Utility of ^{211}At -trastuzumab for the Treatment of Metastatic Gastric Cancer in the Liver: Evaluation of a Preclinical α -Radioimmunotherapy Approach in a Clinically-relevant Mouse Model

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ABSTRACT

A liver metastasis from a primary gastric cancer (LMGC) is relatively common and results in an extremely poor prognosis due to a lack of effective therapeutics. We here demonstrate in a clinically-relevant mouse model that an α -radioimmunotherapy (α -RIT) approach with astatine-211-labeled-trastuzumab (^{211}At -trastuzumab) has efficacy against LMGCs that are positive for human epidermal growth factor receptor 2 (HER2).

Methods: ^{211}At was produced in a cyclotron via a $^{209}\text{Bi} (\alpha, 2n) ^{211}\text{At}$ reaction. ^{211}At -trastuzumab was subsequently generated using a single-step labelling method. NCI-N87 cells (HER2-positive human GC cells) carrying a luciferase gene were intrasplenically transplanted into severe combined immunodeficiency mice to generate a HER2-positive LMGC model. A bio-distribution study was then conducted through the intravenous injection of ^{211}At -trastuzumab (1 MBq) into these LMGC xenograft mice. In parallel with this experimental therapy, PBS, intact trastuzumab or ^{211}At -non-specific human IgG (1MBq) were injected into control groups. The therapeutic efficacy was evaluated by monitoring tumor changes by chemiluminescence imaging. Monitoring of body weights, white blood

cell counts, and serum markers of tissue damage were conducted at regular intervals.

Microdosimetry using a CR39 plastic detector was also performed.

Results: The biodistribution analysis revealed an increased uptake of ^{211}At -trastuzumab in the metastasized tumors that reached approximately 12% of the injected dose per gram of tissue (%ID/g) at 24 hours. In contrast, its uptake to the surrounding liver was about 4%ID/g.

The LMGCs in the mouse model reduced dramatically at 1 week after the single systemic injection of ^{211}At -trastuzumab. No recurrences were observed in six of eight mice treated with this single injection and their survival time was significantly prolonged compared to the control groups, including the animals treated with ^{211}At -non-specific antibodies. No severe toxicities or abnormalities in terms of body weight, white blood cell number, liver function, or kidney parameters were observed in the ^{211}At -trastuzumab group. Microdosimetric studies further revealed that ^{211}At -trastuzumab had been delivered at an 11.5- fold higher dose to the LMGC lesions compared to the normal liver.

Conclusion: α -RIT with ^{211}At -trastuzumab has considerable potential as an effective and safe therapeutic option for LMGC.

Keywords: cancer metastasis; gastric cancer; HER2; α -RIT

INTRODUCTION

Gastric cancer (GC) is the fifth most frequently diagnosed cancer and the third leading cause of cancer-related death worldwide (1). Significantly, 35% of GC patients develop a distant metastasis at the time of diagnosis, with 4-14% of GCs developing a liver metastasis (LMGC) (2). No satisfactory therapeutics have yet been established for LMGCs and the prognosis is therefore dismal with a 5-year survival rate of 0-10%. Human epidermal growth factor receptor 2 (HER2) has shown promise as a therapeutic target for GC as HER overexpression is observed in 7-34% of patients (3). Of note in this regard, previous studies have reported a higher HER2 positivity in LMGCs (37.2%) and a correlation of this overexpression with the poor prognosis in these patients (4,5). Indeed, a recombinant humanized monoclonal antibody targeting the HER2 protein, trastuzumab, has now been clinically used for HER2-overexpressing GCs (6). However, despite the clinical benefits of this drug in combination with chemotherapy, the long-term survival of LMGC patients remains unsatisfactory (7).

α -particle radioimmunotherapy (α -RIT) is a targeted radionuclide treatment regimen that utilizes monoclonal antibodies for the specific delivery of radionuclide emitting α -

particles (8). Astatine-211 (^{211}At) is one of the currently attractive alpha-particle emitters in terms of clinical use because it has an appropriate half-life (7.2 hours) and can be manufactured using a cyclotron (9). Our prior preclinical studies in mouse models have provided evidence that ^{211}At -labeled antibodies are effective against several cancers without severe toxic effects (10,11). The therapeutic effectiveness of ^{211}At -labeled trastuzumab (^{211}At -trastuzumab) has been reported (12,13). Furthermore, the benefit of single-domain antibody fragments targeting HER2 has been studied (14).

We hypothesized that a targeted delivery of α -particles using a cancer-specific antibody would have therapeutic efficacy against LMGC. We thus investigated the use of ^{211}At -trastuzumab against HER2-overexpressing LMGCs in a preclinical mouse model.

MATERIALS AND METHODS

Cells and Reagents

The human NCI-N87 (N87) cell line was purchased from ATCC (Manassas, VA) and was transfected with RediFect Red-FLuc-Puromycin lentiviral particles to establish N87 cells

carrying luciferase genes (N87/Luc), as described previously (13). The MKN45-Luc (MKN45/Luc) cells were obtained from Japanese Collection of Research Bioresources Cell Bank (Ibaraki, Japan). Cells were cultured in RPMI-1640 medium, supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (Wako, Osaka, Japan), and maintained in humidified atmosphere of 5% CO₂ at 37°C. The anti-HER2 monoclonal antibody trastuzumab and human IgG (HuIgG) were purchased from Chugai Pharmaceutical (Tokyo, Japan) and Invitrogen (Carlsbad, CA), respectively. *N*-succinimidyl-3-(trimethylstannyl)benzoate was obtained from Santa Cruz Biotechnology (Dallas, TX) and stored at -30°C. *N*-chlorosuccinimide was purchased from Tokyo Chemical Industry (Tokyo, Japan).

Antibodies and Radiochemistry

²¹¹At was produced in a cyclotron via a ²⁰⁹Bi (α , 2n)²¹¹At reaction at the National Institute of Radiological Sciences of the National Institutes for Quantum and Radiological Science and Technology (Chiba, Japan), as described previously (15). The ²¹¹At labeling of antibodies

was also carried in accordance with a previously described method (10). Briefly, antibodies (3-5 mg/ml, in 0.2 M sodium carbonate buffer pH 8.5) were conjugated with *N*-succinimidyl-3-(trimethylstannyl)benzoate and this immunoconjugated preparation was isolated in PBS using a Sephadex 50 spin column (GE Healthcare). The tin concentration of the immunoconjugates were determined by inductively coupled plasma mass spectrometry using an Agilent 7500a device (Yokogawa Analytical Systems, Tokyo, Japan) (16). Briefly, the protein solutions (100 μ l, 232.8-296.5 μ g of protein) were digested with 0.5 ml of nitric acid (Tama Chemicals, Kawasaki, Japan) at 90°C for 30 min using a microwave oven, diluted with ultrapure water. Under our experimental conditions, the quantitation limit of tin was 0.042 ng/g. The tin levels of trastuzumab or HuIgG without *N*-succinimidyl-3-(trimethylstannyl)benzoate in the solvent were below this quantitation limit. The immunoconjugate was then adjusted to pH 5.5 by adding citric acid prior to labeling. ^{211}At (55-90 MBq) was dissolved with 0.04 mg/ml *N*-chlorosuccinimide in methanol supplemented with 1% acetic acid for labeling. The immunoconjugate (2-3 mg/ml) was added to ^{211}At and reacted for 1 min followed by another 1 min reaction with 2 mg/ml *N*-

chlorosuccinimide. Finally, sodium ascorbate (50 mg/ml) was added to stop the reaction.

^{211}At -labeled antibodies (^{211}At -trastuzumab and ^{211}At -HuIgG) were isolated in PBS using a Sephadex 50 spin column and verified by high-performance liquid chromatography.

Animal Experiments

All animal experiments conducted in this study were approved by the Animal Care and Use Committee of the National Institute of Radiological Sciences at the National Institutes for Quantum and Radiological Science and Technology and were undertaken in compliance with the institutional guidelines regarding animal care and handling.

LMCG Xenograft Mouse Model

N87/Luc or MKN45/Luc cells (2×10^6 cells in 50 μl medium) were intrasplenically transplanted into C.B17/Icr-scid/scidJcl mice (CLEA Japan, Inc, Tokyo, Japan) under anesthesia to form liver metastases via the splenic vein. The spleen was resected after transplantation to avoid tumor formation in the spleen.

Biodistribution and dose estimation

Biodistribution studies were performed as described previously (10). Briefly, ^{211}At -trastuzumab or ^{211}At -HuIgG (1 MBq/100 μl) was intravenously injected into the LMGC xenograft mice. At 1, 3, and 24 h post injection, a group of 4-5 mice was euthanized and tumor and tissues were dissected. The activities in each tissue were measured using a gamma counter (Aloka, Tokyo, Japan) to calculate the %ID/g. The absorbed dose of tumor and tissues were estimated as described previously (17). Briefly, the doses were estimated using the area under the curve on the basis of the biodistribution data (the trapezoidal method) and the mean energy emitted per transition of ^{211}At and a daughter nuclide ^{211}Po with a correction for the branching ratio (18). A radiation weighting factor of 5 was used (19).

Experimental Therapy

The experimental therapy was performed by giving an injection of ^{211}At -trastuzumab (1MBq) intravenously to the LMGC xenograft mice. PBS, intact trastuzumab or ^{211}At -HuIgG (1MBq) were also injected into control groups. All protein doses were adjusted to the equivalent

amount (10 µg) by adding intact antibody. The therapeutic efficacy was evaluated by monitoring tumor changes via chemiluminescence imaging. Monitoring of body weights, white blood cell counts, and serum markers of tissue damage were conducted at regular intervals to evaluate the toxicity of the treatment. Mice were euthanized at an endpoint in which the chemiluminescence intensity of the tumor reached 1×10^6 or at 120 days post-injection.

Immunohistochemistry and Histological Analysis

The HER2 expression level in the LMGCs was confirmed by immunohistochemical staining using a Histofine® HER2 kit (Nichirei Biosciences) in accordance with the manufacturer's instructions. Histological analysis was performed as described previously (10). Briefly, LMGC xenograft tissues were sampled from the mice at 1, 3, and 24 h post-injection of ^{211}At -trastuzumab, fixed with 10% (v/v) formalin and embedded in paraffin for sectioning. After sectioning, sequential samples were used for HER2 and hematoxylin and eosin (H&E) staining, respectively.

Microdosimetry

α -particle detection and microdosimetry were performed as described previously (20,21).

Briefly, LMGC xenograft tissues containing a normal liver region were dissected at 24 h after the i.v. injection of ^{211}At -trastuzumab (1 MBq), filled with OTC compound, and sectioned at an 8- μm thickness using a cryotome (Leica Biosystems, Tokyo, Japan). Sectioned samples were then placed on a CR-39 plate and exposed for 8 h at room temperature. During the exposure, the same sections were stained with H&E, and histologic images were acquired using a scanning microscope with an x20 objective lens. At 8 h after placement on CR39, the samples were removed and the CR-39 plate was chemically etched for 2 h in 7 M sodium hydroxide solution at 70°C. Microscope images of the α -particle tracks were captured under an FSP-1000 microscope (Seiko Time Systems Inc., Japan) and analyzed with track analysis software (PitFit) (22).

Statistical Analysis

Statcel 3 software (OMS, Tokorozawa, Japan) were used for all statistical analysis. Tumor

volumes and survival data were analyzed using two-way repeated measures ANOVA and the Kaplan-Meier method, respectively. A *P*-value of <0.05 was considered significant.

RESULTS

Radiochemistry

The number of tin groups per antibodies measured using inductively coupled plasma mass spectrometry were 5.2 ± 0.1 ($n = 3$) and 2.6 ± 0.5 ($n = 3$) for trastuzumab and HuIgG immunoconjugates, respectively. The labeling yield and specific activity of our test molecules were $51.6 \pm 9.1\%$ and $95.2\text{--}453.0$ kBq/ μg for ^{211}At -trastuzumab and $56.2 \pm 9.7\%$ and $19.9\text{--}521.7$ kBq/ μg for ^{211}At -HuIgG, respectively. The radiochemical purities of these agents were consistently measured above 95%.

Establishment of an LMGC Mouse Model

An LMGC mouse model was established by transplanting N87/Luc or MKN45/Luc cells via the splenic vein into C.B17/Icr-scid/scidJcl mice (Supplemental Figures 1 and 2). LMGC

lesions in this mouse model were confirmed both by chemiluminescence and visually (Supplemental Figure 1A-D). Immunohistochemical analyses confirmed the overexpression of HER2 in the LMGCs lesions containing N87/Luc cells (Supplemental Figure 1E,F).

Biodistribution and Absorbed Dose of ^{211}At -trastuzumab in the LMGC Mouse Model

Because the *in vitro* cell binding of ^{211}At -trastuzumab to N87 cells and resulting cytotoxicity was reported previously (13), we investigated the biodistribution of this agent in our LMGC mice following the splenic vein implantation of N87/Luc cells into these animals. We measured longitudinal changes in the ^{211}At uptake in each tissue and tumor after the mice received an intravenous (i.v.) injection of ^{211}At -trastuzumab (1 MBq) (Figure 1A). The tumor uptake was $8.8 \pm 5.0\% \text{ID/g}$ 1 hour after injection and increased over time up to $12.3 \pm 2.0\% \text{ID/g}$ at 24 hours post-injection. Because of its systemic administration, ^{211}At uptakes in the blood ($29.8 \pm 1.8\% \text{ID/g}$), heart ($6.7 \pm 0.7\% \text{ID/g}$), lung ($10.2 \pm 1.1\% \text{ID/g}$), and liver ($10.1 \pm 0.9\% \text{ID/g}$) were relatively higher at 1 hour than in other tissues, but then gradually decreased ($15.6 \pm 1.7\% \text{ID/g}$, $4.3 \pm 0.9\% \text{ID/g}$, $8.3 \pm 3.1\% \text{ID/g}$, $4.0 \pm 2.1\% \text{ID/g}$, respectively).

Higher uptakes in the thyroid and stomach ($6.3 \pm 0.9\%ID/g$ and $14.1 \pm 4.4\%ID/g$ at 24 h) were observed, likely due to free ^{211}At . We further observed that ^{211}At -trastuzumab was mainly excreted in the urine ($3.8\%ID$ up to 24 h, Figure 1B). The absorbed dose by each tissue was calculated from the biodistribution data (Table 1). The absorbed dose at the tumor was estimated to be 4.58 Gy up to 24 hours post-injection. The tumor-to-normal liver ratio was 1.59, calculated from the biodistribution data. The ^{211}At uptake decreased in the tumor at 24 hours after the injection of ^{211}At -trastuzumab, compared to a reduction after only 1 or 3 hours in another LMGC model generated using MKN45/Luc cells that have a significantly lower HER2 expression level than N87/Luc cells (Supplemental Figure 2). In addition, ^{211}At uptake was found in our analysis to be decreased in the tumor at 24 hours after the injection of ^{211}At -HuIgG compared to only 1 or 3 hours in the N87/Luc LMGC model (Supplemental Figure 3).

Therapeutic Efficacy of ^{211}At -trastuzumab Against LMGC in the Mouse Model

We next evaluated the therapeutic efficacy of α -RIT using ^{211}At -trastuzumab to treat LMGCs

in our mouse model (Figure 2 and 3). Chemiluminescence imagery revealed that a single injection of ^{211}At -trastuzumab eradicated the LMGC lesions in the mice, whilst these tumors grew aggressively in the animals that received control injections of PBS, unlabeled trastuzumab, or ^{211}At -HuIgG (Figure 2B). The relative chemiluminescence intensity of the LMGCs (Figure 3A) and the tumor changes at 28 days post-treatment (Figure 3B) indicated that these lesions were well controlled by the systemic injection of ^{211}At -trastuzumab. No recurrences were observed in six of eight mice treated with a single injection of ^{211}At -trastuzumab during the period of observation. ^{211}At -trastuzumab also significantly prolonged the survival of the LMGC mice (Figure 3C) compared to the control treatment groups. Histopathological studies revealed necrotic lesions in both untreated and ^{211}At -trastuzumab-treated LMGCs (Figure 3D). However, the percentage of necrotic lesions in the tumors was higher in the ^{211}At -trastuzumab-treated group.

Toxicity

We evaluated the toxicity levels of ^{211}At -trastuzumab in the mouse model by monitoring body

weights and conducting biochemical examinations of liver and kidney functions. The body weights in the animals decreased immediately and started to recover about 1 week after injection of either ^{211}At -HuIgG or ^{211}At -trastuzumab (Figure 4A). No obvious leukocytopenia was observed during the period of observation (Figure 4B). Biochemical examinations indicated no significant changes in the GOT, GPT, BUN, or creatinine levels before and after treatment (Figure 4C). No atrophy, apoptosis or noticeable damage was observed histopathologically in either the liver or stomach at 3 hours after α -RIT treatment (Figure 4D).

Microdosimetry

Figure 5 shows an intensity map of the α -particle tracks emitted from ^{211}At on the metastatic tumors in an LMGC mouse liver at 24 hours post-injection of ^{211}At -trastuzumab. We extracted the tumor region of interest (Figure 5A, dark pink) from a frozen section that included metastatic tumors by binarization with setting band-pass thresholds for the 24-bit color levels (red, 255; green, 163; blue, 173), and the number of α -tracks located in the tumor

region of interest in the tissue sample was counted in a binarized image (Figure 5A). High density α -tracks were observed in the tumor region compared to the normal tissue region; the numbers of α -tracks per unit area of tumor and normal liver were $49,849 \pm 975 / \text{cm}^2$ and $5,125 \pm 126 / \text{cm}^2$, respectively. The mean absorbed doses per section in the tumor and normal tissue regions at 24 hours were 54.1 ± 1.1 mGy and 4.7 ± 0.1 mGy, respectively, suggesting that the tumor region received an 11.5-fold higher dose compared to the normal liver (Figure 5B). This dosimetry was performed in an 8 μm -thick tissue sample from which we visualized a very localized dose distribution in the section that distinguished the area in which the α -particles were concentrated in the tumor.

DISCUSSION

The results of our present analysis indicate that a systemic injection of ^{211}At -trastuzumab achieves dramatic tumor control in a clinically-relevant mouse model of HER2-overexpressing LMGCs. In the mice treated with ^{211}At -trastuzumab, the LMGCs were eradicated without recurrence during the monitored period in six out of eight animals.

Previous studies have indicated that α -particles induce huge clusters of irreparable DNA double strand breaks and thereby cause targeted cancer cell death (10,11). Consistent with these findings, we found in our present analyses that broad necrotic regions arise in LMGCs treated with ^{211}At -trastuzumab. The survival of LMGC mice treated with ^{211}At -trastuzumab was also significantly prolonged compared to the control-treated animals. These findings are particularly relevant because despite the continuing improvements in chemotherapy regimens, current treatments for LMGC patients are still limited in terms of extending survival, and the prognosis in these cases thus remains dismal. Hepatic resection and radiofrequency ablation are recommended in these cases if the metastasis is limited to the liver, but patients with LMGC often have multiple metastatic tumors in the liver and further metastases in the peritoneum, lymph nodes and adjacent organs (23-25). Hence, our current therapeutic strategy holds promise for the future management of LMGCs that are positive for HER2.

Targeting α -particle therapy or α -RIT using ^{211}At has shown to be therapeutically effective for HER2-positive cancers in various mouse xenograft systems. For example, ^{211}At -

trastuzumab has shown to be therapeutically effective in a mouse model of radioresistant ovarian cancer (12). A previous study reported the effectiveness of single-domain antibody fragments as an attractive platform for ^{211}At delivery targeting HER2 because of their prolonged tumor targeting and rapid clearance from normal tissues (14). In addition, α -emitters other than ^{211}At are also utilized for α -RIT against cancer. Thorium-227-labeled trastuzumab has shown significant therapeutic effects in a HER2-positive ovarian cancer xenograft (26). Recently also, α -RIT using bismuth-213 has been shown to be effective for experimental pancreatic ductal adenocarcinoma (27).

Since α -particles are highly cytotoxic, the safety of the α -RIT approach is an important consideration for possible clinical applications. Damage to normal liver tissues will be one of the top issues of concern if this treatment approach is being tested for metastatic tumors in the liver. Hence, an effective and accurate dose estimation in the normal liver and metastatic tumors is of particular importance. We have here estimated the appropriate radiation dose for the normal liver and metastatic tumors using two different approaches; a biodistribution-based dose estimation and microdosimetry using a CR-39 plastic detector that

we have previously reported (21). The tumor to liver ratios in in absorbed dose at 24 hours after injection were 1.6 and 11.5 in the biodistribution-based dose estimation and the microdosimetry, respectively (Table 1 and Figure 5B). LMGCs were carefully dissected to separate them from normal liver in the former approach but normal liver tissues were inevitably mixed in with the LMGC samples measured, which would affect the result. Notably, ^{211}At or ^{211}At -trastuzumab would be expected to be localized in tissues with a non-homogenous distribution. Our microdosimetric analyses revealed that a 10-fold higher dose had been given to the tumor compared to the normal liver, suggesting that the α -particles had been successfully targeted using the antibody and that our strategy minimized the damage to the surrounding normal tissue (Figure 5). This is highly relevant to the established and continuing concerns about systemic drug administration, including hematological toxicity levels, damage to excretion pathways (liver and kidney), and the accumulation of free ^{211}At in normal tissues.

Although a transient but recoverable loss of body weight was observed in our α -RIT-treated LMGC mice, no apparent toxicities were evident over the monitoring period.

Moreover, higher uptakes of ^{211}At -trastuzumab were observed in the stomach (Figure 1A) but we found no apparent histopathological abnormalities (Figure 4J). These data are supportive of the safety of ^{211}At -trastuzumab in terms of its translation to clinical use.

There were some limitations of this study of note. Although our LMGC model is highly relevant to the clinical manifestations of LMGC, the severe combined deficiency mice we used have of course severely defective immunity and the effects of the immune system in tumor eradication via ^{211}At -trastuzumab are unknown. Tumor-bearing mouse models with a functional immune system may be desirable for future studies of this treatment approach. In addition, we only used a single injection of ^{211}At -trastuzumab in our current mouse experiments but because ^{211}At has a short half-life, this agent could conceivably be administered via multiple injections to improve its efficacy. Further studies would be needed to evaluate the therapeutic efficacy and toxicity associated with a multi-injection approach.

In conclusion, α -RIT using ^{211}At -trastuzumab is efficacious in a clinically-relevant mouse model of LMGC lesions that overexpress HER2. We propose that this strategy holds promise as a therapeutic option for LMGC in human patients.

DISCLOSURE

The authors declare no financial or other competing interests.

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KEY POINTS

QUESTION: What is the potential of ^{211}At -trastuzumab for treating HER2-positive LMGC?

PERTINENT FINDINGS: A single intravenous injection of ^{211}At -trastuzumab induces a significant growth inhibition of LMGC in the mouse.

IMPLICATIONS FOR PATIENT CARE: ^{211}At -trastuzumab has potential as a future LMGC treatment in human patients.

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FIGURES AND FIGURE LEGENDS

FIGURE 1. Biodistribution of ^{211}At -trastuzumab in the LMGC mouse model. (A)

Uptake %ID/g (percent injected dose per gram of tissue) values for ^{211}At in the blood, organs and tumor tissues at 1, 3, 24 h post i.v. injection of ^{211}At -trastuzumab (1 MBq). Data represent the mean $\pm$ SD ($n = 5$ for each timepoint). (B) Activity (%ID, percent injected dose) of ^{211}At in the feces and urine of the mice. Five animals were used at each timepoint. The data represent the mean ($n = 5$).

FIGURE 2. Radioimmunotherapy with $^{211}\text{At-trastuzumab}$ in the LMGC mouse model.

(A) Treatment schema. (B) Representative chemiluminescence images of the LMGC lesions

in the model mice on the day before (Day 0) and 1, 4, 8 weeks (wk) after treatment. Mice were treated with either PBS (control), trastuzumab (10 μ g), ^{211}At -HuIgG (1MBq, 10 μ g) or ^{211}At -trastuzumab (1MBq, 10 μ g). The color scale indicates the chemiluminescence intensity per pixel.

FIGURE 3. Results of ^{211}At -trastuzumab radioimmunotherapy in the LMGC mouse model.

(A) Waterfall plot of the percent tumor chemiluminescence intensity change in each mouse at 28 days post-treatment. The tumor chemiluminescence intensity at Day 0 was set as the

baseline ($n=8$ in each group). (B) Relative chemiluminescence intensity of the tumor in each mouse. The chemiluminescence intensity at Day 0 was considered to be 100% ($n = 8$ in each group). (C) Kaplan-Meier survival curves for the LMGC mice. * $P<0.05$ versus control; ** $P<0.05$ versus trastuzumab; *** $P<0.05$ versus $^{211}\text{At-HuIgG}$ ($n = 8$ in each group). (D) Histopathological analysis by H&E staining of LMGC lesions that were untreated and were treated for 3 h with α -RIT. Scale bars, 50 μm . Percentages of necrosis in the tumors are shown in the right. Two (for untreated) or five (for treated with $^{211}\text{At-trastuzumab}$) mice were used. Data represent the mean $\pm$ SD.

FIGURE 4. Toxicity evaluation of ^{211}At -trastuzumab radioimmunotherapy in the LMGC mouse model. (A) Body weights and (B) white blood cell counts of the LMGC mice after treatment are shown. Plots are interrupted if the mouse reached the endpoint. Data represent the mean $\pm$ SD. (C) Glutamic oxaloacetic transaminase (GOT), glutamic pyruvate transaminase (GPT), blood urea nitrogen (BUN) and creatinine (CRE) levels measured up to 30 days after ^{211}At -trastuzumab radioimmunotherapy. Data for each mouse are shown in the graph. UT; untreated mice. (D) Histopathological analysis by H&E staining of the mouse liver and stomach in animals that were untreated or treated with ^{211}At -trastuzumab for 3 h with α -RIT, respectively. Scale bars, 50 μm .

FIGURE 5. Microdosimetry. (A) Microscopic images of a sectioned-sample of mouse liver tissue, including metastasized tumors, and contour map of the α -particle track density at binned positions (ΔX , ΔY) with 50 μm intervals at 24 h post treatment. Scale bars, 2 mm. (B) Estimated local absorbed dose per frozen section at the tumor and normal liver regions at 24 h post treatment.

TABLE 1

Absorbed does (Gy) by each of the indicated tissue types at 24 h after the i.v. injection of

^{211}At -trastuzumab (1 MBq)

Tissue	Absorbed dose (Gy)
Whole blood	10.11
Heart	2.96
Lung	4.39
Salivary glands	1.61
Thyroid (Neck)	2.95
Liver	2.88
Pancreas	0.85
Stomach	5.79
Small intestine	1.20
Large intestine	0.66
Kidney	2.90
Muscle	0.27
Bone	0.87
Tumor	4.58

SUPPLEMENTAL FIGURE 1. Chemiluminescence images of the LMGC lesions in the model mice with the abdomen closed (A) and opened (B). Color scale indicates the luminescence intensity per pixel. (C) Visual images of the data shown in (B). (D) Liver dissected from the same mouse. Arrows in (C) and (D) highlight the visible lesions. (E) H&E and (F) HER2 immunohistochemical staining of an LMGC dissected from the xenograft mouse model.

SUPPLEMENTAL FIGURE 2. An LMGC mouse model generated using MKN45/Luc cells. (A) Western blotting analysis. (B) Visual image of the LMGCs. The yellow arrow indicates the visible tumor. (C) Biodistribution of ^{211}At -trastuzumab in the MKN45/Luc-LMGC model mice.

SUPPLEMENTAL FIGURE 3. Biodistribution of ^{211}At -HuIgG in the N87/Luc-LMGC model mice.

Graphical Abstract