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**INTERIM REPORT ON INTRATHORACIC RADIOTHERAPY
OF HUMAN SMALL-CELL LUNG CARCINOMA IN NUDE MICE
WITH Re-188-RC-160, A RADIOLABELED SOMATOSTATIN ANALOGUE**

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INTERIM REPORT ON INTRATHORACIC RADIOTHERAPY OF HUMAN SMALL-CELL LUNG CARCINOMA IN NUDE MICE WITH Re-188-RC-160

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INTRODUCTION

The purpose of this study was to evaluate the therapeutic efficacy of Re-188-RC-160 in experimental models of human small cell lung carcinomas which mimic the clinical presentation. In the experimental model, cells from the human small cell lung carcinoma cell line NCI-H69 cells were inoculated into the thoracic cavity of athymic mice and rats. Subsequently, the biodistribution of Re-188-RC-160 after injection into the pleural cavity, a radiolabeled somatostatin analogue, was monitored as was the effect on the subsequent growth of tumors. The results presented here, and which are a part of a larger series of studies, suggest that Re-188-RC-160 can be effectively used in this animal model to restrict the growth of small cell lung carcinoma in the thoracic cavity.

The cell line NCI-H69 was derived from a human small cell lung carcinoma and has been used in concert with experimental, therapeutic radiopharmaceuticals (radiolabeled antibodies) [1,5]. Additionally, in nude mice NCI-H69 tumors exhibit reduced tumor volumes when treated intra-lesionally with unlabeled somatostatin analogues, including RC-160, 12, 111. The cell line produces tumors when implanted subcutaneously or

introduced into the thoracic cavity or lung parenchyma [9].

RC-160 is a somatostatin analogue which has been extensively studied for its ability to restrict the growth of a variety of tumors [12]. RC-160 has been reported to be more potent than other analogues such as octreotide and BIM-23014 [4]. RC-160 can be directly radiolabeled with Tc-99m and Re-188 [3]. Rhenium-RC-160 has been reported to maintain the spatial topography of the side chains essential for somatostatin-receptor-binding as determined by NMR and computer modeling conformational analysis [13].

In a series of foundation studies to be presented elsewhere, the therapeutic potential of the somatostatin analogue RC-160 radiolabeled with ¹⁸⁸Re has been evaluated in *nu/nu* mice bearing xenografts of the human prostate adenocarcinoma PC-3, which is receptor-positive but poorly responsive to unlabeled RC-160. ¹⁸⁸Re-RC-160 was selectively retained in the tumor following direct intra-tumor injection and resulted in protracted reductions in tumor volume and an increase in survival time. Based on these initial successes the present studies were initiated to examine the feasibility of treating somatostatin-receptor-positive tumors using regional administration.

METHODS AND MATERIALS

Peptide labeling. RC-160 was synthesized by classical synthesis and supplied by DeBiopharm S.A. (Lausanne, Switzerland). ¹⁸⁸Re was obtained from a clinical prototype ¹⁸⁸W/¹⁸⁸Re generator [6] fabricated at Oak Ridge National Laboratory (Oak Ridge, TN, USA). The generator was eluted in 20 ml of oxidant-free 0.9% saline and collected in 5 ml aliquots. The second and third aliquots were used in experiments, and subsequently filtered through a 0.22 micron filter into a sterile evacuated vial. After use, the generator was flushed with approximately 20 ml of air and stored under a nitrogen atmosphere.

The RC-160 radiolabeling kits were prepared in 6 ml capacity amber vials and

contained a final volume of 2.0 ml. Each kit contained 500 μ g peptide in tartrate/phthalate buffer, pH 5.2, containing stannous tartrate to reduce the perrhenate plus excipients. All kits were prepared using nitrogen purged solutions and the head space gas was similarly purged with nitrogen gas. Vials were stored frozen at -30°C. For labeling, 2.0 ml of a 188 Re-perrhenate solution (15-20 mCi) was added (final labeling volume 4 ml), and the vial heated at 80°C - 90°C for 30 minutes with periodic mixing. At the end of the incubation period, the solution was allowed to cool slightly and an aliquot removed for radiochemical analysis. Prior to use in animals aliquots were mixed 1:1 with 20% human serum albumin (clinical-grade).

Radiochemical analysis. Radiochemical analysis was performed using thin layer chromatography (TLC) and reverse phase (RP) HPLC. TLC with a 0.9% saline mobile phase was used to measure the unreduced or unbound radionuclide which migrated with the solvent front. The amount of Re-188 at the solvent front was less than 3%. TLC with a mobile phase consisting of 85% ethanol:15% aqueous acetic acid pH 3.5 was used to determine the amount of radiocolloid. Under these chromatographic conditions radiocolloid was retained at the origin while the radiolabeled peptide migrated with the solvent front. RP-HPLC was performed using a C₁₈ column (250 mm X 4 mm, 7 μ average particle size) in a Kontron chromatographic system with the effluent tubing attached in-line to a Raytest radioactivity detector. A linear gradient was used to elute the peptide [100% A to 66% B in 30 minutes using a flow rate of 1 ml/minute where A = 0.05 M triethyl ammonium phosphate (TEAP), pH 2.5, and B = acetonitrile (2:3)]. The elution profile revealed a main peak (approximately 90%) which eluted near 23 minutes and a smaller peak which eluted at a slightly earlier time.

Biodistribution studies. Biodistribution studies were performed in adult female

nu/nu mice at selected times after injection into the pleural cavity. Each experimental group was composed of at least five animals, with each animal receiving 0.1 ml containing approximately 4 μ Ci. Animals were sacrificed by ether overdose, and selected organs dissected, weighed, and associated radioactivity determined. The data was calculated as the percentage dose per gram of tissue, although in some cases the data was also calculated as the percentage dose per organ.

Effects on tumors. The tumor model using NCI-H69 cells inoculated in the thoracic cavity followed generally the model described by McLemore et al. [9]. In these studies animals were inoculated with $5-7.5 \times 10^6$ NCI-H69 cells in 0.1 ml of serum-free RPMI medium. The cells were introduced by injection with a 26 guage needle from a position ventral and midline over the liver and under the rib-cage. Proper positioning was previously confirmed using dye injection.

The test materials (RC-160 and Re-188-RC-160) were similarly injected into the pleural cavity with a 26 guage needle from a position ventral and midline over the liver and under the rib-cage. Each injection contained approximately 5 μ g of peptide in a volume of 0.1 ml and a radioactive dose (when used) of 200 μ Ci.

RESULTS

Animal model. Cells from the cell line NCI-H69 were injected into the thoracic cavity of athymic mice and athymic rats. Tumor development was more consistent in athymic mice as compared to athymic rats, and tumor growth appeared to be slower than that described in the literature [9]. In both mice and rats tumors could be found on diaphragm, heart, vena cava, pleural surface, pleural and pericardial membranes, and on the rib cage wall. The surfaces of the lungs frequently evidenced erythema and blood vessels on the heart surface were frequently pronouncedly large. A pleural effusion was

found in one rat and a volume of 8 ml of effusion was recovered (5% of total body weight).

Biodistribution. Biodistributions were conducted in athymic mice inoculated into the thoracic cavity 24 hours previously with 7.5×10^6 tumor cells. After 4 hours significant accumulations of radioactivity were found associated with the lungs, heart, intestines, and chest wall (Figure 1, panel a). A 1 ml wash of the thoracic cavity (prior to organ removal) recovered nearly 5% of the total injected dose. Lesser amounts of radioactivity were associated with the liver and kidneys. After 24 hours, the lung retained the highest percentage of the injected dose/gram, although significant accumulations were found associated with the chest wall, heart, and in a wash of the thoracic cavity.

A comparison was made of the amount of Re-188-RC-160 associated with the thoracic cavity in animals which had been inoculated with NCI-H69 cells in the thoracic cavity compared to that found in animals which received no tumor cells (Figure 2). Tumored animals had a markedly higher retention, especially after 24 hours.

Effects on tumors. In an initial study, athymic mice were injected with 7.5×10^6 tumor cells into the thoracic cavity. The animals were a) treated on 1 day and 5 days with $200 \mu\text{Ci}$ doses of Re-188-RC-160 or b) received no treatment. After 28 days the animals were euthanized and the thoracic cavity examined. In the group treated with Re-188-RC-160, no evidence of tumors was found in 8/10 animals, while 2/10 animals had minimal disease. In the group with no treatment, 7/7 exhibited local disease restricted to the thoracic cavity. In all cases the visible tumor burdens were low. No alterations in overall lung morphology were observed in "normal" animals administered similar dose regimens of Re-188-RC-160.

In a second study, athymic mice were injected with 5.0×10^6 tumor cells into the

thoracic cavity. The animals were then a) treated with Re-188-RC-160 on days 14, 17, and 25, b) treated with RC-160 alone (on the same days and with the same amount of peptide), or c) left with no treatment. Results are found in Figure 3 and Table 1. Figure 3 illustrates the average body weight following the treatments while Table 1 illustrates the effect of the intra-thoracic treatment on the tumor occurrence. Animals treated with RC-160 and Re-188-RC-160 exhibited an initial loss of weight following treatments. This loss of weight appears to resolve with time. In the animal group treated with Re-188-RC-160, either no evidence of tumor or minimal tumor burdens were found in 5/5 animals at 48 days after initial inoculation with tumor cells. On the other hand, 3/3 of the animals treated with only RC-160 had tumors and 5/5 of the animals which received no treatment had tumors.

DISCUSSION

In the present interim report, data are presented on the experimental evaluation of Re-188-RC-160, a radiolabeled somatostatin analogue, for treatment of small cell lung carcinoma using an animal model with direct relevance to clinical settings. The results presented here are a part of a larger study and are consistent with tumor targeting and consequent therapeutic effect of the radiolabel.

Small-cell lung cancer represents 20-25% of all lung cancers and has a tendency for metastasing [10]. In terms of local disease, approximately 33% of all small lung cell carcinomas exhibit metastasis to the pleura; 13% to the chest wall and diaphragm; 18% to the pericardium; and nearly 95% have lymph node involvement. Small-cell lung carcinomas are initially radiation- and chemo-sensitive, however, post-therapy patients frequently undergo recurrence and the tumors become refractory to repeated therapy.

Salvage radiotherapy for intrathoracic small cell lung carcinomas that has progressed on

combination chemotherapy is not very effective. Short-term palliation and occasional long-term survivors have been reported, but most patients die within a few months. Generally, the long-term survival of patients with small cell lung carcinoma is bleak; and until recently it was unusual for a patient to survive for 5 years regardless of the treatment modality. The improvements in the therapy of small cell lung carcinoma have come with high-dose, combination-chemotherapy and combined-modality-chemotherapy plus radiotherapy; however these regimens result in significant toxicity. Third line therapeutic options would be of substantial benefit in improving the life expectancy and quality of life of patients with small cell lung carcinoma.

Radiolabeled somatostatin analogues appear to bind *in vivo* to the majority of small cell lung carcinomas examined and *in vitro* these tumors generally express somatostatin receptors [7]. Such studies indicate that small cell lung carcinoma may be an excellent target for somatostatin mediated therapy. However, unlabeled somatostatin appears to have a limited ability to restrict the growth of small cell lung carcinoma in experimental or clinical settings [8] when delivered systemically. Additionally, the tumor uptake of somatostatin analogues when delivered systemically is quite low, due at least in part to the rapid clearance rate.

The low absolute tumor uptake of somatostatin analogues when delivered systemically clearly suggests that alternative administration strategies are needed for effective therapeutic use. Thus, we have focused on the local and regional administration of Re-188-RC-160. In this report, an anti-tumor response was observed using Re-188-RC-160 administered into the pleural cavity. Comparison with results using RC-160 demonstrates that the therapeutic response is due to the Re-188-RC-160, and not to just the peptide alone. Transient weight loss was the only visible evidence of treatment.

It is interesting to consider the implications of Re-188 energy deposition ($r_{50} = 3.3$ mm) when Re-188-RC-160 is administered in the thoracic cavity. The energy deposition is locally restricted. Upon initial administration, the energy would be expected to be dispersed across the pleural and visceral surfaces. Any radiolabeled peptide which clears in the blood pool could re-access the tumor via the vascular supply. With localization, the energy would be deposited in tumor zones accessed by the radiolabeled peptide. Unbound peptide in the blood pool is cleared rapidly, thereby minimizing the radiation dose to the normal lung and other normal organs.

CONCLUSIONS

Cells from the human small cell lung carcinoma cell line NCI-H69 cells were inoculated into the thoracic cavity of athymic mice and rats. Subsequently, the biodistribution of Re-188-RC-160, a radiolabeled somatostatin analogue, was monitored, as was the effect on the subsequent growth of tumors. The results suggest that Re-188-RC-160 can be effectively used in this animal model to restrict the growth of small cell lung carcinoma in the thoracic cavity. The results are consistent with tumor targeting and a general lack of retention of Re-188-RC-160 in normal organs such as liver, spleen, and kidney. Ongoing studies continue to focus on this animal model system and be extended to include histology, autoradiography, and additional biodistribution studies.

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FIGURE 1. Biodistribution of Re-188-RC-160 following injection into the pleural cavity of *nu/nu* mice xenografted with NCI-H69 small cell lung carcinoma cells. Inocula of 7.5×10^6 NCI-69 small cell lung carcinoma cells in 0.1 ml were injected into the pleural cavity. After 24 hours, approximately 4 μ Ci of Re-188-RC-160 was similarly injected. The top panel shows the average % injected dose/gram of specimen at 4 hours with the bottom panel showing results at 24 hours. The thorax was washed with 1 ml of 0.9% saline prior to removal of the organs. (n = 5).

- * Figure 2. Comparison of the retention of Re-188-RC-160 administered in the thoracic cavity in tumored and normal animals after either 4 hours or 24 hours. The data was calculated from the % injected dose/gram with 5 animals used in each experimental group at each time point.

- Figure 3. Comparasion of the total body weights of *nu/nu* mice inoculated in the thoracic cavity with 5.0×10^6 NCI-H69 cells and subsequently either not treated (control); treated with Re-188-RC-160; or treated with Re-160. Re-188-RC-160 was used at a radioactive dose of 200 μ Ci. RC-160 was used at a comparable dose of peptide as with Re-188-RC-160 and in a similar formulation, however, no radioactivity was used. The arrows indicate the days of treatment (days 14, 17, 25). The data points are the average from 10 animals in each group.

TABLE 1 *Effect of intra-thoracic treatment of athymic mice initiated two weeks after inoculation in the thoracic cavity of 5.0 X 10⁶ NCI-H69 small cell lung carcinoma cells. Each X marks the response from an individual animal and - indicates that no animal was observed to exhibit this response.*

	TYPE OF TREATMENT		
	Re-188-RC-160	RC-160	NONE
no evidence of tumor	XXXXXX	-	
minimal tumor burden	XXX	X	X
confined tumor burden	X	XXXX	XXXXXX
extended tumor burden	-	XXX	XXX
death from tumor	-	-	-

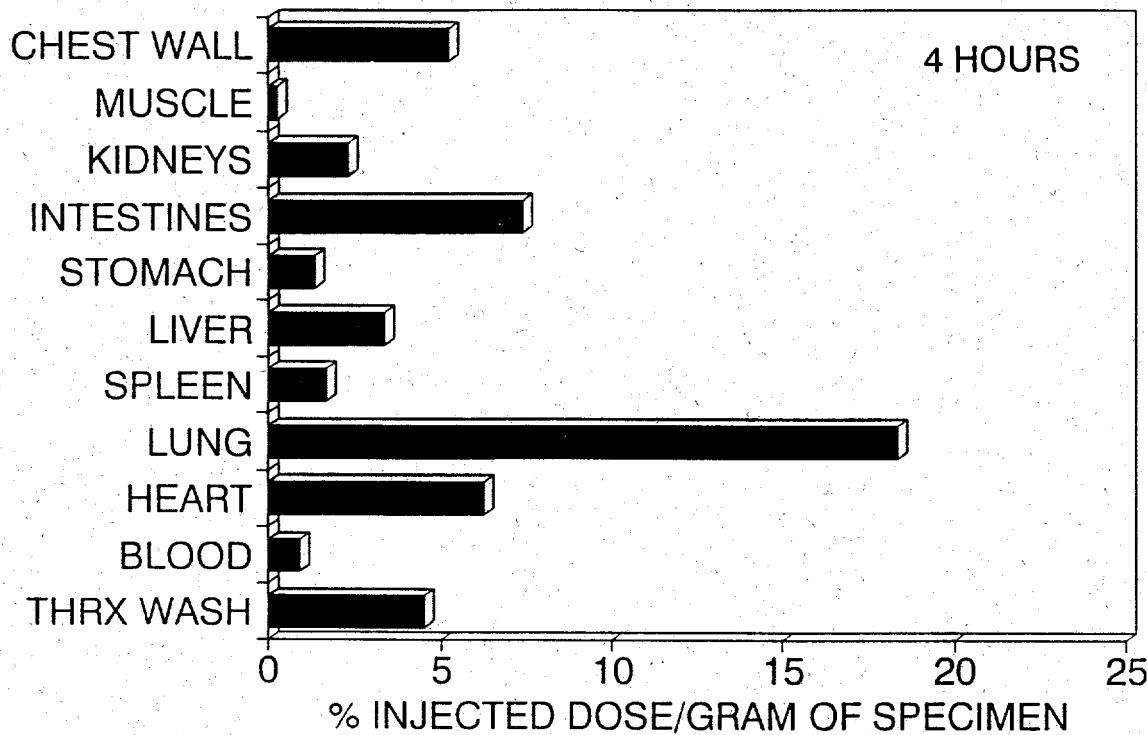
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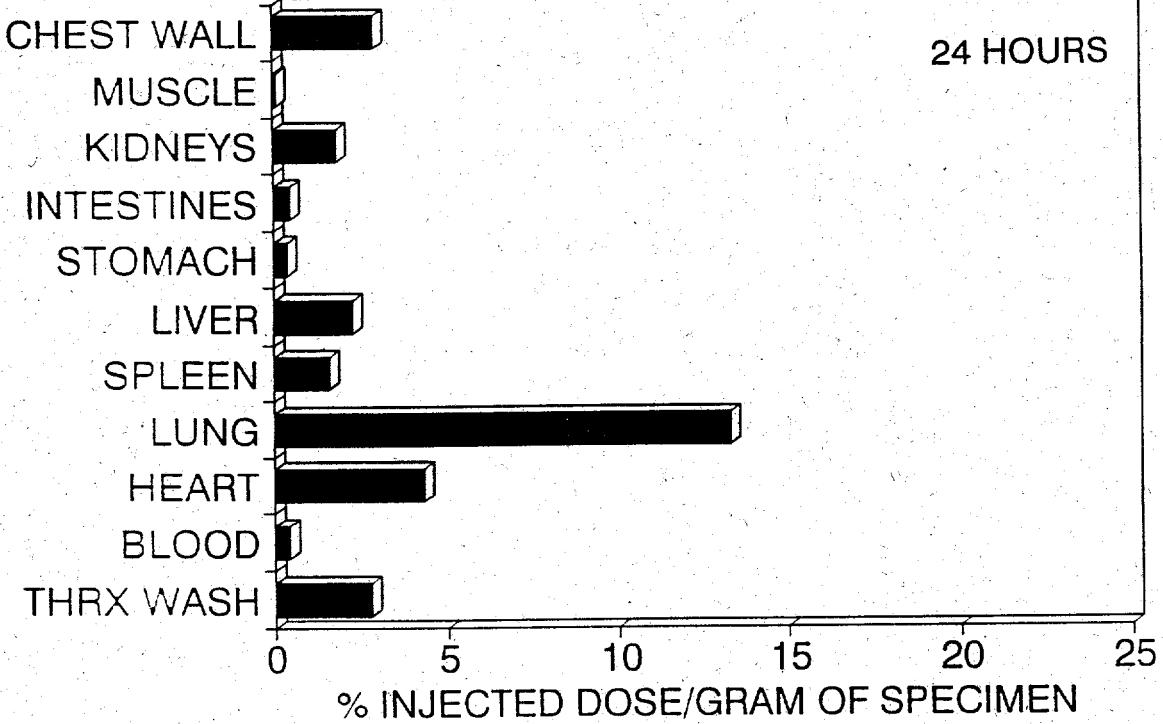
day 0 -- 5 x 10⁶ cells injected in 0.1 ml serum-free medium

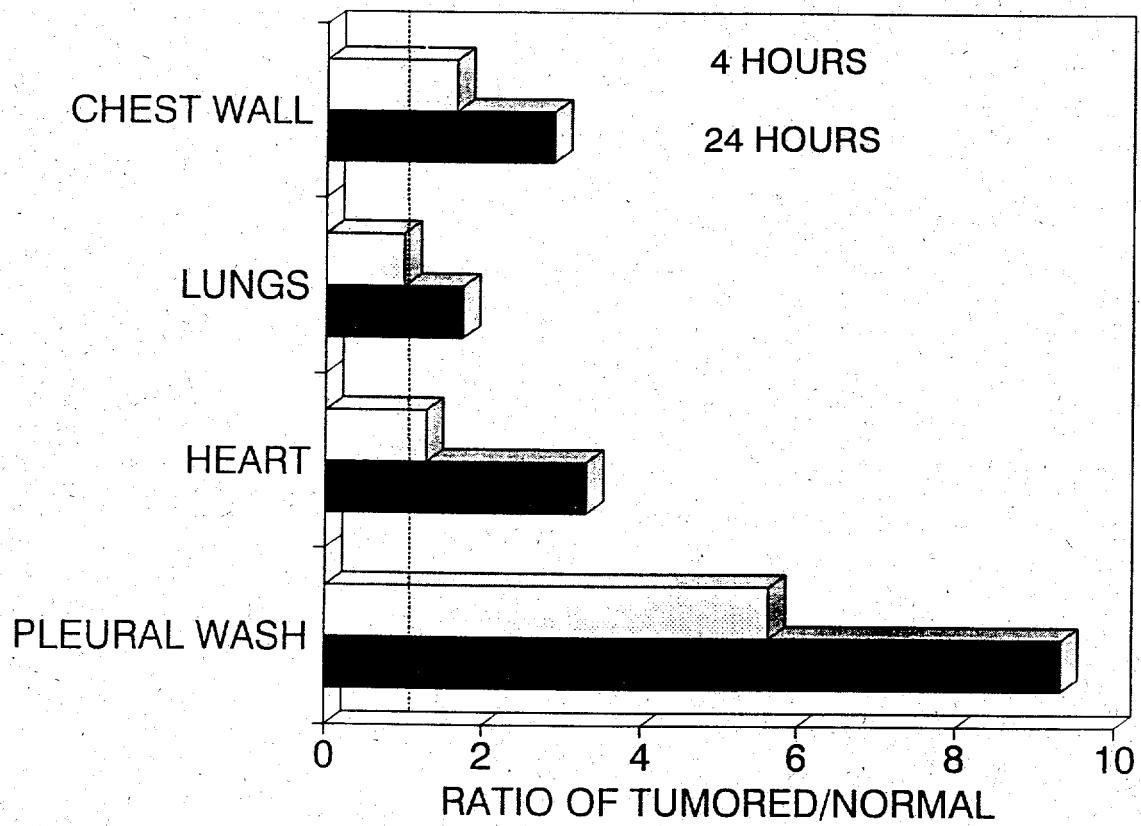
day 14, 17, 25 -- animals injected with 0.1 ml Re-188-RC-160 or RC-160

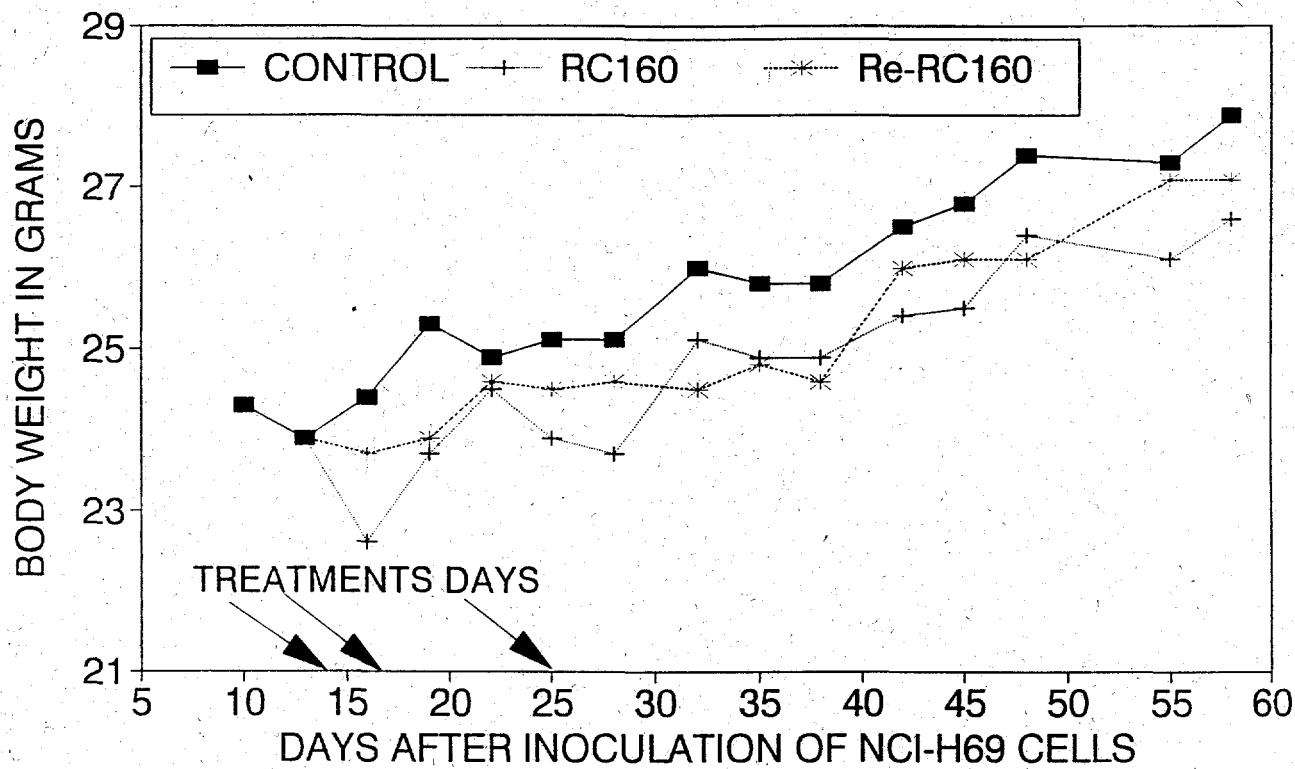
day 58 -- experiment ended

Minimal tumor burden -- local tumor with no evidence of invasion; confined tumor burden -- tumor confined to thorax and present in multiple sites; extended tumor burden -- tumor in thorax with invasion or metastasis to sites outside of the thorax









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