



# Inhibition of Pulmonary Metastasis of Melanoma B16FO Cells in C57BL/6 Mice by a Nutrient Mixture Consisting of Ascorbic Acid, Lysine, Proline, Arginine, and Green Tea Extract

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## **Summary:**

Tumor metastasis is a major reason for treatment failure in cancer patients. Melanoma is one of the types of cancer that most frequently metastasize, especially in the lung. Currently used therapies are ineffective with a high level of toxicity and mortality.

Cancer metastasis is accompanied by degradation of connective tissue necessary to facilitate the spread and invasion of cancer cells from one organ to other parts of the body. Our previous studies have shown that specific mixture of micronutrients essential for the synthesis and stability of collagen and connective tissue in the body can inhibit invasion of cancer cells through this tissue. The primary objective of this study was to investigate whether these natural components applied as a synergistic mixture (NM) could inhibit experimentally induced lung metastases in C57BL/6 female mice injected with melanoma B16FO cells. Several studies have demonstrated that melanoma cells injected into the vein of C57BL/6 mice form metastasis in the lungs. We used this model to assess the effectiveness of NM containing ascorbic acid, lysine, proline, arginine and green tea extract on melanoma cells ability to form metastasis in the lungs and growth of tumors. Mice were divided into equal groups (#1-6) and injected via tail vein with melanoma B16FO cells (Group1-4), the same number of melanoma cells pre-incubated for 24 hours with NM (Group 5), and the same number of melanoma cells pre-incubated with saline (Group 6). Groups 1, 3, 4, 5, and 6 were fed the normal diet and Group 2 the diet supplemented with 0.5% NM. Animals in Groups 3 and 4 received NM by ip and iv injections, respectively. Two weeks later, melanoma metastatic colonies in the lungs were identified and counted.

In mice supplemented with NM through the diet the number of metastatic colonies in the lungs was reduced by 63%. In mice who received NM through ip and iv injection the number of lung metastases was reduced by 86%. In mice injected with melanoma cells pretreated with NM no lung metastases were detected, the metastasis was inhibited by 100%.

The results of this study show that this nutrient mixture administered in a diet was effective in a significant inhibition of metastasis of B16FO melanoma cells to the lungs.

By exposure to higher concentrations of NM, such as obtained through iv or ip delivery, an enhanced inhibitory effect was obtained (up to 86%). It is important to note that the exposure of tumor cells to NM before their injection to the mice completely prevented development of lung tumors (100% inhibition of metastasis).

These findings together with our earlier results clearly indicate that this nutrient combination (NM) has a high potential in preventing cancer metastasis. Furthermore, use of this nutrient mixture would not pose any toxic effects clinically, especially in the relevant doses. This can be supported by our previously published in vivo safety studies and a general safety record of these micronutrients, even if used in a very high doses. Taking into account that there is no effective treatment to stop cancer metastasis, there is an urgent need to confirm the effectiveness of this approach in clinical settings.