**FIG. 12A, FIG. 12B, FIG. 12C, and FIG. 12D** show representative images of mammospheres and wound healing assay in shRNA-mediated NOS2 knockdown cells. Illustrative images of primary and secondary mammospheres **(FIG. 12A and FIG. 12B)** and migration (wound healing assay) **(FIG. 12C and FIG. 12D)** in SUM159 and MDA-MB-231 cells transfected with two different NOS2-directed shRNAs (shRNA1, shRNA2) or empty vector (sRNA-EV);

**FIG. 13A, FIG. 13B, FIG. 13C, FIG. 13D, and FIG. 13E**show the i*n vivo* effects of L-NMMA in SUM159 xenografts. **FIG. 13A:** Tumor volume of SUM159 breast xenografts ($n$ = 10/group) treated with vehicle, L-NMMA, chemotherapy and combination. Two-way ANOVA and Bonferroni's *post-hoc* test. **FIG. 13B:** Illustrative images of Ki67 staining in vehicle, L-NMMA, chemotherapy (docetaxel), and combination groups. Original optical objective: 10×. Counterstain: hematoxylin. **(FIG. 13C)** Cell proliferation of tumor xenografts is depicted as Ki67 positive cells. 1,000 cells were counted from 10 different fields and percentage was determined. One-way ANOVA and Bonferroni's *post-hoc* test. Primary and secondary MSFE of breast cancer cells isolated from tumor tissue **(FIG. 13D)**. One-way ANOVA and Bonferroni's *post-hoc* test. **FIG. 13E:** Tumor-initiating capacity of tumor cells assayed by the limiting dilution method. Fisher's exact test. Results were normalized to vehicle. Data are presented as mean ± SEM. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

**FIG. 14A, FIG. 14B, FIG. 14C, FIG. 14D, FIG. 14E, and FIG. 14F** show NMMA levels in plasma and tumor tissue. $CD44^{+}/CD24^{-/low}$ population in L-NMMA-treated xenografts. Flow cytometric analysis (% Parent) of $CD44^{+}/CD24^{-/low}$ cells isolated from SUM159 **(FIG. 14A)** and MDA-MB-231 **(FIG. 14B)** xenograft tumor tissue of mice treated with vehicle, L-NMMA, docetaxel and combination (docetaxel + L-NMMA). **FIG. 14C and FIG. 14D:** Ratiometric quantification of methylarginine in plasma and tumor tissue (MDA-MB-231 and SUM159 xenografts) by LC-MS/MS (Student's *t*-test). **FIG. 14E:** iNOS catalyzes the reaction of L-Arginine to L-Citrulline + nitric oxide (NO). Ratiometric quantification of citrulline SUM159 xenograft tissue LC-MS/MS (Student's *t*-test). **FIG. 14F:** Total nitric oxide production in SUM159 cells treated with L-NMMA and 1400W (4 mM) for 0.5, 2, 6 and 24 hrs. Results were normalized to vehicle. Data are presented as mean ± SEM. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, One-way ANOVA and Bonferroni's *post-hoc* test;

increased to 200 mg/kg. The tumor growth was efficiently blocked by L-NMMA administered alone or in combination with docetaxel (FIG. 13A). The inventor correlated these results with tumor cell proliferation by immunohistochemistry (Ki67). Higher proliferating rate in vehicle and chemotherapy groups compared to L-NMMA and combination groups (FIG. 13B and FIG. 13C) was observed. The inventor next analyzed the impact on CSC self-renewal by the MSFE assay. Docetaxel showed a dramatic increase in the primary and secondary MSFE of single tumor cells isolated from breast tumor tissue. This increment was efficiently blocked by addition of L-NMMA (combination group) (FIG. 13D). Flow cytometric analysis showed slight increase in $CD44^{+}/CD24^{-/low}$ population after chemotherapy (FIG. 14A). LDA showed that vehicle, L-NMMA, docetaxel and combination groups exhibited 12/12, 4/12, 12/12, 6/12 tumors, respectively, at 7 weeks with $5 \times 10^4$ cells. At week nine a significant reduction in tumor-initiating capacity was observed as different groups with $2 \times 10^4$ cells yielded 7/12, 0/12, 3/12, 0/12 tumors in vehicle, L-NMMA, docetaxel and combination, respectively ($p < 0.05$, Fisher's exact test) (FIG. 13E).

The role of L-NMMA was then investigated, either alone or in combination with docetaxel in a different TNBC mouse model. Docetaxel (20 mg/kg at days 0 and 21) and 200 mg/kg L-NMMA (daily) were given to MDA-MB-231 xenograft-bearing mice for 31 days. Firstly, reduced tumor growth was found in L-NMMA group compared to vehicle, but there was no change between docetaxel and combination groups (FIG. 5A). These results were further correlated with lower proliferation rate in L-NMMA and combination groups as seen by immunohistochemistry (FIG. 5B and FIG. 5C). Additionally, higher apoptosis levels in docetaxel-treated xenografts were found; these results might offset the high proliferation rate relative to the combination group (FIG. 5D). Primary MS was less for both L-NMMA and combination, compared to vehicle and chemotherapy alone groups. L-NMMA treatment was able to reduce secondary MS, but no change was observed for the combination group (FIG. 5E). Flow cytometric analysis showed no changes in $CD44^{+}/CD24^{-/low}$ population (FIG. 14B). LDA showed that vehicle-treated and docetaxel-treated groups presented 12/12 and 8/12 tumors, respectively, with a significant decrease in L-NMMA-treated (1/12) and combination-treated (4/12) xenografts at 5 weeks with $5 \times 10^4$ cells. After six weeks, a decrease in both L-NMMA and combination groups (3/12 and 5/12, respectively) compared to vehicle and docetaxel groups (6/12 and 8/12, respectively) was observed ($p < 0.05$, Fisher's exact test) (FIG. 5F). The investigations demonstrate that L-NMMA plasma levels are cleared rapidly (FIG. 14C), whereas it

## CLAIMS:

**WHAT IS CLAIMED IS:**

1. A pharmaceutical composition, comprising:

1) a chemotherapeutically-effective amount of a first iNOS inhibitor selected from the group consisting of $N^G$-monomethyl-L-arginine [L-NMMA], (N-[[3-(aminomethyl)phenyl] methyl]-ethanimidamide) [1400W], and ($N^5$-[imino(nitroamino)methyl]-L-ornithine methyl ester) [L-NAME] ; and

2) a therapeutically-effective amount of:

a first antihypertensive agent that comprises a first calcium channel antagonist.

2. The pharmaceutical composition in accordance with claim 1, wherein the first calcium channel antagonist is selected from the group consisting of amlodipine, aranidipine, azelnidipine, barnidipine, benidipine, clinidipine, clevidipine, diltiazem, efonidipine, fendiline, felodipine, gallopamil, isradipine, lacidipine, lercanidipine, manidipine, nicardipine, nifedipine, nimodipine, nisoldipine, nitrendipine, nivaldipine, pranidipine, and verapamil.

3. The pharmaceutical composition in accordance with claim 1 or 2, wherein the first calcium channel antagonist is amlodipine.

4. The pharmaceutical composition in accordance with any preceding claim, further comprising a therapeutically-effective amount of a first chemotherapeutic agent, or a second, distinct iNOS inhibitor.

5. The pharmaceutical composition in accordance with any preceding claim, further comprising one or more of an immunomodulating agent, a neuroactive agent, an anti-inflammatory agent, an anti-lipidemic agent, a hormone, a receptor agonist, a receptor antagonist, an anti-infective agent, a protein, a peptide an antibody, an antigen-

binding fragment, an enzyme, an RNA, a DNA, an siRNA, an mRNA, a ribozyme, a hormone, a cofactor, a steroid, an antisense molecule, a second distinct antihypertensive agent, a second distinct chemotherapeutic agent, or any combination thereof.

6. The pharmaceutical composition in accordance with claim 4, wherein the first chemotherapeutic agent is selected from the group consisting of cyclophosphamide, doxorubicin, 5-fluorouracil, docetaxel, paclitaxel, trastuzumab, methotrexate, epirubicin, cisplatin, carboplatin, vinorelbine, capecitabine, gemcitabine, mitoxantrone, isabepilone, eribulin, lapatinib, carmustine, a nitrogen mustard, a sulfur mustard, a platin tetranitrate, vinblastine, etoposide, camptothecin, and any combination thereof.

7. The pharmaceutical composition in accordance with any preceding claim, wherein i) the first iNOS inhibitor comprises L-NMMA; ii) the first antihypertensive agent comprises amlodipine, and the first chemotherapeutic agent comprises docetaxel.

8. The pharmaceutical composition in accordance with any preceding claim, further comprising a liposome, a surfactant, a niosome, an ethosome, a transferosome, a phospholipid, a sphingosome, a nanoparticle, a microparticle, or any combination thereof.

9. The pharmaceutical composition in accordance with any preceding claim, further comprising a pharmaceutically-acceptable carrier, buffer, diluent, vehicle, excipient, or any combination thereof.

10. The pharmaceutical composition in accordance with any preceding claim, formulated for mammalian administration, and preferably, for human administration.

11. The pharmaceutical composition in accordance with any preceding claim, adapted and configured as part of a therapeutic kit that comprises the composition, and at least a first set of instructions for administration of the composition to a human in need thereof.

12. The pharmaceutical composition in accordance with any preceding claim, for use in therapy, prophylaxis, or amelioration of one or more symptoms of a mammalian cancer.

13. The pharmaceutical composition in accordance with any preceding claim, for use in the therapy, prophylaxis, or amelioration of one or more symptoms of a human breast cancer, and in particular, a human therapy-resistant, metastatic, or triple-negative breast cancer.

14. A pharmaceutical composition in accordance with any one of claims 1 to 13, for use in therapy.

15. A pharmaceutical composition in accordance with any one of claims 1 to 13, for use in the treatment of cancer in a mammalian subject.

16. Use of a pharmaceutical composition in accordance with any one of claims 1 to 10, in the manufacture of a medicament for treating or ameliorating one or more symptoms of cancer in a mammal.

17. Use in accordance with claim 16, in the manufacture of a medicament for treating human breast cancer, and in particular, a refractory, a treatment-resistant, a relapsed, a metastatic, or a triple-negative breast cancer.

18. Use of a pharmaceutical composition in accordance with any one of claims 1 to 13, in the manufacture of a medicament for treating or ameliorating one or more symptoms of cancer in a mammal.

19. The use in accordance with claim 18, wherein the cancer is diagnosed as, or is identified as, a refractory, a metastatic, a relapsed, or a treatment-resistant cancer.

20. The use in accordance with claim 18 or claim 19, wherein the cancer is diagnosed as, or is identified as, a treatment-resistant or a metastatic cancer, and in particular, a treatment-resistant, triple-negative human breast cancer.

21. The use in accordance with any one of claims 18 to 20, wherein a therapeutically-effective amount of radiation is to be further administered to the animal.

22. The use in accordance with any one of claims 16 to 21, wherein the pharmaceutical composition is to be administered systemically to the animal, in a single administration,

or in a series of multiple administrations over a period of from one or more days, over a period of one or more weeks, or over a period of one or more months or longer.