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**Efficacy Evaluation of a Twelve Peptide Mixture Against
Established, Estrogen supplemented MCF-7 Human Mammary Carcinoma
Xenografts**



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Executive Digest

A twelve peptide mixture was administered either intravenously or intratumorally once daily for 14 consecutive days against established, estrogen supplemented MCF-7 human mammary carcinoma xenografts. Treatment was well tolerated but inactive regardless of dosage level or route of administration.

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Introduction

The purpose of this study was to evaluate the efficacy of a twelve peptide mixture against established, estradiol supplemented MCF-7 human mammary carcinoma xenografts. The peptide mixture was administered, intravenously or intratumorally, once daily for fourteen consecutive days.

Materials and Methods

Chemicals

Twelve peptides (labeled in consecutive order from 4061559-4061570) were obtained from Sanare as white powders. The intent was to administer an amount of each peptide in the mix such that the final aggregate concentration in the mouse blood volume (~2ml) was 0.8mg/ml after each dose. The peptide mix was prepared by first adding 5ml of phosphate buffered saline (PBS) to each vial of powder to achieve a concentration of 2mg/ml for each peptide. A further dilution was made to account for each of the peptide's molecular weight to provide equimolar concentrations of each peptide at 0.35 μ M. Equal amounts of each of the twelve peptides were then combined to form the top dose solution (Appendix 10). The top dose solution was aliquoted out for each dosing day and frozen at -80°C until use. On each day of treatment, one frozen vial of top dose solution was removed from the freezer and thawed at room temperature. The dosing solution was clear and colorless with a pH value of 8. Lower dosages were prepared by direct dilution of the top dose with the appropriate amount of PBS diluent.

Animals and Husbandry

Female athymic mice (nu/nu) were obtained from Harlan. They were 6-7 weeks old on Day 1 of the experiment. The animals were fed irradiated Rodent Diet 5053 (LabDiet™) and water *ad libitum*. Mice were housed in static cages with Bed-O'Cobs™ bedding inside Biobubble® Clean Rooms that provide H.E.P.A filtered air into the bubble environment at 100 complete air changes per hour. All treatments, body weight determinations, and tumor measurements were carried out in the bubble environment. The environment was controlled to a temperature range of 70° \pm 2°F and a humidity range of 30-70%.

MCF-7, human mammary carcinoma, is an estrogen-dependent tumor model, and all of the animals were implanted with 17- β estradiol pellets (1.7mg/pellet, 90 day release) from Innovative Research of America (Cat.#NE-121). The pellets were implanted subcutaneously with a 10-gauge trocar needle one day (Day -1) prior to tumor implant. Blood levels of 17- β estradiol were not monitored during the experiment.

Test animals were implanted subcutaneously on Day 0 with 30 to 60mg tumor fragments using an 11-gauge trocar needle. All animals were observed for clinical signs at least once daily. Animals with tumors in excess of 1g or with ulcerated tumors were euthanized, as were those found in obvious distress or in a moribund condition. All procedures carried out in this experiment were conducted in compliance with all the laws, regulations and guidelines of the National Institutes of Health (NIH/PHS) and with the approval of MIR's Animal Care and Use Committee.

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Treatment

Treatments began on Day 8, when the mean estimated tumor mass for all groups in the experiment was 122mg (range of group means, 120-125mg). All animals weighed $\geq 18.8\text{g}$ at the initiation of therapy. Mean group body weights at first treatment were well-matched (range of group means, 21.6-25.7g). All animals were dosed according to individual body weight on the day of treatment (0.2ml/20g) as indicated in the protocol (Appendix 1).

Measurements and Endpoints

Testing in this experiment was generally carried out adhering to the general principles established by the groups of Schabel, Skipper, Griswold, Corbett, Leopold, Ross and the NCI (1-7). Body weights and tumor measurements were recorded twice weekly. Tumor burden (mg) was estimated from caliper measurements by the formula for the volume of a prolate ellipsoid assuming unit density as: Tumor burden (mg) = $(L \times W^2)/2$, where L and W are the respective orthogonal tumor length and width measurements (mm).

The primary endpoints used to evaluate efficacy were: tumor growth delay, complete and partial tumor response, and the number of tumor-free survivors at the end of the study. A complete response (CR) is defined as a decrease in tumor mass to an undetectable size ($<50\text{mg}$), and a partial response (PR) is defined as a $\geq 50\%$ decrease in tumor mass from that at first treatment. PRs are exclusive of CRs, as are Tumor-Free Survivors (TFS). Tumor Growth Delay (T-C) was also used to quantify efficacy. Tumor growth delay for this experiment was expressed as a T-C value, where T and C are the median times in days required for the treatment and control group tumors, respectively, to grow to a selected evaluation size, 750mg.

Net Log_{10} Tumor Cell Kill (Net Kill) was used as a secondary efficacy endpoint. Net Kill is the change in tumor burden (logs) over the treatment period. Traditionally Net Kill is estimated by the method of Schabel et al (1,3) but this method requires that initial and post treatment regrowth rates be equal, a condition that may not be met by certain differentiation therapies or those that induce significant tumor bed effects. We instead use a modification of the method of Ross et al (7), that can accommodate any regrowth rate and which simply assumes that the tumor regrowth rate between dosage levels are similar to that observed post treatment. This method simply extrapolates (or interpolates) the regrowth curve back to the last day of treatment for the calculation of Net Kill. The two methods converge to the same result if initial and regrowth rates are similar. The Net Kill value allows quantitative comparison of efficacy across multiple experimental protocols and across models by normalizing the efficacy data for treatment regimens of varied duration and differences in tumor growth rates between experiments or models. Positive values indicate that an actual reduction of tumor burden had occurred at the end of therapy relative to the pretreatment burden. Negative values indicate the tumor grew (although possibly more slowly than the control tumors) during treatment. Thus negative Net Kill values do not necessarily imply a complete lack of activity. Tumor-free survivors and tumor-bearing survivors whose tumors had not reached the Evaluation Size by the last day of the experiment were assigned a Time to Evaluation Size of $>$ the last day of the experiment and were included in calculations of Tumor Growth Delay and Net Kill.

The calculation of Net Kill by either method requires the establishment of the tumor doubling time (Td), which is the time in days for the tumor burden to double. Doubling time (Td) was estimated from the least squares best-fit straight line from a log-linear plot

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of tumor burden vs. time over the period of exponential growth (~200 to ~800mg range). This parameter was calculated and tabulated in Table 1 for every animal in the experiment.

Assessment of Side Effects

All animals were observed for clinical signs at least once daily. Animals were weighed on each day of treatment and at least twice weekly thereafter. Individual body weights were recorded twice weekly.

Treatment-related weight loss in excess of 20% is generally considered unacceptably toxic. In this report, a dosage level is described as tolerated if treatment-related weight loss (during and two weeks after treatment) is <20% and mortality during this period in the absence of potentially lethal tumor burdens is ≤10%.

Upon death or euthanasia, all animals were necropsied to provide a general assessment of potential cause of death and perhaps target organs for toxicity. The presence or absence of metastases was also noted. Remarkable observations of clinical signs and necropsy findings have been tabulated in Appendix 4. Individual and group toxicity findings have been summarized in Tables 1-3 and Figure 3.

Statistics

The median times to evaluation size for all study groups were first analyzed by application of the Kruskal-Wallis rank sum test analysis to determine if any significant differences existed between any study groups. Upon identification that significant differences exist, post-hoc multiple comparisons to a specified control group were conducted using an additional Kruskal-Wallis test followed by the multiple comparison procedure of Dunn.

Statistical significance was determined using built-in Microsoft Excel data analysis tools, SigmaStat 3.0, and the R-Project (8).

Data Retrieval

MIR Preclinical Services retains permanent “active” copies (on CD) of all experiments unless instructed otherwise, including active graphics applications.

Results/Discussion

Tumor Growth/General Observations/Controls

The mean estimated tumor burden for all groups in the experiment on the first day of treatment was 122mg and all of the groups in the experiment were well-matched (range, 120-125mg). All animals weighed ≥18.8g at the initiation of therapy. Mean group body weights at first treatment were also well-matched (range, 21.6-25.7g). A tumor burden of 750mg was chosen for evaluation of efficacy by tumor growth delay. The Median Control Tumor reached evaluation size on Day 17.8, and the tumor volume doubling time for the Control Group was 3.8 days (Table 2). Control animals experienced a 0.8g mean weight loss during the treatment regimen. There were no spontaneous regressions in the Control Group. Thioglycolate cultures of donor (2/2) tumors used for implantation of this study were negative for gross bacterial contamination. Based on

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historical data for this model, the biology of the Control Group was judged to be within the normal range.

Toxicity

Treatment with the peptide mixture was well tolerated at 80mg/kg (IV) resulting in no treatment-related mortalities and a treatment-related weight loss of 7.4% (1.9g). The lost weight was not recovered prior to the end of the study (Tables 1 and 2, Figure 3). One animal died on Day 24, a necropsy could not be conducted due to the body being too decomposed. Another animal had an ulcerated tumor and was removed from the study. Upon necropsy of the remaining animals, there were no remarkable findings (Appendix 4).

Animals #1 and #2 were euthanized on Study Day 26 due to tumor burdens >1g. At the client's request, the tumors were excised and flash frozen in liquid nitrogen. They were then stored at -80°C until shipment to the client.

Treatment with the peptide mix was well tolerated at 40mg/kg (IV) resulting in no treatment-related mortality and a maximum treatment-related body weight loss of 6.4% (1.5g). The lost weight was not recovered prior to the end of the study (Tables 1 and 2, Figure 3). There were no remarkable findings upon necropsy (Appendix 4).

Treatment with the peptide mix at 20mg/kg dosed intravenously and at 80mg/kg dosed intratumorally was well tolerated resulting in neither treatment-related mortalities nor weight loss associated with the treatment (Tables 1 and 2, Figure 3). Upon necropsy, there were no remarkable findings (Appendix 4).

Efficacy

Daily intravenous treatments with the twelve peptide mixture at all dosage levels (80, 40, and 20mg/kg) was ineffective, producing minimal tumor growth delays ranging from 1.7 to 0.4 days and negative net tumor cell kill values ranging from -0.48 to -0.55logs, which indicate tumor progression during treatment. Treatment produced neither complete/partial tumor regressions nor tumor-free survivors (Tables 1, 2 and 3).

Daily intratumoral treatments with the twelve peptide mixture at 80mg/kg, was also inactive producing a tumor growth delay of 1.1 days with an associated net tumor cell kill of -0.53log. Treatment produced neither complete/partial tumor regressions nor tumor-free survivors (Tables 1, 2 and 3).

Treatment with the twelve peptide mixture was well tolerated but completely ineffective against established, estradiol supplemented MCF-7 human mammary carcinoma xenografts. The anti-cancer activity produced was minimal at best, with virtually identical activity across all dosage levels and routes of administration. Weight loss was minimal when compared to that of the vehicle-treated control group; therefore, a follow-up study should be considered with higher dosage levels.

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Glossary

Apparent Net Tumor Cell Kill – The net change in tumor burden (measured in logs) between the first and last treatments. An equation is derived for the tumor growth of each mouse in the experiment from a least squares best fit of the exponential portion of the tumor growth curve post treatment. This equation is solved to give the apparent tumor burden surviving the last day of treatment for each animal. Median values are used for comparison of treatment groups to the controls to derive the net reduction in tumor burden during treatment (Net log₁₀ Kill, or NK_{apparent}). This modification of the methods of Schabel et al. (1, 3) and Ross et al (7) allows an estimate of reduction in tumor burden even in the circumstance of tumor regrowth rates that are significantly different from the control growth rates. In general the method of Schabel overestimates net reduction in tumor burden (Net Kill) values. NK_{apparent} values faithfully estimate the tumor burden at last treatment, but they don't really estimate how much of the effect was due to cell killing and how much was due to hindered regrowth during treatment if the regrowth rates are dissimilar to initial or control growth rates. The term Net Kill is actually a misnomer for the combined effects of cell killing and hindered tumor growth between treatments. With the long treatment regimens in common use today, the relative contribution of hindered regrowth to the NK_{apparent} can be large. However the relative contributions of cell killing and hindered regrowth can be estimated. These questions can be important to those designing cytotoxic therapies or those dealing with compounds of mixed mechanisms. For this reason we also report fractional effect values that estimate the relative contributions of cell killing and slowed regrowth to the net change in tumor burden during therapy (NK_{apparent}). As always NK_{apparent} values normalize the data for differences in duration of treatment and also tumor doubling times across tumor models and experiments, allowing quantitative comparison between experiments. Two underlying assumptions are required for our calculations: (1) all treatment fractions produce equivalent cell killing, and (2) the regrowth rate between fractions is identical to the post treatment regrowth rate. These assumptions are also required for the traditional Schabel calculations, but those carry the additional assumption that the regrowth and initial growth rates are equal. (*Group efficacy parameter*)

Complete Regression (CR) – An animal is credited a complete regression if the tumor burden is reduced to an immeasurable volume at any point after the first treatment. Our convention is to record any tumor measurement less than 3 mm as a "0". This is in keeping with the convention of the NCI and reflects the inherent and unacceptably high mechanical error in such measurements in addition to the uncertain biology of what is measured at those small sizes (4). (*Individual efficacy parameter*)

Day 0 – The day tumors are implanted into the animals (Not to be confused with the first day of treatment which is always indicated relative to Day 0).

Day of Max. Body Wt. Loss – The day of body weight nadir (if any) between the first day of treatment and 2 weeks after the last day of treatment. Reported as "NA" if no weight loss occurred. (*Group toxicity parameter*)

Evaluation Size – The tumor burden (mg) selected for calculation of tumor growth delay. The Evaluation Size is selected from the exponential portion of the control tumor growth curve where the error of measurement tends to be minimal (usually between 500 and 1000mg).

Fractional Effect - When initial and post-treatment regrowth rates are different it is possible to estimate the relative contributions of hindered regrowth rate and tumor cell killing to the observed net reduction in tumor burden (NK_{apparent}). This is accomplished by first calculating the Gross Kill for the group (Schabel (1)) based on the median value calculated from best fit equations for the actual regrowth data for each mouse in the experiment. The Gross Kill is then used to calculate a theoretical Net Kill value (NK_{corrected}) that would have been obtained if the tumors had re-grown at their original (pretreatment) growth rates. The ratio of the NK_{corrected} and NK_{apparent} values provides an estimate of the percentage of the therapeutic effect that was due to actual reductions in cell number (Tumor cell kill (%)). The rest of the effect (Hindered Regrowth (%)) is due to hindered post treatment regrowth rates during the treatment regimen. In studies with long durations of treatment, the relative contribution of hindered regrowth to NK_{apparent}

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can be large, even for small differences between growth and regrowth rates. (*Group efficacy parameter*)

Maximum Rx-Related Body Wt. Loss – This is meant to reflect treatment-related weight change. Calculated as the maximum weight loss occurring anytime between the first day of treatment and two weeks from the last day of treatment, expressed as a percentage of the weight on the first day of treatment. (*Group toxicity parameter*)

Partial Regression (PR) – An animal is credited with a partial regression if its tumor burden decreases to less than half of the tumor burden at first treatment. PRs are tabulated exclusive of CRs. (*Individual efficacy parameter*)

Percent Body Wt. Change – Change in body weight between first and last days of treatment expressed as a percentage of the body weight on the first day of treatment. If actual body weight measurements were not recorded on precisely the first and last days of treatment, these values are calculated by interpolation between the closest measurements on either side of the target date. (*Individual toxicity parameter*)

% Rx Related Deaths – The percentage of animals presumed to have succumbed to treatment-related toxicity expressed as a percentage of the number of evaluable animals in the group. An animal is presumed to experience a treatment-related death if it is found dead or is euthanized in moribund condition within 2 weeks of the last treatment with a tumor burden less than half that of the smallest lethal tumor in the control group and shows no evidence of infection, mechanical dosing trauma, or other obvious causes of morbidity at necropsy. Animals dying from non-treatment-related causes prior to reaching an evaluable efficacy endpoint are excluded from this evaluation and the number of animals in the group is reduced accordingly. (*Group toxicity parameter*)

% Tumor Free Survivors (TFS) – Any animal with no measurable evidence of disease on the last day of the experiment. This value is exclusive of CRs. These animals may or may not represent “cures”, depending on when the experiment was terminated. We recommend that no animal be assumed cured unless the animal has been held for a period of time past the last treatment equal to twice the time required to allow a single surviving cell to grow to a tumor burden of 500mg. That time in days can be calculated as $(57.6 \times Td)$.

Recovery Time – The length of time in days for treatment-related weight loss to be recovered. Measured as the time from the nadir of body weight to return to the pretreatment value. This parameter is an important monitor of lingering or delayed toxicity. (*Group toxicity parameter*)

Rx Related Death – An animal is presumed to experience a treatment-related death if it is found dead or is euthanized in moribund condition within 2 weeks of the last treatment with a tumor burden less than half that of the smallest lethal tumor in the control group and shows no evidence of infection, mechanical dosing trauma, or other obvious causes of morbidity at necropsy. (*Individual toxicity parameter*)

Td (Tumor Doubling Time) – The growth rate of the tumor expressed as the volume doubling time (days). Calculated from a log-linear least squares regression of the exponential portion of the tumor growth curve. These values are used to compute tumor cell kill, fractional effect, and surviving fraction estimates. They are also used to assess the appropriateness of the biology of the tumor in this experiment against historical values.

Therapeutic Index – We define therapeutic index as simply the range of tolerated dosage levels that produce substantial anticancer activity. Substantial activity for this purpose is defined as a tumor growth delay that is \geq the duration of treatment and that is also statistically different from the control at the $P \leq 0.05$ level.

Time to Evaluation Size – The time (days) it takes a tumor to reach the specified Evaluation Size. Calculated from a log-linear least squares best fit of tumor burden versus time for the exponential portion of the final (post-treatment) tumor growth curve. This value is calculated for every animal in the experiment. The group medians are then used to calculate the Tumor Growth Delay. (*Individual efficacy parameter*)

Time to Fold Growth End Point – Occasionally (usually when initial mean tumor burdens across all groups are not well-matched) it is advantageous to display efficacy parameters in terms of fold growth, where the selected endpoint is the time it takes to reach a selected multiple of

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initial tumor burden. This increases the probability of uncovering a statistically significant therapeutic effect by eliminating the confounding effects of disparity in initial tumor sizes. Calculated as described for Time to Evaluation Size from the fold growth data (Appendix 9). (*Individual efficacy parameter*)

Tumor Burden at Last Rx – The tumor burden on the last day of treatment. This value is calculated from a log-linear least squares best fit of tumor burden versus time for the exponential portion of the final (post-treatment) tumor growth curve. (Presented to facilitate T/C comparisons. Additional T/C information is presented in Appendix 8. T/C values presented in Appendix 8 are simple ratios, not the NCI convention)

Tumor Growth Delay – Tumor Growth Delay (T-C) is the difference between the median times it takes the treated group and the control group (always the first group in Table 2) to reach the stated evaluation size. This is calculated from the median times to evaluation size for each animal in the group, not from interpolation of the median growth curve. Net kill values are not presented when the T-C is negative. (*Group efficacy parameter*)

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Table 1 - Group Toxicity and Response Summary

Group Summary

Group #	Treatment	Dose (mg/kg/inj)	Schedule	Route	Toxicity				Efficacy				
					Maximum Treatment-related Weight Loss (%)	Day of Max. Treatment-related Weight Loss	Recovery Time (days)	% Rx Related Deaths	Tumor Growth Delay (days)	Apparent Net Tumor Cell Kill (logs)	% CR	% PR	% TFS
1	Vehicle Treated Control	0.2ml/20g	QDx7	IV	3.4	22	NA	0	NA	NA	0	0	0
2	Peptide	80.00	QDx7	IV	7.4	22	NA	0	1.7	-0.48	0	0	0
3	Peptide	40.00	QDx7	IV	6.4	22	NA	0	1.2	-0.51	0	0	0
4	Peptide	20.00	QDx7	IV	GAIN	NA	NA	0	0.4	-0.55	0	0	0
5	Peptide	80.00	QDx7	IT	GAIN	NA	NA	0	1.1	-0.53	0	0	0

GROUP SUMMARY ENDPOINT DEFINITIONS AND CALCULATION METHODS

Maximum Mean BW Loss (%)	Calculated from the minimum of the mean BW curve (while there are greater than half the animals still surviving) for each group within the Rx period and out to 2 weeks after the end of Rx.
Day of Maximum Mean BW Loss	Calculated from the minimum of the mean BW curve (while there are greater than half the animals still surviving) for each group within the Rx period and out to 2 weeks after the end of Rx.
Recovery Time (days)	The number of days from the time of the minimum mean BW to recover the lost BW. Calculated only for animals which lost BW and later recovered the lost BW.
% Rx Related Deaths	% of mice in each group with treatment-related deaths.
Tumor Growth Delay (days)	The median time to evaluation size is calculated for each group (see Table 1). The tumor growth delay (TGD) is calculated by subtracting the median TGD for the control group from the median TGD for each treatment group.
Apparent Net Tumor Cell Kill (logs)	By interpolation, the log-linear regression line for each animal is used to calculate the tumor weight (TW) at the start of treatment, and the TW at the end of treatment, for control and treatment groups, respectively. For each treatment group, the median TW (end of treatment) is subtracted from the median control TW (start of treatment) to calculate the apparent net cell kill (in log units).
% CR	% of mice in each group that experienced complete regressions.
% PR	% of mice in each group that experienced partial regressions.
% TFS	% of mice in each group that were tumor free survivors.

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Table 2 – Individual Animal Toxicity and Response Summary

Calculated End Points

Evaluation Size		750 mg	Last Day of Expt.		33						
Fold Growth End Point		4									
Group # 1		Treatment Vehicle Treated Control			Growth Endpoints						
Dose 0.2ml/20g											
Animal	BW Change During Rx (%)	Max. BW Loss (%)	Rx-related Death	Td (days)	Tumor Burden @ Last Rx	Time to Evaluation Size (days)	Time to Fold Growth EP (days)	Complete Regression	Partial Regression	Tumor Free Survivor	
1	-2.9	15.7	no	3.7	394	18.1	15.2	no	no	no	
2	0.1	0.4	no	3.6	429	17.3	15.2	no	no	no	
3	-2.6	2.9	no	3.0	361	16.2	14.5	no	no	no	
4	-8.0	10.6	no	3.9	335	18.7	15.8	no	no	no	
5	10.2	BW GAIN	no	3.9	400	17.2	15.4	no	no	no	
6	-7.9	11.5	no	3.7	490	17.6	15.4	no	no	no	
7	0.9	1.1	no	3.9	317	19.1	16.8	no	no	no	
8	-1.6	2.5	no	4.6	331	20.4	16.2	no	no	no	
Mean			Total	3.8	382	18.1	15.6	Total	Total	Total	
SD			Rx Deaths:	0.5	58	1.3	0.7	CR:	PR:	TFS:	
Median			0	3.8	378	17.8	15.4	0	0	0	
Group # 2		Treatment Peptide			Growth Endpoints						
Dose 80.00											
Animal	BW Change During Rx (%)	Max. BW Loss (%)	Rx-related Death	Td (days)	Tumor Burden @ Last Rx	Time to Evaluation Size (days)	Time to Fold Growth EP (days)	Complete Regression	Partial Regression	Tumor Free Survivor	
1	0.3	12.2	no	7.3	308	22.3	14.4	no	no	no	
2	-1.3	9.4	no	4.5	207	22.9	19.6	no	no	no	
3	1.7	1.5	no	5.2	351	19.1	15.3	no	no	no	
4	1.2	0.9	no	5.1	288	19.5	18.5	no	no	no	
5	-0.4	6.3	no	4.8	346	18.9	15.3	no	no	no	
6	1.5	11.4	no	5.4	280	21.6	17.7	no	no	no	
7	-0.5	7.4	no	15.4	260	NA	21.3	no	no	no	
8	1.5	BW GAIN	no	4.1	522	16.1	15.3	no	no	no	
Mean			Total	6.5	320	20.1	17.2	Total	Total	Total	
SD			Rx Deaths:	3.7	94	2.4	2.5	CR:	PR:	TFS:	
Median			0	5.1	298	19.5	16.5	0	0	0	
Group # 3		Treatment Peptide			Growth Endpoints						
Dose 40.00											
Animal	BW Change During Rx (%)	Max. BW Loss (%)	Rx-related Death	Td (days)	Tumor Burden @ Last Rx	Time to Evaluation Size (days)	Time to Fold Growth EP (days)	Complete Regression	Partial Regression	Tumor Free Survivor	
1	-1.3	2.4	no	5.8	277	21.1	16.5	no	no	no	
2	0.0	0.4	no	17.3	233	36.2	20.5	no	no	no	
3	-0.8	5.4	no	7.6	470	16.7	12.4	no	no	no	
4	-1.9	2.7	no	4.7	305	18.7	16.0	no	no	no	
5	-0.7	12.7	no	10.4	340	20.3	14.3	no	no	no	
6	3.3	BW GAIN	no	4.5	360	18.7	16.2	no	no	no	
7	-0.3	2.7	no	5.5	659	15.5	12.4	no	no	no	
8	-1.8	2.5	no	3.9	286	19.4	17.1	no	no	no	
Mean			Total	7.4	366	20.8	15.7	Total	Total	Total	
SD			Rx Deaths:	4.5	138	6.5	2.7	CR:	PR:	TFS:	
Median			0	5.6	322	19.1	16.1	0	0	0	
Group # 4		Treatment Peptide			Growth Endpoints						
Dose 20.00											
Animal	BW Change During Rx (%)	Max. BW Loss (%)	Rx-related Death	Td (days)	Tumor Burden @ Last Rx	Time to Evaluation Size (days)	Time to Fold Growth EP (days)	Complete Regression	Partial Regression	Tumor Free Survivor	
1	6.2	BW GAIN	no	5.8	233	22.3	15.9	no	no	no	
2	3.6	BW GAIN	no	3.0	339	17.6	15.2	no	no	no	
3	4.0	BW GAIN	no	4.7	394	17.9	15.1	no	no	no	
4	1.2	BW GAIN	no	3.7	535	16.4	14.8	no	no	no	
5	3.0	BW GAIN	no	4.3	253	19.3	16.2	no	no	no	
6	2.6	BW GAIN	no	6.4	338	20.3	18.3	no	no	no	
7	3.5	3.2	no	6.6	422	18.4	14.6	no	no	no	
8	-4.3	5.7	no	7.0	431	18.2	15.1	no	no	no	
Mean			Total	5.2	368	18.8	15.6	Total	Total	Total	
SD			Rx Deaths:	1.5	99	1.8	1.2	CR:	PR:	TFS:	
Median			0	5.3	367	18.3	15.2	0	0	0	

Group #		5									
Treatment Peptide											
Dose 80.00		Growth Endpoints									
Animal	BW Change During Rx (%)	Max. BW Loss (%)	Rx-related Death	Td (days)	Tumor Burden @ Last Rx	Time to Evaluation Size (days)	Time to Fold Growth EP (days)	Complete Regression	Partial Regression	Tumor Free Survivor	
1	-1.5	6.5	no	6.9	378	18.9	14.9	no	no	no	
2	0.5	0.5	no	3.4	411	17.3	15.3	no	no	no	
3	-1.4	2.3	no	3.7	374	17.6	16.4	no	no	no	
4	11.7	BW GAIN	no	5.3	195	24.7	20.9	no	no	no	
5	5.7	BW GAIN	no	4.9	352	18.9	16.0	no	no	no	
6	3.7	3.4	no	5.5	363	19.1	15.1	no	no	no	
Mean			Total	4.9	345	19.4	16.4	Total	Total	Total	
SD			Rx Deaths:	1.3	76	2.7	2.2	CR:	PR:	TFS:	
Median			0	5.1	369	18.9	15.7	0	0	0	

ENDPOINT DEFINITIONS AND CALCULATION METHODS

BW Change During Rx (%)	Body weight change during treatment = (End Rx BW - Start Rx BW)
Max. BW Loss (%)	Maximum body weight loss during treatment, and out to 2 weeks following the end of treatment.
Rx-related Death	Treatment-related death, as determined by clinical signs observations (see Comments table), body weight measurements and behavior of other mice within the group.
Td (days)	Tumor doubling time calculated from log-linear regressions over growth data.
Tumor Burden @ Last Rx	Tumor size on day of last treatment as estimated by caliper measurement.
Time to Evaluation Size (days)	Calculated by interpolation of the log-linear regression line on the tumor growth data, to the evaluation size.
Time to Fold Growth EP (days)	Calculated by interpolation of the log-linear regression line on the fold growth data, to the evaluation size.
Complete Regression	Animals for which the tumor size decreased below 50mg.
Partial Regression	Animals for which the tumor size decreased to < 50% of the initial size.
Tumor Free Survivor	Animals for which a complete regression persisted to the end of the study.

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Table 3 – Group Statistics

Kruskal-Wallis One Way Analysis of Variance on Ranks

Data source: Time to Evaluation Size Data in MIR948.SNB

Normality Test: Failed (P = 0.002)

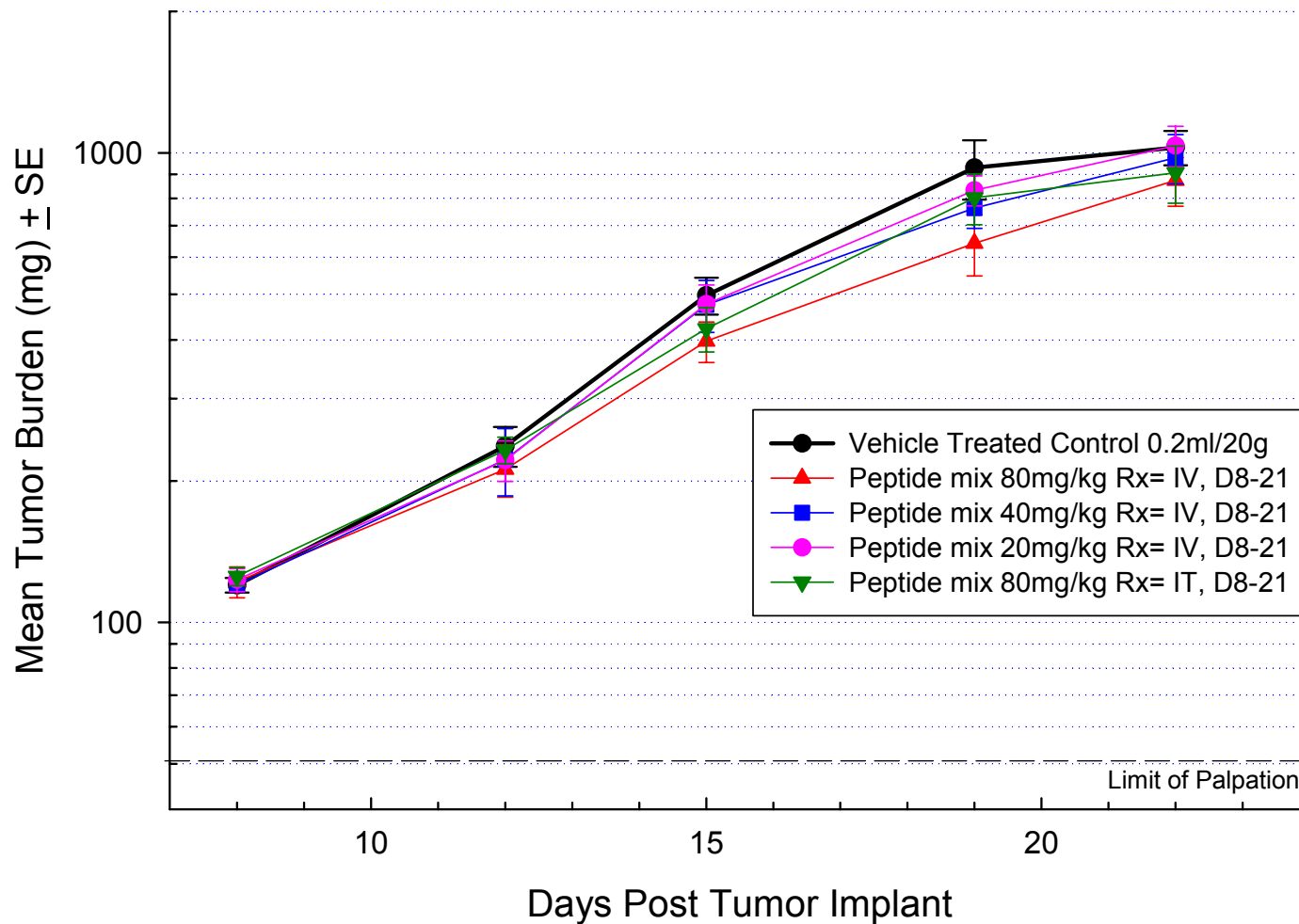
Group	N	Missing	Median	25%	75%
1.000	8	0	17.843	17.226	18.889
2.000	7	0	19.539	18.915	22.147
3.000	8	0	19.064	17.723	20.695
4.000	8	0	18.287	17.728	19.817
5.000	6	0	18.896	17.593	19.125

H = 3.938 with 4 degrees of freedom. (P = 0.414)

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.414)

Figure 1– Group Comparisons with Std. Error

UCP-0200802R1 (MIR948)
MCF-7 Tumor Burden
Group Comparison with Std. Error



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Figure 2 - Group Comparisons by Median

UCP-0200802R1 (MIR948)
MCF-7 Tumor Burden
Group Median Comparison

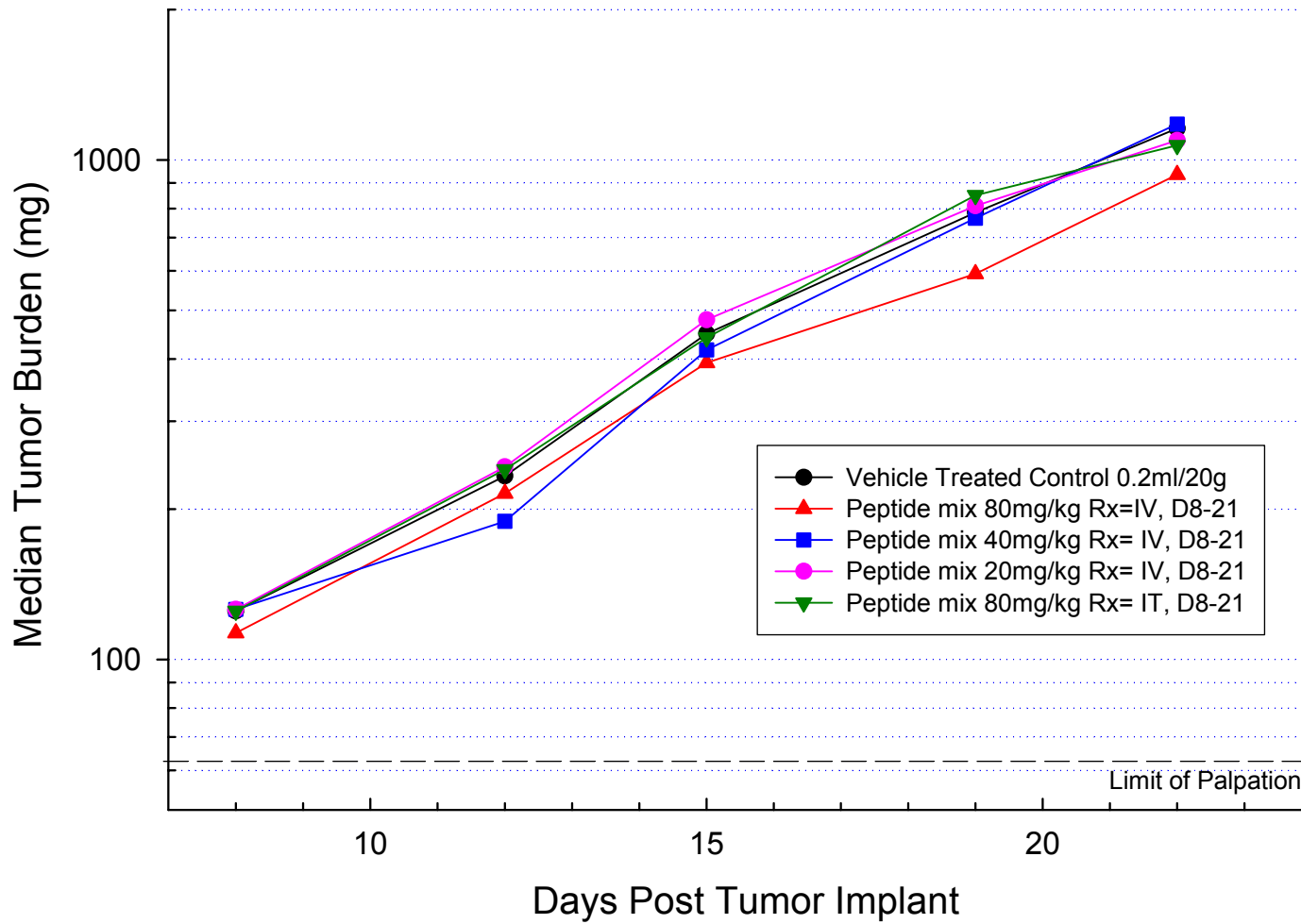
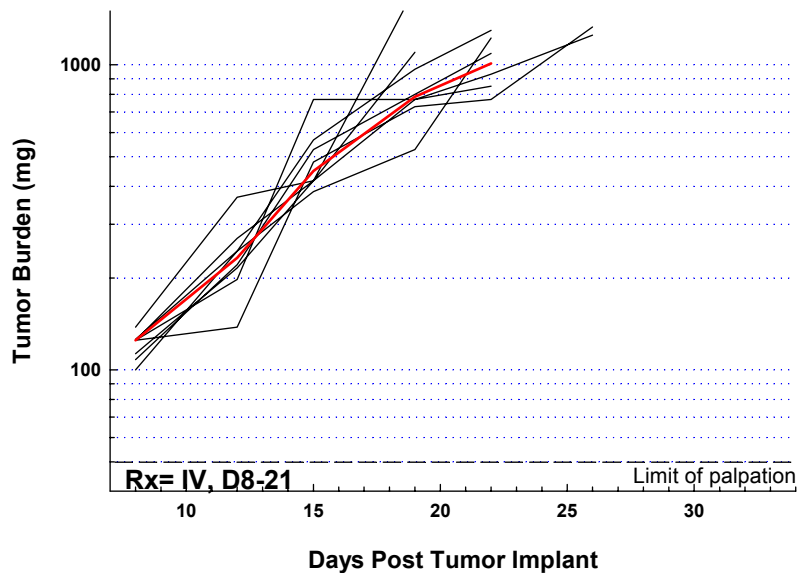
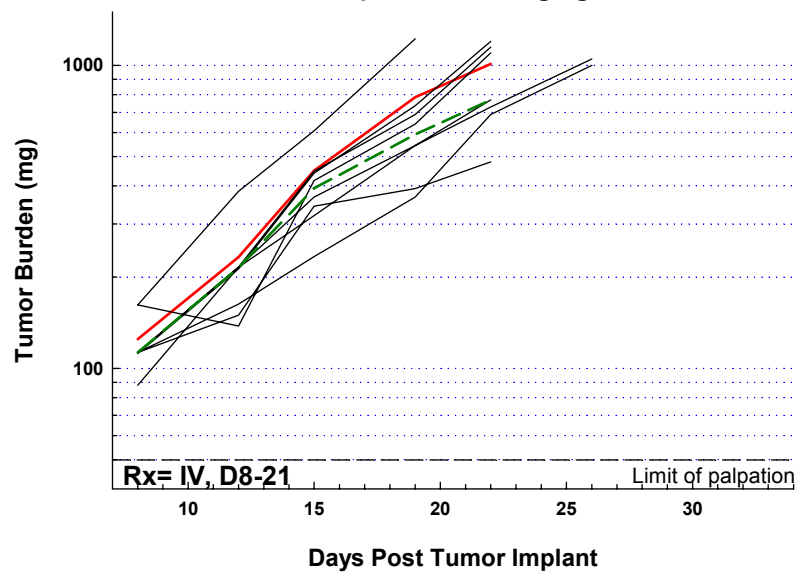


Figure 3– Individual Tumor Growth Curves by Group

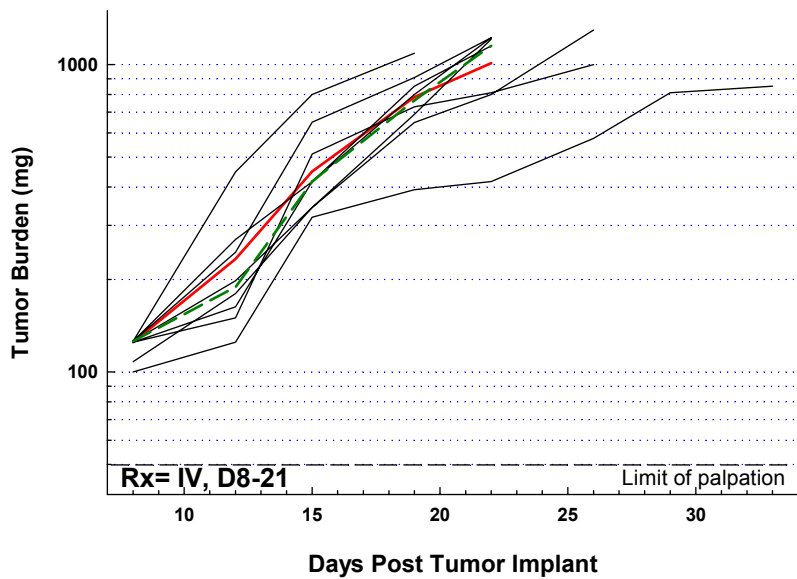
Group 1
Vehicle Treated Control 0.2ml/20g



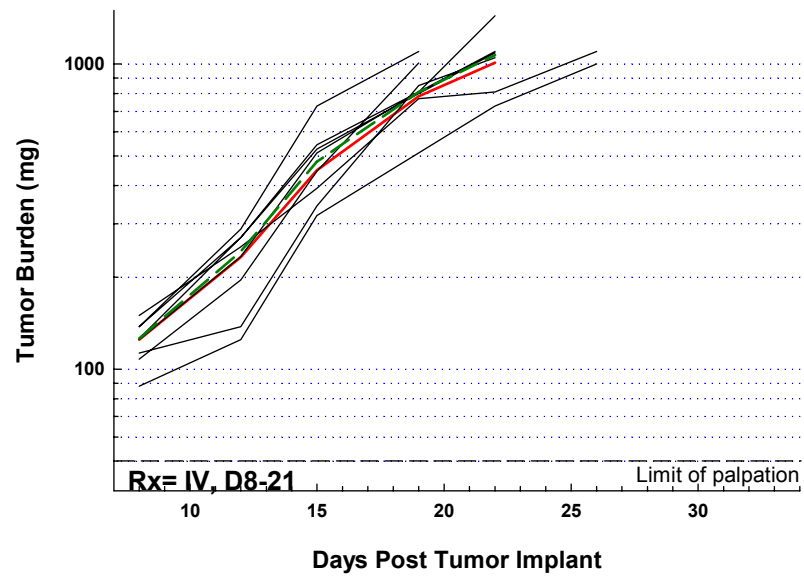
Group 2
Peptide Mix 80mg/kg



Group 3
Peptide Mix 40mg/kg



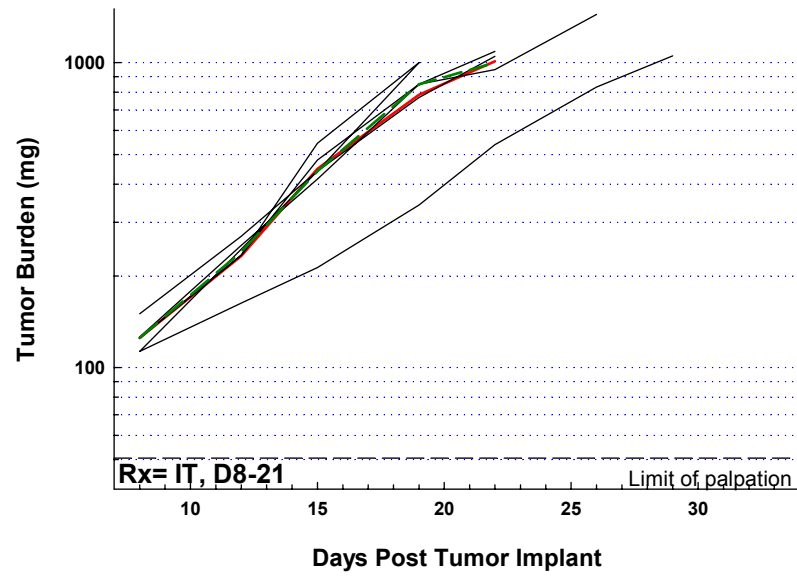
Group 4
Peptide Mix 20mg/kg



— Control Median

- - - Group Median

Group 5
Peptide Mix 80mg/kg

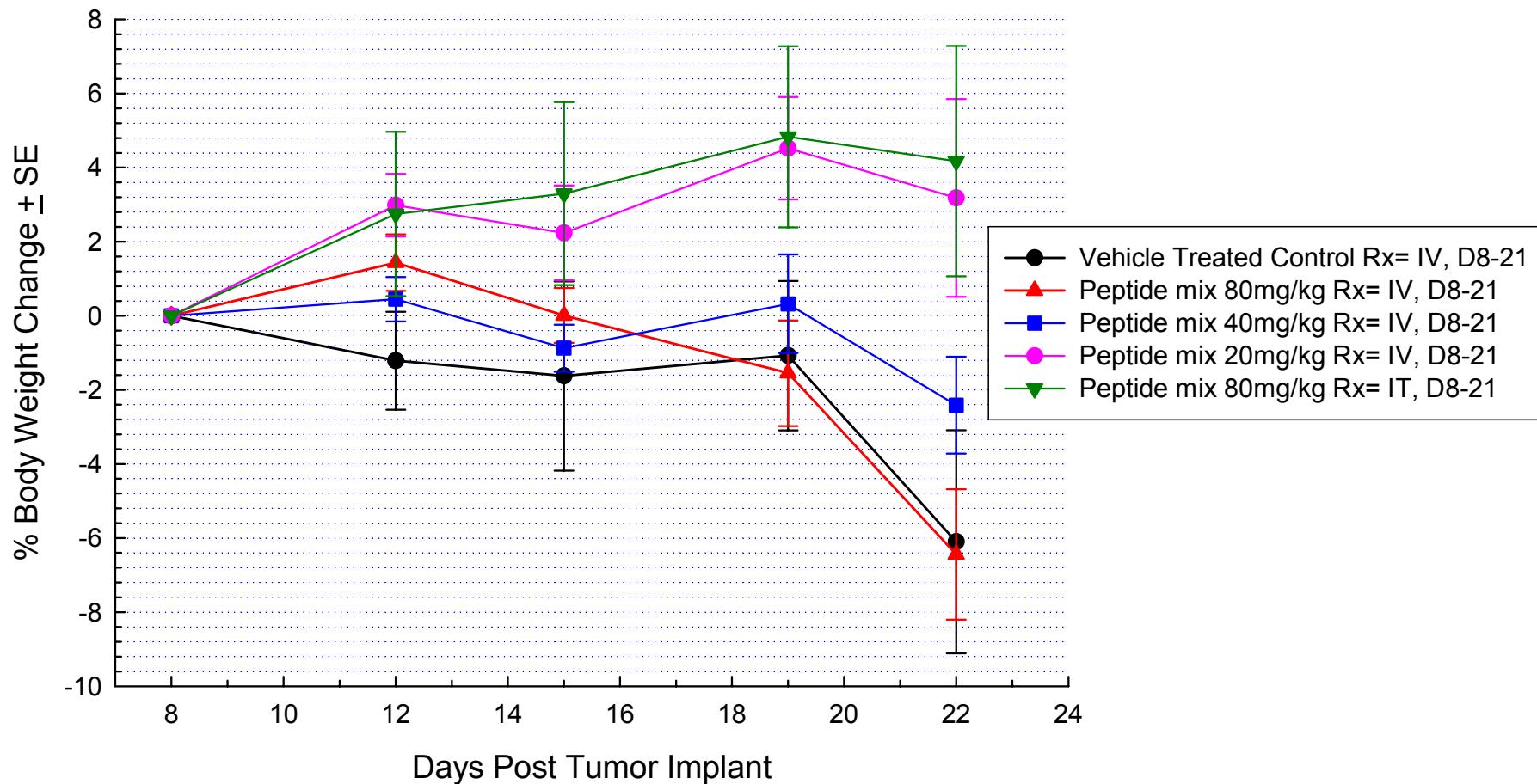


— Control Median

- - - Group Median

Figure 4 – % Body Weight Change Summary with Std. Error

UCP-0200802R1 (MIR948)
Mean Body Weight Change by Group with Std. Error



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Appendix 1 – Protocol Summary

Study # **G5 B5 200802R1**
MIR # 948

Study Title **Efficacy Evaluation of Peptide Against MCF-7 (+estradiol) Ovarian Carcinoma Xenograft**
Principal Scientist M. Baugher

Tumor = MCF-7
Location = SC
Animal = mouse
Strain = outbred nu/nu

Gender = F
Test Animals = 38
Implanted Animals = 55
Implant Date = 8/27/2008

Evaluation Size = 750 mg
Fold Growth End Point = 4 x

Group	# Animals	Compound	Route	Schedule	Dose (mg/kg/Inj)	Rx Start	Rx End	Treatment Days	
								Start	End
1	8	Vehicle Treated Control	IV	QDx14	0.2ml/20g	4-Sep-08	17-Sep-08	8	21
2	8	Peptide	IV	QDx14	80.00	4-Sep-08	17-Sep-08	8	21
3	8	Peptide	IV	QDx14	40.00	4-Sep-08	17-Sep-08	8	21
4	8	Peptide	IV	QDx14	20.00	4-Sep-08	17-Sep-08	8	21
5	6	Peptide	IT	QDx14	80.00	4-Sep-08	17-Sep-08	8	21

Drugs					
Drug Name	Vehicle	Source	Lot #	Amount Needed (mg)	Stability
peptide mix	PBS	Sanare	multiple (12 different lots)		2hr.

Measurement Frequencies	General Procedures
<p>Body Weights: 2x/wk</p> <p>Tumor Weights: 2x/wk</p> <p>Sac. Tumor Weight (g): >1g</p>	<p>Allow animals to acclimate for 5 days. 24hrs prior to tumor implant, each mouse needs to be supplemented with 17-β-estradiol pellet (SC). Implant tumor fragments (30 to 70mg) into mice high in the axilla (just under arm). Triage mice into treatment groups when mean tumor burden is 100-150mg (target 125mg). Distribute animals to treatment groups such that the mean tumor burden in each group is within 10% of the overall mean. Body weights and tumor measure are to be recorded 2x/week, clinical signs daily. Mice dosed individually by body weight on the day of treatment as described above. Animals with tumor burdens greater than 1g or found in a moribund condition will be euthanized. Hold animals for Tumor Growth Delay Endpoint and complete regression/tumor free survivor determination. Provide weekly interim reports. Full report will be provided to the client with detailed methods, statistical multi-endpoint analysis, publication quality graphics, discussion of results and all raw data.</p>

General Comments
<p>The purpose of this experiment is to evaluate of the Peptide against established MCF-7 (+estradiol) in outbred nu/nu mice. The cost for this experiment as written is \$16,650 (with a full report).</p>

Inoculum			Thioglycolate		
Trocar	% Brei	Number of Cells Injected	Tumor Source Code	Number of positives	Total tubes
<input checked="" type="checkbox"/>			MCF-7/SRI-27 (T,8-5-08)	0	2

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Appendix 2 – Raw Data (Tumor Measurements and Body Weights)

Group #1 Treatment: **Vehicle Treated Control** Dose: **0.2ml/20g**

Animal	Date 9/4/08 Day 8			Date 9/8/08 Day 12			Date 9/11/08 Day 15			Date 9/15/08 Day 19			Date 9/18/08 Day 22			Date 9/22/08 Day 26			Date 9/25/08 Day 29			Date 9/29/08 Day 33			Date Day			Date Day		
	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)
1	6	6	22.9	7	9	22.7	9	13	22	10	16	20.8	11	18	19.3															
2	6	7	23.8	7	10	24.1	9	14	23.7	11	16	24.7	12	18	24.1															
3	5	10	20.6	6	15	20	7	17	20.1	13	21	20.7																		
4	5	9	25.4	6	12	23.1	7	17	23.5	9	19	23.7	9	21	22.7															
5	5	11	22.6	7	15	23.3	7	17	25.7	10	22	23.9																		
6	5	10	26.1	6	11	25.5	9	19	23.3	9	19	24.3	9	23	23.1	10	25	23.9												
7	5	10	27.2	5	11	27.5	8	15	27.4	9	18	27.4	9	19	26.9	11	22	27												
8	5	8	20.4	7	10	20.4	8	12	19.9	9	13	21.1	12	17	20.6															

Group #2 Treatment: **Peptide** Dose: **80**

Animal	Date 9/4/08 Day 8			Date 9/8/08 Day 12			Date 9/11/08 Day 15			Date 9/15/08 Day 19			Date 9/18/08 Day 22			Date 9/22/08 Day 26			Date 9/25/08 Day 29			Date 9/29/08 Day 33			Date Day			Date Day			
	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	
1	5	7	25.5	6	12	25.9	7	15	25.4	8	17	24.5	9	18	22.4	10	21	23.2													
2	5	9	22.3	5	13	23	6	13	21.5	7	15	20.3	9	17	20.8	10	20	20.2													
3	5	9	26.9	6	12	26.9	8	14	27.6	9	17	27	11	19	26.5																
4	6	9	22.4	5	11	22.2	7	17	22.9	8	20	23.1	10	22	22.5																
5	4	14	23.9	5	17	24	7	18	23.7	8	23	23.7	10	24	22.4																
6	5	9	29	6	12	30.7	7	13	28.8	8	17	27.8	9	19	25.7																
7	5	9	28.4	5	12	28.6	7	14	28.1	7	16	28.1	8	15	26.3																
8	6	9	27	8	12	27.2	9	15	27.5	12	17	27.8																			

Group #3 Treatment: **Peptide** Dose: **40**

Animal	Date 9/4/08 Day 8			Date 9/8/08 Day 12			Date 9/11/08 Day 15			Date 9/15/08 Day 19			Date 9/18/08 Day 22			Date 9/22/08 Day 26			Date 9/25/08 Day 29			Date 9/29/08 Day 33			Date Day			Date Day			
	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	
1	6	6	21	6	10	21.2	7	14	20.5	9	16	21	10	16	20.5	12	18	21.6													
2	5	8	23.7	5	10	23.7	7	13	23.7	7	16	24.4	7	17	23.6	8	18	24.5	9	20	25.5	9	21	25.6							
3	6	7	24	7	10	23.8	10	13	23.8	11	15	24	12	17	22.7																
4	5	10	22.5	5	13	22.2	7	17	22	9	21	22.7	10	23	21.9																
5	5	10	24.4	5	12	24.3	8	16	24.2	9	18	22.8	9	20	22.6	10	20	21.3													
6	6	7	23.3	7	11	24.2	8	13	24	10	16	24.7	12	17	24.1																
7	6	7	30	8	14	30.5	10	16	29.6	11	18	29.2																			
8	6	7	20	6	11	19.9	7	14	19.5	9	17	20.4	11	20	19.6																

Group #4

Treatment: **Peptide**

Dose: **20**

Animal	Date 9/4/08 Day 8			Date 9/8/08 Day 12			Date 9/11/08 Day 15			Date 9/15/08 Day 19			Date 9/18/08 Day 22			Date 9/22/08 Day 26			Date 9/25/08 Day 29			Date 9/29/08 Day 33			Date Day			Date Day			Date Day					
	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)			
1	5	7	25.4	5	10	26.5	7	13	27.2	8	16	27.9	9	18	28.5	10	20	25.7																		
2	6	6	28.7	7	8	30	9	11	29.6	12	14	30.2																								
3	5	10	25.6	6	13	26.9	8	16	26.5	9	20	27.2	12	20	26.7																					
4	5	11	21.9	6	16	21.9	9	18	22.3	10	22	22.7																								
5	5	9	18.8	5	11	19.7	7	14	19.2	9	21	20.2	10	21	20.4																					
6	5	12	26.6	6	14	27.5	7	16	27.2	9	19	27.6	9	20	27.1	10	22	27.5																		
7	6	7	21.7	7	11	22.4	9	13	22.5	10	16	22.5	11	18	21																					
8	5	11	28	6	15	27.6	8	17	26.4	9	20	27	10	22	26.7																					

Group #5

Treatment: **Peptide**

Dose: **80**

Animal	Date 9/4/08 Day 8			Date 9/8/08 Day 12			Date 9/11/08 Day 15			Date 9/15/08 Day 19			Date 9/18/08 Day 22			Date 9/22/08 Day 26			Date 9/25/08 Day 29			Date 9/29/08 Day 33			Date Day			Date Day			Date Day							
	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)	Width (mm)	Length (mm)	Body Weight (g)		
1	5	10	20.1	6	13	19.8	8	15	19.8	10	17	19.6	10	19	19.3	12	20	18.8																				
2	5	10	20.7	6	13	21.2	8	17	20.6	10	20	21.3																										
3	5	12	25.8	6	15	25.9	7	18	25.2	10	20	25.8																										
4	5	9	22.7	5	13	24.7	5	17	25.7	6	19	25.9	7	22	25	8	26	26.7	9	26	25.8																	
5	6	7	19.4	6	14	21.3	8	13	20.1	10	17	20.9	11	18	20.9																							
6	5	9	20.6	7	10	19.9	7	18	22.1	9	19	22	10	21	21.2																							

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Appendix 3 – Raw Data (Daily Census)

LEGEND: Sacrificed (s) Dead in cage (d) Missing (m) Accidental death (a)

Group	Date 9/4/08		Date 9/5/08		Date 9/6/08		Date 9/7/08		Date 9/8/08		Date 9/9/08		Date 9/10/08		Date 9/11/08		Date 9/12/08		Date 9/13/08		Date 9/14/08		Date 9/15/08		Date 9/16/08				
	Change	Alive	Change	Alive	Change	Alive	Change	Alive	Change	Alive	Change	Alive	Change	Alive	Change	Alive	Change	Alive	Change	Alive	Change	Alive	Change	Alive	Change	Alive	Change	Alive	
1		8		8		8		8		8		8		8		8		8		8		8		8		8		s2	6
2		8		8		8		8		8		8		8		8		8		8		8		8		8		s1	7
3		8		8		8		8		8		8		8		8		8		8		8		8		8		s1	7
4		8		8		8		8		8		8		8		8		8		8		8		8		8		s2	6
5		6		6		6		6		6		6		6		6		6		6		6		6		6		s2	4

Group	Date 9/17/08		Date 9/18/08		Date 9/19/08		Date 9/20/08		Date 9/21/08		Date 9/22/08		Date 9/23/08		Date 9/24/08		Date 9/25/08		Date 9/26/08		Date 9/27/08		Date 9/28/08		Date 9/29/08				
	Change	Alive	Change	Alive	Change	Alive	Change	Alive	Change	Alive	Change	Alive	Change	Alive	Change	Alive	Change	Alive	Change	Alive	Change	Alive	Change	Alive	Change	Alive	Change	Alive	
1		6	s3	3		3	d1	2		2	s2	0		0		0		0		0		0		0		0		0	
2		7	s4	3		3	d1	2		2	s2	0		0		0		0		0		0		0		0		0	
3		7	s4	3		3		3		3	s2	1		1		1		1		1		1		1		1		s1	0
4		6	s4	2		2		2		2	s2	0		0		0		0		0		0		0		0		0	
5		4	s2	2		2		2		2	s1	1		1		1		s1	0		0		0		0		0		0

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Appendix 4 – Raw Data (Clinical Signs, Observations, and Comments)

Date	Day	Group	Animal	Tech	Comment
22-Sep-08	26	1	4	MB	Animal found dead on 9-20-08. Too decomposed to necropsy.
18-Sep-08	22	1	1,2,8	MB	Animals euthanized due to tumor burdens >1g. Animals necropsied, no remarkable findings.
16-Sep-08	20	1	3,5	MB	Animals euthanized due to tumor burdens >1g. Animals necropsied, no remarkable findings.
22-Sep-08	26	1	6,7	MB	Animals euthanized due to tumor burdens >1g. Tumors excised and snap frozen in liquid nitrogen per clients request. Stored at -80C. Animals necropsied, no remarkable findings.
22-Sep-08	26	2	6	MB	Animal found dead on 9-20-08. Too decomposed to necropsy.
18-Sep-08	22	2	7	MB	Animal euthanized due to ulcerated tumor. Necropsy performed, no remarkable findings.
16-Sep-08	20	2	8	MB	Animal euthanized due to tumor burden >1g. Animal necropsied, no remarkable findings.
22-Sep-08	26	2	1,2	MB	Animals euthanized due to tumor burdens >1g. Tumors excised and snap frozen in liquid nitrogen per clients request. Stored at -80C. Animals necropsied, no remarkable findings.
18-Sep-08	22	2	3,4,5	MB	Animals euthanized due to tumor burdens >1g. Animals necropsied, no remarkable findings.
29-Sep-08	33	3	2	MB	Animal euthanized due to tumor burden >1g. Animal necropsied, no remarkable findings.
16-Sep-08	20	3	7	MB	Animal euthanized due to tumor burden >1g. Animal necropsied, no remarkable findings.
22-Sep-08	26	3	1,5	MB	Animals euthanized due to tumor burdens >1g. Animals necropsied, no remarkable findings.
18-Sep-08	22	3	3,4,6,8	MB	Animals euthanized due to tumor burdens >1g. Animals necropsied, no remarkable findings.
22-Sep-08	26	4	1,6	MB	Animals euthanized due to tumor burdens >1g. Animals necropsied, no remarkable findings.
16-Sep-08	20	4	2,4	MB	Animals euthanized due to tumor burdens >1g. Animals necropsied, no remarkable findings.
18-Sep-08	22	4	3,5,7,8	MB	Animals euthanized due to tumor burdens >1g. Animals necropsied, no remarkable findings.
22-Sep-08	26	5	1	MB	Animal euthanized due to tumor burden >1g. Animal necropsied, no remarkable findings.
25-Sep-08	29	5	4	MB	Animal euthanized due to tumor burden >1g. Animal necropsied, no remarkable findings.
16-Sep-08	20	5	2,3	MB	Animals euthanized due to tumor burdens >1g. Animals necropsied, no remarkable findings.
18-Sep-08	22	5	5,6	MB	Animals euthanized due to tumor burdens >1g. Animals necropsied, no remarkable findings.

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Appendix 5 – Consolidated Tumor Burdens

Estimated Tumor Weights (mg)

Group #		Treatment Vehicle Treated Control								Route IV		Rx Start Day	Rx End Day
		Dose 0.2ml/20g								Schedule QDx14		8	21
Animal	Day	8	12	15	19	22	26	29	33				
1	108	221	527	800	1089								
2	126	245	567	968	1296								
3	125	270	417	1775									
4	113	216	417	770	851								
5	138	368	417	1100									
6	125	198	770	770	932	1250							
7	125	138	480	729	770	1331							
8	100	245	384	527	1224								
Mean	120	238	497	930	1027	-	-	-	-	-	-	-	-
SD	12	66	127	381	210	-	-	-	-	-	-	-	-
Median	125	233	449	785	1011	-	-	-	-	-	-	-	-

Group #		Treatment Peptide								Route IV		Rx Start Day	Rx End Day
		Dose 80.00								Schedule QDx14		8	21
Animal	Day	8	12	15	19	22	26	29	33				
1	88	216	368	544	729	1050							
2	113	163	234	368	689	1000							
3	113	216	448	689	1150								
4	162	138	417	640	1100								
5	112	213	441	736	1200								
6	113	216	319	544	770								
7	113	150	343	392	480								
8	162	384	608	1224									
Mean	122	212	397	642	874	-	-	-	-	-	-	-	-
SD	26	77	111	269	275	-	-	-	-	-	-	-	-
Median	113	215	393	592	770	-	-	-	-	-	-	-	-

Group #		Treatment Peptide								Route IV		Rx Start Day	Rx End Day
		Dose 40.00								Schedule QDx14		8	21
Animal	Day	8	12	15	19	22	26	29	33				
1	108	180	343	648	800	1296							
2	100	125	319	392	417	576	810	851					
3	126	245	650	908	1224								
4	125	163	417	851	1150								
5	125	150	512	729	810	1000							
6	126	270	416	800	1224								
7	126	448	800	1089									
8	126	198	343	689	1210								
Mean	120	222	475	763	976	-	-	-	-	-	-	-	-
SD	10	103	171	205	311	-	-	-	-	-	-	-	-
Median	126	189	417	765	1150	-	-	-	-	-	-	-	-

Group #		Treatment Peptide								Route IV		Rx Start Day	Rx End Day
		Dose 20.00								Schedule QDx14		8	21
Animal\Day	8	12	15	19	22	26	29	33					
1	88	125	319	512	729	1000							
2	108	196	446	1008									
3	125	234	512	810	1440								
4	138	288	729	1100									
5	113	138	343	851	1050								
6	150	252	392	770	810	1100							
7	126	270	527	800	1089								
8	138	270	544	810	1100								
Mean	123	222	477	833	1036	-	-	-	-	-	-	-	
SD	20	62	132	174	251	-	-	-	-	-	-	-	
Median	126	243	479	810	1070	-	-	-	-	-	-	-	

Group #		Treatment Peptide								Route IT		Rx Start Day	Rx End Day
		Dose 80.00								Schedule QDx14		8	21
Animal\Day	8	12	15	19	22	26	29	33					
1	125	234	480	850	950	1440							
2	125	234	544	1000									
3	150	270	441	1000									
4	113	163	213	342	539	832	1053						
5	126	252	416	850	1089								
6	113	245	441	770	1050								
Mean	125	233	423	802	907	-	-	-	-	-	-	-	
SD	14	37	112	243	252	-	-	-	-	-	-	-	
Median	125	240	441	850	1000	-	-	-	-	-	-	-	

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Appendix 6 – Consolidated Body Weights

Animal Body Weights (g)

Group #	Treatment	Route IV								Rx Start Day	Rx End Day
	Vehicle Treated Control	Schedule QDx14								8	21
	Dose 0.2ml/20g	8	12	15	19	22	26	29	33		
1		22.9	22.7	22.0	20.8	19.3					
2		23.8	24.1	23.7	24.7	24.1					
3		20.6	20.0	20.1	20.7						
4		25.4	23.1	23.5	23.7	22.7					
5		22.6	23.3	25.7	23.9						
6		26.1	25.5	23.3	24.3	23.1	23.9				
7		27.2	27.5	27.4	27.4	26.9	27.0				
8		20.4	20.4	19.9	21.1	20.6					
Mean		23.6	23.3	23.2	23.3	22.8	-	-	-	-	-
SD		2.5	2.5	2.6	2.3	2.7	-	-	-	-	-
Median		23.4	23.2	23.4	23.8	22.9	-	-	-	-	-
Group #	Treatment	Route IV								Rx Start Day	Rx End Day
	Peptide	Schedule QDx14								8	21
	Dose 80.00	8	12	15	19	22	26	29	33		
1		25.5	25.9	25.4	24.5	22.4	23.2				
2		22.3	23.0	21.5	20.3	20.8	20.2				
3		26.9	26.9	27.6	27.0	26.5					
4		22.4	22.2	22.9	23.1	22.5					
5		23.9	24.0	23.7	23.7	22.4					
6		29.0	30.7	28.8	27.8	25.7					
7		28.4	28.6	28.1	28.1	26.3					
8		27.0	27.2	27.5	27.8						
Mean		25.7	26.1	25.7	25.3	23.8	-	-	-	-	-
SD		2.6	2.9	2.7	2.8	2.3	-	-	-	-	-
Median		26.2	26.4	26.5	25.8	22.5	-	-	-	-	-
Group #	Treatment	Route IV								Rx Start Day	Rx End Day
	Peptide	Schedule QDx14								8	21
	Dose 40.00	8	12	15	19	22	26	29	33		
1		21.0	21.2	20.5	21.0	20.5	21.6				
2		23.7	23.7	23.7	24.4	23.6	24.5	25.5	25.6		
3		24.0	23.8	23.8	24.0	22.7					
4		22.5	22.2	22.0	22.7	21.9					
5		24.4	24.3	24.2	22.8	22.6	21.3				
6		23.3	24.2	24.0	24.7	24.1					
7		30.0	30.5	29.6	29.2						
8		20.0	19.9	19.5	20.4	19.6					
Mean		23.6	23.7	23.4	23.7	22.1	-	-	-	-	-
SD		3.0	3.2	3.1	2.7	1.6	-	-	-	-	-
Median		23.5	23.8	23.8	23.4	22.6	-	-	-	-	-

Group #		Treatment Peptide		Route IV		Rx Start Day		Rx End Day	
4		20.00		QDx14		8		21	
Animal\Day	8	12	15	19	22	26	29	33	
1	25.4	26.5	27.2	27.9	28.5	25.7			
2	28.7	30.0	29.6	30.2					
3	25.6	26.9	26.5	27.2	26.7				
4	21.9	21.9	22.3	22.7					
5	18.8	19.7	19.2	20.2	20.4				
6	26.6	27.5	27.2	27.6	27.1	27.5			
7	21.7	22.4	22.5	22.5	21.0				
8	28.0	27.6	26.4	27.0	26.7				
Mean	24.6	25.3	25.1	25.7	25.1	-	-	-	-
SD	3.5	3.5	3.4	3.4	3.5	-	-	-	-
Median	25.5	26.7	26.5	27.1	26.7	-	-	-	-

Group #		Treatment Peptide		Route IT		Rx Start Day		Rx End Day	
5		80.00		QDx14		8		21	
Animal\Day	8	12	15	19	22	26	29	33	
1	20.1	19.8	19.8	19.6	19.3	18.8			
2	20.7	21.2	20.6	21.3					
3	25.8	25.9	25.2	25.8					
4	22.7	24.7	25.7	25.9	25.0	26.7	25.8		
5	19.4	21.3	20.1	20.9	20.9				
6	20.6	19.9	22.1	22.0	21.2				
Mean	21.6	22.1	22.3	22.6	21.6	-	-	-	-
SD	2.4	2.6	2.6	2.6	2.4	-	-	-	-
Median	20.7	21.3	21.4	21.7	21.1	-	-	-	-

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Appendix 7 – Consolidated % Body Weight Loss

% Body Weight Change

Group # 1		Treatment Vehicle Treated Control								Route IV				Rx Start Day 8	Rx End Day 21
		Dose 0.2ml/20g								Schedule QDx14					
Animal	Day	8	12	15	19	22	26	29	33						
1	0.0	-0.9	-3.9	-9.2	-15.7										
2	0.0	1.3	-0.4	3.8	1.3										
3	0.0	-2.9	-2.4	0.5											
4	0.0	-9.1	-7.5	-6.7	-10.6										
5	0.0	3.1	13.7	5.8											
6	0.0	-2.3	-10.7	-6.9	-11.5	-8.4									
7	0.0	1.1	0.7	0.7	-1.1	-0.7									
8	0.0	0.0	-2.5	3.4	1.0										
Mean	0.0	-1.2	-1.6	-1.1	-6.1	-	-	-	-	-	-	-	-	-	-
SD	0.0	3.7	7.2	5.7	7.4	-	-	-	-	-	-	-	-	-	-
Median	0.0	-0.5	-2.5	0.6	-5.9	-	-	-	-	-	-	-	-	-	-

Group # 2		Treatment Peptide								Route IV				Rx Start Day 8	Rx End Day 21
		Dose 80.00								Schedule QDx14					
Animal	Day	8	12	15	19	22	26	29	33						
1	0.0	1.6	-0.4	-3.9	-12.2	-9.0									
2	0.0	3.1	-3.6	-9.0	-6.7	-9.4									
3	0.0	0.0	2.6	0.4	-1.5										
4	0.0	-0.9	2.2	3.1	0.4										
5	0.0	0.4	-0.8	-0.8	-6.3										
6	0.0	5.9	-0.7	-4.1	-11.4										
7	0.0	0.7	-1.1	-1.1	-7.4										
8	0.0	0.7	1.9	3.0											
Mean	0.0	1.4	0.0	-1.6	-6.4	-	-	-	-	-	-	-	-	-	-
SD	0.0	2.2	2.1	4.0	4.7	-	-	-	-	-	-	-	-	-	-
Median	0.0	0.7	-0.6	-1.0	-6.7	-	-	-	-	-	-	-	-	-	-

Group # 3		Treatment Peptide								Route IV				Rx Start Day 8	Rx End Day 21
		Dose 40.00								Schedule QDx14					
Animal	Day	8	12	15	19	22	26	29	33						
1	0.0	1.0	-2.4	0.0	-2.4	2.9									
2	0.0	0.0	0.0	3.0	-0.4	3.4	7.6	8.0							
3	0.0	-0.8	-0.8	0.0	-5.4										
4	0.0	-1.3	-2.2	0.9	-2.7										
5	0.0	-0.4	-0.8	-6.6	-7.4	-12.7									
6	0.0	3.9	3.0	6.0	3.4										
7	0.0	1.7	-1.3	-2.7											
8	0.0	-0.5	-2.5	2.0	-2.0										
Mean	0.0	0.5	-0.9	0.3	-2.4	-	-	-	-	-	-	-	-	-	-
SD	0.0	1.7	1.8	3.8	3.5	-	-	-	-	-	-	-	-	-	-
Median	0.0	-0.2	-1.1	0.5	-2.4	-	-	-	-	-	-	-	-	-	-

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Appendix 8 – Group T/C Values

Calculated T/C Values (%)

Group# \ Day	8	12	15	19	22	26	29	33
1	100	100	100	100	100			
2	90	92	88	75	76			
3	101	81	93	97	114			
4	101	104	107	103	106			
5	100	103	98	108	99			

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Appendix 9 – Individual and Group Fold-Growth Values

Calculated Fold Growth

Group #	Treatment	Dose	Route IV Schedule								Rx Start Day	Rx End Day
1	Vehicle Treated Control	0.2ml/20g	QDx14								8	21
Animal\Day	8	12	15	19	22	26	29	33				
1	1.0	2.0	4.9	7.4	10.1							
2	1.0	1.9	4.5	7.7	10.3							
3	1.0	2.2	3.3	14.2								
4	1.0	1.9	3.7	6.8	7.5							
5	1.0	2.7	3.0	8.0								
6	1.0	1.6	6.2	6.2	7.5	10.0						
7	1.0	1.1	3.8	5.8	6.2	10.6						
8	1.0	2.5	3.8	5.3	12.2							
Mean	1.0	2.0	4.2	7.7	9.0	-	-	-	-	-		
SD	0.0	0.5	1.0	2.8	2.3	-	-	-	-	-		
Median	1.0	2.0	3.8	7.1	8.8	-	-	-	-	-		
2	Peptide	80.00	QDx14								8	21
Animal\Day	8	12	15	19	22	26	29	33				
1	1.0	2.5	4.2	6.2	8.3	11.9						
2	1.0	1.4	2.1	3.3	6.1	8.9						
3	1.0	1.9	4.0	6.1	10.2							
4	1.0	0.9	2.6	4.0	6.8							
5	1.0	1.9	3.9	6.6	10.7							
6	1.0	1.9	2.8	4.8	6.8							
7	1.0	1.3	3.0	3.5	4.2							
8	1.0	2.4	3.8	7.6								
Mean	1.0	1.8	3.3	5.2	7.6	-	-	-	-	-		
SD	0.0	0.5	0.8	1.6	2.3	-	-	-	-	-		
Median	1.0	1.9	3.4	5.5	6.8	-	-	-	-	-		
3	Peptide	40.00	QDx14								8	21
Animal\Day	8	12	15	19	22	26	29	33				
1	1.0	1.7	3.2	6.0	7.4	12.0						
2	1.0	1.3	3.2	3.9	4.2	5.8	8.1	8.5				
3	1.0	1.9	5.2	7.2	9.7							
4	1.0	1.3	3.3	6.8	9.2							
5	1.0	1.2	4.1	5.8	6.5	8.0						
6	1.0	2.1	3.3	6.3	9.7							
7	1.0	3.6	6.3	8.6								
8	1.0	1.6	2.7	5.5	9.6							
Mean	1.0	1.8	3.9	6.3	8.0	-	-	-	-	-		
SD	0.0	0.8	1.2	1.4	2.1	-	-	-	-	-		
Median	1.0	1.6	3.3	6.2	9.2	-	-	-	-	-		

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Appendix 10 - Peptide Prep Procedure

2mg/ml dilution and Freeze Scheme

Peptide	MW	mg provided	ml PBS to add	conc mg/ml	conc needed for 0.000347mmol/ml	volume of 2mg/ml needed	dilute 2.0 ml to a final volume of X	ml of PBS to add	dosing days	withdraw volume (ml)	amt remainin g	# 1.0 ml aliquots
59	3125.5	10	5	2	1.0845485	2.2	4.057	1.857	7	3.9	0.157	0
60	1735.9	10	5	2	0.6023573	2.2	7.3046	5.1046	7	3.9	3.4046	3
61	1910.2	10	5	2	0.6628394	2.2	6.6381	4.4381	7	3.9	2.7381	3
62	1909.3	10	5	2	0.6625271	2.2	6.6412	4.4412	7	3.9	2.7412	3
63	2399	10	5	2	0.832453	2.2	5.2856	3.0856	7	3.9	1.3856	1
64	1326.5	10	5	2	0.4602955	2.2	9.5591	7.3591	7	3.9	5.6591	6
65	1129.3	10	5	2	0.3918671	2.2	11.2283	9.0283	7	3.9	7.3283	7
66	2223.7	10	5	2	0.7716239	2.2	5.7023	3.5023	7	3.9	1.8023	2
67	1746.2	10	5	2	0.6059314	2.2	7.2615	5.0615	7	3.9	3.3615	3
68	2070.3	10	5	2	0.7183941	2.2	6.1248	3.9248	7	3.9	2.2248	2
69	1570.8	10	5	2	0.5450676	2.2	8.0724	5.8724	7	3.9	4.1724	4
70	1886.2	10	5	2	0.6545114	2.2	6.7226	4.5226	7	3.9	2.8226	3
freeze remaining 2mg/ml soln in 1.0ml aliquots= 3 each								1.857	46.8 ml total 6.685714 freeze volume			
freeze aliquot size =1.0 ml for the mix remaining												

peptide prep and freezing procedure.xls