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# Comparative Studies on the Therapeutic Benefit of Targeted α-Particle Radiation Therapy for the Treatment of Disseminated Intraperitoneal Disease

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Supplementary Information

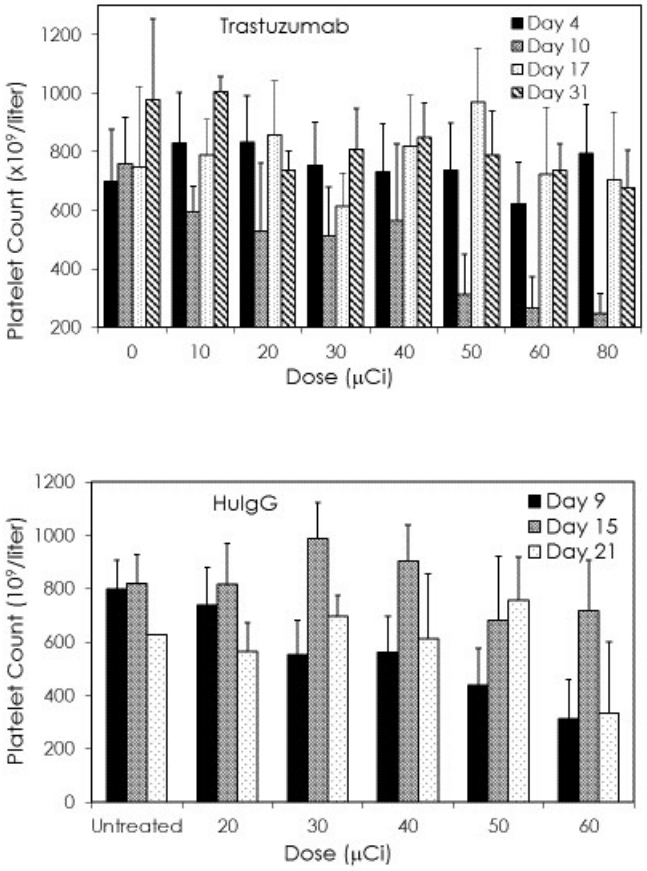


Figure S1 Effect of increasing doses (μCi) of $^{211}$At-RIT on platelet levels. Athymic mice bearing 3 d i.p. LS-174T tumor xenografts were injected with 10, 20, 30, 40, 50, 60 or 80 μCi of $^{211}$At-trastuzumab. Additional groups of mice were treated with 20, 30, 40, 50 or 60 μCi of $^{211}$At-HuIgG. Bloods (50 μL) were collected from 5 mice in each group, diluted 1:1 with K-EDTA (1.5mg/mL) and cell counts analyzed on a VetScan VM5.

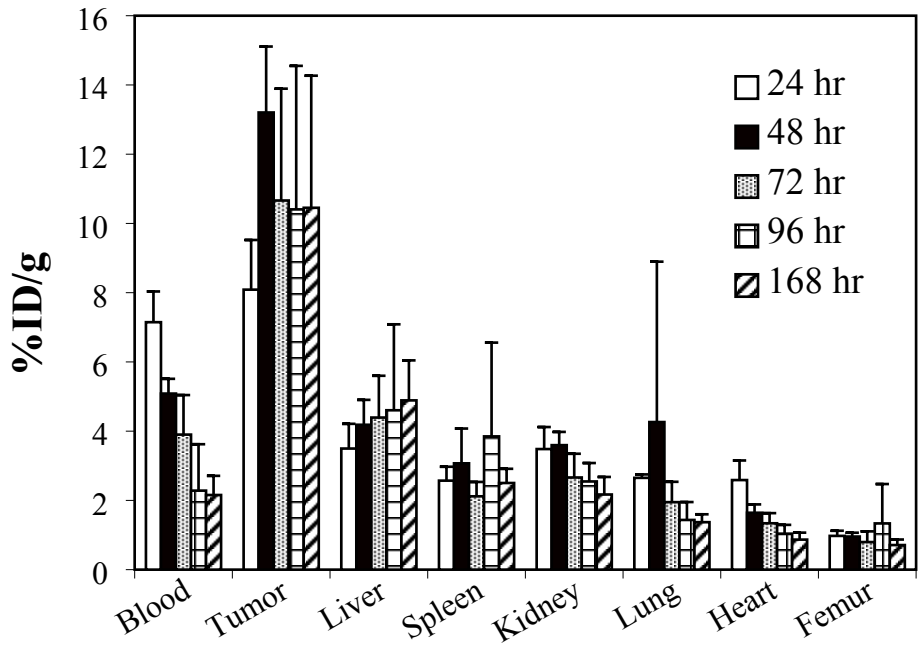


Figure S2 Tumor targeting and normal organ distribution of $^{111}$In-trastuzumab. Athymic mice bearing s.c. LS-174T tumor xenografts were injected with i.v. with $^{111}$In-trastuzumab (~5 μCi) when tumors were 0.2-0.4 mm in diameter. The mice were euthanized at 24, 48, 72, 96, and 168 h by $CO_2$ inhalation for harvesting of the blood, tumor, and major organs. The tumor and normal organs were wet-weighed and counted in a γ-scintillation counter. The percent injected dose per gram (%ID $g^{-1}$) was determined for each tissue; the averages and standard deviations are presented.

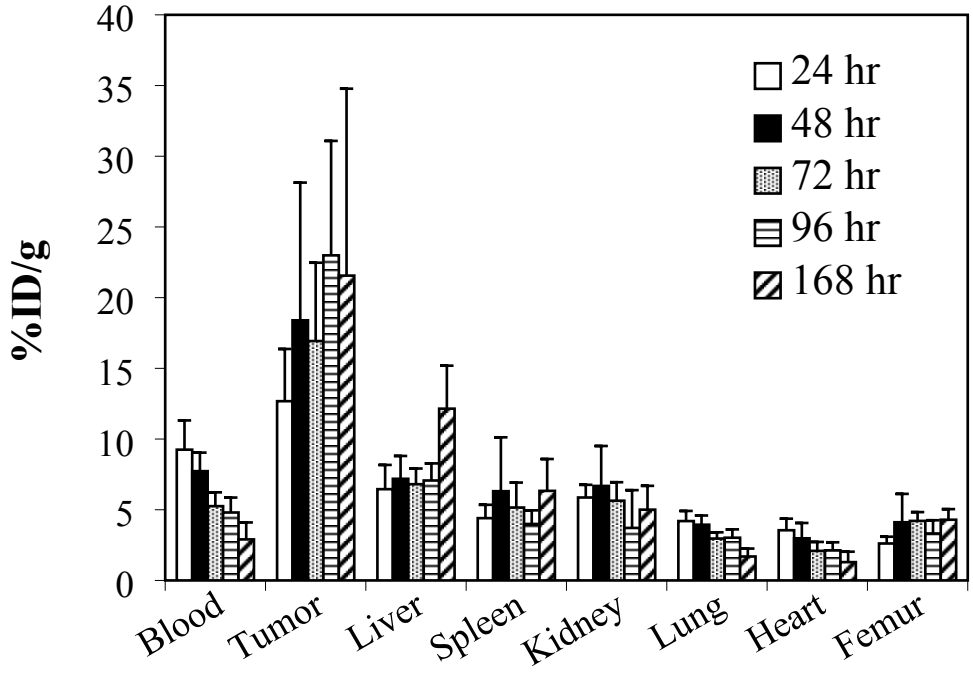


Figure S3 Tumor targeting and normal organ distribution of $^{111}$In-trastuzumab. Athymic mice bearing s.c. LS-174T tumor xenografts were injected with i.v. with $^{227}$Th-trastuzumab (~0.5 μCi) when tumors were 0.2-0.4 mm in diameter. The mice were euthanized at 24, 48, 72, 96, and 168 h by $CO_2$ inhalation for harvesting of the blood, tumor, and major organs. The tumor and normal organs were wet-weighed and stored at -20 $^0$C for 2 mo to allow for equilibrium to be reached. After 2 mo, the samples were counted in a γ-scintillation counter. The percent injected dose per gram (%ID g$^{-1}$) was determined for each tissue; the averages and standard deviations are presented.

**Table S1.** Effect of Increasing $^{211}$At-Radioimmunotherapy Doses on the Weights of Athymic Mice Bearing i.p. LS174T Tumor Xenografts.

| | | Days Post Radioimmunotherapy | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| RIT | Dose (μCi) | 0 | 5 | 10 | 13 | 17 | 21 | 27 | 33 |
| None | None | 24.7±1.7 | 25.0±1.9 | 25.6±2.3 | 25.0±2.6 | 24.3±0.5 | 24.9±1.8 | 25.0±1.2 | 25.5 |
| Trastuzumab | 10 | 25.5±1.5 | 24.5±1.4 | 25.6±1.5 | 24.6±1.6 | 24.8±2.0 | 24.5±1.0 | 25.3±1.6 | 23.7±1.4 |
| | 20 | 25.5±1.7 | 24.3±2.4 | 23.9±3.0 | 24.3±2.5 | 25.0±1.3 | 25.6±1.7 | 25.5±1.8 | 24.5±1.9 |
| | 30 | 26.4±1.6 | 24.7±1.9 | 23.9±2.4 | 24.0±2.2 | 23.4±2.5 | 23.3±2.3 | 25.0±2.7 | 24.3±3.7 |
| | 40 | 25.6±2.3 | 23.4±2.8 | 22.7±2.6 | 22.4±2.6 | 22.5±2.6 | 22.2±2.7 | 23.7±2.5 | 23.1±2.5 |
| | 50 | 25.9±2.4 | 22.1±3.0 | 23.0±2.6 | 23.1±2.8 | 24.0±3.0 | 24.7±3.2 | 25.4±2.9 | 24.6±3.2 |
| | 60 | 26.5±2.5 | 22.7±3.5 | 23.7±3.1 | 24.3±2.0 | 25.0±2.4 | 25.3±1.6 | 26.9±1.7 | 25.9±1.9 |
| | 80 | 25.9±2.5 | 20.3±3.3 | 21.5±2.6 | 21.5±2.3 | 21.6±2.1 | 22.9±1.3 | 24.3±1.7 | 25.1±2.4 |

| | | Days Post Radioimmunotherapy | | | | |
|---|---|---|---|---|---|---|
| RIT | Dose (μCi) | 0 | 6 | 11 | 18 | 20 |
| None | None | 29.2±3.7 | 29.4±3.5 | 31.0±2.9 | 26.9 | |
| HuIgG | 20 | 28.3±2.1 | 26.9±2.2 | 26.7±2.2 | 27.5±1.2 | 27.5±2.1 |
| | 30 | 28.8±1.7 | 26.2±1.6 | 26.4±2.0 | 27.5±2.3 | 27.5±1.5 |
| | 40 | 27.2±2.1 | 24.3±2.5 | 23.6±2.3 | 26.1±1.8 | 26.1±2.4 |
| | 50 | 27.8±1.9 | 22.5±7.4 | 24.2±2.0 | 25.3±2.3 | 25.4±1.4 |
| | 60 | 26.8±1.4 | 21.0±3.0 | 22.3±0.8 | 24.6±0.3 | 23.6±0.8 |

Athymic mice (n=9-15) bearing 3 d i.p. LS-174T tumor xenografts were injected with doses of $^{211}$At-trastuzumab from 10-80 μCi to establish the effective, suboptimal, therapeutic dose for subsequent studies with chemotherapeutics. Animal weights were monitored 1-2 times per week for 5 weeks as an indicator of toxicity. Additional groups included those that received no treatment and those that were injected with 20, 30, 40 or 60 μCi of the non-specific control, $^{211}$At-HuIgG.

**Table S2.** Effect of Increasing $^{211}$At-Radioimmunotherapy Doses on the Weights of Athymic Mice Bearing i.p. LS174T Tumor Xenografts.

| | | Days Post Radioimmunotherapy | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| RIT | Dose (μCi) | -1 | 7 | 12 | 15 | 19 | 22 | 28 | 32 |
| None | None | 22.6±2.0 | 23.3±2.3 | 23.7±4.7 | 23.9±3.8 | 25.2±4.8 | 29.4 | 29.1 | 28.3 |
| Trastuzumab | 20 | 24.1±2.6 | 22.3±2.1 | 21.6±2.2 | 21.8±2.1 | 22.6±2.4 | 22.5±2.6 | 23.4±2.2 | 24.1±2.6 |
| | 30 | 23.7±1.5 | 21.5±1.6 | 20.9±1.8 | 21.5±2.0 | 22.3±2.1 | 21.8±2.4 | 21.4±2.2 | 22.2±2.0 |
| | 40 | 24.3±2.7 | 21.3±3.2 | 21.4±3.0 | 21.7±3.0 | 22.0±3.2 | 21.5±3.1 | 21.1±3.2 | 21.5±3.9 |
| | | Days Post Radioimmunotherapy | | | | | | | |
| RIT | Dose (μCi) | 0 | 4 | 7 | 11 | 14 | 20 | 24 | 27 |
| None | None | 24.4±1.8 | 23.8±2.1 | 24.6±1.9 | 25.1±2.4 | 26.2±2.0 | | | |
| HuIgG | 20 | 24.8±2.3 | 22.9±2.3 | 23.3±2.3 | 23.4±2.5 | 23.5±2.8 | 24.4±3.7 | | |
| | 30 | 24.5±2.1 | 21.9±2.0 | 21.6±1.9 | 21.9±2.3 | 21.7±2.5 | 22.0±1.8 | 23.3±3.1 | 21.9 |
| | 40 | 23.1±5.2 | 20.6±4.7 | 20.0±4.6 | 20.3±4.6 | 20.5±4.8 | 20.9±6.0 | 20.5±6.8 | 22.3±0.6 |

Athymic mice (n=15-30) bearing 3 d i.p. LS-174T tumor xenografts were injected with 20, 30 or 40 μCi $^{211}$At-trastuzumab to validate the effective, suboptimal, therapeutic dose for subsequent studies with chemotherapeutics. Animal weights were monitored 1-2 times per week for ~5 weeks as an indicator of toxicity. Additional groups included those that received no treatment and those that were injected with 20, 30, or 40 μCi of the non-specific control, $^{211}$At-HuIgG.

**Table S3.** Tumor targeting and normal organ distribution of $^{111}$In-trastuzumab following i.v. administration in athymic mice bearing s.c. LS-174T xenografts

| | Time points (d) | | | | |
|---|---|---|---|---|---|
| Tissue | 24 | 48 | 72 | 96 | 168 |
| Blood | 7.15±0.89 | 5.09±0.43 | 3.90±1.14 | 2.28±1.34 | 2.16±0.55 |
| Tumor | 8.09±1.43 | 13.20±1.91 | 10.66±3.23 | 10.41±4.15 | 10.45±3.82 |
| Liver | 3.50±0.71 | 4.18±0.72 | 4.39±1.21 | 4.60±2.48 | 4.89±1.16 |
| Spleen | 2.57±0.40 | 3.07±1.00 | 2.12±0.42 | 3.85±2.71 | 2.50±0.41 |
| Kidney | 3.48±0.64 | 3.60±0.38 | 2.66±0.69 | 2.55±0.53 | 2.17±0.51 |
| Lung | 2.65±0.10 | 4.26±4.63 | 1.95±0.59 | 1.44±0.52 | 1.37±0.23 |
| Heart | 2.59±0.57 | 1.64±0.24 | 1.34±0.29 | 1.04±0.25 | 0.87±0.20 |
| Femur | 0.97±0.15 | 0.96±0.11 | 0.80±0.31 | 1.34±1.14 | 0.71±0.16 |

Athymic mice bearing s.c. LS-174T xenografts and non-tumor bearing mice were injected (i.v.) with $^{111}$In-trastuzumab (~7.5 μCi). The mice were sacrificed by exsanguination, the blood and tissues were harvested, wet-weighed and the radioactivity measured. The values represent the average percent injected dose per gram of tissue (%ID/g). The standard deviations were also calculated and are given in parentheses.

**Table S4.** Tumor targeting and normal organ distribution of $^{227}$Th-trastuzumab following i.v. administration in athymic mice bearing s.c. LS-174T xenografts

| | Time points (d) | | | | |
|---|---|---|---|---|---|
| Tissue | 24 | 48 | 72 | 96 | 168 |
| Blood | 9.25±2.07 | 7.73±1.31 | 5.25±0.98 | 4.80±1.06 | 2.90±1.20 |
| Tumor | 12.68±3.69 | 18.40±9.74 | 16.93±5.54 | 22.99±8.09 | 21.56±13.22 |
| Liver | 6.45±1.71 | 7.19±1.62 | 6.80±1.11 | 7.07±1.21 | 12.15±3.04 |
| Spleen | 4.40±0.94 | 6.31±3.81 | 5.16±1.77 | 3.99±0.96 | 6.33±2.25 |
| Kidney | 5.87±0.90 | 6.68±2.83 | 5.64±1.31 | 3.71±2.67 | 5.01±1.69 |
| Lung | 4.20±0.72 | 3.94±0.65 | 2.96±0.44 | 3.02±0.59 | 1.69±0.56 |
| Heart | 3.55±0.82 | 2.98±1.08 | 2.09±0.63 | 2.13±0.57 | 1.29±0.74 |
| Femur | 2.61±0.48 | 4.11±2.01 | 4.21±0.62 | 3.32±0.94 | 4.29±0.75 |

Athymic mice bearing s.c. LS-174T xenografts and non-tumor bearing mice were injected (i.v.) with $^{227}$Th-trastuzumab (~7.5 μCi). The mice were sacrificed by exsanguination, the blood and tissues were harvested, wet-weighed and the radioactivity measured. The values represent the average percent injected dose per gram of tissue (%ID/g). The standard deviations were also calculated and are given in parentheses.

**Table S5.** Effect of Increasing $^{227}$Th-Radioimmunotherapy Doses on the Weights of Athymic Mice Bearing i.p. LS174T Tumor Xenografts.

| | | Days Post Radioimmunotherapy | | | | | |
|---|---|---|---|---|---|---|---|
| RIT | Dose | 0 | 7 | 11 | 14 | 18 | 27 |

| | (μCi) | | | | | | |
|---|---|---|---|---|---|---|---|
| None | None | 26.8±2.7 | 27.7±2.7 | 28.0±4.0 | | | |
| Trastuzumab | 0.25 | 28.0±2.4 | 26.7±2.2 | 26.3±2.2 | 25.8±2.4 | 25.6±2.3 | |
| | 0.5 | 28.2±2.1 | 25.8±2.4 | 26.0±2.9 | 25.7±3.7 | 26.2±3.5 | 27.5±1.1 |
| | 1.0 | 28.2±2.7 | 25.5±2.3 | 24.4±4.0 | 24.8±3.0 | 25.6±2.0 | |
| | 2.0 | 27.9±1.6 | 25.2±1.1 | 22.8±2.1 | 21.9 | | |
| | 5.0 | 28.2±2.5 | 23.9±2.7 | | | | |
| Panitumumab | 0.25 | 26.4±1.8 | 24.8±1.3 | 25.3±1.2 | 25.0±1.8 | 24.3±0.4 | 26.1 |
| | 0.5 | 28.5±1.9 | 26.0±1.7 | 26.3±1.5 | 26.0±1.8 | 26.5±1.6 | 27.3±2.3 |
| | 1.0 | 28.1±2.5 | 25.5±2.8 | 24.9±3.0 | 23.6±2.2 | 25.5±2.7 | 25.0±2.9 |
| | 2.0 | 28.0±0.9 | 25.5±1.3 | 24.7±1.7 | 23.8±1.9 | 24.9±1.3 | 22.7±3.1 |
| | 5.0 | 28.6±2.3 | 25.3±2.3 | 20.2±1.8 | | | |
| HuM195 | 0.25 | 27.7±1.2 | 28.2±1.1 | 29.5±2.3 | 29.1± | | |
| | 0.5 | 26.9±2.7 | 26.9±2.8 | 26.8±2.0 | 24.9±1.4 | 24.3±1.1 | 24.7±1.1 |
| | 1.0 | 26.0±2.0 | 25.1±2.4 | 25.5±1.5 | 27.0 | 26.7 | 26.7 |
| | 2.0 | 27.6±1.6 | 26.7±2.3 | 26.0±2.3 | 24.9 | | |
| | 5.0 | 27.8±1.5 | 26.0±1.6 | 22.8±2.6 | | | |

Athymic mice (n=10) bearing 3 d i.p. LS-174T tumor xenografts were injected with doses of $^{227}$Th-trastuzumab or $^{227}$Th-panitumumab from 0.25-5.0 μCi to establish the effective, suboptimal, therapeutic dose for subsequent studies with chemotherapeutics. Animal weights were monitored 1-2 times per week for 4 weeks as an indicator of toxicity. Additional groups included those that received no treatment and those that were injected with the non-specific control, $^{227}$Th-HuM195.