge of how to prepare liposome formulations and their use in delivering nucleic acids. Such a person would be a lipid formulation chemist, who might have a PhD and background in organic chemistry or, like myself, membrane biophysics, and/or a biochemist; and a person having knowledge in the formulation and production of nucleic acid-based protein expression generally, including protein replacement therapies or vaccines. Given my background in biophysics, my experience and knowledge of gene therapy and targeted drug delivery, and my time working in the field for many years, I view myself as a skilled person familiar with the roles of each member of such a team and with a particular familiarity with lipid formulations and nucleic acid delivery.

26. I was then asked to provide my views on what was common general knowledge in the field immediately prior to 01 December 2009.

27. To assist me in my role as an independent expert, I have been provided with a copy of an extract from Justice Emmett's decision in *ICI Chemicals & Polymers Ltd v Lubrizol Corp Inc.* Exhibited hereto

and marked **Exhibit LH-3** is a copy of this document. I have read the document and understand that it explains what is meant by the term "common general knowledge". I understand that the common general knowledge is the technical background to the hypothetical skilled worker in the relevant field, that it is not limited to material which might be memorized and retained at the front of the skilled worker's mind but also includes material in the field in which they are working which they know exists and to which they would refer as a matter of course. I have been further advised that such material may include, for example:

- Standard texts and handbooks;
- Standard English dictionaries;
- Technical dictionaries relevant to the field;
- Magazines, journals, technical bulletins, and other publications specific to the field; and
- Technical material readily accessible on the Internet.

28. Unless stated otherwise, my comments below concern information of which I was aware immediately prior to 01 December 2009, and which I consider to have been common general knowledge for a person of skill in the art immediately prior to 01 December 2009, looking to develop compositions having a nucleic acid formulated within a liposome.

29. As at 01 December 2009, there were many labs working on nucleic acid delivery approaches. Pieter Cullis' lab was one of the best known and his and many other of these groups had worked on DNA or RNA delivery. One of the most common approaches was to use liposomes or similar types of lipid vehicles including stabilised plasmid-lipid particles (SPLP), stabilised nucleic acid-lipid particles (SNALP), and stabilized antisense-lipid particles (SALP). The naming of these vehicles often related to the specific cargo being encapsulated rather than a significant difference in structure of the lipid vehicle itself. Other delivery systems such as polymeric particles and protamine cores encased in lipids were also being tested and developed.

30. Lipid delivery platforms had proven successful in DNA and siRNA delivery and so were a reasonably well-developed technology within certain bounds. Early research also showed that mRNA could be encapsulated within a cationic liposome, delivered and expressed. One particular cationic lipid, first published by my team in the early 90's, known as DC-cholesterol ((3β-[N-(N',N'-dimethylamino-ethane)carbamoyl]-cholesterol) is an ionizable cationic lipid which can be used in the preparation of liposomes in combination with DOPE (dioleoylphosphatidylethanolamine) to deliver nucleic acids into various cell types *in vitro*, as well as *in vivo*. To demonstrate this I have included a copy of a 1992 Human Gene Therapy journal article and a copy of a 1996 Journal of Controlled Release article

including myself as an author which speaks to this. Now shown to me and respectively marked **Exhibit LH-4** and **Exhibit LH-5** is a copy of each document.

31. The advent of the ionizable cationic lipid (with a pKa typically around 6.2-6.5) was important in allowing for development of a lipid delivery system which could efficiently complex the nucleic acid with this lipid at a pH at which the ionizable lipid was charged, for example pH 4 or thereabouts, and then have the ionizable lipid be neutral when delivered as part of the lipid particle inside the body at physiological pH. This allowed for improved circulation in the body and reduced toxicity. The eventual uptake in the cell occurs by endocytosis and the entrapment within the endosome results in the ionizable lipid encountering a pH drop again to become charged. This causes a change in conformation, with pairing with endosomal membrane anionic lipids, resulting in disruption of the endosomal membrane and nucleic acid release into the cytoplasm. The ionizable lipid development therefore brought about a great interest in lipid delivery and many groups did significant work in developing improved ionizable cationic lipids. That work continues to this day given the importance of this lipid and the changes needed for different applications.

32. I was then asked about the state of knowledge in the field of mRNA delivery immediately prior to 01 December 2009. In simple terms, the principle was well-known to lipid formulation chemists. The challenge was largely in the delivery. It was also understood that it was generally preferable to use modified mRNA (mmRNA) as this would help stabilize the mRNA. Certain delivery vehicles, such as cationic lipid or polymer delivery vehicles were also known to be needed to help protect the mRNA which is otherwise prone to degradation. I identified several publications which support that view. For example, I identified the following documents (Karikó *et al*., WO 2007/024708A2; Karikó *et al*., *Incorporation of Pseudouridine Into mRNA Yields Superior Nonimmunogenic Vector With Increased Translational Capacity and Biological Stability*, Molecular Therapy, 16(11): 1833-1840 (2008); and Karikó *et al*., *In vivo protein expression from mRNA delivered into adult rat brain*, Journal of Neuroscience Methods, 105(1): 77-86 (2001)) that discuss delivery of lipofectin-complexed modified mRNA. Now shown to me and respectively marked **Exhibit LH-6** to **LH-8** is a copy of each of those documents. The documents mention the benefits of mmRNA in terms of reduced innate immunotoxicity and improvements in protein production. This indicates the greatly improved production of luciferase by pseudouridine-modified mRNA along with a dramatic reduction in interferon production.

33. I was asked about the development of cationic lipids immediately prior to 01 December 2009 and, as mentioned, there was significant development work at that time. Pieter Cullis had published quite a bit on this topic, along with Sean Semple and John-Paul Behr, and the general characteristics

of cationic lipids were well-known immediately prior to 01 December 2009. One example of a well-known paper I was very familiar with from this group around this time and which was subsequently considered to be important in this field was Semple, S., Akinc, A., Chen, J. *et al.* Rational design of cationic lipids for siRNA delivery. *Nat Biotechnol* 28, 172–176 (2010) https://doi.org/10.1038/nbt.1602. The article was published 17 January 2010, only a month after the relevant priority date, however it was received 16 September 2009 (before the relevant priority date), and I consider it to be relevant here in terms of representing some of the knowledge in the field. This article described key considerations for cationic lipid design. These include the presence of an ionizable head group, which was generally a tertiary amine head group, with a suitable pKa. A key aspect was also a hydrophobic tail which would generally have two fatty acid chains, such as linoleyl chains and, at that time, they often had a *cis* double-bond in each tail which caused a 'kink' to give an appropriate bulky conformation. The head group and tail would be joined by a linker group. This kind of lipid would be more likely to adopt the inverted hexagonal (H_{II}) phase which is good for lipid fusion and membrane disruption in the endosome for delivery. Now shown to me and marked **Exhibit LH-9** is a copy of that document.

34. Even though these general characteristics were understood there was still a lot of variability between different ionizable or cationic lipids and how they would interact with, formulate and, particularly, deliver nucleic acids to the target cells and so this was an ongoing field of development immediately prior to and after 01 December 2009. For example, it later became common to include more than two lipid chains in the tail to create that preferred lipid conformation which could adopt the inverted hexagonal phase. Many groups and companies are still running large research projects trying to develop ionizable cationic lipids for different applications due to the variability in results.

35. I was asked about other components of a lipid delivery formulation immediately prior to 01 December 2009. These would include a neutral or helper lipid such as a phosphatidylcholine with lipid chains, like DSPC and DOPE which are among the most common. They help stabilise the lipid particle and form the basic outer layer structure. Cholesterol was also a component of many of the later lipid formulations, after the early two component liposomes, and was again considered important from a structural perspective. Finally, a PEG-lipid would be included with the PEG being presented on the outer surface of the particle. Some of the PEG-lipid work was initially developed in my lab. The PEG-lipid is important for achieving appropriate circulation time of the particles in the body by limiting opsonization but also affects the size of the lipid particles. While the PEG-lipid is important it is only present in small amounts otherwise it can lead to mixed micelles that destabilise the lipid particles. It can also increase steric hindrance to the extent the particle will not be able to attach to and enter the cell. There is also a known issue around PEG allergic toxicity. This can

happen when someone has been exposed to PEG, which is of course present in many different everyday hygiene formulations, and they develop anti-PEG antibodies. Delivering higher doses of a PEG-lipid to such a person can result in an allergic toxicity. High levels of PEG-lipid could therefore be problematic and are best avoided. The cholesterol and neutral lipids need to be present in higher amounts because of their structural roles and, of course, you need sufficient ionizable lipid to complex the nucleic acid you want to formulate and to trigger later endosomal release.

Cationic lipids

36. I have mentioned the characteristics of the cationic lipid. The cationic lipid is not only important for complexing with the nucleic acid but also has a critical role in interacting with the negatively charged endosomal membrane lipids. Permanently positively charged cationic lipids, as were initially developed for lipid delivery vehicles, are problematic in that the positive charge can attract negatively charged serum proteins, leading to faster clearance and greatly reduced transfection. Some also demonstrated toxicity issues and so weren't suitable for *in vivo* use.

37. Ionizable cationic lipids were developed to address this issue prior to or around 01 December 2009 and they allowed for condensation with the nucleic acid at an acidic pH but would then display a neutral or low cationic surface charge at physiological pH allowing for efficient transport and cellular particle uptake. As I have described, after endocytosis the pH of the endosomal compartment drops significantly and so the ionizable lipid becomes protonated again and can interact with naturally occurring anionic phospholipids in the endosomal membrane to form ion pairs that induce a conformational change from a bilayer to a hexagonal H_{II} phase, thereby destabilizing the endosomal membrane for nucleic acid release. It was in this endosomal release process which Semple, Cullis and others showed the ionizable cationic lipid could provide real advantages in transfection efficiency. Now shown to me and marked **Exhibit LH-10** is a copy of an article from 2001 which discusses lipid vesicles using ionizable cationic lipids.

Non-Cationic lipid and Cholesterol

38. The lipid delivery formulations would also include a non-cationic (also known as a neutral) lipid, sometimes referred to as a phospholipid, such as DSPC. These lipids play something of a structural role in forming and maintaining a stable lipid particle. They don't all work as well as each other so DSPC is generally more favoured than, for example, dioleyl phosphatidylethanolamine (DOPE). Cholesterol also has a structural role to play in achieving the desired morphology and fluidity of the lipid particle and was typically included in lipid delivery formulations after the early two component (cationic plus neutral lipid) liposomes.

PEG-lipid

39. Surface modification of the lipid particle with a PEG-lipid, as mentioned, stabilizes the particle in the presence of serum and so helps to extend the circulation in the blood due to reduced protein absorption. Inclusion of a small amount of PEG-lipid in the particle is primarily for the prevention of particle aggregation. Most PEG-lipids used for lipid particles would be in the 1500-3500 Da molecular weight range. Commonly used PEG-lipids are selected from PEG-DMG, PEG-ceramides and so on, and may include PEG-CerC14 and PEG-CerC20 for example. The size of the lipid particle is important for cellular uptake and the amount of PEG-lipid in the particle has an influence on particle size. Cullis' lab showed, prior to 01 December 2009, that the PEG shields the cationic lipid and extends circulation time while the lipid could influence the exchange of the polymer from the carrier so that cellular uptake can occur. For example, I identified the following document (Monck *et al*., *Stabilized plasmid-lipid particles: pharmacokinetics and plasmid delivery to distal tumors following intravenous injection*, Journal of Drug Targeting, 7(6): 439-452 (2000)) discussing that variation of acyl chains in the PEG-lipid could modulate biodistribution of the lipid particle (e.g. into the liver). Now shown to me and marked **Exhibit LH-11** is a copy of this document.

40. These four lipid particle components could be formulated in differing relative amounts but within generally understood limits. The cationic lipid (or ionizable cationic lipid) had to be present in a sufficient amount to complex with the nucleic acid but also to be sufficiently available to trigger endosomal release. The neutral lipid and cholesterol could, to a small extent, be balanced with one another but cholesterol was generally present in higher relative amounts. The PEG-lipid couldn't be present in too high a relative amount as it would otherwise present too much of a steric barrier to contacting the cell membrane for endocytosis and could undesirably affect the physical characteristics of the particle.

41. I was asked about the typical size of lipid particles immediately prior to 01 December 2009. In my opinion, and I expect the opinion of others in the field, the typical size of a lipid particle for delivery of a nucleic acid into the human body would be about 100 nm in diameter or less. Generally, you would certainly aim for a liposome diameter of less than 200 nm because of long circulation time and ability to passively and/or actively target a diseased site.

42. Immediately prior to 01 December 2009 I would therefore have been, and was, aware that a lot of groups were working on developing lipid delivery approaches for a variety of nucleic acids, including DNA, siRNA, and mRNA. I would have looked to publications from Pieter Cullis and other key labs as well as industry activity and conference presentations to understand what had been shown to be successful formulations in lipid formation and nucleic acid encapsulation. The materials for lipid

formation were either readily available or were easily synthesized and so it was relatively straightforward to prepare and characterize lipid particles.

43. I was asked about typical methods to actually formulate lipid particles encapsulating a nucleic acid immediately prior to 01 December 2009. In my opinion this was a straightforward matter well before that date, at least on a laboratory scale, and at that time a skilled graduate student could perform the encapsulation and formulation tasks. The formulations, in terms of molar ratios, and a number of individual lipids within each lipid class which had been shown to form lipid particles were described in the literature and generally fell within well-defined ranges. There were also publications on the approaches to batch formation of the encapsulated lipid particles using the nucleic acid in buffer solution and the lipids in an ethanol solution and then mixed in various ways. When microfluidics was later described in the literature then this made the task easier and more reproducible, and so more amenable to industry applications, within defined parameters but it was not particularly problematic to make and test such lipid particles for encapsulation efficiency, morphology etc. immediately prior to 01 December 2009.

44. Summing up, I consider that the following was common general knowledge in the field of providing compositions having a nucleic acid formulated within a liposome, as at 01 December 2009:

- that a delivery vehicle was needed to protect nucleic acid, DNA and RNA, from degradation and to deliver it efficiently to the target cells and particularly so with the labile mRNA;
- that once inside the cell you had to overcome degradation by ensuring a good level of endosomal release;
- that a number of related delivery vehicle options were available such as liposomes, SNLPs, SPLPs, etc.;
- that the same liposome systems could and were used for delivering various nucleic acids, including pDNA, mRNA and siRNA;
- that these liposome systems developed over time and included two-, three-, and four-component liposomes which can interchangeably deliver various nucleic acids;
- that mRNA could be produced, with the option of modifying the nucleosides to increase stability;
- that four standard lipid classes can be formulated together including a cationic (ionizable) lipid, a neutral lipid, cholesterol, and a PEG-lipid;
- that the typical size of the liposome for delivery in the human body was generally around 100 nm or less in diameter;
- a number of commercially available cationic lipids were typically used and ionizable cationic lipids were also well-known through Pieter Cullis, and others, published work;
- the relative amounts of the four lipid classes for *in vivo* liposomal delivery generally fell within fairly well defined ranges which were found in the literature.

45. I was asked whether people working in the field of providing compositions having a nucleic acid formulated within a liposome would have carried out searches for relevant information/publication, and/or kept up to date with developments in the field.

46. My answer is yes. The various researchers and labs I worked and collaborated with over the years kept up-to-date with developments in the literature in the field, as did I. Some important companies working in this space were spun out of the University of British Columbia, from Pieter Cullis' lab amongst others, and so sometimes the first publication on a technology might come from a patent publication. Due to the significant industry interest in this area, people working the field would often look to the patent literature, technical journals, and other non-patent literature when carrying out projects. I, and people from my teams, also regularly attended conferences and meetings in the field.

E. PRIOR ART DOCUMENTS

47. After providing my opinion above, I was provided with a copy of the following documents and asked to provide my views regarding them, in particular regarding any information and teaching they contain that would have been relevant to a person in the Field looking to provide compositions having a nucleic acid formulated within a liposome as at 01 December 2009:

- WO2005/120152A2, Cationic Lipids and Methods of Use (**D1**);
- WO2009/086558A1, Improved Compositions and Methods for the Delivery of Nucleic Acids (**D2**)
- WO2004/002453A1, Method and Apparatus for Producing Liposomes (**D3**);
- WO1998/51278A2, High Efficiency Encapsulation of Charged Therapeutic Agents in Lipid Vesicles (**D4**);
- WO2010/129687A1, Methods of Delivering Oligonucleotides to Immune Cells (**D5**);
- Lu et al., *Cancer Gene Therapy* 1994: 1(4):245-52 (**D12**);
- Maurer N., *Biophys*. *J*. 2001;80:2310–2326 (**D13**);
- Martinon et al., *Eur. J. Immunol.* 1993. 23: 1719-1722 (**D14**);
- Pascolo, S. (2008). Handbook of Experimental Pharmacology, vol 183 (**D15**);
- Hess et al., *Cancer Immunol. Immunother.* (2006) 55: 672-683 (**D16)**;
- Fenske DB et al. *Toxicol. Pathol.* 2008 Jan; 36(1):21-9 (**D17**);
- Malone RW et al. *Proc Natl Acad Sci U S A.* 1989 Aug; 86(16):6077-81 (**D18**); and
- WO1990/011092A1, Expression of Exogenous Polynucleotide Sequences in a Vertebrate (**D19**).

48. Exhibited hereto and marked **Exhibits LH-12** to **LH-24** are copies of each of the above documents. I consider that each of these documents would have been identified by and would have been of interest to a skilled person looking to provide a nucleic acid formulated within a liposome immediately prior to 01 December 2009. I have been told that although **D5** has a publication date of 11 November 2010, it is still relevant here as the earliest valid priority date of the Opposed Application may be found to be 30 November 2010.

49. I was asked to review these documents and explain what each would have disclosed to me (or, if I had read the document following its publication, what it disclosed to me) regarding nucleic acids formulated within a liposome as at the date the document was published and, if different, immediately prior to 01 December 2009. Having reviewed the documents, I provide my comments on them below.

Document "D1"

50. I have been provided with a copy of WO2005/120152 A2 entitled "Cationic Lipids and Methods of Use" (**D1**) which forms **Exhibit LH-12** to this declaration.

51. **D1** begins by setting out the need for effective and safe gene delivery systems for gene therapy. The document then discusses recent work that has shown stabilized plasmid-lipid particles (SPLPs) to be a good option. As I have already mentioned above, typically, SPLPs and liposomes comprise cationic lipids. This document addresses some of those problems and generally discusses the preparation of the ionizable cationic lipids, DLin-DMA and DLen-DMA, for liposomes and nucleic acid-lipid particles, in order to increase flexibility over the commonly used cationic lipids, such as DODAC and DODMA, that can lack sufficient membrane fluidity, thereby impacting the efficiency of delivery of a bioactive agent. DLin-DMA is a an ionizable cationic lipid 1,2-dilinoleyloxy-3-dimethylaminopropane (DLin-DMA), and a key lipid component of stable nucleic acid lipid particles (SNALP). As I mentioned above when discussing the common general knowledge, DLin-DMA was the early benchmark lipid of a series of DLin-DMA-based lipids that have been shown to be successfully formulated in nucleic acid-lipid particles, and were known to me before 01 December 2009, (see paragraph 36 above).

52. Following the summary section, **D1** provides a number of definitions in paragraphs [0031] to [0046]. These definitions appear to be standard definitions known in the field.

53. The structure of the cationic lipids (i.e. Formula I and Formula II) are then defined in section III, which is followed by a description of lipid-based carrier systems containing cationic lipids (section

IV). Paragraphs [0051] and [0052] describe the components that form lipid-based carriers and their typical ranges. For example, the document describes the lipid-nucleic acid particles typically comprise a nucleic acid, a cationic lipid (2%-60%), a non-cationic lipid (5% to 90%), a PEG-lipid conjugate (1% to 20%), and cholesterol (10% to 60%), and typically have a mean diameter of 50 nm to 150 nm. This is consistent with my understanding of what was well-known in the field at the relevant date, see paragraph 44 above.

54. Examples of cationic lipids (e.g. DLin-DMA, DODMA, DOTAP, etc.), non-cationic lipids (DSPC, DOPE), PEG-lipids (e.g. PEG-DMA), and the types of nucleic acids (e.g. single stranded or double stranded DNA, single of double stranded RNA, RNAi, siRNA, etc.) are described at some length in the paragraphs that follow. Sterols such as cholesterol may also be present. Again these components were all known to me prior to 01 December 2009 and were common lipid components used to formulate a nucleic acid-lipid particle or liposome.

55. **D1** includes significant discussion around all aspects of the nucleic acid (paragraphs [0082] to [0108]). For example, paragraph [0082] highlights a number of nucleic acids that would be suitable for formulation with the SPLPs and SNALPs including those encoding a therapeutic product. And also that the SPLPs and SNALPs can be used to deliver the nucleic acid to a cell for expression of a protein (e.g. mRNA) *or* for gene silencing (e.g. siRNA). The document specifically mentions nucleic acids encoding therapeutic (gene) products of interest (paragraph [0083]), for example, genes associated with metabolic diseases and disorders (e.g. diseases that are expressed in the liver (paragraph [0085]) which I understand this to mean that proteins or peptides can be encoded for by mRNA.

56. In particular, I note that paragraph [0084] of **D1** highlights the nucleic acid is a siRNA molecule that silences the gene of interest and the next embodiment provided recites the following

"*the nucleic acid encodes a polypeptide expressed or overexpressed in a subject with a particular disease or disorder (e.g., a pathogenic infection or a neoplastic disorder) and can conveniently be used to generate an immune response against the polypeptide expressed by the gene*"

and then even further, in other embodiments

"*the nucleic acid encodes a polypeptide that is underexpressed or not expressed in subjects with a particular disease or disorder (e.g., a metabolic disease or disorder)*…".

In my view, this would indicate the nucleic acid is a mRNA molecule as I understand this to be describing protein expression, including protein replacement therapy approaches and vaccines.

Paragraph [0087] further discusses treating metabolic diseases and disorders including those related to the liver and liver-targeting.

57. Typical methods for the preparation of nucleic acid-lipid particles, such as continuous mixing, detergent dialysis, etc. (paragraphs [0109] to [0150]), and typical methods for the preparation of liposomes containing cationic lipids, such as sonication, extrusion, etc. (paragraphs [0163] to [0169] are discussed. The use of liposomes is described, including as delivery vehicles (paragraphs [0170] to [0182]) and diagnostic agents (paragraph [0183] to [0184]), followed by discussion of loading the liposomes (paragraphs [0185] to [0190]), all which I was generally aware of prior to 01 December 2009.

58. The Examples of **D1** mainly focus around the use of siRNA for the purpose of gene silencing. The document describes SNALP preparation with a lipid composition of DSPC:Chol:PEG-C-DMA:Cationic Lipid at a ratio of 20:48:2:30 molar percent (paragraph [0196]). The synthesis of a cationic lipid, DLin-DMA, is then discussed (paragraph [0207]). Of note, is the silencing of gene expression following the delivery of an SNALP in Examples 7 and 8 (paragraphs [0216]-[0219]) of which the cationic lipid in the SNALP compositions were varied, which was shown to be an important part of the formulation approach. Examples 9 and 10 discuss *in vivo* transfection of organs/tumors by various SPLP compositions as shown in paragraph [0220] and [0222], again varying the cationic lipid.

59. I was asked what this document would have taught a skilled addressee looking to provide nucleic acids formulated within a liposome as at 01 December 2009.

60. Overall, **D1** demonstrates that nucleic acid-lipid particles (SPLPs or SNALPs) and other lipid-based carrier systems (e.g. a liposome) containing cationic lipids can encapsulate a number of nucleic acids, such as DNA, mRNA and siRNA. These various types of particles, including lipid-based particles, such as liposomes, which are described extensively throughout the document and formulated within the Examples in what I consider to have the typical four-components using the standard relative molar ratios known in the field at the relevant date, and so I believe this would teach a skilled addressee that each of these formulation approaches could be used to deliver a nucleic acid for therapy.

Document "D2"

61. I have been provided with a copy of WO2009/086558A1 entitled "Improved Compositions and Methods for the Delivery of Nucleic Acids" (**D2**) which forms **Exhibit LH-13** to this declaration. This

document is directed to the delivery of encapsulated nucleic acids in lipid particles which are said to allow effective knock-down of specific target proteins at relatively low dosage and toxicity.

62. In terms of the formulation, Summary pages 8 to 9 describe DLin-K-DMA, as the preferred cationic lipid. As mentioned above when discussing **D1** (see paragraph 51 above), DLin-K-DMA was known to me before 01 December 2009 as an effective ionizable cationic lipid and was one of a series of DLin-DMA-based lipids. Further on page 9, **D2** describes the lipid particle further comprising neutral lipids and lipids capable of reducing particle aggregation. As mentioned above when providing my views on the common general knowledge at paragraph 41, the PEG-lipid is important in conferring appropriate colloidal stability to the lipid particle and reducing particle aggregation. A particularly preferred lipid particle is described on page 9 with DLin-K-DMA in combination with a neutral lipid selected from DSPC, POPC, DOPE, and SM, cholesterol, and PEG-S-DMG, PEG-C-DOMG or PEG-DMA, in a molar ratio of about 20-60% DLin-K-DMA:5-25% neutral lipid:25-55% Chol:0.5-15% PEG-S-DMG, PEG-C-DOMG or PEG-DMA, which are all standard components and ratios of lipid particles known to me at 01 December 2009.

63. **D2** has a section discussing the ionizable cationic lipids, which were a significant development in the field of lipid delivery (see pages 14 to 27). **D2** also provides significant discussion on the other components of the lipid particles from pages 27 to 35. The end of page 34 and beginning of page 35 of **D2** describes that the preferred lipid particle will have a combination of cationic lipids, non-cationic lipids, neutral/helper lipids and PEG-modified lipids, which as I mentioned above are all familiar to me as the basic building blocks of lipid particles extending beyond the more basic two component liposomes used for cell work.

64. After the discussion on the lipids themselves, **D2** describes on pages 36 to 37 that their lipid particles could be used to deliver any type of active agent. It states that their lipid particles could be associated with a nucleic acid, whereby the nucleic acid is fully encapsulated in the lipid particle. The nucleic acid is defined as being any nucleic acid and includes any oligonucleotide or polynucleotide. The nucleic acids can be single- or double-stranded DNA or RNA, or DNA-RNA hybrids. Further, this document describes the nucleic acids being of varying lengths dependent on the form of the nucleic acid. There is considerable discussion in **D2** around the nucleic acid types (see pages 36 to 53) and the possible modification of the nucleic acid (see pages 53 to 64).

65. Page 60 onwards describes the encapsulation of the therapeutic agent, such as nucleic acid, into the lipid particle formulations. Particle size is mentioned as having a diameter of from about 70 to about 200 nm, preferably about 90 to about 130 nm. From page 74 onwards, **D2** mentions that

their compositions can be used to deliver a therapeutic agent, i.e. a nucleic acid, to a cell, *in vitro* or *in vivo*. On one hand, at the top of page 76, including "*typical applications include using well-known procedures to provide intracellular delivery of siRNA to knock down or silence specific cellular targets"*. And on the other hand, including "*delivery of DNA or mRNA sequences that code for therapeutically useful polypeptides. In this manner, therapy is provided for genetic diseases by supplying deficient or absent gene products"*, such as for Duchenne's dystrophy and for cystic fibrosis. Again, these two alternative applications suggest the use of different nucleic acids which can both be formulated in the lipid particles of the invention. It was generally known at the time that siRNA-lipid particles could be employed for gene silencing applications and mRNA or DNA for gene expression or replacement (i.e. gene therapy).

66. The Examples of **D2** start on page 85 and for several pages present the synthetic schemes for the preparation of the various lipids, including cationic and PEG lipids. In Example 20, they formulate and characterise liposomal siRNA formulations using various cationic lipids (40%): DSPC (10%): cholesterol (40%): PEG-C-DOMG (10%).

67. I was asked what this document would have taught a skilled addressee looking to provide nucleic acids formulated within a liposome as at 01 December 2009. **D2** teaches that it is possible to formulate any nucleic acid, including DNA, mRNA and siRNA, within their lipid particles and shows that approaches that were known prior to 01 December 2009 can be used to form these lipid delivery vehicles.

Document "D3"

68. I have been provided with a copy of WO2004/002453A1 entitled "Method and Apparatus for Producing Liposomes" (**D3**), which forms **Exhibit LH-14** to this declaration.

69. This document largely focuses on a mixing apparatus and describes processes for preparing lipid formulations, e.g. for stabilized plasmid-lipid particles (SPLP). In simple terms it's really just an early microfluidic mixing approach to achieve the mixing of a nucleic acid and lipid components which was already known to result in liposomal formation.

70. **D3** talks about the aqueous solution being a buffer comprising a therapeutic product, such that upon mixing with the lipid formulation the therapeutic product is encapsulated in the liposome. At paragraph [09] the therapeutic product is defined as including anything from a protein, a nucleic acid, an antisense nucleic acid, tRNA, snRNA, siRNA, etc… the therapeutic product preferably being

selected from a nucleic acid. The definition of nucleic acid is broadly described at paragraphs [27] and [28] as being DNA (such as antisense, pDNA, etc.) or RNA (such as tRNA, snRNA, rRNA, mRNA, siRNA, etc.). Further the nucleic acid may encode for various proteins (see paragraph [66]).

71. In terms of the disclosure of **D3**, it describes the use of standard lipids already well-known in the field at that time to form lipid particles including DODMA, DSPC, cholesterol, and PEG-lipid (PEG-DSG) which are shown as formulated in a 25:20:45:10 ratio (see paragraph [47]), which I consider to be within fairly standard ranges in the field at that time.

72. A lot of discussion in **D3** is then around the different microfluidic arrangements (also shown in the figures), concentration of components, and the particles formed, including their size (e.g. preferably less than 200 nm, about 100 nm at paragraph [46]) and their characteristics.

73. I was asked what this document would have taught a skilled addressee looking to provide nucleic acids formulated within a liposome as at 01 December 2009. While the Examples of **D3** focus on DNA formulation and the encapsulation of other small molecules, the discussion is around many types of nucleic acids with pDNA, mRNA and siRNA also being suitable for encapsulation. The teaching of the document is really around how to form lipid particles, whatever nucleic acid is encapsulated. My view is that this document conveys that the microfluidic mixing approach is a useful one. The lipids used to form the lipid vehicles and their relative molar ratios appear to be standard selections from those known at that time.

Document "D4"

74. I have been provided with a copy of WO1998/51278A2 entitled "High Efficiency Encapsulation of Charged Therapeutic Agents in Lipid Vesicles" (**D4**), which forms **Exhibit LH-15** to this declaration.

75. This document is directed to methods for the preparation of a lipid-nucleic acid composition. In particular, as described at page 11, this document describes "*methods and compositions for producing lipid-encapsulated therapeutic agent particles in which charged therapeutic agents are encapsulated within a lipid layer*". This document was published in 1998 and so well before the 01 December 2009 date, and the method described is very familiar to me, and I consider would have been familiar to others in the field. I note that Pieter Cullis is one of the inventors on this patent publication. Pieter Cullis and his group were among the leaders in the field at the relevant date.

76. The disclosure of **D4** is largely around evaluation of the quality of the lipid-nucleic acid particles based on drug/lipid ratio, encapsulation efficiency, nuclease resistance and serum stability and controllable particle size (e.g. less than 200 nm, preferably about 90 to about 130 nm). The documents goes into some detail as to how this criteria is evaluated on pages 12 and 13.

77. **D4** then goes into further detail on the various lipids which may be suitable for formation of the lipid-nucleic acid composition, which are all familiar to me as the basic building blocks of liposomal formulations. PEG-modified lipids are discussed on page 18 and neutral lipids, such as DOPE, DSPC and POPC, and sterols are discussed on page 19. The end of page 19 describes that the composition will have a mixture of amino (cationic) lipids, neutral lipids, a sterol and PEG-modified lipids. Particular formulations are also taught on page 20 with 10-35% DODAP: 25-45% DSPC: 35-55% cholesterol: 0.5-15% PEG-CerC14.

78. After the discussion on the lipids themselves, **D4** describes on page 20 that their lipid-nucleic acid particles could be used to deliver any form of nucleic acid that is known, including DNA or RNA (see page 21) and that DNA could be genomic DNA or cDNA and that and that the RNA could be mRNA (see last paragraph page 24).

79. **D4** also mentions the typical applications of their lipid-nucleic acid particles (page 31) using standard transfection methods to deliver DNA or mRNA sequences which encode for therapeutically useful polypeptides. I understand the document to specifically refer to protein replacement therapies for genetic diseases such as Duchenne's dystrophy and cystic fibrosis, which are mentioned in the same paragraph.

80. I was asked what this document would have taught a skilled addressee looking to provide nucleic acids formulated within a liposome as at 01 December 2009.

81. Overall, the central teaching of this document is around high efficiency encapsulation of nucleic acids in lipid particles and it clearly discusses formation of lipid-based particles such as liposomes having a combination of cationic lipids, non-cationic lipids, cholesterol and PEG-modified lipids, which are all familiar to me and provided in standard relative molar ratios. Although the Examples of **D4** describe the results of antisense encapsulation, I believe the document would clearly teach a skilled addressee that these lipid formulations could be used to deliver *any* nucleic acid based on such statements made within the document.

Document "D5"

82. I have been provided with a copy of WO2010/129687A1 entitled "Methods of Delivering Oligonucleotides to Immune Cells" (**D5**), which forms **Exhibit LH-16** to this declaration.

83. The disclosure of **D5** is largely around new lipids that have been developed and their use in producing liposomal vehicles for nucleic acid delivery. The bottom of page 8 mentions a method of preparing a liposome involves mixing a sterol, a neutral lipid, and a cationic lipid. PEG or a PEG-modified lipid is an optional addition to the liposomes. The last paragraph of page 13 mentions a lipid formulation of 10-75% cationic lipid; 0.5-50% neutral lipid; 5-60% of the sterol; and 0.1-20% of the PEG or PEG-modified lipid. Other formulations are described on pages 14 and 15. The lipid particles are described as including liposomes having an aqueous core, and having an average particle size (either containing or not containing a nucleic acid) of at least about 100 nm in diameter (see pages 17 and 18, for example). They provide a number of different Examples of such cationic lipids varying in the nature of the head group and hydrophobic tails. They also discuss additional cationic lipids which look like those that were commonly known at the time, e.g. DLin-MC3-DMA being one of the most commercially successful early ionizable lipids. The document describes the delivery vehicles having a combination of the prepared cationic lipids with a sterol, a neutral lipid and a PEG-modified lipid. The molar amounts of each component is described at multiple places throughout the document. I consider **D5** clearly discloses liposomal formulations including the four well-known lipid classes within standard ranges, to provide a delivery vehicle for nucleic acids.

84. The document describes the nucleic acids which can be encapsulated within and delivered by the lipid particles and the main one of interest in **D5** is siRNA although they say any nucleic acid could be delivered. On that, I note that on page 45 both DNA and mRNA are mentioned as alternative nucleic acids for delivery with the lipid particles. The statement described is very similar to that highlighted above in **D4** such that lipid particles described in **D5** can be used for the delivery of DNA or mRNA sequences which encode for therapeutically useful polypeptides. In a further statement below this, **D5** suggests that the lipid particles containing nucleic acids can be used in the treatment of infectious diseases which I consider to be discussion of a vaccine formulation.

85. Further on in the document, modifications of the nucleosides and other components of the RNA are described and these appear fairly standard for the time this document was published.

86. I was asked what this document would have taught a skilled addressee looking to provide nucleic acids formulated within a liposome as at 01 December 2009. While the Examples of **D5** focus on

siRNA formulation and delivery, the discussion is around many types of nucleic acid and DNA and mRNA is mentioned in the context of providing an alternative nucleic acid that can be delivered in this type of liposomal formulation for application to gene therapy. The core teaching of the document is really around new lipids formulated with the other well-known lipid classes to provide a four-component lipid delivery vehicle within standard ranges, to provide a delivery vehicle for nucleic acids.

Document "D12"

87. I have been provided with a copy of Lu et al., *Cancer Gene Therapy* 1994: 1(4):245-52 (**D12**), which forms **Exhibit LH-17** to this declaration.

88. The document generally discusses the comparison of the efficiency of a range of commercially available cationic liposome formulations in mRNA transfection. **D12** talks about the advantages of delivering mRNA over DNA in terms of the safety benefits in the context of gene therapy.

89. In terms of the disclosure of **D12**, it describes the preparation of cationic liposome-mRNA complexes. The document describes using lipids such as DOTAP, as well as commercial formulations such as Lipofectin, Lipofectamine, and Transfectam. These commercially available formulations were well-known as at 01 December 2009 and generally contained a mixture of a cationic lipid and a neutral lipid which, when in an aqueous solution, would form liposomes. The mRNA used is luciferase mRNA, in both capped and uncapped forms. These kinds of liposomes were further developed, as shown in **D1**-**D4** for example, into four lipid component liposomes more appropriate for *in vivo* applications.

90. The results indicate that the levels of gene expression achievable by liposome-mRNA-mediated transfection were comparable to the levels observed for liposome-DNA complexes. I read this as showing cationic liposome-mRNA complexes with improved profiles related to the efficiency and toxicity as compared to liposome-DNA complexes.

91. I was asked what this document would have taught a skilled addressee looking to provide a nucleic acids formulated within a liposome as at 01 December 2009. Given the time that this document was published, the focus of **D12** is clearly on demonstrating, as a proof of concept of the approach, that cationic liposomes can be used to deliver mRNA and showing results that are comparable to liposome-DNA complexes. This shows that even as far back as in 1994 it was known that liposomes,

of one form or another, could be used to encapsulate and deliver mRNA in addition to their previous use in delivering the larger DNA.

Document "D13"

92. I have been provided with a copy of Maurer N., *Biophys*. *J*. 2001;80:2310–2326 (**D13**), which forms **Exhibit LH-18** to this declaration. I was familiar with this document shortly after its publication as Pieter Cullis and Sean Semple are two of the authors. This document discusses ethanol-induced lipid mixing to create liposomes encapsulating oligonucleotides. It also discusses factors influencing stability of the formed liposomes including ethanol concentration. In other words, this document is particularly focused on the effect of ethanol to destabilize the bilayer of the liposome.

93. The lipid components of choice in this study was DODAP/DSPC/Chol/PEG-CerC14 at molar ratios of 25:20:45:10, respectively (page 2311). Again, I consider this to be a fairly typical four component liposome within standard ranges for the time this document was published. Page 2314, under 'Results' describes the process of selection of the lipids used based on their effect in the final cationic liposome. In terms of the size, the document describes the lipid particle having a particle diameter of between 70 nm to 120 nm, which is influenced by the addition of PEG-Cer (page 2315, left hand column).

94. The results presented in **D13** show that they successfully encapsulated a number of oligonucleotides as well as plasmid DNA at high entrapment efficiencies (see Figure 9). They conclude that the liposomes can be used to entrap negatively charged polyelectrolytes, generally (bottom of page 2320).

95. I was asked what this document would have taught a skilled addressee looking to provide nucleic acids formulated within a liposome as at 01 December 2009. The focus of **D13** is clearly on demonstrating the use of ethanol as membrane-destabilizing agent in conjunction with a PEG-lipid to control and modulate the interaction between highly charged polymers such as nucleic acids and cationic liposomes, and also the size of the liposome.

96. Overall, the central teaching of this document is that nucleic acid can be entrapped within cationic liposomes, not only showing the versatility of these four-component systems but also validating their utility for liposomal delivery of macromolecular drugs.

Document "D14"

97. I have been provided with a copy of Martinon et al., *Eur. J. Immunol.* 1993. 23: 1719-1722 (**D14**), which forms **Exhibit LH-19** to this declaration. I note that this paper was published quite early in the field of nucleic acid delivery.

98. This document describes the delivery of mRNA encapsulated within a three-component liposome of, cholesterol/dipalmitoylphosphatidylcholine/phosphatidylserine, and specifically discusses mRNA encoding an influenza virus nucleoprotein. The document shows the successful delivery of the mRNA and its expression to induce a CTL response in three strains of mice.

99. I was asked what this document would have taught a skilled addressee looking to provide nucleic acids formulated within a liposome as at 01 December 2009. Overall, the central teaching of this study is to show that mRNA can be encapsulated within liposomes and, in this very early study, administration of mRNA *in vivo* demonstrates virus specific induction of lymphocytes, highlighting that mRNA delivery systems can be useful for immunization when coupled with the protective liposome delivery approach. The document talks to the advantages of this approach for vaccination purposes.

Document "D15"

100. I have been provided with a copy of Pascolo, S. (2008). Handbook of Experimental Pharmacology, vol 183 (**D15**), which forms **Exhibit LH-20** to this declaration.

101. This document is a book chapter out of the "Handbook of Experimental Pharmacology" and is entitled "Vaccination with Messenger RNA (mRNA)". I understand this book was published just before the priority date and talks about vaccination with mRNA.

102. In **D15**, the introduction sets out the advantages of mRNA when compared to plasmid DNA, including the potential safety hazards with direct, *in vivo*, delivery of plasmid DNA. It finishes by highlighting that the following are discussed: the features of mRNA that are required for vaccine formulation, the delivery methods developed and validated in mice, and the result of clinical trials.

103. Section 2.3 of **D15** provides a significant amount of discussion around various modifications of mRNA, such as the nature of the 3' and 5' UTRs, optimization of the 5' cap structure, poly-A tail residue length, etc. which are all components which need to be considered in designing a mRNA

therapeutic and which I consider to be known factors. I have previously mentioned that modified nucleosides are useful in designing mRNA for delivery. They can also provide for very significant gains in the efficiency of protein production as compared with unmodified mRNA and enhance stability, which I already discussed in relation to the Karikó 2008 Molecular Therapy article above in paragraph 32.

104. **D15** also provides significant discussion around mRNA delivery methods in Section 3. In particular, Section 3.1. discusses four different mRNA based formulations that have been shown to trigger an immune response in mice against the coded antigen. The categories included for these vaccines are encapsulation of mRNA in cationic liposomes, direct injection of naked mRNA, needleless delivery of gold particles coated by mRNA, and transfection of in vitro generated autologous APCs. Of note is reference to the Martinon (1993) paper which I have discussed above and is referenced as **D14** and the Hess (2006) paper which I will discuss below (referenced as **D16**). One of the most common approaches was to use liposomes or other types of lipid particles to deliver nucleic acids, although other delivery systems such as polymeric particles and protamine cores encased in lipids were also being tested and developed, as highlighted in this Section. At the end of Section 3.1, this document mentions that not all methods have been tested in humans yet, maybe because of stability or toxicity issues, but indicates that methods such as encapsulation of mRNA within cationic liposomes are useful for achieving an immune response.

105. The document also mentions different types of approaches you can take in delivering mRNA (see Section 3.2 on adjuvants), including using different liposome-encapsulated mRNA forms co-administered which could enhance immunogenicity. At the end of the document, **D15** highlights that the design of efficacious vaccination strategies will help turn mRNA-based vaccine strategies into potent therapeutic and prophylactic modalities.

106. I was asked what this document would have taught a skilled addressee looking to provide nucleic acids formulated within a liposome as at 01 December 2009. My view is that this document sets out that mRNA, modified and unmodified, can be formulated and delivered to induce an immune response. With the discussion around antigens and inducing an immune response it is clear that **D15** describes vaccine strategies that shows that mRNA can be administered to mice in order to achieve an immune response. One viable delivery method being encapsulation of mRNA within cationic liposomes.

Document "D16"

107. I have been provided with a copy of Hess et al., *Cancer Immunol. Immunother.* (2006) 55: 672-683 (**D16**), which forms **Exhibit LH-21** to this declaration.

108. This documents discloses studies into tumor-associated antigen encoding mRNA, encapsulated within a cationic liposome, and the response achieved from cytotoxic T lymphocytes, in particular whether they could function as a cancer immunotherapy. I consider the emphasis of this document is essentially to show that the mRNA could induce an immune response and have at least some potential as a cancer vaccine.

109. The document talks about *in vitro* transcribed (IVT) mRNA usage and specifically mentions the Martinon paper, which I have already discussed above, in the context of it being the first publication that demonstrated that injection of an mRNA encapsulated in a liposome could elicit an immune response. The results found in **D16** suggest that "*vaccinating with liposome-encapsulated, nonreplicating IVT mRNA encoding model TAA ovalbumin (OVA) mRNA primed vigorous CTL responses that inhibited the growth of OVA-expressing tumors*". In terms of the liposome, cationic liposomes were formed from, DOTAP, DOSPER, and DOTAP-DOPE, and studied and it was found that the efficacy of the vaccination was dependent on the liposome used and the route of administration. The cationic lipid-neutral lipid combination of DOTAP-DOPE was found to be best.

110. I was asked what this document would have taught a skilled addressee looking to provide nucleic acids formulated within a liposome as at 01 December 2009. In my view this study is another example showing that mRNA can be encapsulated within a variety of cationic liposomes and demonstrates feasibility of vaccinating with nonreplicating IVT mRNA as a cancer immunotherapy. Again, this highlights that mRNA delivery systems can be useful for inducing an immune response and that liposomes of some form were demonstrated to work for mRNA delivery.

Document "D17"

111. I have been provided with a copy of Fenske DB et al. *Toxicol. Pathol.* 2008 Jan; 36(1):21-9 (**D17**), which forms **Exhibit LH-22** to this declaration. I note this document is a review article published not long before the relevant date listing Pieter Cullis as an author and relates to therapeutic agents (e.g. nucleic acids) encapsulated within lipid particles.

112. This document essentially discusses the evolution of liposomal formulations for the delivery of nucleic acids, dating back as early as the 1980's and 1990's which found the emergence of lipid classes, such as cationic lipids and PEG-lipids, for successful encapsulation of any type of DNA and RNA. These liposomal formulations include the early two- and three-component lipid systems, to the four-component lipid systems that are most typical now and that were discussed above in relation to a number of the prior art documents I have reviewed. This statement is supported by my views expressed earlier as to what was known in the field at the relevant time and also discussed in many of the documents I have discussed above.

113. **D17** also sets out the importance of each of the lipid classes, including the challenges faced along the way and the solutions to those challenges as at 2008. I generally agree with the discussion in this document. As I have already discussed in relation to the nature of the component lipids, they all have their role to play and I would have expected that changes in the nature of these lipids, and reasonably significant changes in the relative proportions in which they are present, would have a significant impact on the ability of the particle to encapsulate the nucleic acid, undergo endocytosis and then achieve endosomal release to have the nucleic acid enter the cytoplasm.

114. The document also mentions that "*cationic lipids were a crucial component, as they facilitated interaction with the nucleic acid, charge neutralization, and condensation of large polyanionic macromolecules*". As I have mentioned above (see paragraphs 36 and 37 above), the cationic lipid is a key component of the formulation and must be successful in both complexing with the nucleic acid, DNA or RNA, during the initial lipid particle formation and, subsequently, in triggering endosomal release due to the pH drop within the endosome. Those cationic lipids which display a permanent charge at physiological pH were known to have problems in terms of the cationic charge on the lipid particle, inherent toxicities and reduced circulation times. The solution at the time was the addition of PEG-lipids to the formulation, as discussed in this document, the PEG-lipid *would be an essential component of any long-circulating lipid particle*.

115. **D17** also discloses that particle size is another important parameter in nucleic acid delivery and mentions (page 23) that lipid particles have the *greatest utility* when the particles are formed around 100 nm in diameter as this provides for extravasation at the disease site. As I have mentioned previously, particle size is important in terms of the ability of the particle to undergo endocytosis into the target cell by slipping through leaky endothelial junctions.

116. The importance of cholesterol is also discussed (page 23, left hand column). The role of cholesterol in the lipid particle is to stabilize the bilayer, which indirectly leads to longer circulation times.

117. Toward the end of the document, while it specifically discusses the emergence of siRNA and its delivery (page 27), **D17** also discusses the development of SPLP and SALP liposomes comprising DSPC (a neutral lipid), Chol (a sterol), DODMA (a cationic lipid), and a PEG-lipid (either PEG-S-DSG for formation of SPLPs or PEG-Cer for the formation of SALPs), i.e. typical four component lipid system. The various types of lipid particles can be formed using different methods, such as batch formation using the well-known ethanol dilution approach, the commonly used detergent dialysis approach, and then the emergence of microfluidics. When microfluidics was described in the literature then this made the task of encapsulating nucleic acids easier and more reproducible, and so more amenable to industry applications. **D17** discloses that microfluidics can be used to "*prepare SPLPs with similar properties to those formed by detergent-dialysis, but its strength stems from its effectiveness at encapsulating a variety of different nucleic acid molecules: both RNA and DNA, ranging from small fragments to large plasmids*" (page 27, left hand column). I was asked to briefly comment on this statement. My recollection of the change that microfluidic mixing brought to the field meant that lipid particle formation could be formed at greater scale with more precise control over the parameters such as particle size, and generally simplified encapsulation of nucleic acids, meaning that this mixing approach could be used to encapsulate any type of nucleic acid, including plasmid DNA, mRNA, and siRNA.

118. I was asked what this document would have taught a skilled addressee looking to provide nucleic acids formulated within a liposome as at 01 December 2009. The teaching of the document is really around the evolution of various lipid particles, whatever nucleic acid is encapsulated. The lipids used to form the lipid particles appear to be standard selections from those known at that time, ranging from the early two-component lipid systems to the more typical three- and four component lipid systems. This document therefore provides something of a guide and also conveys that the microfluidic approach is a very useful one for rapid formulation of any chosen nucleic acid within lipid particles.

Document "D18"

119. I have been provided with a copy of Malone RW et al. *Proc Natl Acad Sci U S A.* 1989 Aug; 86(16):6077-81 (**D18**), which forms **Exhibit LH-23** to this declaration.

120. This document describes the delivery of mRNA encapsulated within a specific two-component liposome, DOTMA/DOPE (bottom of page 6077, right-hand column). The same system has

previously been used to transfect DNA into cells. Given this is an article published in the late 1980's, this was a very simple lipid delivery approach showing effective transfection of mRNA *in vitro*.

121. **D18** also discusses the benefits of some modification to the mRNA, and showed that at least capping was important for stability and translation efficiency of the luciferase mRNA (page 6081). The results indicating that the m^7G-capped mRNA binds more efficiently to the ribosomes. In later years it became well understood that it was generally preferable to use modified mRNA (mmRNA) as this would often result in better expression of the protein. I already discussed this above in paragraph 32 in relation to the Karikó 2008 Molecular Therapy article.

122. This document concludes by suggesting that this early work raises the possibility for an alternative nucleic acid, i.e. mRNA, that can be delivered in cationic liposomal formulations and be used for application in gene therapy (page 6081, right-hand column).

123. I was asked what this document would have taught a skilled addressee looking to provide nucleic acids formulated within a liposome as at 01 December 2009. The focus of **D18** is clearly on demonstrating, as a proof of concept of the approach, that cationic liposomes can be used to deliver mRNA. The results showed that a high-efficiency mRNA transfection system using the commercially available cationic liposome formulation, LIPOFECTIN, provided results that are comparable to liposome-DNA complexes. These formulations, however, are not suitable for administration in humans and so the teaching, at this early stage, was really that liposomes of some form could be an effective delivery vehicle.

Document "D19"

124. I have been provided with a copy of WO1990/011092A1 entitled "Expression of Exogenous Polynucleotide Sequences in a Vertebrate" (**D19**), which forms **Exhibit LH-24** to this declaration. I note this patent document published around the same time as document (**D18**) I just discussed and shares some of the same authors/inventors, such as Robert Malone and Philip Felgner. As I mentioned, it was common for groups at that time to file patent applications and so sometimes the patent application may be the first discussion of a technique if the group didn't publish quickly after patent filing.

125. This document discloses the delivery of a nucleic acid within a liposome to induce an immune response (vaccination) or for gene therapy. For example, one of the focuses of **D19** is the treatment of muscular dystrophy and that mRNA encoding for a protein (e.g. dystrophin) can be delivered for

gene therapy (e.g. see last paragraph on page 7). Throughout the document, a number of proteins which can be encoded for or conditions which can be treated with encoded therapeutic proteins are discussed (e.g. see pages 12 to 13 and 25 to 26). In an alternative, the document also discusses delivery of mRNA (or DNA) as part of a vaccine composition for the production of a polypeptide to generate immunity to a pathogen (e.g. see pages 13 to 15 and 35 to 36).

126. The mRNA was produced in a standard manner using phage RNA polymerases to prepare mRNA from DNA templates in the presence of the individual ribonucleoside triphosphates (e.g. see last paragraph on page 19). The document also discusses the advantages of capping mRNA (e.g. see pages 20 to 21). As I have discussed above, capping is known to be useful in stabilizing the mRNA.

127. **D19** also discusses liposomes and their preparation in some detail (e.g. see pages 45 to 49, and Example 6 on page 53). Given the publication date of this document, the liposomes formed are very simple and prepared via conventional methods using commercially available and common cationic lipids. For example, the document discloses DOTAP and DOTMA liposomes with or without the addition of phosphatidylcholine, cholesterol, phosphatidylethanolamine, etc.

128. The document ends with several examples. The first few examples discuss the preparation of the cationic lipid, DOTAP, followed by preparation of the polynucleotides including preparation of the mRNA, and then the liposomal formulations (Example 6, page 53). Example 6 also briefly mentions that the cationic liposomes will spontaneously deliver the polynucleotides into cells *in vivo*, and variation of the ratio of polynucleotide to liposome is adjusted depending on application (page 53, third paragraph). Several examples are then disclosed showing *in vivo* expression of the mRNA or DNA in mice, for example, using the liposome formulation prepared in Example 6.

129. I was asked what this document would have taught a skilled addressee looking to provide nucleic acids formulated within a liposome as at 01 December 2009. As I've mentioned, **D19** is another example of an early publication describing simple cationic liposomes formed using commercially available cationic lipids where the delivery an encapsulated mRNA was demonstrated to result in functional protein expression. This document would have indicated to me that mRNA can be encapsulated and protected from degradation in a liposomal formulation, in this case with the intention of gene therapy and vaccination applications.

F. THE OPPOSED APPLICATION

130. After I provided my comments in relation to the documents discussed above, I was provided with a copy of Australian Patent Application No. 2020202755 (the "**Opposed Application**"), a copy of which is exhibited hereto and marked **Exhibit LH-25**. I was asked to consider the Opposed Application and provide my views on its disclosure.

131. The Opposed Application is titled "*Delivery of mRNA for the Augmentation of Proteins and Enzymes in Human Genetic Diseases*".

132. The background of the invention from pages 1 to 2 very clearly sets out that the invention is all about protein and enzyme replacement therapy. In particular, a specific focus being placed on urea cycle metabolic diseases such as ornithine transcarbamylase (OTC) deficiency, which I know can be a very severe genetic disease.

133. The summary of the invention from pages 2 to 6a specifies that "*methods of intracellular delivery of nucleic acids that are capable of correcting existing genetic defects and/or providing beneficial functions to one or more target cells*" (page 2) are disclosed. The document goes on to disclose that the compositions and nucleic acids, and methods, described are useful in the management and treatment of a large number of diseases, in particular diseases requiring expression of a protein or enzyme of which the disease involves a deficiency, specifically useful for the treatment of urea cycle metabolic disorders, such as OTC. Compositions and methods for increasing expression of a functional protein or enzyme in a target cell are also provided, as well as methods of treating a subject having a protein or enzyme deficiency (page 4), and compositions and methods useful for facilitating the transfection and delivery of one or more nucleic acids. The summary highlights mRNA as an example nucleic acid throughout the summary section, I take this to mean that it is the preferred nucleic acid. The RNA is stated as preferably being an mRNA encoding a functional protein or enzyme. They also mention that the mRNA may be modified to confer stability. I have already discussed that this was typical at the time.

134. Moving through the summary of the invention section, I see that four component liposomes are described as the preferred transfer vehicle for the delivery of mRNA to the target cells. For example, page 4 says the transfer vehicle may comprise one or more cationic lipids, non-cationic lipids, and/or PEG-modified lipids, and provides the example mixture of the following lipids: *CHOL, DOPE, DLinDMA and DMG-PEG-2000*. I am very familiar with this type of combination of lipids, which Semple, Cullis and others identified as an effective liposomal formulation for the delivery of nucleic

acids as at 01 December 2009. Further, the 'DLin' series was developed and discussed in the key publication I already referred to in paragraph 35 - *Nat Biotechnol* 28, 172–176 (2010). Other mixtures are also disclosed here, such as a three component system: ICE, DOPE, and DMG-PEG-2000, and also a disclosure suggesting the mixture *may comprise any one of more lipids selected from the group consisting of ICE, DSPC, CHOL, DODAP, DOTAP and C8-PEG-2000 ceramide*.

135. The remainder of the summary of invention section appears to provide embodiments that I understand relate to the main claims. I will discuss the claims in depth in the Claims section.

136. Turning to the detailed description section of the Opposed Application at pages 7 to 8, it appears much of the discussion here is what was defined in the summary of invention section and describes the intent of the invention is to provide compositions and methods that are useful for the treatment of diseases which result from the deficient production of proteins or enzymes. The compositions, methods and uses refer to the same compositions, methods and uses as defined above, including the disclosure of mRNA-liposomal compositions for modulating the expression of a protein in a target cell, mRNA-liposomal compositions for increasing expression of a urea cycle enzyme in a target cell, and methods of expressing a urea cycle enzyme in a target cell / methods of treating a subject with a urea cycle deficiency by delivering/administering a liposomal-mRNA composition to the cell such that the mRNA encodes a urea cycle enzyme. This suggests that the liposomal formulations described using the typical three or four lipid component classes could be used to deliver mRNA to result in functional protein or enzyme expression, in particular the expression of a functional protein or enzyme in which a subject is deficient (e.g. a urea cycle enzyme), i.e. defining gene therapy and protein replacement. Pages 7 and 8 detail this is achieved by the nature of the compositions which facilitate the delivery of the nucleic acid to 'target cells' for subsequent transfection. It is these target cells which then produce the functional version of the gene product to rectify the deficiency of that product caused by the disease state.

137. Several terms are then defined from the bottom of page 8 to page 20. The terms such as "nucleic acid, "functional" and "expression", "encapsulation", etc. all appear to have their ordinary meaning known by a skilled person in the field at the time. Page 9 again reinforces the nature of the invention being described in the Opposed Application stating that "The present invention contemplates the use of such nucleic acids (and in particular RNA or stabilized RNA) as a therapeutic capable of facilitating the expression of a functional enzyme or protein, and preferably the expression of a functional enzyme of [sic] protein in which a subject is deficient (e.g., a urea cycle enzyme)". I note that the term stabilized RNA is mentioned and on pages 10 to 16 of the Opposed Application it is stated that the mRNA may be modified (e.g. chemically and/or biologically) which

can improve stability and/or half-life of the mRNA or improve or otherwise facilitate translation. This suggests to me that a chemically or biologically modified mRNA-liposomal formulation would be the most effective and considered the preferred approach. As I discussed in relation to the prior art, the use of some form of modified RNA was common and the benefits of using it in this kind of formulation was well-known prior to 01 December 2009. I would expect that modified mRNA would have been chosen and used in the liposome formulations because it is more effective than unmodified mRNA as (i) it can be considered less immunologically 'toxic' and (ii) can produce more encoded protein and often over a longer period of time . Again, the 2008 Molecular Therapy article I have referred to earlier shows these points were well-understood around 2008. Page 11 states that a preferred outcome of the invention is that the activity of the nucleic acid, such as the mRNA encoding a functional protein or enzyme, is prolonged over an extended period of time.

138. Page 18 discusses transfer vehicles for the nucleic acid. Liposomes are initially mentioned but a range of very different delivery technologies are then described as being suitable including proteoliposomes, silicate nanoparticulates, semiconductor nanoparticulates, plasmids, viruses, and so on. I find this confusing in so far as the Opposed Application is saying, which appears to be the case, that all of these delivery approaches are considered to be encompassed by the invention when used to deliver nucleic acids. To my understanding it would be considered a very different approach indeed to use a classic liposomal formulation versus a silicon dioxide nanoparticle or one of the biological delivery vehicles described. The implication seems to be that all of these delivery vehicles would work in the same way in terms of the delivery of nucleic acid to target cells for transfection to address a protein deficiency, but I don't see how that could be the case. Based on the discussion in other parts of the Opposed Application and the Examples, my assumption is that liposomes are the true delivery vehicles of interest.

139. The importance of delivery to target cells and tailoring of the transfer vehicle with that in mind, is discussed on page 19. Hepatocytes appear to be preferred target cells based on comments here and elsewhere. The Examples and page 19 sets out that the properties of size, charge and/or pH can be optimized to ensure effective delivery and reduce immune clearance. The structure of the preferred liposomal delivery vehicle is described as having an interior aqua space which is separated from the external medium by one or more lipid bilayers. This is consistent with my understanding of classical liposome morphology. Page 20 describes that the payload, such as a nucleic acid, may be carried within interior aqueous space or within the bilayer or even on the outer surface of the bilayer.

140. Pages 21 to 25 specify variations of the liposomal formulation comprising the standard four lipid classes, including variations of the relative amounts of each. Page 21 starts by defining their use of

the term “cationic lipid” as covering any lipid species that carries a net positive charge at a selected pH, such as physiological pH. Interestingly, halfway down page 22 the Opposed Application states that several commercially available reagents are available “to enhance transfection efficacy”. As I have discussed from the prior art above, such liposome-forming reagents as these (LIPOFECTIN, LIPOFECTAMINE etc.) had already previously been used for RNA delivery and these passage seems to acknowledge this fact and that such liposomes are already known for transfection. This makes the nature of the invention being described a little unclear. Each class of lipid is subsequently defined by their relative amounts and also some specific examples. I note that one specific type of cationic lipid is discussed in detail, the imidazole cholesterol ester or “ICE” lipid, which I understand could be the preferred cationic lipid in this invention. Page 23 shows this structure and states that imidazole-based cationic lipids are characterized by reduced toxicity relative to other cationic lipids. Given how broadly “cationic lipid” was defined on page 21 this raises the question in my mind of which of the more toxic cationic lipids are not considered to form part of the liposome compositions of the Opposed Application. I don’t see any further discussion on that point to provide guidance on which cationic lipids to avoid. The amount of cationic lipid which might be used is presented in an extremely wide molar ratio range of 1% to 90% on page 23. PEG-lipids are then described briefly followed by “non-cationic lipids” on page 24. These are described as including the usual examples I would have been familiar with at the time, mainly DOPE and DSPC, but also includes cholesterol (line 24 of page 24) in the definition of a non-cationic lipid. The non-cationic lipid may have a molar ratio of 5% to 90% when used in combination with a cationic lipid. This leaves a very wide range of molar ratio combinations of even these two lipid classes and would seem to be very close in composition to the earlier LIPOFECTIN and such classes, depending on any other lipid classes which are present.

141. A liposomal formulation defining a broad definition of the relative amounts of the four lipids is specified at the bottom of page 24 to the top of page 25 as being prepared using: *DSPC/CHOL/DODAP/C8-PEG-500 ceramide in a molar ratio of about 1 to 50 : 5 to 65 : 5 to 90 : 1 to 25, respectively*. These ranges are extremely broad and, particularly, I’m surprised the cationic lipid could be present even at 60 molar % in such a four-lipid system, never mind up to 90 molar %, and still provide for a workable liposomal formulation as these levels don’t leave much formulation room at all for the structural lipids to perform their role. More specific molar amounts and combinations of the four lipids are specified from lines 1 to 7 of page 25. For example, *DSPC/CHOL/DODAP/mPEG-5000* at a molar ratio of about 33:40:25:2, *DSPC/CHOL/D0DAP/C8 PEG-2000-Cer* at a molar ratio of about 31:40:25:4, *POPC/D0DAP/C8-PEG-2000-Cer* at a molar ratio of about 75-87:3-14:10, or *DSPC/CHOL/DOTAP/C8 PEG-2000-Cer* at a molar ratio of about 31:40:25:4.

142. Immediately following this, the Opposed Application sets out that the selection of the lipids from the different lipid classes as well as the relative molar ratio of each "*is based upon the characteristics of the selected lipid(s), the nature of the intended target cells or tissues and the characteristics of the nucleic acids to be delivered by the liposomal transfer vehicle. Additional considerations include, for example, the saturation of the alkyl chain, as well as the size, charge, pH, pKa, fusogenicity and toxicity of the selected lipids*". I assume reference to saturation of the alkyl chain probably refers to the tail(s) of the cationic lipid as double bonds can influence the role this lipid plays, particularly in endosomal release. The statement, overall, is one I agree when the aim is to tailor the liposome for delivery to particular target cells, uptake and subsequent endosomal release and expression of a functional replacement peptide or enzyme from an mRNA. To achieve this, all of these factors, and others, would have to be considered in the liposome design, tested and many rounds of this repeated to achieve the desired delivery and expression.

143. From page 25, line 15 to page 28, line 10, the Opposed Application provides a number of citations, including some which are incorporated by reference, to describe liposome production, nucleic acid encapsulation, and sizing of the liposome. The techniques and methods described are familiar to me and would have been considered standard techniques and methods known by those skilled in the field before the relevant date. I note that the description provided here is again consistent with my understanding of a classic liposome, i.e. having a lipid bilayer and aqueous core.

144. Particle size is another important parameter in the delivery of liposomal formulations in terms of the ability of the particle to undergo endocytosis into the target cell. This is discussed in the Opposed Application from page 27, line 18, to page 28, line 10. Standard sizing techniques are discussed as appropriate to achieve the desired liposome size. I consider the ranges provided on page 28, lines 8 to 10, are fairly standard ranges and as I have previously mentioned above in paragraph 41, for example, the diameter of the particle is generally around 100 nm such that it can pass through the endothelium of the target tissue (e.g. liver). Page 27, line 18 onwards, again clarifies that size selection has to be tailored for the site of the target cell or tissue and to some extent the actual application. This seems to me to teach that a variety of liposome sizes would be required to treat different conditions which require delivery to different target cells or tissues. I don't see any more discussion around how to judge which size is appropriate for different tissues other than hepatocytes, which seems to be a focus, and so presumably this would need to be determined for each application.

145. The pages that follow discuss production of functional proteins and enzymes. Page 29 discusses the advantages of having a subject produce the deficient protein endogenously including avoiding the

need for delivery of recombinant therapeutics. Passive and active targeting are then discussed with mention of approaches which were known at the relevant date. I don't believe the Opposed Application is claiming to have invented any of these targeting approaches. Pages 31 and 32 then define and discuss the target cell and clarify that it is a cell or tissue to which the compositions are directed or targeted. That is, as a I have discussed above, the invention seems to include designing of the liposome, and tailoring, for delivery to a selected target cell or tissue. A significant amount of discussion then follows on particular disease states resulting from protein deficiency with OTC being a focus. This again reinforces the invention is described as being for replacing protein or enzyme deficiencies in genetic diseases based on targeted delivery of the liposomes to the target cell or tissue in which the enzyme or protein should be produced.

146. I note on page 35, line 28 to page 26, first line, the following passage is recited "*Alternatively, the nucleic acids may encode full length antibodies or smaller antibodies (e.g. both heavy and light chains) to confer immunity to a subject. While one embodiment of the present invention relates to methods and compositions useful for conferring immunity to a subject (e.g., via the translation of mRNA nucleic acids encoding functional antibodies), the inventions disclosed herein and contemplated hereby are broadly applicable*.". I was asked what this conveyed to me. I consider this statement does not mean a protective immune response can be achieved. It is clear the Opposed Application is merely suggesting that the mRNA being delivered may encode a functional antibody in keeping with the rest of the description regarding enzyme or protein replacement therapies. This is similar to administration of a functional antibody to the subject but instead of delivering the functional antibody, delivering mRNA which will encode that antibody. It is essentially a description of what is known as "passive immunization", achieved transiently *via* the delivery of the appropriate mRNA, as opposed to true "active immunization". This is not a disclosure of a vaccine formulation that produces a protective immune response generating immunological memory in a subject.

147. The detailed description ends with discussion of what would be considered standard administration routes and dosages. It doesn't appear to be particularly addressed to the earlier discussion of liposome delivery as all sorts of administration routes are considered which would not be appropriate. I understand this is not uncommon in patent applications.

148. As a general comment, the Opposed Application contains a lot of disclosure of things which were already well-known prior to 01 December 2009 with a clear focus on delivery of mRNA encapsulated in classic liposomal formulations for treating urea cycle metabolic disorders (e.g. OTC deficiency). In particular, it addresses delivery of mRNA to the liver to result in functional protein or

enzyme expression, i.e. the expression of a functional protein or enzyme in which a subject is deficient (e.g. a urea cycle enzyme). In other words, for gene therapy and/or protein replacement.

149. I was asked to comment on the Examples of the Opposed Application. Example 1 provides a general method for preparation of the liposomal formulations containing mRNA. I note that the approach used is the solvent dilution approach. The solvent (e.g. alcohol such as ethanol) dilution method was a well-established procedure for encapsulating nucleic acids in lipid vehicles. In this method, lipids are dissolved in a water-miscible alcohol such as ethanol, which is then diluted by an acidic buffer containing the nucleic acid. I note that the particles generated are described as multilamellar vesicles (MLVs). This means they would have more than one lipid bilayer in a sort of concentric pattern around the aqueous core. The MLVs are then extruded which is a standard approach. A different assay approach is described for DOTAP-containing liposomes which makes sense based on the permanently charged quaternary amine nature of DOTAP.

150. I will not discuss Examples 2 to 5 in any detail as they simply relate to what I understand to be liposomal formulations of standard lipids already well-known in the field at that time, including DSPC, cholesterol, DODAP (or DOTAP in Ex. 3) and C8-PEG-2000 ceramide which are shown as formulated in a 31:40:25:4 ratio, which I consider to be within fairly standard ranges in the field at that time, containing a few different mRNAs. I do note, however, that Example 3 mentions the use of a buffer but this is somewhat confusing because DOTAP is a quaternary amine cationic lipid and there is no need to use an acidic buffer, whereas Examples 2, 4 and 5 all use the ionizable lipid, DODAP. I have briefly summarized the formulations and encapsulated mRNA of Examples 2 to 5 below, for convenience. One comment I will make is that I am surprised the molar ratio ranges of the lipid components in the description (as I discussed earlier) are so very broad when only a single molar ratio formulation of a four lipid liposome at a 31:40:25:4 ratio has been made and tested. I'm not sure how the Opposed Application could be confident in 2009 of other molar ratio formulations working which differ significantly from this one example. All of Examples 2 to 5 describe producing MLVs which are then extruded to produce LUVs being large unilamellar vesicles i.e., liposomes having a single lipid bilayer surrounding an aqueous interior. I note that LUVs are typically considered to have a diameter of greater than 100 nm up to a point at which they might be considered to be giant unilamellar vesicles (GUVs) at, say, 1 micron and upwards.

Example no.	Lipids Used	Molar ratio	mRNA
2	DSPC/Chol/DODAP/C8-PEG-2000 ceramide	**31:40:25:4**	Renilla Luciferase
3	DSPC/Chol/DOTAP/C8-PEG-2000 ceramide	**31:40:25:4**	Renilla Luciferase
4	DSPC/Chol/DODAP/C8-PEG-2000 ceramide	**31:40:25:4**	Firefly Luciferase

5	DSPC/Chol/DODAP/C8-PEG-2000 ceramide	**31:40:25:4***	Murine OTC

*Example 5 states '2' for the DODAP molar ratio but I have assumed this should be '25' as that matches the other examples and the molar ratio otherwise would not add up to 100.

151. I note Examples 6 and 7 provide the production method and formulation of a three-component lipid formulation using the ICE lipid in combination with DOPE and DMG-PEG-2000 formulated in a 70:25:5 ratio. Biodistribution and other techniques are then briefly described. With regard to the *in vivo* bioluminescence study, it is impossible to understand the results from Figure 2 as no information is provided as to what the results equate to. While I note the graph shows significant variations between different groups, it's not entirely clear which formulations are used even though the figure legend mentions different sample names, so I can't draw any conclusions on exactly what was administered in the best performing formulations. Further down, under the heading "*In Situ Hybridization Results*", again, all these specialized liver cells or anatomical structures are only loosely identified. More specific markers for each cell type has to be used for positive identification. Overall, I find it hard to see how any firm conclusions can be drawn from the studies described in Example 7.

152. In Example 8, the lipid formulation is cholesterol, DOPE, DLin-DMA, and DMG-PEG2000 containing FFL mRNA, wherein the lipid formulation is prepared in a "*manner similar to that described*" above. Presumably, this means the same preparation method and ratios as disclosed above for Examples 2 to 5, i.e. at a molar ratio of about 31:40:25:4, given the similar four-component lipid formulations, but I cannot be certain of that. Of note, is that DLin-DMA is an ionizable lipid which was invented by others. As I mentioned above when discussing background knowledge (see paragraph 34 above), DLin-DMA was the early benchmark lipid of a series of DLin-DMA-based lipids that have been shown to be successfully formulated in nucleic acid-lipid particles, and were known to me before 01 December 2009.

153. In short, it appears to me that the Examples of the Opposed Application demonstrate the use of one three-component ICE lipid formulation produced in Example 6 with some results presented in Example 7. Examples 1-5 and 8 describe different four-component lipid formulations, however, the molar ratio of each of those four-component lipid formulations are identical and the lipid components are exactly the same apart from the change in the cationic lipid between the DODAP (Ex.'s 2, 4 and 5), DOTAP (Ex. 3) and DLin-DMA (Ex.8), and the change from DSPC to DOPE (Ex. 8) for the non-cationic lipid with one change in PEG-lipid in Example 8. I was aware of these lipids prior to 01 December 2009 as they had been well studied in the field of nucleic acid delivery. Although questionable, the only Example I consider to show something new is Example 6 and 7, which as I

have said describes the production of the three-component ICE lipid formulation containing an mRNA and resulted in "*enriched delivery of mRNA to liver versus spleen in vivo*".

154. Finally, a discussion section follows the Examples and this appears to go to the heart of what the Opposed Application is suggesting the invention is useful for. The bottom of page 49 describes that the mRNA in the lipid transfer vehicle "*can be used for the delivery and expression of genes in vivo in liver including hepatocytes*". The ICE lipid is referred to as having given enriched delivery of mRNA to the liver versus the spleen. The final conclusion and summing up of the invention being described appears to be on page 50 where it is stated "*The successful delivery of such mRNA to the liver and in particular to hepatocytes supports the conclusion that the methods, formulations and compositions of the present invention can be used for the treatment and the correction of in-born errors of metabolism that are localized to the liver*". This is consistent with my earlier observations that the focus of the Opposed Application is clearly on treating diseases related to protein deficiency and specifically those which are capable of being treated *via* delivery of liposomes encapsulating an mRNA to the liver for expression of the protein or peptide.

155. I was then asked to comment on claim 1 of the Opposed Application and compare that with the technical information provided in the Examples, which I have just discussed. To simplify the comparison for the liposomal formulation aspect I have included a table below which compares the ranges of the lipid components in claim 1 and those of the liposomal formulations demonstrated in the Examples, without mention of the particular mRNA. I understand claim 1 to require a four-lipid liposome and so I have not included in the table the liposome of Examples 6 and 7 which is a three-lipid liposome. I have then added below the table the components of claim 1 as I see it in terms of technical or functional aspects.

Lipid Component	**Molar ratio in claim 1**	**Specific lipids used in Examples**	**Molar ratio represented in Examples**
Cationic lipid	20-70%	(I) DODAP (Ex. 2, 4, 5) (II) DOTAP (ex. 3) (III) DLin-DMA (Ex. 8)	25% 25% *25%
Non-cationic lipid	5-90%	(I) DSPC (Ex. 2-5) (II) DOPE (Ex. 8)	31% *31%
PEG-modified lipid	0.5-20%	(I) C8-PEG-2000 cer (Ex. 2-5) (II) DMG-PEG-2000 (Ex.8)	4% *4%
Cholesterol	No limitation	Cholesterol (Ex. 2-5, 8)	40%

* Value not recited for Example 8 but value assumed based on formulation being described as similar to earlier ones.

Claim 1 components:

a) A composition including an mRNA encapsulated within a liposome in which the mRNA encodes a suitable protein or peptide

b) the liposome has a size less than 150 nm and comprises the following lipid components which can be present in a molar ratio amount between the ranges set out:

i. a cationic lipid at 20 – 70%

ii. a non-cationic lipid at 5 - 90%

iii. a PEG-modified lipid at 0.5 – 20%

iv. cholesterol

c) the mRNA comprises

i. a 5'-UTR

ii. a 3'-UTR

iii. a 5' cap structure

iv. a poly A tail.

156. As I have already discussed, and consistent with the definition in the Opposed Application, I consider that claim 1 requires that the mRNA is encapsulated within a liposome which is a lipid transfer vehicle having at least one lipid bilayer surrounding an aqueous interior. I noted before that the particles generated in the Examples have not been fully characterised, in fact, even the particle size was not determined or at least the data was not provided. Nonetheless, all of the four-lipid liposome formulations did describe the formation of MLVs followed by extrusion to generate LUVs. I understand that the mRNA can be one that encodes for literally any protein or peptide, even though very limited data was presented on this aspect. This seems strange to me as the entire focus of the patent was on the delivery of the mRNA to produce a therapeutically functional protein. That is, the focus is on protein replacement therapies and particularly those around urea cycle disorders, such as OTC deficiency. I don't see how the Opposed Application can claim delivery of an mRNA encoding for anything, such as an antigenic polypeptide, when this would seem to be completely outside of all discussion and Examples it presents. I would have concerns around the ability of this approach to treat any disease of protein deficiency but claim 1 seems to go even well beyond that in terms of the mRNA being delivered. Finally, I understand that the nature of the lipid components, in addition to the ranges which are listed, are only limited by the nature of the class described in claim 1. That is, any lipid which can present an overall positive charge is included in the "cationic lipid" term even despite its size, toxicity pKa etc.; any lipid which does not present a positive charge

in this manner, including anionic lipids and neutral lipids, are included within the "non-cationic lipid" term; any lipid which has been synthetically modified to present a PEG moiety is included in the "PEG-modified lipid" term; and cholesterol is included.

157. This being the case my understanding is that the Opposed Application, and particularly claim 1, is saying that any mRNA encoding a protein or peptide can be encapsulated into a cationic liposome formed from any combination of molar ratio values between: 20-70% of any cationic lipid; with 5-90% of any non-cationic lipid; with 0.5-20% of any PEG-modified lipid; and any relative amount of cholesterol. I find this extremely surprising based on a number of issues but not least that the Examples of the Opposed Application demonstrate a very small number of liposomal formulations, one of which I consider a three-component lipid formulation, and the others being very similar four-component systems using similar non-cationic lipids, two PEG-modified lipids, and cholesterol and at exactly the same molar ratios. The main point of variation is a swapping of one cationic lipid for a somewhat related one without any change in the molar ratio of that component, with the exception of the three-component lipid formulation. That seems to me to be a very small experimental set indeed from which to say that such a wide range of liposomal formulations, made from what would be a vast number of the four lipid component combinations, would be suitable for delivery of any mRNA encoding a protein or peptide. The other question I have is whether the claims include compositions with no cholesterol, since that is not clear to me. This is particularly so because what I consider to be the main Example, is a three component lipid formulation which does not include cholesterol in the typical sense, but merely as the body of the cationic lipid itself, which I do not consider a separate component as it required by claim 1. I will discuss some of the other specific issues below which will help explain my view.

Cationic Lipid

158. The cationic lipid is a key component of the formulation and its key function is to complex with the mRNA during the initial liposome formation and, subsequently, trigger endosomal release due to the pH drop within the endosome, as discussed above in paragraph 37.

159. In terms of the molar ratio range it is clearly important to have a suitable amount to complex with the mRNA and also to ensure sufficient availability for the subsequent endosomal membrane fusion event for release into the cytoplasm. The four-component liposomal formulations discussed in the prior art section above, for example for D1-D5 and D13, tend to use a cationic lipid in the 25 to 30 molar % range. This is consistent with the molar % ratio used in Examples 2 to 5 and 8 of the Opposed Application. Based on this, it would be unusual, and this would have been understood immediately prior to 01 December 2009, to go much outside of that range. In my view, very low

amounts such as below 20 mol% would not be sufficient to complex with the nucleic acid and still be available to trigger endosomal release. I believe 70 mol% would be problematic as this leaves too little 'room' for both the neutral lipid and cholesterol to actually form stable particles with the desired morphology. I therefore find it surprising that the Opposed Application suggests that the cationic lipid could be present in as low a relative amount as 20 % or as high as 70 %. Again, I note that the Opposed Application only shows the cationic lipid present in a four-lipid liposome formulation at 25 molar % and so hasn't shown whether those formulations having significantly different levels are effective.

Non-cationic Lipid

160. As I mentioned above, the term "non-cationic lipid" seems to me to be very broad indeed and covers all sorts of lipids that I would not necessarily expect to see in a liposomal formulation. This is particularly so because this lipid class, in the majority of formulations I am aware of and which were described throughout the literature prior to 01 December 2009, have generally been limited not just to the phosphatidylcholine/ethanolamine class, but to a very small number of actual neutral helper lipids typically including DSPC, DPPC, DOPE and closely related compounds. As I've mentioned before, DOPE is known to bring with it stability challenges for the final lipid particle and is often avoided even though this drawback can bring with it better endosomal release. I actually published on the particular characteristics of DOPE versus DOPC, as a helper lipid, back in 1995.

161. The typically used non-cationic lipids which are employed in four-component lipid particle formation for nucleic acid delivery, and which were used immediately prior to 01 December 2009, can carry a high degree of variability in outcome. I can't really imagine what other lipid types are supposed to be covered by the term "non-cationic lipid" and expected to work in the same way as, for example, DSPC and DOPE. The Examples of the Opposed Application certainly didn't give any exemplification as to what other types of non-cationic lipid would work well in a liposomal formulation in this same role.

162. In terms of the molar ratio, there can be some degree of variation between the non-cationic lipid and cholesterol so this allows a small amount of give and take, if you like. Nonetheless, it would have been very unusual before 01 December 2009, and still would be, to formulate a liposome with as little as 5% of the non-cationic lipid when in combination with those other components. That just doesn't appear to me to be a sufficient amount for this lipid to fulfil its structural role. Again, I note that the Opposed Application only shows two non-cationic lipids, DSPC and DOPE, present in the liposomal formulation at 31 molar % in the exemplified four-lipid formulations and so hasn't shown

how to make a four-lipid liposome formulation outside of 31 molar % or with any other non-cationic lipids.

PEG-modified Lipid

163. The PEG-lipid plays a number of roles and represents a sort of balancing act in the formulation. The nature of the PEG-lipid was known to influence the rate at which the PEG can depart the lipid particle and so has a knock-on effect on circulation time. The amount of PEG-lipid in the liposomal formation has a strong influence on the resulting size of the lipid particle with increasing amounts in the formulation providing smaller particle sizes. Particle size is another important parameter in the lipid particle delivery field in terms of the ability of the particle to be directed to and then undergo endocytosis into, the target cell. You can't, for example, produce lipid particles which are comparable in terms of the nature of the lipid components and particle size and compare them with increasing PEG-lipid amounts. This is because a lipid particle with around 1-5 % PEG-lipid will have a very different particle size to one with, say, 10-20 % PEG-lipid. This is indicative of the variability you get with lipid formulations when you start altering the relative amounts of the main lipid components.

164. The PEG-lipid is important in conferring appropriate colloidal stability to the liposomal formulation and reducing particle aggregation. Again, you need a sufficient amount to achieve that stability but if you have too much PEG-lipid present then it creates a high degree of steric hindrance on the particle surface. This can be detrimental in terms of docking of the particle with the cell and also in preventing appropriate contact between the ionizable cationic lipid and the endogenous anionic phospholipids of the endosomal membrane for cytoplasmic release. Also, if you have too much PEG-lipid in the formulation then the particle becomes unstable and results in mixed micelles which are to be avoided for this sort of formulation. Certainly at levels approaching 20 molar % I doubt that the liposome could be functional for *in vivo* delivery in that, even after some PEG-lipid is lost from the liposome, as occurs naturally systemically, there would be such a dense layer of PEG on the outside of the particle that the interactions with the cell and endosomal membranes would be blocked or limited such that not enough mRNA could be delivered. The particle size would also decrease significantly with PEG-lipid levels at 20 molar % and would likely be functionally limiting. Finally, with levels at 20 molar % you would likely get a significant degree of mixed micelles forming and so you lose a lot of the uniformity and functionality of the lipid particles that you are trying to achieve. I would have been aware of these issues at 01 December 2009 and would not have considered adding a PEG-modified lipid at much above 4-5 molar % for the reasons I have discussed. Again, I note that the Opposed Application only shows the PEG-modified lipids, DMG-PEG-2000 and C8-PEG-200 ceramide, present in the liposome formulation at 4 molar % and so hasn't showed how

to make a liposomal formulation with different PEG-modified lipids or significantly outside of this molar ratio value.

Cholesterol

165. As I have mentioned above, the Examples only use cholesterol in some of the formulations and the Example formulation that does not is described as a useful one showing delivery and expression of mRNA in the liver. This is confusing to me. In my opinion, I would consider cholesterol a required feature in claim 1, but in my mind this would then exclude the three component lipid formulation produced and studied in Examples 6 and 7, leaving the other formulations which simply relate to what I understand to describe liposomal formulations of standard lipids already well-known in the field.

166. In terms of the relative molar ratio for this component, there is no limitation placed on it in the claims. I would not have expected as at 01 December 2009 to see a four-component liposomal formulation to have a level of cholesterol much lower than the low 30% region without a negative impact on the liposome structure. While there may be some limited capacity to compensate for a somewhat low level of cholesterol with a slightly higher than usual level of DSPC or DOPE, anything in the very low 30% region (or less) would seem to me to be at a high risk of not having the appropriate particle architecture for nucleic acid encapsulation and delivery. The 40% used in the formulations of the Examples of the Opposed Application is what I would consider to be a classic or commonly accepted level for liposomal formulations for nucleic acid delivery prior to 01 December 2009. Once again, the Opposed Application only exemplifies the use of cholesterol in some Examples, not the main Example, and only at a single molar % value of 40%.

167. In short, it appears to me that the Examples of the Opposed Application only demonstrate a very small number of very similar formulations which were known both in terms of the actual lipids used and their general relative amounts, and which were able to be formulated and delivered with mRNA as part of a liposomal formulation. I don't see how that can be extrapolated out to the various combinations of claim 1 based on the data I have reviewed. Also, I am confused as to how the liposomal formulation of Examples 6 and 7 can even be encompassed by claim 1. I don't see how the results presented in the Examples justify claim 1 covering the delivery of any mRNA polynucleotide which encodes for a protein or peptide with all of the various combinations of the four lipids forming a liposomal transfer vehicle.

168. I was asked to briefly comment on the other claims of the Opposed Application, grouping them when I felt that to be appropriate. I see there are 43 claims in total.

169. Claim 2 appears to be a variation on claim 1. As with claim 1, claim 2 is saying that any mRNA encoding a protein or enzyme is encapsulated into a classic cationic liposome formed from all molar ratio values of any cationic lipid between 20-70%; any non-cationic lipid; any PEG-modified lipid; and one or more cholesterol-based lipids. This in some respects is broader than claim 1, as now the only lipid restricted by molar amount is the cationic lipid, the other three components can essentially be present in any relative amounts. I'm not sure exactly what lipids are intended to be included by the term "cholesterol-based lipid". The term clearly doesn't include cholesterol itself as it is only to be "cholesterol-based". In checking the Opposed Application I noted this is defined on page 22 as being cholesterol-based cationic lipids such as DC-Chol which I mentioned I have great familiarity with. This can be in combination with other cationic or non-cationic lipids. I therefore consider this term in claim 2 to mean a molecule other than cholesterol but which is cholesterol 'like' in some manner with cationic examples seeming to be preferred. Perhaps this claim was intended to cover the formulation of Example 7 with the ICE lipid although that would then include both the cationic lipid and the cholesterol-based lipid which seems confusing. If not then I can't see any Example which uses a separate cationic lipid along with a cholesterol-based lipid, a PEG-modified lipid and DSPC or DOPE. In any event, this claim seems to allow for almost any amount of the PEG-modified lipid along with any relative amount of DSPC or DOPE and, any amount of the cholesterol-based lipid, separate to the cationic lipid which is itself present within a very broad range. Claim 2 does require that the non-cationic lipid is either DSPC or DOPE. I note that the only non-cationic lipids exemplified in the Opposed Application as part of a liposomal formulation are these very commonly known and used DSPC and DOPE. I see that claims 4 and 5 then claim each of these non-cationic lipids separately. Claim 18 falls back onto claim 2 and defines the liposome formulation in terms of a specific molar ratio for the non-cationic lipid, which is the same molar ratio as defined in claim 1.

170. Claim 3 limits the protein encoded by the mRNA to any enzyme. Claims 16 and 17 further define the nature of the enzyme to focus more around what I understand to be the aim of the invention, i.e. specifically useful for the treatment of urea cycle metabolic disorders, such as OTC.

171. Claims 6 and 15 define the liposome formulation in terms of a specific PEG-modified lipid and the molar ratio (for claim 2), which is the same molar ratio as defined in claim 1. The specific PEG-modified lipid being DMG-PEG-2000, which is the PEG-modified lipid used in Example 7 and briefly mentioned as a component of the formulation disclosed in Example 8 of the Opposed Application. Again, this is a very commonly known and used PEG-modified lipid.

172. Claims 7 and 10 to 14 define specific features of the mRNA component, including features of the poly A tail and 5' cap structure, presence of a 5' UTR and/or 3' UTR, modified or unmodified mRNA, etc. I didn't see any discussion on these features as part of the Examples or anything in the Opposed Application which would suggest these features had been invented and so I understand these to be limitations brought in from knowledge or prior art available at the relevant date. This is consistent with my own understanding and knowledge regarding these features being well-known prior to the relevant date.

173. Claims 8 and 9 define the size of the liposome. As I have previously discussed, the ideal or typical lipid particle diameter for use in the human body is about 100 nm or in some instances less. In any case, my understanding of these claims is that they add features which were already known in the art and aren't considered within the Opposed Application to be new or part of the 'invention' themselves.

174. Claims 19 to 21 defines the liposome formulation in terms of an additional nucleic acid, being any other RNA that is a non-coding RNA. I didn't see any Examples of the delivery of more than one kind of mRNA within the Opposed Application.

175. Claims 22, 23 and 24 appear to define three slightly different methods of delivery of mRNA, comprising administering to a subject in need of delivery a composition as defined by claims 1 to 21. For example, claim 22 appears to be directed to systemically administering the composition such that the composition results in prolonged stable expression of the protein encoded by the mRNA in the liver. Claim 23 appears to be directed to administering the composition such that the composition results in systemic expression of the protein or peptide encoded by the mRNA. I didn't see any guidance in the Opposed Application as to how you would tailor the formulation to achieve one or the other of these outcomes. Claim 24 appears to be directed to administering the composition such that the composition results in expression of the protein or peptide encoded by the mRNA in the liver, but specifically wherein the protein or peptide is an enzyme.

176. These claims are followed by claims 25, 26 and 27, which appear be corresponding uses of the compositions in claims 1 to 21. It was then explained to me that claims 25 to 27 have a focus on the process of manufacture of the medicament, being the composition of claims 1 to 21, but limited in their scope by the purpose of that medicament which, in these three claims, is prolonged stable expression, systemic expression, and modulating expression of the protein or the peptide encoded by the mRNA, respectively. I do not believe that administering a specific composition resulting in a

prolonged stable expression has been shown in the Examples. Otherwise, my comments for claims 22, 23 and 24 are also relevant here.

177. Claims 28 to 43 fall back onto any one of the method or use claims 22 to 27 and appear to set out additional features of the treatment method and delivery approach. Of particular note is claim 33. This claim falls back onto any one of the method or use claims and defines that the administration of the *composition confers immunity to a subject*. This claim is inconsistent with my views on what is described by the Opposed Application and what the focus of the invention is all about. Claim 33 is suggesting that a protective immune response is required which will be specific to the particular antigen produced and to result in immunological memory against that antigen and so this appears to be describing a vaccine formulation for the purposes of conferring immunity. However, the Opposed Application does not provide any guidance relating to providing immunity to infectious diseases. As I have discussed in paragraph 146, the Opposed Application only mentions passive immunization and is merely suggesting that the delivery of mRNA may encode a functional antibody.

178. Following my consideration of the Opposed Application I was provided with copies of: (i) Gómez-Aguado, I., et al. "Nanomedicines to Deliver mRNA: State of the Art and Future Perspectives". *Nanomaterials*. Feb 2020; 10: 364, which forms **Exhibit LH-26** to this declaration; (ii) Buschmann, M.D., et al. "Nanomaterial Delivery Systems for mRNA Vaccines". *Vaccines*. Jan 2021; 9: 65, which forms **Exhibit LH-27** to this declaration; and (iii) WO2012/170930A1 titled Lipid Nanoparticle Compositions and Methods for mRNA Delivery, which forms **Exhibit LH-28** to this declaration. I note the Gómez-Aguado and Buschmann articles were published well after the relevant date in 2020 and 2021 respectively. I understand WO2012/170930A1 is a Shire Human Genetic Therapies, Inc. patent publication filed after the Opposed Application priority date which relates to a similar technology to that described in the Opposed Application. I was asked to particularly consider section 4.2 from the Gómez-Aguado article and pages 5 and 6 of the Buschmann article, but in the context of the articles as a whole. I was asked to particularly consider the Examples of WO2012/170930A1 and also to give my views on the data presented in tables 1 and 2.

179. Addressing the Gómez-Aguado article first, I note that section 4.2 focuses on protein replacement therapy using mRNA, as is the focus of the Opposed Application. I understand from this section that a lot of research is ongoing to try to address various diseases of protein deficiency, which is also my understanding. Delivery approaches for the liver, lungs and heart are described as the most common for accessibility reasons. Table 3 of section 4.2 discusses some clinical trials on protein replacement therapies and mentions an OTC deficiency trial which, as I have discussed, is clearly the

focus of the Opposed Application. The end of section 4.2 discusses this trial, which was run by Translate Bio, and states that it was discontinued as the preclinical work did not support the required pharmacokinetics and safety profile. It then states that "investors" related those poor results to the non-optimal features of the "first-generation LNPs designed to target the liver". I was informed that related Australian patents to the Opposed Application have been assigned to Translate Bio and that they may have acquired the technology. I can't comment on the authors' view on this clinical trial failure, or what the investors referred to meant by first-generation LNP technology, although the term lipid nanoparticle (LNP) is often used very loosely in the media and non-scientific or patent literature, but the outcome certainly raises concerns. I am informed the trial had an estimated start date in 2019 and so it is firstly surprising that it took so long to start this early preclinical work given the relevant date of the Opposed Application 10 years earlier. Of course, sometimes there can be commercial reasons for slow progression of a technology into the clinic. More concerning is that the lipid transfer vehicle was not appropriate for human use. I have already commented that I did not believe the data presented in the Opposed Application actually indicated any real likelihood of success in treating a disease of protein deficiency and this failed trial would seem to show that ultimately the approach could not be made to work. Without knowing the details of the trial I can't really comment in any more detail.

180. The Buschmann article is a good summary of mRNA vaccine delivery in the context of its accelerated development during the COVID-19 pandemic. It talks about the many factors involved in developing a successful vaccine and offers some history on the development of the technology. Page 5 discusses the development of the lipid nanoparticle technology which was successfully developed and used in the Moderna and Pfizer/BioNTech vaccines. I was asked about the development of the ionizable cationic lipid as mentioned on pages 5 and 6 of Buschmann. I have already mentioned the importance of this development above and that the D-Lin series was particularly important at the time. Buschmann describes the development of the earlier DLin-DMA lipid to DLin-MC3-DMA which is a commercially successful ionizable lipid as having required more than 300 lipids to be screened and then this placed within thousands of different formulations to optimize the approach. I have already mentioned some of the factors which can affect the properties of a cationic or ionizable cationic lipid and certainly relatively small seeming structural changes can significantly affect pKa and also the conformation changes the lipid undergoes for endosomal release and so I don't find this comment surprising. A lot of large companies have invested huge amounts of resources to developing lipid delivery technologies which will work well in various indication and clearly, in recent times, including lipid delivery of mRNA vaccines. As I've indicated, it is unrealistic to expect any cationic lipid to work in such delivery formulations for any application.

181. Finally, having reviewed WO2012/170930A1, I find it a little surprising that much of the discussion seems to relate to the same technology and the same approach as described in the Opposed Application. The Examples seem to describe a number of four lipid component formulations, such as DODAP/DOPE/Cholesterol/DMG-PEG2K, which are broadly equivalent (with variation in relative amounts) to those in the Opposed Application. Turning to tables 1 and 2, and the underlying experiments, I was asked about the outcome they demonstrate. It seems like the experiments weren't particularly successful. While I understand the comment in WO2012/170930A1, above table 1, relating to the observed increase in EPO protein, it is clear that this did not translate at all into an increase in hematocrit, which indicates a therapeutic outcome couldn't be achieved. I was asked about the ICE formulation particularly, and I see that this is the same formulation as is described and tested in Examples 6 and 7 of the Opposed Application. While this is a different mRNA to that tested for delivery in the Opposed Application it does strongly suggest that the approach will not work for all forms of mRNA or, to put it another way, will not be capable of treating all diseases of protein deficiency.

G. THE OPPOSED APPLICATION IN VIEW OF THE PRIOR ART

182. I understand that, in order to be patentable, what is defined by the claims of a patent application needs to be new and inventive over what was previously commonly known and what was disclosed and taught in the prior art. I was asked to consider the claims of the Opposed Application in view of what was commonly known relating to lipid formulations for delivery of mRNA therapeutics immediately prior to 01 December 2009 and the prior art, and consider whether what is claimed is different, unexpected, surprising or inventive, or not. Unless stated otherwise, my comments are directed as of that 01 December 2009 date.

The Opposed Application and Document D1

183. Overall, I consider that the claims of the Opposed Application do not present anything new over what is described in **D1**.

184. Taking claim 1 first, which is broadly directed to a composition comprising a liposomal formation encapsulating an mRNA in which the mRNA encodes a suitable protein or peptide, it seems to me that the components of the liposomal formulation, are either very standard components of nucleic acid-lipid formulations, or are disclosed by **D1**. I consider that the molar ratio ranges of the lipid components in claim 1 encompass molar ratios taught by **D1** as being employed for liposomal

formulations. I also consider the size of the liposome in claim 1 encompasses liposome sizes taught by **D1**. See for example paragraph [0052] of **D1** which highlights that the nucleic acid-lipid particles typically have mean diameter of about 50 nm to about 150 nm.

185. The components of the liposomal formulation, i.e. (**b**)(**i**) – (**b**)(**iv**) as I set out at paragraph 155, are very clearly disclosed by **D1**. As I have set out above, **D1** demonstrates that nucleic acid-lipid particles (SPLPs or SNALPs) and other lipid-based carrier systems (e.g. a liposome) containing cationic lipids can encapsulate a number of nucleic acids, such as mRNA. These various types of lipid-based particles, such as liposomes, are described extensively and formulated within the Examples in what I consider to have the typical four lipid components using standard relative molar ratios.

186. The document describes SNALP preparation with a lipid composition of DSPC:Chol:PEG-C-DMA:Cationic Lipid at a ratio of 20:48:2:30 molar percent (see [0196]), within the claimed ranges. Example formulations of **D1** describe the formulation of a number of lipid particles, including members of the DLin series. See for example a table provided at [0224] of **D1** using the cationic lipid, DLin-DMA, in combination with DSPC, Chol, and PEG-C-DMA at a ratio of 30:20:48:2 molar percent. I regard that as a clear description of the liposomal formulation approach claimed by the Opposed Application.

187. **D1** includes significant discussion around all aspects of the nucleic acid encoding a polypeptide (paragraphs [0082] to [0108]). The document specifically mentions nucleic acids encoding therapeutic (gene) products of interest (paragraphs [0082] and [0083]), for example, genes associated with metabolic diseases and disorders (paragraph [0085]). I understand this to mean that proteins or peptides can be encoded for by mRNA and a disclosure that a liposomal formulation as used in the Examples of the Opposed Application will formulate an mRNA encoding a suitable protein or peptide.

188. Essentially, by following the core teaching of **D1**, and in view of what was commonly known, I believe a skilled person looking to make a composition, such as a mRNA encapsulated in a liposomal formulation, would have made a liposome composition falling within claim 1.

189. Teams involved in making lipid delivery formulations encapsulating therapeutic nucleic acids at the relevant time would already have been using the liposomal formulation containing the four required lipid classes, which had been demonstrated as successful by a number of groups. For these reasons I regard compositions falling within claim 1 as entirely self-evident and obvious to make

based on what **D1** describes. **D1** and other documents being specifically directed to mRNA-lipid particles and describing relevant cationic liposome delivery vehicles within the ranges of claim 1 of the Opposed Application and identical or extremely similar to those exemplified in the Opposed Application.

190. The same applies to claim 2, as I have mentioned before the main feature that is different in claim 2 when compared to the features of claim 1, is that the non-cationic lipid is defined as being DSPC or DOPE. I do not think that limiting the non-cationic lipid those that are well-known in the field, changes anything. Especially given at least one of these non-cationic lipids, DSPC, is specifically mentioned in the Examples of **D1**. There is nothing new or inventive associated with claim 2 in my opinion. For the same reasons, I do not believe there is anything new or inventive in claim 4 or claim 5. As just mentioned, the non-cationic lipids, DSPC and DOPE, as defined in claims 4 and 5, respectively, are well-known in the field.

191. Claims 3, 16 and 17 limit the protein encoded by the mRNA to any enzyme and further define the nature of the enzyme. Whilst **D1** does not disclose the specific enzymes defined in claim 16 that are specifically useful for the treatment of urea cycle metabolic disorders, such as OTC, **D1** does extensively disclose a very wide range of genes associated with metabolic diseases and disorders and specifically describes disorders in which the liver is a target in addition to liver diseases and disorders (see [0087] of **D1**).

192. Claims 6 and 15 do not appear to add anything new in that **D1** uses PEG-modified lipid in its liposomal formulations. **D1** specifically exemplifies use of PEG-2000-C-DMA and the molar ratio within the range of claim 15 of the Opposed Application (see [0196] and [0224, for example).

193. Claims 7 and 10 to 14 focus on the features of the mRNA. **D1** has disclosure on nucleic acid modifications at [0046] in **D1**. I have already mentioned the benefits of using modified mRNA were well-known.

194. In my view, and given **D1** addresses the development and use of nucleic acid-based liposome formulations within the scope of the claims of the Opposed Application, as I have already discussed, claims 1 to 43 addressing delivery of a nucleic acid to encode a protein or a peptide are also necessarily disclosed in these documents and so are not new.

195. Consistent with my review and comments, I consider that claims 1-43 of the Opposed Application are not new or do not provide an inventive step in view of what was commonly known, and what was disclosed and taught in **D1**. I consider it would have been obvious for a skilled person in the

field, in view of this document addressing as it does nucleic acids encapsulated within liposomal formulations, to have arrived at the composition falling within those claimed in the Opposed Application.

196. I believe the teaching in the Opposed Application and in **D1** is largely the same as a matter of practical outcome. The skilled person or team would not need to locate or add any missing information. Instead, they would be able to find in a straightforward manner all the features within claims 1 to 43 and make a composition comprising an mRNA encoding a protein or peptide, wherein the mRNA is encapsulated within a liposome, within those claims utilising common general knowledge and routine skill.

The Opposed Application and Document D2

197. I also consider that the claims of the Opposed Application do not present anything new over what is described in **D2**.

198. As I have discussed before, this document highlights the delivery of encapsulated nucleic acids in lipid particles which are said to allow effective knock-down of specific target proteins at relatively low dosage and toxicity. Specifically, I consider that the document discloses the use of a cationic liposome having those four lipid components and even discloses a formulation with DLin-K-DMA in combination with a neutral lipid selected from DSPC, POPC, DOPE, and SM, along with cholesterol, and PEG-S-DMG, PEG-C-DOMG or PEG-DMA, in a molar ratio of about 20-60% DLin-K-DMA:5-25% neutral lipid:25-55% Chol:0.5-15% PEG-S-DMG, PEG-C-DOMG or PEG-DMA, which is within the ranges of each of those components in claim 1 of the Opposed Application. There is considerable discussion in **D2** around the type of nucleic acids (see pages 36 to 53) and the possible modification of the nucleic acid (see pages 53 to 64). Page 60 onwards in **D2** describes the encapsulation of the therapeutic agent, such as a nucleic acid, into their lipid particle or liposome formulations. Particle size is mentioned as having a diameter of from about 70 to about 200 nm. Therefore, claims 7 to 14 and 19 to 21 appear to simply cover various features of the mRNA and further defined sizes for the lipid particles which don't appear to be things that the Opposed Application claims to have invented.

199. It seems to me that **D2** discloses each component of claim 1 of the Opposed Application in that delivery of mRNA, which can encode for proteins and peptides, is disclosed as encapsulated in a liposomal formulation prepared from the four lipid components of claim 1 within the ranges in that claim. I can't see anything additional in claim 1 that is not found in **D2**.

200. In my view, a skilled person looking to provide a formulation for delivery of a composition comprising mRNA encapsulated in a liposome prior to 01 December 2009 would, in view of their common general knowledge of such formulations, and on reading **D2**, have understood that a nucleic acid-lipid particle, as taught by this document, would be appropriate for formulating mRNA encoding for a protein or peptide. The skilled person or team would not need to locate or add any missing information and the essential features of claim 1 would present themselves, as a matter of course, by utilising common general knowledge and routine skill and judgment.

201. My views above also apply to claim 2. **D2** discloses the use of neutral lipids, including DSPC and DOPE, in the formulations I have discussed in paragraph 63 and so nothing in claim 2 appears to me to be new. This equally applies to claims 4 and 5 in which the non-cationic lipids, DSPC and DOPE are separately claimed. Similarly, **D2** discloses the use of PEG-modified lipids, including PEG-DMG also in the formulation I have discussed in paragraph 63 and so again nothing in claim 6 appears to me to be new either. In terms of claim 2, the features of claims 15 and 18, i.e. limiting the molar ratios of the PEG-modified lipid and the non-cationic lipid to those ranges that are well-known in the field, changes nothing, and are specifically disclosed in **D2**.

202. **D2** discloses that protein encoded by the mRNA can be an enzyme (see page 76) which would seem to disclose the subject of at least claim 3. As I have discussed above at paragraphs 67 and 68, the delivery of mRNA sequences that encode for therapeutically useful polypeptides is discussed for the purpose of providing gene therapy for genetic diseases by supplying deficient or absent gene products (see page 76). In fact, **D2** provides disclosure of a cationic liposome delivering an mRNA that encodes an enzyme for the purpose of gene therapy on page 76. As I understand it, all of this disclosure is, if not already explicitly in **D2**, is incorporated by reference into that document.

203. The disclosure in **D2** around administering mRNA encoding for a protein relating to genetic diseases, within the cationic liposome are clearly directed to production of proteins and expression of genes and so discloses claims 22 to 27 in the manner I have already described.

204. In view of my above review and comments, I consider that claims 1 to 43 of the Opposed Application are not different from or would be obvious in view of what was commonly known and what was disclosed and taught in **D2**. The Examples of **D2** start on page 85 and for several pages present the synthetic schemes for the preparation of the various lipids, including cationic and PEG lipids. In Example 20, they formulate and characterise siRNA liposomal formulations using various cationic lipids (40%): DSPC (10%): cholesterol (40%): PEG-C-DOMG (10%). Although, this example is directed to the encapsulation of siRNA, I consider it equally applicable to other nucleic acids, such as the larger DNA and mRNA, which had been demonstrated as successful by a number of groups.

Example 21 on pages 138 to 142 then described *in vivo* regulation of gene expression using their liposomal formulations, which is a similar approach to the Examples of the Opposed Application.

205. Given the above, I do not see that there is anything different or inventive in what is claimed in the claims of the Opposed Application over **D2**. In my view, a skilled addressee reproducing what is described in **D2** would have made a composition comprising mRNA encoding a protein that is encapsulated within a liposome within the claims of the Opposed Application.

The Opposed Application and Document D3

206. I also consider that the claims of the Opposed Application do not present anything new over what is described in **D3**.

207. As I have previously discussed, **D3** largely focuses on a mixing apparatus and describes processes using this for preparing lipid formulations, e.g. stabilized plasmid-lipid particles (SPLP), and discusses mixing approaches to achieve the mixing of a nucleic acid and lipid components which was already known to result in liposomal formation. **D3** describes the approach of using lipid formulations with four lipid component classes to formulate delivery vehicles for nucleic acids which are within the ranges of claim 1 of the Opposed Application. This also applies to the subject matter of claim 2. Similarly, **D3** discloses various cationic lipids, and other non-cationic lipids, including DSPC and DOPE, which are the subject of claims 4 and 5, respectively. For example, at [47] **D3** describes a DSPC:cholesterol:PEG-DSG:DODMA SPLP formulation prepared at respective molar ratios of 20:45:10:25. For claims 8 and 9, **D3** discloses a preferred particle size less than 150 nm (e.g. about 100 nm) and so nothing in these claims appears to me to be new.

208. Messenger RNA is clearly described as one of the nucleic acids which can be delivered using the approach of **D3** and it even further discloses that the nucleic acid may encode for various proteins, which I understood to refer to mRNA. This appears to be the same approach as is claimed in claims 22 to 27 of the Opposed Application. Certain of the other claims describe specific features of the mRNA and enzymes which are not found in **D3** but appear to have been commonly known in the field immediately prior to 01 December 2009.

209. While the Examples of **D3** focus on DNA formulation and the encapsulation of other molecules, the discussion is around many types of nucleic acids and pDNA, mRNA and siRNA are mentioned. That is, **D3** appears to indicate that their approach can be used irrespective of the size, within reason, of the therapeutic being delivered. The teaching of the document is really around how to form lipid particles, whatever nucleic acid is encapsulated.

210. Given the above, I do not see that there is anything different or inventive in what is claimed in the claims of the Opposed Application over **D3**. In my view, a skilled addressee reproducing what is described in **D3** would have made compositions comprising mRNA encoding for a protein encapsulated within a liposome within the claims of the Opposed Application.

The Opposed Application and Document D4

211. I also consider that the claims of the Opposed Application do not present anything new over what is described in **D4**.

212. **D4** discloses encapsulating antisense oligonucleotides with a liposomal formulation including DSPC, cholesterol, DODAP, and PEG-CerC14 in a 25:45:20:10 ratio. This is exactly the cationic lipid, cholesterol and neutral lipid used in many of the formulations exemplified in the Opposed Application and all these lipid components are within the ranges of claim 1. The only difference being in the nature of the PEG-lipid which, in most of the Opposed Application's Examples, is the closely related C8-PEG-2000 ceramide. I believe that this would have shown a skilled team looking to produce a mRNA encoding a protein encapsulated within a liposome at 01 December 2009 that this sort of formulation could be used. This also applies to the subject matter of claim 2. Similarly, **D4**, at page 19, discloses various preferable neutral lipids, including DSPC and DOPE, which is the subject of claims 4 and 5, respectively. For claims 8 and 9, **D4** discloses preferred particle sizes of about 90 nm to about 130 nm and so nothing in the claims appears to me to be new. I didn't see that the Opposed Application attributed any special significance or benefit to the liposomal formulations that were specifically described in the Examples.

213. **D4** discloses that protein encoded by the mRNA can be an enzyme (see page 31) which would seem to disclose the subject of at least claim 3. As I have discussed above at paragraph 60, the delivery of mRNA sequences that encode for therapeutically useful polypeptides is discussed for the purpose of providing gene therapy for genetic diseases by supplying deficient or absent gene products (see page 31). In fact, **D4** provides disclosure of a cationic liposome delivering an mRNA that encodes an enzyme for the purpose of gene therapy. As I understand it, all of this disclosure is, if not already explicitly in **D4**, is incorporated by reference into that document.

214. The disclosure in **D4** around administering mRNA encoding for a protein relating to genetic diseases, within the cationic liposome are clearly directed to production of proteins and expression of genes and so discloses claims 22 to 27 in the manner I have already described.

215. It seems to me that the knowledge that **D4** supplies around the liposomal formulation, nucleic acid encapsulation and the production of nucleic acid encapsulated within liposomes would have lead a

skilled team seeking to produce a composition as claims by the Opposed Application, immediately prior to 01 December 2009.

216. Given the above, I do not see that there is anything inventive in what is claimed in the Opposed Application over **D4**.

The Opposed Application and Document D5

217. I also consider that the claims of the Opposed Application do not present anything new over what is described in **D5**.

218. **D5** only demonstrates the encapsulation of siRNA in the Examples, however it clearly states other forms of nucleic acid can be formulated and it specifically mentions mRNA. Delivery of mRNA that codes for therapeutically useful polypeptides is also mentioned in the context of gene therapy. The disclosure in **D5** around administering mRNA encoding for a protein relating to genetic diseases, within the cationic liposome, are clearly directed to production of proteins and expression of genes and so discloses claims 22 to 27 in the manner I have already described.

219. **D5** discloses encapsulating nucleic acids with a liposomal formulation comprising the four lipid components of claim 1 within the ranges in that claim. I can't see anything additional in claim 1 that is not found in **D5**. Specifically, I consider that the document discloses the use of a cationic liposome having those four lipid components and even discloses a formulation of DLin-DMA:DSPC:cholesterol:PEG-DMG of 40:10:40:10 which is within the ranges of each of those components in claim 1 of the Opposed Application. This is exactly the cationic lipid, cholesterol and neutral lipid used in various of the formulations exemplified in the Opposed Application and all these lipid components are within the ranges of claim 1. I believe that this would have shown a skilled team looking to produce a mRNA encoding a protein encapsulated within a liposome at 01 December 2009 that this sort of formulation could be used. This also applies to the subject matter of claim 2. Similarly, **D5** discloses various preferable neutral lipids, including DSPC and DOPE, which is the subject of claims 4 and 5, respectively.

220. Given the above, I do not see that there is anything different or inventive in what is claimed in the Opposed Application over the **D5**. In my view, a skilled addressee reproducing what is described in **D5** would have made liposomes encapsulating mRNA according to the claims of the Opposed Application.

The Opposed Application and Document D12

221. The document is an early publication showing the efficiency of a range of commercially available cationic liposomes in mRNA transfection. As I have already discussed, **D12** discusses the advantages of delivering mRNA over DNA in terms of the safety benefits in the context of gene therapy.

222. While **D12** describes the preparation of cationic liposome-mRNA complexes, the lipids used are all commercially available cationic liposomes. The mRNA encapsulated is luciferase mRNA, in both capped and uncapped forms. The results indicate that the levels of gene expression achievable by liposome-mRNA-mediated transfection were comparable to the levels observed for liposome-DNA complexes. I read this as showing cationic liposome-mRNA complexes with improved profiles related to efficiency and toxicity as compared to liposome-DNA complexes. It also indicates that the larger DNA as well as mRNA can be delivered by the same approach, that is, liposomes can deliver each in addition to siRNA as exemplified in documents I have already discussed.

223. The principle of the use of cationic liposome-mRNA complexes for gene therapy has been known for a long time. The focus of **D12** is clearly on demonstrating, as a proof of concept of the approach, that cationic liposomes can be used to deliver mRNA showing results that are comparable to liposome-DNA complexes.

224. I believe that **D12**, and many other documents which were already published on cationic liposome-mRNA complexes, and the common general knowledge immediately prior to 01 December 2009 made it clear that mRNA was highly desirable for use in gene therapy and protein replacement and that mRNA could be formulated with a cationic liposome. As I have already discussed, it was commonly known at the relevant date that the same liposome systems could and were used for delivering various nucleic acids, including pDNA, mRNA and siRNA and that these liposome systems included two-, three-, and four-component liposomes could interchangeably deliver various nucleic acids. That much is demonstrated in the documents I have already discussed and so the size of the nucleic acid was not a barrier to liposomal delivery.

225. I do not see anything surprising in the Opposed Application's description or the Examples that indicates they discovered anything new. The lipid classes, and in some instances exactly the same lipids at exactly the same molar ratios as in the Examples of the Opposed Application, have already been published in the documents I have discussed. The only difference between Examples of the respective documents is often the nature of the nucleic acid itself but, as I have just mentioned, the same liposome systems could and had been used for delivering various nucleic acids. With the possible exception of the lipid formulation described in Example 6 and Example 7 of the Opposed

Application and the ICE lipid used there, I can't see that there is any invention in simply using the same lipid formulation with a different nucleic acid. Regarding Examples 6 and 7 of the Opposed Application, I note that three-component lipid formulation does not appear to be the subject of the claims.

The Opposed Application and Document D13

226. The central teaching of **D13** is that DNA and oligonucleotides can be entrapped within liposomes, not only showing the versatility of these four-component systems but also validating their utility for liposomal delivery of therapeutic nucleic acids.

227. The lipid components of choice in this study were DODAP/DSPC/Chol/PEG-CerC14 at molar ratios of 25:20:45:10, respectively, which is within the ranges of claim 1 and claim 2 of the Opposed Application. Again, I consider this to be a fairly typical four component liposome within standard ranges for the time this document was published. In fact the lipid components are the same lipid components used in many of the Examples of the Opposed Application with the exception of the slight difference between the PEG-CerC14 described here and the PEG-CerC8 used in the Opposed Application. In terms of the size, the document describes the lipid particle having a particle diameter of between 70 nm to 120 nm, which is also within the ranges of the subject claims. **D13** also discusses the influence of the PEG-lipid on the size of the lipid particle, which is also something I have discussed previously and which was well-known in the field.

228. Certain of the other claims describe specific proteins, features of the mRNA etc. which are not found in **D13** but appear to have been commonly known in the field immediately prior to 01 December 2009. The results presented in **D13** show that they successfully encapsulated a number of oligonucleotides as well as plasmid DNA at high entrapment efficiencies (see Figure 9). I consider that D13 would teach a person of skill in the art that the same formulation approach they use for antisense and DNA would be suitable for mRNA as I would, immediately prior to 01 December 2009, have expected that to be the case.

229. Given the above, I do not see that there is anything different or inventive in what is claimed in the claims of the Opposed Application over **D13**. In my view, a skilled addressee reproducing what is described in **D13** would have made compositions within the claims of the Opposed Application.

The Opposed Application and Document D14

230. This document has a focus on the delivery of mRNA encapsulated within a specific three-component liposome, cholesterol/dipalmitoylphosphatidylcholine/phosphatidylserine, and

specifically discusses mRNA encoding an influenza virus nucleoprotein. The central teaching of this study is to show that mRNA can be encapsulated within liposomes and in this very early study administration of mRNA *in vivo* demonstrates virus specific induction of lymphocytes, highlighting that mRNA delivery systems can be useful for immunization.

231. I believe that **D14**, and many other documents which were already published on delivery of mRNA encapsulated within liposomes, and the common general knowledge immediately prior to 01 December 2009 made it clear that mRNA delivery was highly desirable. The discussion of mRNA, encoding proteins, that are encapsulated within typical well-known four-component lipid formulations within the Opposed Application is of advancements which were already known. It is my view that the claims of the Opposed Application are not inventive on this basis.

The Opposed Application and Document D15

232. As I have already discussed, this document is a book chapter out of the "Handbook of Experimental Pharmacology" and entitled "Vaccination with Messenger RNA (mRNA)". I believe a skilled person looking to provide a lipid formulation for delivery of a mRNA would, immediately prior to 01 December 2009, in view of their knowledge of such formulations, and on reading **D15**, have understood that mRNA-based formulations and delivery methods, as taught by this document, would be appropriate, and indeed were recommended, for formulating mRNA encoding for a protein, particularly in the context of inducing an immune response.

233. This document sets out that mRNA, modified and unmodified, can be formulated and delivered to induce an immune response. It is clear that **D15** describes vaccine strategies that show that mRNA can be administered to mice in order to achieve an immune response. One viable delivery method being encapsulation of mRNA within cationic liposomes.

234. In my view, a skilled person looking to provide a delivery system for mRNA would, in view of their general knowledge of lipid formulations, and on reading **D15**, have used the four-component lipid formulation approach. By doing that, and by using commonly employed and typical molar ratios, they would have made a composition falling within claim 1 of the Opposed Application. The skilled person or team would not need to locate or add any missing information and the essential features of claim 1 would present themselves, as a matter of course, by utilising their general knowledge and routine skill and judgment.

235. Given the above, I do not see that there is anything different or inventive in what is claimed in the Opposed Application over the **D15**. In my view, a skilled addressee reproducing what is described in **D15** would have made a composition according to the claims of the Opposed Application.

The Opposed Application and Document D16

236. The discussion in this document provides another example showing that mRNA can be encapsulated within cationic liposomes and demonstrates feasibility of vaccinating with nonreplicating IVT mRNA for cancer immunotherapy. Again, this highlights that mRNA delivery systems can be useful for inducing an immune response and that liposomes that have been successfully used for DNA were expected to work for mRNA or were demonstrated to work for mRNA. This document shows mRNA liposome encapsulation, IV administration and successful delivery and expression with DOTAP:DOPE liposomes which are a cationic lipid and non-cationic lipid from the Examples of the Opposed Application.

237. I believe that **D16**, and many other documents which were already published on delivery of mRNA encapsulated within liposomes, and the common knowledge in the field immediately prior to 01 December 2009 made it clear that mRNA delivery was highly desirable and feasible with the use of liposomes. The discussion of mRNA for encoding proteins that are encapsulated within typical well-known four-component lipid formulations within the Opposed Application is of advancements which were already known. It is simply the encapsulation of mRNA in yet another liposome form which was itself well-known. It is my view that the claims of the Opposed Application are not inventive on this basis.

238. Given the above, I do not see that there is anything inventive in what is claimed in the Opposed Application over the **D16**. In my view, a skilled addressee reproducing what is described in **D16** and in light of other documents I have discussed above would have added cholesterol and a PEG-lipid to improve circulation time and stability of the liposomes and have made a composition according to the claims of the Opposed Application.

The Opposed Application and Document D17

239. As I have previously discussed, the teaching of **D17** is really around the evolution of various lipid particles, whatever nucleic acid is encapsulated, including both DNA and RNA, ranging from small fragments to larger plasmids. The lipids used to form the lipid particles appear to be standard selections from those known at that time, ranging from the early two-component lipid systems to the more typical three- and four component lipid systems.

240. While **D17** specifically discusses the emergence of siRNA and its delivery, it also addresses the development of SPLP and SALP liposomes comprising DSPC (a neutral lipid), Chol (a sterol), DODMA (a cationic lipid), and a PEG-lipid (either PEG-S-DSG for formation of SPLPs or PEG-Cer for the formation of SALPs), i.e. a liposome formed of the four lipid components of claim 1 and claim 2. **D17**

also discloses that particle size is another important parameter in nucleic acid delivery and mentions that lipid particles have the *greatest utility* when the particles are formed around 100 nm in diameter, as is the case in the Opposed Application. The discussion on siRNA and DNA indicates that nucleic acid of varying sizes could and had been delivered by various lipid transfer vehicles.

241. The skilled person or team would not need to locate or add any missing information and the essential features of claim 1 would present themselves, as a matter of course, by utilising common knowledge in the field and routine skill and judgment.

242. Given the above, I do not see that there is anything different or inventive in what is claimed in the Opposed Application over the **D17**. In my view, a skilled addressee reproducing what is described in **D17** would have made a composition according to the claims of the Opposed Application.

The Opposed Application and Document D18

243. This document describes the delivery of mRNA encapsulated within a specific two-component liposome, DOTMA/DOPE. The same system has previously been shown to transfect DNA into cells. As I have already discussed, it was common general knowledge at the relevant date that the same liposome systems could and were used for delivering various nucleic acids, including pDNA, mRNA and siRNA and that these liposome systems included two-, three-, and four-component liposomes which could interchangeably deliver various nucleic acids.

244. **D18** also discusses the benefits of some modification to the mRNA, and showed that at least capping was important for stability and translation efficiency of the luciferase mRNA. The results indicating that the m^7G-capped mRNA binds more efficiently to the ribosomes.

245. This document concludes by suggesting that this early work raises the possibility for an alternative nucleic acid, i.e. mRNA, that can be delivered in cationic liposomal formulations and be used for application to gene therapy.

246. I believe that **D18**, and many other documents which were already published on delivery of mRNA encapsulated within liposomes, and the common general knowledge immediately prior to 01 December 2009 made it clear that delivery and use of mRNA was highly desirable. The discussion of mRNA for encoding proteins that are encapsulated within typical well-known four-component lipid formulations within the Opposed Application is of advancements which were already known. It is my view that the claims of the Opposed Application are not inventive on this basis.

The Opposed Application and Document D19

247. This document discloses the delivery of a nucleic acid within a liposome to induce an immune response (vaccination) or for gene therapy. For example, one of the focuses of **D19** is the treatment of muscular dystrophy and the document clearly discloses that mRNA encoding for a protein (e.g. dystrophin) can be delivered for gene therapy (e.g. see last paragraph on page 7).

248. While **D19** only shows the encapsulation of mRNA in simple lipid formulations such as complexes comprising cationic lipids (DOTAP and DOTMA) and non-cationic lipids (DOPE), I believe a skilled person looking to provide a lipid formulation for delivery of a mRNA would, immediately prior to 01 December 2009, in view of their common general knowledge of such formulations, and on reading **D19**, have understood that mRNA-based formulations and delivery methods, as taught by this document, would be appropriate for formulating mRNA encoding for a protein for delivery. Again, the discussion in this document validates that mRNA had been delivered by a variety of liposome types. There would have been no reason to feel that a different type, such as those four lipid component formulations of **D1**-**D5** and others discussed above, would not also be successful in delivering mRNA.

249. In my view, a skilled person looking to provide a delivery system for mRNA would, in view of their existing knowledge of lipid formulations such as those discussed in the prior art documents I have addressed above, and on reading **D19**, have used a four-component lipid formulation approach. By doing that, and by using commonly employed and typical molar ratios, they would have made a composition falling within claim 1. The skilled person or team would not need to locate or add any missing information and the essential features of claim 1 would present themselves, as a matter of course, by utilising common knowledge and routine skill and judgment.

250. Given the above, I do not see that there is anything different or inventive in what is claimed in the Opposed Application over the **D19**. In my view, a skilled addressee reproducing what is described in **D19** would have made a composition according to the claims of the Opposed Application.

Overall comments on the impact of the prior art

251. I consider the disclosure and teaching in all of the prior art documents I have referred to, to be highly relevant to the field, and I believe they would have been identified and considered as highly relevant by others skilled in the field. The documents, whether considered individually or together in any way, clearly disclose and teach using a liposomal formulation including well-known lipid classes within standard ranges, to provide an effective delivery vehicle for *any* nucleic acid. In particular, the prior art documents essentially provide analogous disclosure and teaching

concerning the delivery of nucleic acids in liposomal formulations. I consider that a skilled person looking to produce a composition comprising mRNA encapsulated in a liposome would have considered these documents individually, and also together.

252. As discussed above, the components, and some of their respective molar ratios, of the liposomal formulations covered by the Opposed Application are standard and well-known in the therapeutics delivery industry and were routinely used in the delivery of DNA and RNA therapeutics immediately prior to 01 December 2009. As I have already discussed, it was the common knowledge at the relevant date that the same liposome systems could and were used for delivering various nucleic acids, including pDNA, mRNA and siRNA and that these liposome systems included two-, three-, and four-component liposomes could interchangeably deliver various nucleic acids. When a large number of two and three lipid component liposomes had been shown to successfully encapsulate and deliver large and small nucleic acids, such as DNA, mRNA and siRNA, I don't see how it could be inventive to take a further known four lipid component vehicle and apply it to delivery of mRNA. I don't see that any hurdle was overcome in this work.

253. Based on the disclosure and teaching in any the above prior art documents, at the relevant time I, and I believe other skilled persons also, would have considered it a straightforward and routine exercise to provide a composition within the claims of the Opposed Application, and would have regarded it as self-evident that they would provide such a formulation using the standard methods and uses claimed also.

254. I have made all the inquiries that I believe are desirable and appropriate and no matters of significance that I regard as relevant have, to my knowledge, been withheld.

I make this declaration conscientiously believing the statements contained in this declaration to be true in every particular.

DECLARED at Chapel Hill, NC, USA on this 5th day of September 2023

(Place) (Day) (Month)

Signature of declarant _____

LEAF HUANG