

Inhibition of Lung Cancer Metastasis in mice by inhaled aerosolized anti-MARCKS peptides

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Abstract

We have previously reported that intraperitoneal administration of peptides that inhibit the function of MARCKS (myristoylated alanine-rich C Kinase Substrate) protein also inhibit metastasis of human lung cancer cells orthotopically-injected into the left lobe of SCID mice [1,2]. Here, we administered an anti-MARCKS peptide, BIO 11006, (the active portion of the MANS peptide identical to the N-terminal region of MARCKS) via the inhaled aerosol route in 2 models of lung cancer in SCID mice: PC-9 cells orthotopically injected into the left lobe, and A549 cells injected into the tail vein which go on to seed in the lung and form metastatic tumors. In the orthotopic model, initiation of treatment with BIO 11006 at day 4 post injection of cells and treatment every day thereafter for 4 weeks resulted in significant attenuation of tumor metastasis into the lung, heart or diaphragm, with almost identical attenuation whether the peptides were administered IP or via inhaled aerosol. In additional studies with the orthotopic model, administration of the peptide IP starting at day 15 post orthotopic injection of cells, a point where metastasis had already started, resulted in apparent inhibition of further metastasis. Carrying this study further, we waited until day 26 post orthotopic cell injection to begin daily treatment with BIO 11006 via inhaled aerosol, and found that, even at this advanced time of metastasis, there appeared to be complete inhibition of further tumor metastasis when the animals were examined at day 42 post orthotopic injection of cells. Similar results with the tail vein model were observed: when the peptide was administered by aerosol starting at day 3 post tail vein inoculation, metastasis observed 8 weeks later in distal organs was inhibited by ~ 95% by peptide treatments, either with MANS or BIO 11006. The results suggest that inhaled aerosolized BIO 11006, already approved and utilized in clinical trials in patients with COPD, appears to have potent anti-metastatic effects in two different mouse models of lung cancer.

Introduction

Cancer is a leading cause of death in America. Over the years, significant progress has been made in understanding cancer biology and many therapies are currently utilized to limit cancer's deleterious effects. Most therapies are aimed at destroying cancerous cells. This is the first report of an approach to limiting the metastatic component of lung cancer progression using an inhaled aerosolized anti-MARCKS peptide, BIO-11006.

The Adler lab has reported previously that Myristoylated Alanine-Rich C Kinase Substrate (MARCKS) is a key molecule regulating multiple cellular processes, including the migration of various cell types [3-6] and that use of peptides designed matching the beginning amino acid sequence of MARCKS can effect these processes. Inflammation, another hallmark in cancer, is also linked to MARCKS protein [7]. Application of these data to the problem of cancer metastasis led to the trial of MARCKS-like peptide BIO-11006 in murine lung cancer models.

Methods

Cell culture All procedures were performed in laminar flow hood following sterile techniques. PC9, a cell line derived from a human lung adenocarcinoma of epithelial phenotype was grown on plastic culture dishes in RPMI 1640 media with 2mM Glutamine and 10% Fetal Bovine Serum (FBS) until 80% confluence was observed. A549 cells, a human lung adenocarcinoma cell line with a high viability, was chosen for the study. Cells were cultured in Dulbecco's MEM media with 10% FBS.

Peptide The 11006 peptide (synthesized by Genemed Synthesis, Inc., San Francisco, CA) consists of a sequence identical to the first amino acids of human MARCKS protein; GAQFSKTAAK.

Orthotopic Injections In the orthotopic lung cancer model, 1~2 x 10⁵ human NSCLC cells (PC-9 non small cell lung cancer cell line) are suspended in 40 ul PBS containing 0.5 mg/ml Matrigel at RT and injected into the left lobe of SCID mice (immunodeficient, N=5/group). Groups were untreated control, treated starting at target day, and untreated control sacrificed at target day. In the first set of experiments, mice were sacrificed at day 15 post orthotopic injection then examined for visible tumors in all organs. Group 2 were sacrificed at day 30 post orthotopic injection and examined for visible tumors in all organs. Group 3 were started AT DAY 15 Post orthotopic injection of cells on treatment with inhaled aerosolized BIO-11006 (30 minutes every day, 100mM) for 15 days. The second set of experiments were carried out over a longer time course, 26 days before treatment began. Tumor growth and metastasis to lung, heart and diaphragm were counted post injection in each grouping in each experimental set.

Tail Vein Injections 2.5 X 10⁶ A549 cells were suspended in 200 µl of sterile PBS and injected intravenously via the tail vein of SCID mice (immunodeficient, N=8/group). The control group received aerosolized PBS while the second group received the aerosolized BIO-11006 (from -1 day before the cell line injection) and the third group received test compound BIO-11006 (from +3 days after the cell line injection) every other day for 7 weeks using a nebulizer. The empty chamber was filled with aerosol for 5 minutes before placing the mice in it. Mice remained in the chamber until the 5 mL test compound solution was completely aerosolized. The mice were monitored for body weight every other day for 7 weeks. A separate group of animals (n=7) from the same of batch of experimental animals served as normal control. Normal control animals neither received cell line injection nor the dosing. They served as monitoring group for the health status progression under the same experimental conditions with the other study groups. All animals from the normal group were sacrificed on Day 53 along with the other experimental groups.

Results

Total Number of Metastatic Tumors per Mouse

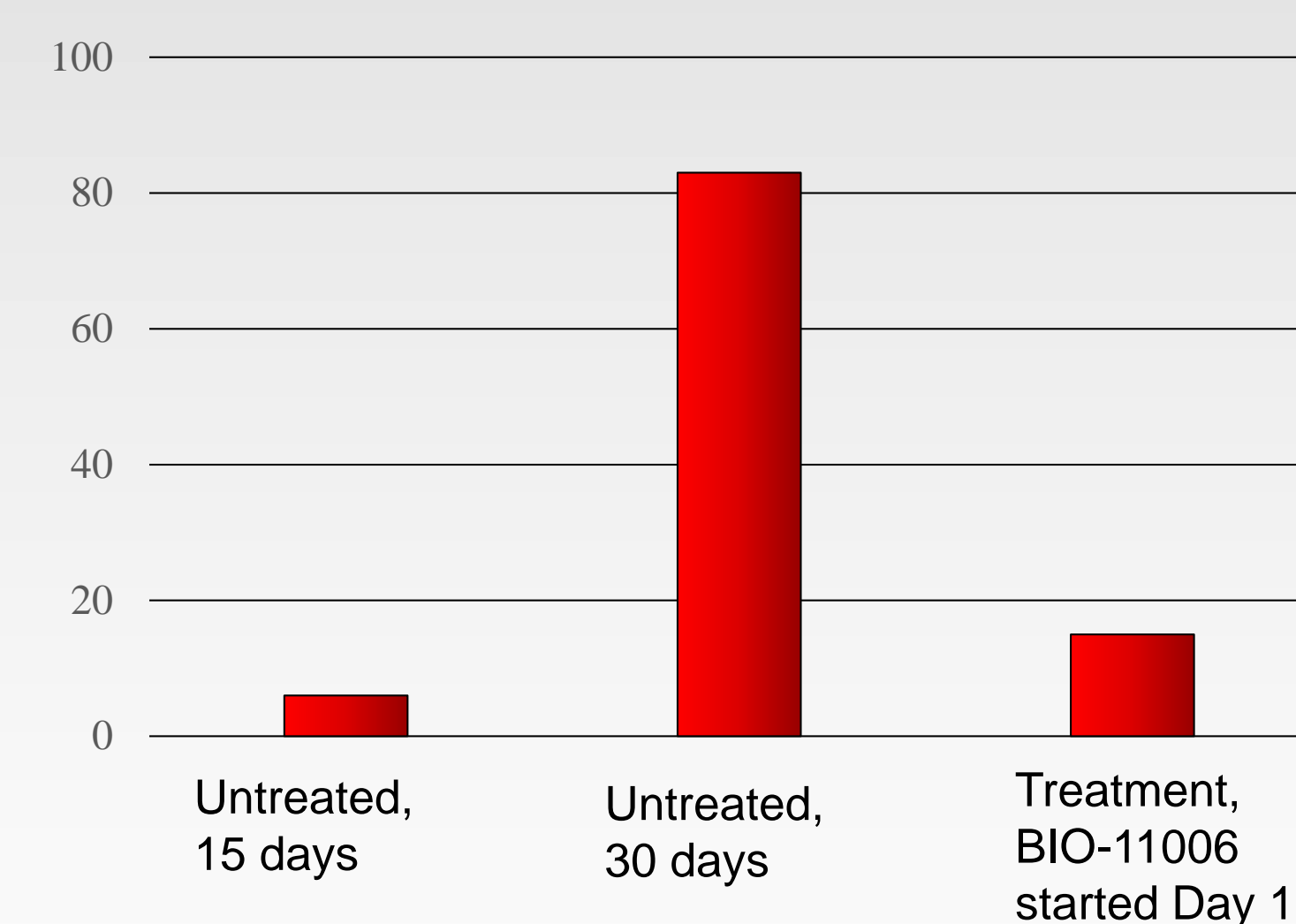


Figure 2. The number of metastatic tumors formed by PC9 cells orthotopically injected into the left lung lobe of SCID mice are shown. Treatment with peptide BIO-11006, starting on Day 27, significantly reduced the total number of tumors compared with untreated controls for the same 42 day time period.

Average Size (mm) of Primary Tumor

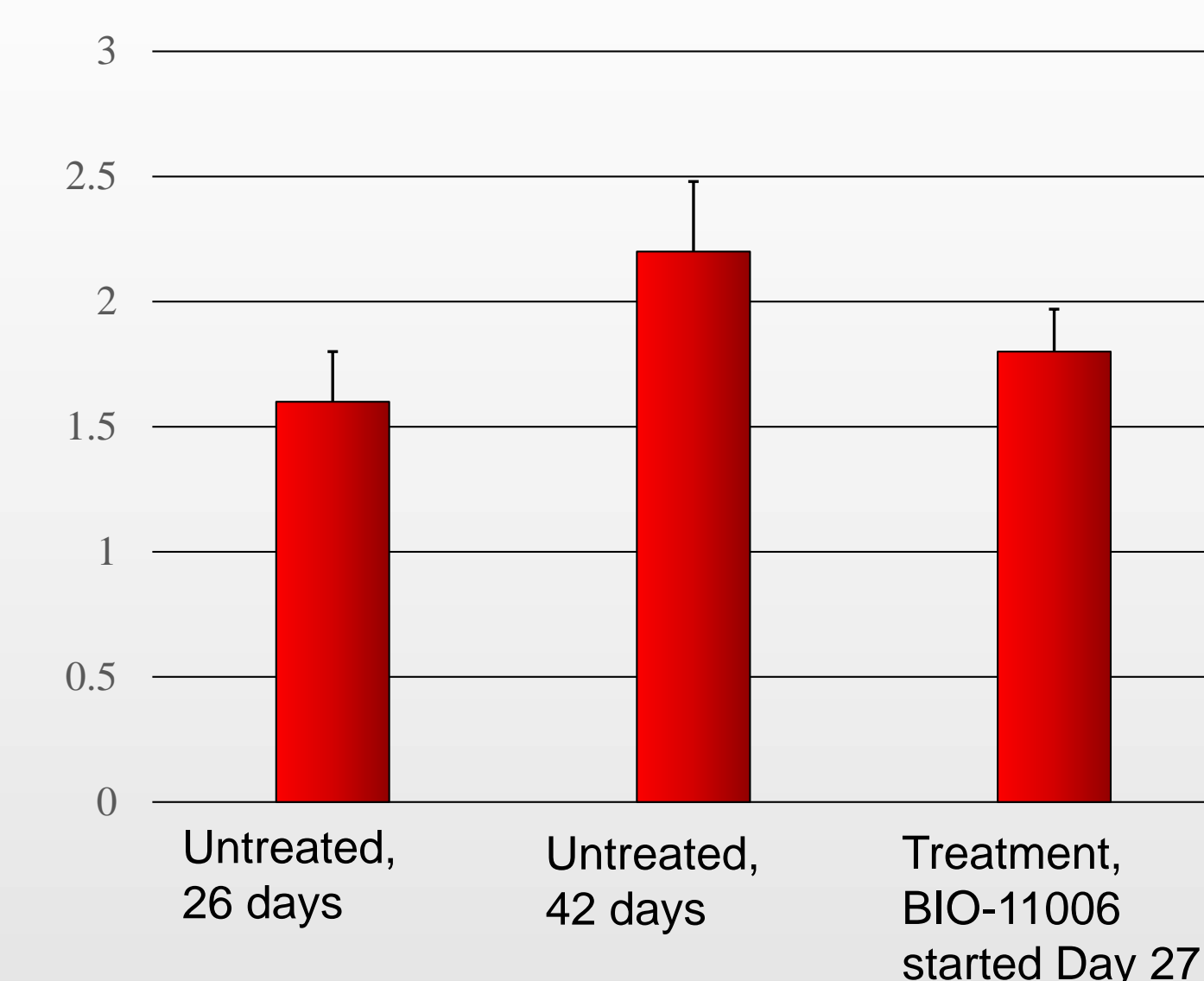


Figure 4. The total number of metastatic tumors formed by A549 cells that were tail vein injected decreased significantly with treatment of peptide BIO-11006. This decrease was evident whether treatment began before the instillation of the cancer cells or 3 days afterward.

Figure 1. The number of metastatic tumors formed by PC9 cells orthotopically injected into the left lung lobe of SCID mice are shown. Treatment with peptide BIO-11006, starting on Day 15, significantly reduced the total number of tumors compared with untreated controls for the same 30 day time period.

Average Number of Tumors per Mouse

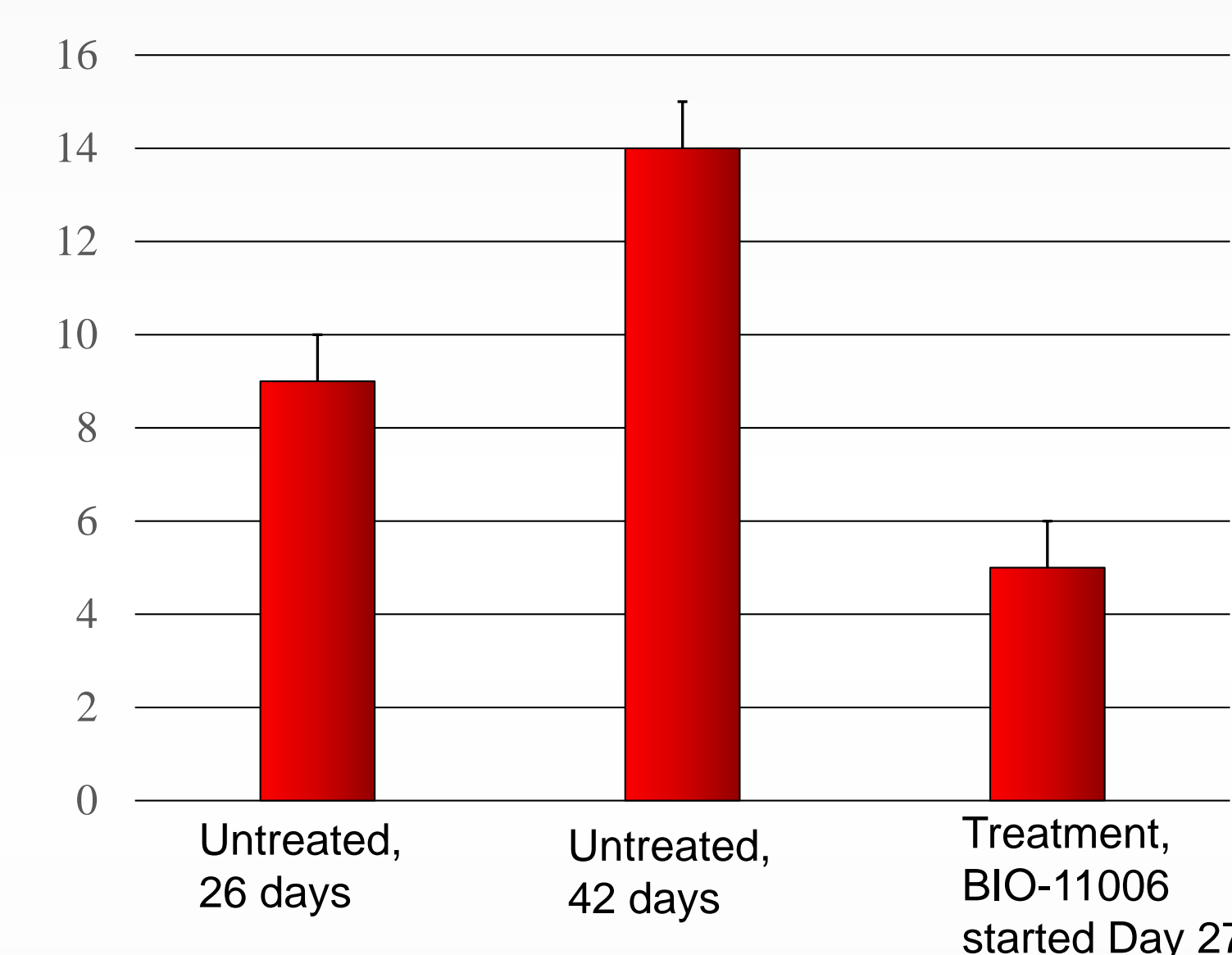
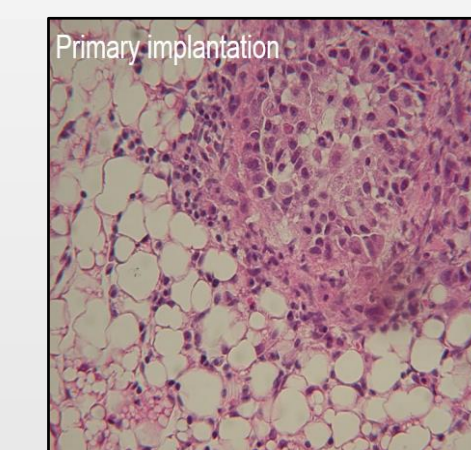
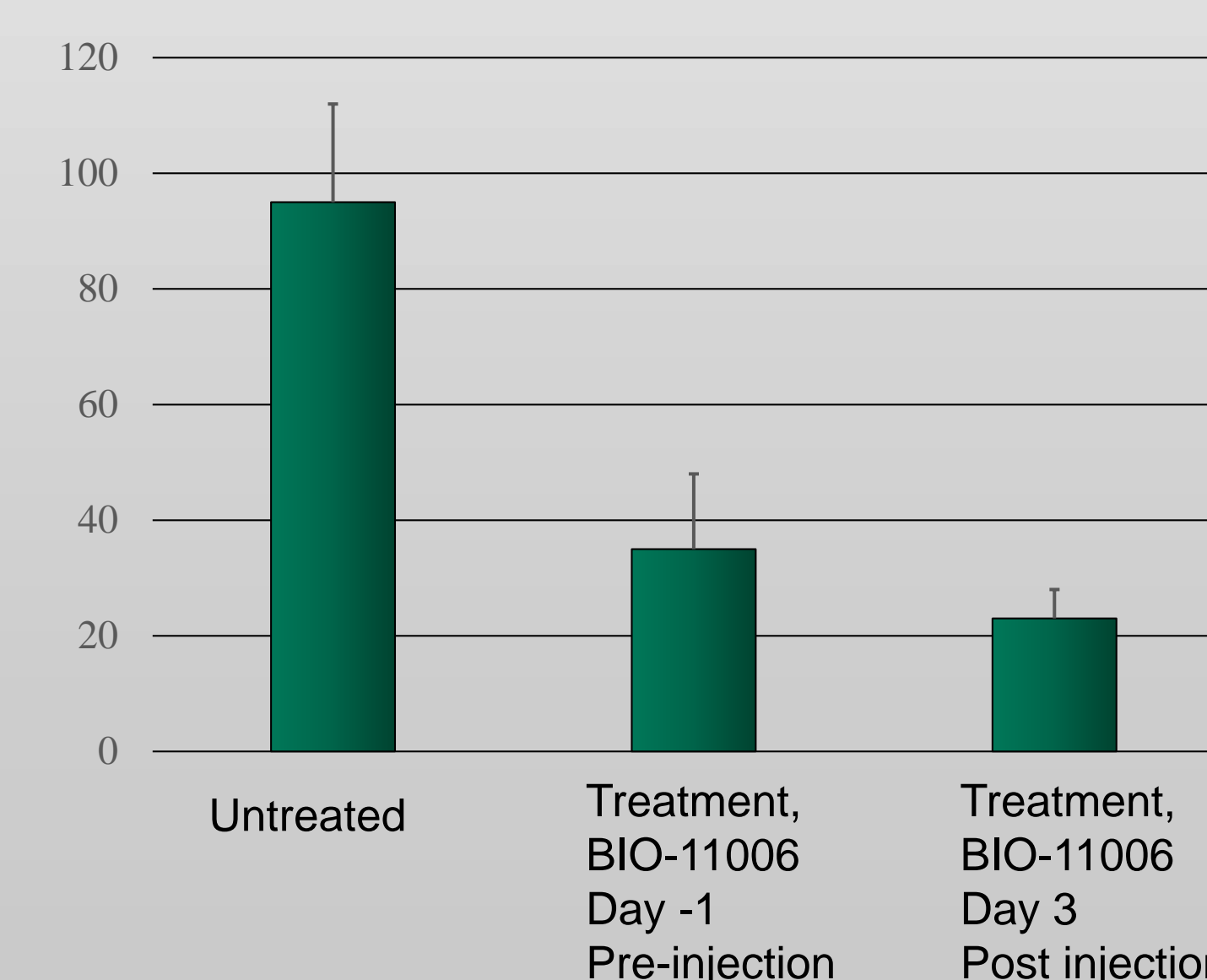


Figure 3. Measurements of the primary tumor, established in the left lung using PC9 cells, were made over the experimental time course. Primary tumors are readily distinguished histologically (inset). Their size is significantly decreased by treatment with BIO-11006.



Total Number of Metastatic Lung Tumors per Treatment Group



Conclusions

- Aerosolized treatment with peptide BIO-11006 is effective at limiting the development of metastatic tumors that form from orthotopically-injected PC9 cells and tail vein-injected A549 cells.
- Treatment of the orthotopically-injected mice starting when metastasis is widespread at 27 days appears to stop metastasis and stop the growth of the primary tumor.

Discussion

We have reported previously that MARCKS protein is a key molecule regulating cellular processes such as secretion, degranulation, and cell migration. We have also shown previously that MARCKS-like peptides inhibit or attenuate many of these processes. In the present study, BIO-11006 peptide, delivered as an aerosol, decreased the metastasis of cancer cells (PC9s) in two murine models.

Our goal was to test the efficacy of treating the induced lung cancers in mice well into the tumor growth and metastasis phase to mimic a human cancer patient presenting with metastatic NSCLC. In each of the murine models explored, peptide BIO 11006 significantly decreased the number of metastatic tumors. Although there was little change in the size of the primary tumor established in the orthotopically injected lung cancer model, the effect on secondary tumor formation was consistent.

Future experiments will add a chemotherapy drug to peptide BIO 11006 and test the effectiveness of the two types of drugs when combined. Combination therapy to address the primary tumor while halting its spread to other organs could prove a valuable new tool for cancer therapy.

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