Introduction
Breast cancer patients often have detectable or occult metastases at diagnosis and most patients will develop metastatic lesions during the course of the disease.

Objective
We investigated the effect of a nutrient mixture (NM) containing ascorbic acid, lysine, proline, and green tea extract on murine breast cancer 4T1, a unique metastatic breast cancer model that has the capacity to metastasize efficiently to sites affected in human breast cancer.

Materials and Methods
1. After one week of isolation, 5-6 week old female Balb/C mice were inoculated with 5x10^3 4T1 cells into the mammary pad and randomly divided into two groups; group A was fed a regular diet and group B a regular diet supplemented with 0.5% NM. 2. After four weeks, the mice were sacrificed and their tumors, livers, kidneys, hearts, and spleens were excised and processed for histology.

3. Dimensions (length and width) of tumors were measured using a digital caliper, and the tumor burden was calculated using the following formula: 0.5 x length x width.

4. We also tested the effect of NM in vitro on 4T1 cells, measuring cell proliferation by MTT assay. MMP secretion by zymography, invasion through Matrigel, migration by scratch test and morphology by H&E staining.

Composition of Nutrient Mixture (NM)
- Vitamin C (as ascorbic acid and as Mg, Ca and palmitate ascorbate) 700 mg
- L-Lysine 1000 mg
- L-Proline 750 mg
- L-Arginine 500 mg
- N-Acetyl Cysteine 200 mg
- Standardized Green Tea Extract (80% polyphenols) 1000 mg
- Selenium 30 µg
- Copper 2 mg
- Manganese 1 mg

Results
1. NM inhibited tumor weight and burden of 4T1 tumors by 50% (p<0.002) and 53.4% (p<0.0001), respectively, as shown in Figure 1.

2. Histologically, both groups demonstrated irregularly round subcutaneous tumors with large central areas of tumor necrosis involving 70% of the tumor mass in the control mice and 50-70% in the supplemented mice. See Figure 2.

3. Lung metastasis was profoundly inhibited by NM supplementation: mean number of colonies was reduced by 87% (p<0.0001) and mean weight of lungs by 60% (p<0.0001) compared to control mice. See Figure 3.

4. Histopathology confirms the inhibition of lung metastasis in NM supplemented mice, as shown in Figure 4.

5. In vitro, NM exhibited dose dependent inhibition of cell proliferation (p<0.0001), with 50% toxicity over the control at 250 and 500 µg/ml concentrations, and 61% at 1000 µg/ml, as shown in Figure 5.

6. Zymography demonstrated MMP-2 and MMP-9 secretion by 4T1 cells which was inhibited by NM in a dose dependent fashion, with virtual total inhibition of both at 1000 µg/ml.

7. Invasion of 4T1 cells through Matrigel and migration through scratch test were inhibited by NM in a dose dependent manner, with total block of invasion at 250 µg/ml and of migration at 1000 µg/ml, as shown in Figures 11 and 12, respectively.

Conclusions
These results suggest that NM has therapeutic potential in treatment of breast cancer.