endoplasmic reticular transport, and interfere with the membrane lipid raft function. *See, e.g.*, Blitterswijk. W., et al., *Biochim. Biophys. Acta.* **1831**, 663-674 (2013). Lipid rafts are specialized plasma membrane microdomains having concentrations of cholesterol and sphingomyelins which spatially organize signaling pathways and regulate cell proliferation and apoptosis. *See, e.g.*, van der Luit, A., et al., *Mol. Cancer Ther.* **6**, 2337-2345 (2007). Lipid rafts are more abundant in cancer cells relative to normal cells and have been proposed to serve as entry points for cytotoxic agents into the cells. *See, e.g.*, Li, Y., et al., *Am. J. Pathol.* **168**, 1107-1118 (2006).

[0006] In addition to potential anti-neoplastic properties, it has also been reported that ischemic chick embryonic tissue extract enriched to 30% 1-*O*-octadecyl-2-oleoyl-*sn*-glycero-3-phospho-(*N*-palmitoyl)ethanolamine shows significant anti-inflammatory and immunomodulatory effects. *See*, Vicenova, M. et al., *Complementary and Alternative Medicine* **14** 339 (2014). In *in vivo* studies of bacterially induced acute pneumonia it was demonstrated that BAP mixtures enriched with NAEPE had a positive effect on disease progression by lowering levels of IL-1β, IL-8 in sera and lowering white blood count, as well as reducing lung parenchyma. *In vitro* studies on the transcriptional activity of proinflammatory cytokine genes related to the activation of intracellular signaling pathways associated with inflammation showed the ability of certain BAP mixtures to influence the immune response of macrophages. *Id.* Moreover, the NAEPE enriched egg yolk extracts have activities which inhibit the phosphorylation of protein kinase C epsilon. *Id.*

[0007] It is an object of the present invention to identify biologically active lipids, including phosphatidylcholine (PC) and/or sphingomyelin (SPH) and/or lysophosphatidylcholine (LPC) compounds, and mixtures thereof, possessing therapeutic properties, including antiproliferative or antineoplastic properties. Moreover, it is an object of the invention to provide compositions and methods of using these compositions to treat proliferative diseases, including cancer.

SUMMARY

[0008] Bioactive phospholipids (BAP) can be extracted from hen egg yolk (*e.g.*, ischemic chick embryos) with alcohol and can be purified using acetone precipitation, as disclosed in Gladkowski, W, et al., *J. Am. Oil Chem.* **89** 179-182 (2012), hereby incorporated by reference in its entirety. Such an egg yolk extract (designated herein as "BAP(-)") contains a wide range of phospholipids but, as demonstrated herein, does not show significant effects in *in*

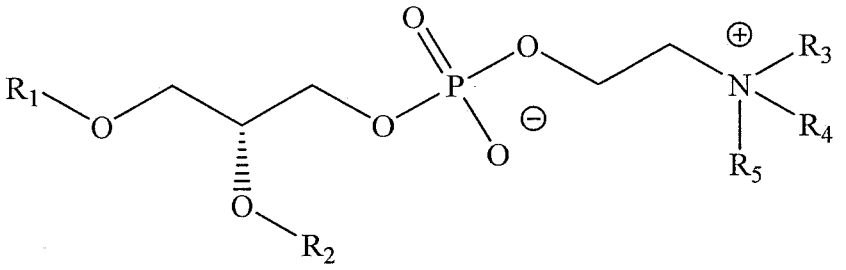
vitro and *in vivo* studies of cancer. It is known to treat BAP(-) mixtures with palmitoyl chloride to produce a material known as BAP(+). It has heretofore been believed that palmitoylization of the BAP(-) extract (producing BAP(+)) is required to yield therapeutic activity of the BAP mixture due to the creation of NAEPE's (or "PNAE") from the corresponding endogenous ethanolamines. As discussed previously, NAEPE's were thought to be the active constituent in BAP(+). *See, e.g.*, Karafiát V., et al., *Folia Biologica (Praha)* **60,** 220-227 (2014) and Kara, J. et. al, *Neoplasma* **33**, 187-205 (1986).

[0009] However, it has surprisingly been discovered that certain lipid components of chemically treated hen egg yolk provide anti-cancer effects, even in the absence or substantial absence (*e.g.*, less than 5% (w/w), less than 1% (w/w), less than 0.1% (w/w), less than 0.01% (w/w), etc.) of the known active NAEPE's. Accordingly, the disclosure provides novel mixtures of lipids and pharmaceutical compositions thereof which are contemplated to be useful in the treatment of various diseases and conditions. The disclosure provides compositions comprising egg yolk extracts (*e.g.*, hen egg yolk extracts, typically from *Gallus gallus domesticus*) enriched with one or more lipid components, such as phosphatidylcholine (PC) and/or sphingomyelin (SPH) and/or lysophosphatidylcholine (LPC) compounds. The disclosure is not limited to hen egg yolk extracts, but embraces extracts from any animal source or synthetic mixtures of lipids, having the same or substantially similar constituents. By "substantially similar" constituents is meant that at least the most abundant PC and/or SPH components are within 25% (w/w), preferably 15% and ideally 10% of the abundance in the hen egg yolk extracts defined herein. In some implementations, one or more lipids according to the disclosure are inhibitors of kinases, including protein kinases, notably tyrosine kinases, such as TTK. These compositions may be used to treat, for example, inflammation or proliferative diseases such as cancer.

[0009a] In one aspect, the disclosure provides a pharmaceutical composition comprising a plurality of bioactive phospholipids derived from hen egg yolk and one or more pharmaceutically acceptable diluents, excipients and/or carriers, wherein said plurality of bioactive phospholipids is substantially free of N-acyl ether phosphatidylethanolamines (NAEPE).

[0009b] In another aspect, the disclosure provides a pharmaceutical composition comprising a plurality of bioactive phospholipids derived from egg yolk and one or more pharmaceutically acceptable diluents, excipients, and/or carriers, wherein said plurality of phospholipids is enriched in one or more lipids selected from the group consisting of:

(i) one or more phosphatidylethanolamine (PE) and/or phosphatidylcholine (PC) derivatives having the structure:

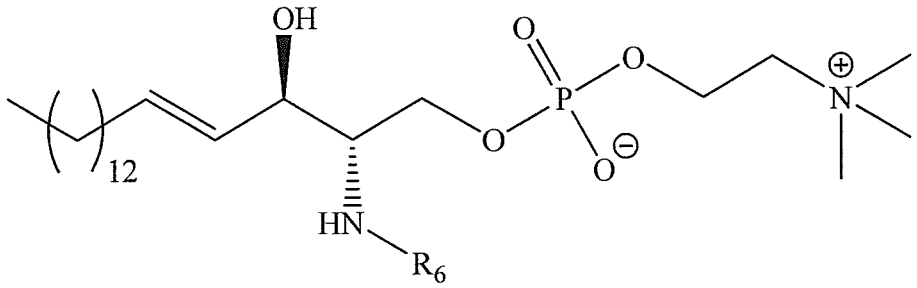


where R_1 or R_2 are independently selected from the group consisting of hydrogen, palmitoyl (C16:0), hexadecenoyl (C16:1), palmitoleoyl (C16:1), stearoyl (C18:0), octadecenoyl (C18:1), oleoyl (C18:1), octadecadienoyl (C18:2), linoleoyl (C18:2), α-linolenoyl (C18:3), octadecatrienoyl (C18:3), arachidonoyl (C20:4), eicosatetraenoyl (C20:4), docosapentaenoyl (C22:5), and docosahexaenoyl (C22:6) radicals;

R_3 is selected from hydrogen, methyl, or a C_2-C_{18} acyl group; wherein if R_3 is an acyl group then the R_4 or R_5 is absent and the nitrogen bonded to R_3 is uncharged;

R_4 and R_5 are independently selected from the group consisting of hydrogen or methyl, or pharmaceutically acceptable salts thereof; and/or

(ii) one or more sphingomyelin (SPH) derivatives having the structure



wherein R_6 is palmitoyl (C16:0), stearoyl (C18:0), oleoyl (C18:1), tetradecanoyl (C14:0), heptadecanoyl (C17:0), nonadecanoyl (C19:0), eicosanoyl (C20:0), docosanoyl (C22:0), docosenoyl (C22:1), henicosanoyl (C23:0), tetracosadienoic (C24:2), or tetracosanoyl (C14:0); and pharmaceutically acceptable salts thereof.

[0009c] In another aspect, the disclosure provides a pharmaceutical composition comprising a plurality of phosphatidylcholine (PC) compounds and one or more pharmaceutically acceptable diluents, excipients and/or carriers; wherein at least 50% (w/w) of said phosphatidylcholine (PC) compounds are selected from the group consisting of:

1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-α-linolenoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine,

1-linoleoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine,

1-α-linolenoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine,

1-palmitoleoyl-2-oleoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-clupanodonoyl-*sn*-glycero-3-phosphocholine,

1-palmitoleoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-osbondoyl-*sn*-glycero-3-phosphocholine,

1-oleoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine,

1-oleoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine,

1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine,

1-hydroxy-2-palmitoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, and

1-oleoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine; and

pharmaceutically acceptable salts thereof.

[0009d] In another aspect, the disclosure provides a pharmaceutical composition comprising an effective amount of (R)-2-(((9Z,12Z)-octadeca-9,12-dienoyl)oxy)-3-(palmitoyloxy)propyl (2-(trimethylammonio)ethyl) phosphate ("MPC9") and an effective amount of (2S,3R,E)-2-heptadecanamido-3-hydroxyoctadec-4-en-1-yl (2-(trimethylammonio)ethyl) phosphate ("SPH1") together with one or more pharmaceutically acceptable excipients, diluents, and/or carriers.

[0009e] In another aspect, the disclosure provides a pharmaceutical composition comprising an effective amount of (R)-2-(((9Z,12Z)-octadeca-9,12-dienoyl)oxy)-3-(oleoyloxy)propyl (2-(trimethylammonio)ethyl) phosphate ("MPC11") and an effective amount of (2S,3R,E)-2-heptadecanamido-3-hydroxyoctadec-4-en-1-yl (2-(trimethylammonio)ethyl) phosphate ("SPH1") together with one or more pharmaceutically acceptable excipients, diluents, and/or carriers.

[0010] Methods of treating proliferative diseases are also provided. A method for the treatment of a mammal (*e.g.*, a human) suffering from cancer is provided comprising administering therapeutically effective amounts of any of the lipid mixtures described herein, or pharmaceutical compositions thereof. In particular, the lipid mixtures are derived from egg yolk extracts (*e.g.*, hen egg yolk extracts, etc.) enriched in phosphatidylcholine and/or sphingomyelin compounds and/or lysophosphatidylcholine compounds or synthetically derived mixtures that are substantially similar thereto. In various implementations, the compositions are administered (*e.g.*, orally) for the treatment of a cancer, including, without limitation, bladder, blood (*e.g.* lymphoma, Jurkat cancer cell line, etc.) brain (*e.g.* T98G

cancer cell line, etc.), breast (*e.g.* 231 cancer cell line, etc.), cervical (*e.g.* HeLa cancer cell line, etc.), colorectal (*e.g.* HCT116 cancer cell line, etc.), esophageal, kidney, liver (*e.g.* HepG2 cancer cell line, etc.), lung (*e.g.* H358 cancer cell line, A549 cancer cell line, etc.), ovarian (*e.g.* SK-OV-3 cancer cell line, etc.), pancreatic (*e.g.* Panc1 cancer cell line, Capan-2 cancer cell line, etc.), skin (*e.g.* M14 cancer cell line, etc.), prostate (*e.g.* DU-145 cancer cell line, etc.), thyroid, or uterine cancers.

[0010a] In another aspect, the disclosure provides the pharmaceutical composition as described herein for use in treating a proliferative disease in a human patient in need thereof.

BRIEF DESCRIPTION OF THE FIGURES

[0011] FIG. 1 illustrates an isolation of specific set of phospholipids derived from an egg extract. The initial egg yolk mixture comprises O-alkyl PE's, SPH's, PC's and other constituents. It will be understood that "R" groups on any compound class in FIG. 1 may be any aliphatic radical, and more typically, the monovalent hydrocarbon radical of a naturally occuring fatty acid. In the derivation illustrated, phospholipids are extracted from egg yolk using butanol (step 1) and further purified by adding the butanol extract to chilled acetone and collecting the lipids which precipitate from the mixture (step 2). These precipitated lipids are then reacted with CDI and an acid (for example, using an ethyl acetate:triethanolamine solvent comprising an amount of about triethanolamine for complete solvation of the precipitated lipids). Such reaction may produce the ten lipid classes shown. Medium pressure chromatography (step 4) is then used to further isolate only specific lipid classes (*e.g.* SPH, PC, LPC) and the final product may then be further purified using water/chloroform extraction to remove residual degradation products produced during chromatographic separation (step 4).

[0012] FIG. 2A shows TLC plates for a thin layer chromatographic separation of BAP(+), BAP(-) into various fractions from the left side of the plate to the solvent front on the right. Stains from fractions F1-F5 of BAP (+) and the corresponding fraction F5 of BAP(-) ("F5(-)") may be seen. As can be seen, the acylation BAP(-) to produce BAP(+) converts the PE component of BAP(-) into an NAEPE component present in BAP(+). Also shown are SPH, LPC and PC controls illustrating where those phospholipid classes elute. As can be seen, fraction F5 comprises a sphingomyelin component and a phosphatidylcholine component. FIG. 2B shows a similar chromatographic separation of MP1000 (top trace) with LPC, SPH

and BAP(+) controls. As can be seen, MP1000 is composed of the F5 fraction but an LPC component is substantially absent. FIG. 2C shows the chromatographic comparison of MP1000, to BAP(+), and BAP(-) controls.

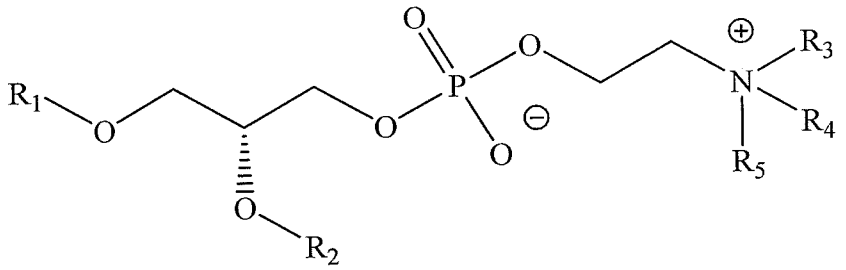
CLAIMS

1. A pharmaceutical composition comprising a plurality of bioactive phospholipids derived from hen egg yolk and one or more pharmaceutically acceptable diluents, excipients and/or carriers, wherein said plurality of bioactive phospholipids is substantially free of N-acyl ether phosphatidylethanolamines (NAEPE).

2. A pharmaceutical composition comprising a plurality of bioactive phospholipids derived from egg yolk and one or more pharmaceutically acceptable diluents, excipients, and/or

carriers, wherein said plurality of phospholipids is enriched in one or more lipids selected from the group consisting of:

(i) one or more phosphatidylethanolamine (PE) and/or phosphatidylcholine (PC) derivatives having the structure:

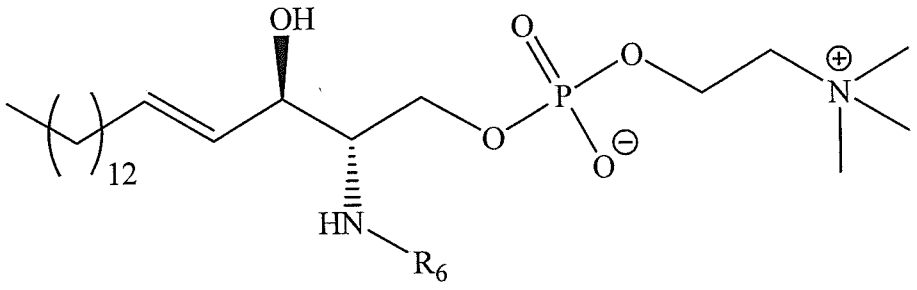


where R_1 or R_2 are independently selected from the group consisting of hydrogen, palmitoyl (C16:0), hexadecenoyl (C16:1), palmitoleoyl (C16:1), stearoyl (C18:0), octadecenoyl (C18:1), oleoyl (C18:1), octadecadienoyl (C18:2), linoleoyl (C18:2), α-linolenoyl (C18:3), octadecatrienoyl (C18:3), arachidonoyl (C20:4), eicosatetraenoyl (C20:4), docosapentaenoyl (C22:5), and docosahexaenoyl (C22:6) radicals;

R_3 is selected from hydrogen, methyl, or a C_2-C_{18} acyl group; wherein if R_3 is an acyl group then the R_4 or R_5 is absent and the nitrogen bonded to R_3 is uncharged;

R_4 and R_5 are independently selected from the group consisting of hydrogen or methyl, or pharmaceutically acceptable salts thereof; and/or

(ii) one or more sphingomyelin (SPH) derivatives having the structure



wherein R_6 is palmitoyl (C16:0), stearoyl (C18:0), oleoyl (C18:1), tetradecanoyl (C14:0), heptadecanoyl (C17:0), nonadecanoyl (C19:0), eicosanoyl (C20:0), docosanoyl (C22:0), docosenoyl (C22:1), henicosanoyl (C23:0), tetracosadienoic (C24:2), or tetracosanoyl (C14:0); and pharmaceutically acceptable salts thereof.

3. The pharmaceutical composition according to claim 2, wherein R_3-R_5 are methyl, such that said plurality of bioactive phospholipids comprises a phosphatidylcholine (PC) component; wherein at least 50% (w/w) of said phosphatidylcholine (PC) component comprises one or more compounds selected from the group consisting of:

1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-α-linolenoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine,

1-linoleoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine,

1-α-linolenoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine,

1-palmitoleoyl-2-oleoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-clupanodonoyl-*sn*-glycero-3-phosphocholine,

1-palmitoleoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-osbondoyl-*sn*-glycero-3-phosphocholine,

1-oleoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine,

1-oleoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine,

1,2-dioleoyl-*sn*-glycero-3-phosphocholine,

1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine,

1-stearoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine,

1-hydroxy-oleoyl-*sn*-glycero-3-phosphocholine

1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, and

1-oleoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine;

and pharmaceutically acceptable salts thereof.

4. The pharmaceutical composition according to claim 3, wherein said docosahexaenoyl group in 1-palmitoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine, 1-α-linolenoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine, 1-palmitoleoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine, and 1-oleoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine is (4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoyl.

5. The pharmaceutical composition according to claim 3, wherein said plurality of bioactive phospholipids comprises a phosphatidylcholine (PC) component; wherein at least 50% (w/w) of said phosphatidylcholine (PC) component comprises one or more compounds selected from the group consisting of:

1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine,

1-palmitoleoyl-2-oleoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine,

1-stearoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine, and

1-hydroxy-2-palmitoyl-*sn*-glycero-3-phosphocholine; and

pharmaceutically acceptable salts thereof.

6. The pharmaceutical composition according to any one of claims 2-5, wherein said plurality of bioactive phospholipids comprises a sphingomyelin (SPH) component, wherein at least 50% (w/w) of said sphingomyelin (SPH) component comprises one or more compounds selected from the group consisting of:

N-palmitoyl-D-erythro-sphingosylphosphorylcholine,

N-stearoyl-D-erythro-sphingosylphosphorylcholine, and

N-oleoyl-D-erythro-sphingosylphosphorylcholine.

7. The pharmaceutical composition according to claim 6, wherein said sphingomyelin (SPH) component comprises N-palmitoyl-D-erythro-sphingosylphosphorylcholine.

8. A pharmaceutical composition comprising a plurality of phosphatidylcholine (PC) compounds and one or more pharmaceutically acceptable diluents, excipients and/or carriers;

wherein at least 50% (w/w) of said phosphatidylcholine (PC) compounds are selected from the group consisting of:

1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-α-linolenoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine,

1-linoleoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine,

1-α-linolenoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine,

1-palmitoleoyl-2-oleoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-clupanodonoyl-*sn*-glycero-3-phosphocholine,

1-palmitoleoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-osbondoyl-*sn*-glycero-3-phosphocholine,

1-oleoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine,

1-oleoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine,

1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine,

1-hydroxy-2-palmitoyl-*sn*-glycero-3-phosphocholine,

1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, and

1-oleoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine; and

pharmaceutically acceptable salts thereof.

9. The pharmaceutical composition according to claim 8, wherein said docosahexaenoyl group in 1-palmitoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine, 1-α-linolenoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine, 1-palmitoleoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine, and 1-oleoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine is (4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoyl.

10. The pharmaceutical composition according to claim 8 or 9 further comprising a sphingomyelin (SPH) component, wherein said sphingomyelin (SPH) component comprises one or more compounds selected from the group consisting of:

N-palmitoyl-D-erythro-sphingosylphosphorylcholine,

N-stearoyl-D-erythro-sphingosylphosphorylcholine, and

N-oleoyl-D-erythro-sphingosylphosphorylcholine.

11. The pharmaceutical composition according to any one of claims 1-10, wherein said plurality of bioactive phospholipids is derived from hen egg yolk by alcohol extraction.

12. The pharmaceutical composition according to any one of claims 1-11, wherein said plurality of bioactive phospholipids is derived from hen egg yolk that has been reacted with an activated carboxylic acid species.

13. The pharmaceutical composition according to claim 12, wherein said carboxylic acid is a fatty acid.

14. A pharmaceutical composition comprising an effective amount of (R)-2-(((9Z,12Z)-octadeca-9,12-dienoyl)oxy)-3-(palmitoyloxy)propyl (2-(trimethylammonio)ethyl) phosphate ("MPC9") and an effective amount of (2S,3R,E)-2-heptadecanamido-3-hydroxyoctadec-4-en-1-yl (2-(trimethylammonio)ethyl) phosphate ("SPH1") together with one or more pharmaceutically acceptable excipients, diluents, and/or carriers.

15. A pharmaceutical composition comprising an effective amount of (R)-2-(((9Z,12Z)-octadeca-9,12-dienoyl)oxy)-3-(oleoyloxy)propyl (2-(trimethylammonio)ethyl) phosphate ("MPC11") and an effective amount of (2S,3R,E)-2-heptadecanamido-3-hydroxyoctadec-4-

en-1-yl (2-(trimethylammonio)ethyl) phosphate ("SPH1") together with one or more pharmaceutically acceptable excipients, diluents, and/or carriers.

16. The pharmaceutical composition according to of claim 14 or 15, wherein said effective amounts comprise from about 1 mg to about 2000 mg.

17. The pharmaceutical composition of any of claims 1-16 for use in treating a proliferative disease in a human patient in need thereof.

18. The pharmaceutical composition according to claim 17, wherein said proliferative disease is cancer.

19. The pharmaceutical composition according to claim 17, wherein said proliferative disease is bladder, brain breast, cervical, colorectal, esophageal, kidney, liver, lung, ovarian, pancreatic, thyroid or uterine cancer.

20. The pharmaceutical composition according to claim 17, wherein said therapeutically effective amount comprises from about 100 mg to about 10 g daily.