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Supplementary Information

for

Synthesis, Quality Control, and *In vivo* Evaluation of ^{153}Sm-Labeled Fe_3O_4@lapatinib Nanoparticles as a Potential Therapeutic Agent for Breast Cancer

1. Determination of radiochemical purity

The assessment of radiochemical purity of Fe_3O_4@lapatinib-^{153}Sm was conducted using a multi-channel gamma spectrometer (ORTEC, USA), with a resolution (FWHM) of 2.3 keV at 1332 keV for ^{60}Co. The detection efficiency was set at 30% using the HPGE GR3019 detector to identify gamma peaks (qualitative analysis) and determine the percentage of radioactive activity of ^{153}Sm within the total radioactive activity of Fe_3O_4@lapatinib-^{153}Sm nanoparticles (quantitative analysis). The procedure was carried out as follows:

Open the sample vial and use a micropipette to carefully place 2 μL of the sample onto the center of a circular blue filter paper with a diameter of 1.5 cm, which is adhered to one side of a transparent adhesive tape. Allow the sample to air dry naturally. Once dry, securely seal the sample by placing another piece of adhesive tape on top. Subsequently, create a circular sample by cutting along the edges of the filter paper, resulting in a diameter of 2 cm.

Next, proceed to measure the counting rate on the gamma spectrum. Identify the radioactive nuclei that emit gamma rays by analyzing the distinct energy peaks that are registered on the gamma spectrum. Utilize the GammaVision – 32 software to compute the peak area corresponding to the gamma-emitting nuclei. The percentage of radiochemical purity attributed to these gamma-emitting nuclei can be determined using the subsequent formula:

$$\text{Radiochemical purity (\%)} = \frac{Peak\ area\ of\ 153_{Sm}}{Total\ peak\ area} x100\% \quad (1)$$

2. Determination of radiolabeling yield

The labeling efficiency of ^{153}Sm on Fe_3O_4@lapatinib was evaluated using paper chromatography with the self-scanning radioisotope device Cyclone, employing a stationary phase of Whatman No.1 filter paper (2x20 cm) and a mobile phase of a 10 mM DTPA solution. The procedure was carried out as follows:

- Place a 5 μL sample to be analyzed on the chromatography paper at the center of the second centimeter.
- Insert the paper into a container with the solvent, seal the container, and allow the solvent to move to the 20th centimeter.
- The chromatography process takes 60 minutes.
- Remove the paper, let it dry, place a thin plastic sheet underneath, and press a phosphor plate onto the chromatography paper.
- After approximately 30-60 seconds, secure the phosphor plate to the rotating carousel, place it in the scanning chamber of the Cyclone device, and set the scanning resolution to 300 dpi (85 micro pixels). The device performs scanning for 5 minutes.
- The percentage of radioactive activity in each radiation area on the chromatography paper is automatically calculated using the OptiQuant 5.0 software. The calculated results are presented in Digital Light Units (DLU/mm^2).
- The results for Fe_3O_4@lapatinib-^{153}Sm nanoparticles are located at the origin of the chromatography paper (R_f = 0.0-0.1), while the free ^{153}Sm portion migrates to positions R_f = 0.7 - 0.9.
- The labeling efficiency of Fe_3O_4@lapatinib-^{153}Sm nanoparticles is determined using the following formula:

Radiolabeling yied (%) = $\frac{A}{A_{tot.}} x100\%$ (2)

where: A represents the activity of Fe_3O_4@lapatinib-^{153}Sm nanoparticles, and A_{tot}. indicates the total activity of the entire chromatography paper.

3. Study of biological distribution in breast cancer xenografted mice

3.1. Tumor induction in mice

Immunodeficient NOD/SCID mice were raised in a controlled environment with filtered air and positive air pressure. The room temperature was maintained at 26 ± 0.5°C, with 55 ± 5% humidity, and automatic lighting cycling from 7:00 AM to 7:00 PM. Both food (Zeigler, USA) and water were sterilized before use. Each mouse cage was placed on a rack system with individual air circulation and HEPA-filtered air to ensure excellent isolation from pathogens and cross-contamination.

All procedures were carried out under aseptic conditions. The mice were housed in cages with air inlet and exhaust in a sterile laminar flow cabinet, at a temperature of 26 ± 2°C and an average humidity of 60 ± 5%. Immunodeficient mice were acclimatized for at least 3 days before conducting experiments. The immunodeficient mice were kept stable and injected with 100 μL of a solution containing 10^6 BT-474 (HER2+) cells per mouse subcutaneously in the right hind flank. After the tumor started to grow, we monitored the tumor size and the body weight of the mice weekly. The tumor size was measured with NSK calipers every 7 days, and the tumor volume (V) was calculated using the formula V = D x R^2 x 0.5 (where D is the length of the tumor, and R is the tumor diameter).

3.2. Biological distribution study

The biodistribution of radiolabeled nanoparticles was investigated in a total of 20 female BT474 (HER2+) xenograft mice (BALB/c), which were evenly distributed into 5 groups. All animals were anesthetized using 2.5% isoflurane in O_2 with a flow rate of 1 L/minute and administered 0.1 mL of Fe_3O_4@lapatinib-^{153}Sm (0.1 mCi) via tail

vein injection. Blood and selected organs were collected at specified time points of 30 minutes, 3 hours, 6 hours, 24 hours, and 48 hours. The collected samples were weighed, and their radioactivity was measured using a PTW Curiementor 4 isotope calibrator (Freiburg, Germany).

Next, the percentage of injected dose (%ID) and percentage of injected dose per gram of tissue (%ID/g) were calculated for various tissues and the tumor using formulas (3) and (4). Based on these results, the targeted delivery capability of Fe_3O_4@lapatinib-^{153}Sm to the breast cancer xenografts in mice was evaluated.

%ID = (Dose in tissue/organ) / (Total injected dose) x 100 (3)

%ID/g = (%ID) / (Organ weight) (4)

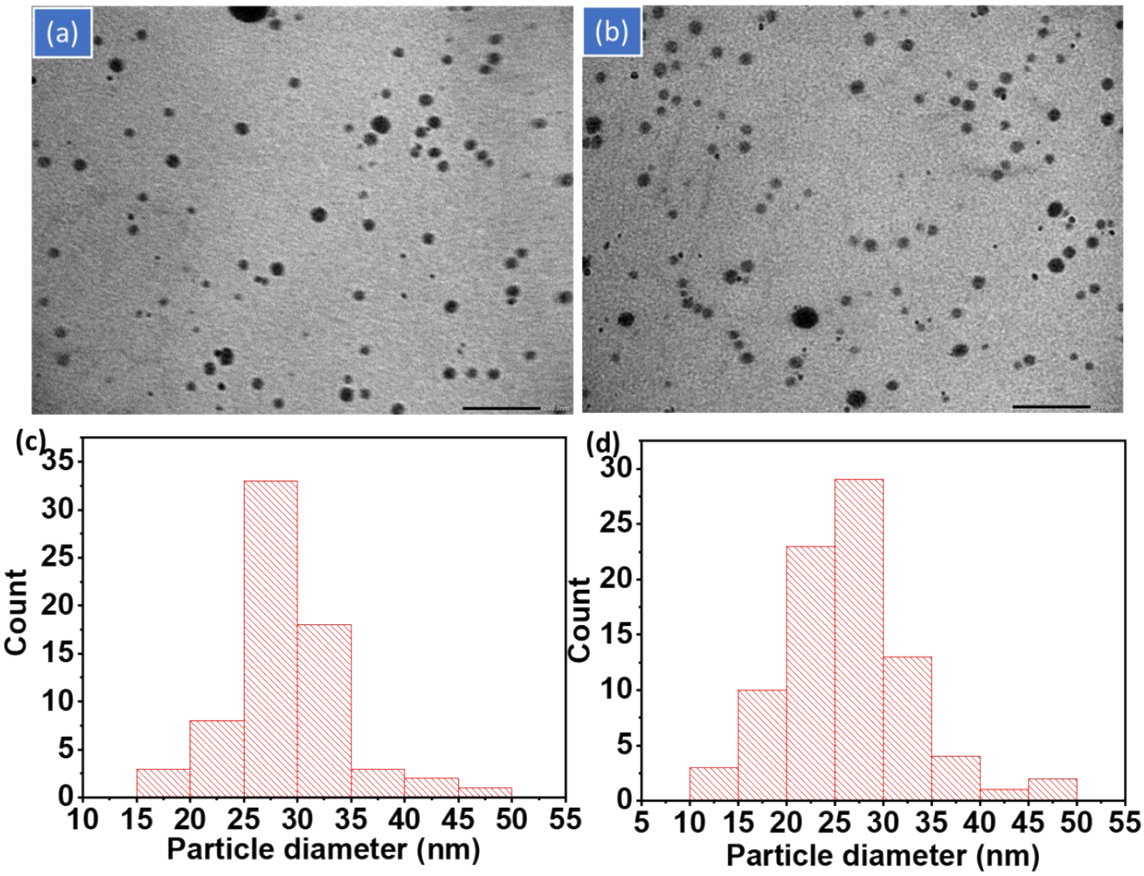

Fig. S1. TEM images of Fe_3O_4 (a) and Fe_3O_4@lapatinib (b), crystaline size distribution of Fe_3O_4 (c) and Fe_3O_4@lapatinib (d)

Table S1. The results of the sterility test of Fe_3O_4@lapatinib-^{153}Sm nanoparticles

Day	FTM				SCDM			
	Sample 1	Sample 2	Positive control	Negative control	Sample 1	Sample 2	Positive control	Negative control
01	-	-	+	-	-	-	+	-
02	-	-	+	-	-	-	+	-
03	-	-	+	-	-	-	+	-
04	-	-	+	-	-	-	+	-
05	-	-	+	-	-	-	+	-
06	-	-	+	-	-	-	+	-
07	-	-	+	-	-	-	+	-
08	-	-	+	-	-	-	+	-
09	-	-	+	-	-	-	+	-
10	-	-	+	-	-	-	+	-
11	-	-	+	-	-	-	+	-
12	-	-	+	-	-	-	+	-
13	-	-	+	-	-	-	+	-
14	-	-	+	-	-	-	+	-

Notes: *(-) : negative; (+): positive;* ***FTM****: Fluid Thioglycollate Medium;* ***SCDM****: Soybean Casein Digest Medium*

Table S2. Ratio and number of dead mice after injection of the prepared Fe_3O_4@lapatinib-^{153}Sm at different dose levels

Experimental goup	Sex	Dosage (mCi/kg)	Total number of mice tested	Number of mice died after 24 hours	Death rate (%)
Treated	Male	100	16	16	100
		80	16	16	100
		40	16	11	68.75
		30	16	6	37.50
		20	16	0	0
	Female	100	16	16	100
		80	16	16	100
		40	16	14	87.50
		30	16	9	56.25
		20	16	0	0
Control	Male and female	-	32	0	0

Table S3. Weight of mice at 24 h after injection

The sex of the mouse	Group	Weight before injection (g)	Weight after injection 24 h (g)
Male	Control (n=10)	20.79 ± 0.54	21.15 ± 0.52
	Treated (n=10)	20.92 ± 0.49	21.43 ± 0.92
Female	Control (n=10)	21.64 ± 0.38	22.91 ± 0.63
	Treated (n=10)	21.42 ± 0.30	22.09 ± 0.37

The data were represented as mean ± SD, and significance was considered wherever applicable as $p < 0.05$

Table S4. Weight of mice at 14 days after injection

The sex of the mouse	Group	Weight before injection (g)	Weight after injection 14 days (g)
Male	Control (n=6)	19.30 ± 0.39	33.47 ± 1.50
	Treated (n=6)	18.53 ± 1.20	26.73 ± 3.70
Female	Control (n=6)	20.33 ± 0.98	33.47 ± 1.50
	Treated (n=6)	22.30 ± 0.95	26.27 ± 2.10

The data were represented as mean ± SD, and significance was considered wherever applicable as $p < 0.05$

Table S5. Hematological parameters of mice after 24 hours of injection

Parameters	Female		Male	
	Treated (n=10)	**Control (n=10)**	**Treated (n=10)**	**Control (n=10)**
WBC (10^9/L)	1.77 ± 0.27*	5.31 ± 0.59	2.26 ± 0.27*	4.55 ± 0.65
RBC (10^{12}/L)	8.89 ± 0.22*	8.14 ± 0.17	8.46 ± 0.16*	7.60 ± 0.40
HGB (g/dL)	14.14 ± 0.48	13.52 ± 0.33	13.28 ± 0.34	12.53 ± 0.75
HCT (%)	46.24 ± 1.40	45.64 ± 0.72	43.64 ± 1.10	42.38 ± 2.40
MCV (fL)	52.00 ± 0.59*	55.13 ± 0.57	51.63 ± 0.72*	55.87 ± 0.94
PLT (10^9/L)	217 ± 28*	1057 ± 67	213 ± 34*	1082 ± 103

Note: **WBC***: White Blood Cell;* **RBC***: Red Blood Cel;* **HGB***: Hemoglobin;* **HCT***: Hematocrit;* **MCV***: Mean Corpuscular Volume;* **PLT***: Platelet. The data were represented as mean ± SD. * indicates statistical significance when comparing the test group to the control group of the same kind and significance was considered wherever applicable as $p < 0.05$*

Table S6. Biochemical parameters of mice 24 hours after injection

Parameters	Female		Male	
	Treated (n=10)	**Control (n=10)**	**Treated (n=10)**	**Control (n=10)**
Glucose (mmol/L)	1.050 ± 0.28*	6.44 ± 0.34	2.08 ± 0.32*	7.14 ± 0.36
Creatinin (µmol /L)	13.01 ± 2.10*	29.81 ± 1.80	16.31 ± 2.70*	29.35 ± 1.30
Protein TP (g/L)	61.97 ± 2.00*	47.20 ± 0.64	75.0 ± 8.10*	47.46 ± 0.97
Cholesterol TP (mmol/L)	15.0 ± 5.20*	2.97 ± 0.41	12.27 ± 2.50*	2.836 ± 0.27
AST (SGOT) (U/L)	351 ± 32*	124.60 ± 13	303 ± 21*	122.30 ± 9.20
ALT (SGPT) (U/L)	88.3 ± 8.90*	50.92 ± 2.80	88.3 ± 9.20*	52.94 ± 2.30

*The data were represented as mean ± SD. * indicates statistical significance when comparing the test group to the control group of the same kind and significance was considered wherever applicable as $p < 0.05$*

Table S7. Organ-to-body weight ratios of injected mice after 24 hours.

Organ-to-body weight ratios (%)	Female		Male	
	Treated (n=10)	**Control (n=10)**	**Treated (n=10)**	**Control (n=10)**
Heart	0.51 ± 0.011	0.53 ± 0.013	0.54 ± 0.02	0.59 ± 0.04
Brain	1.66 ± 0.067	1.59 ± 0.033	1.63 ± 0.06	1.71 ± 0.04
Liver	5.53 ± 0.19*	4.00 ± 0.81	5.19 ± 0.18*	4.50 ± 0.28
Kidney	1.05 ± 0.033	1.15 ± 0.053	1.10 ± 0.03	1.15 ± 0.05
Lungs	0.98 ± 0.052*	0.81 ± 0.051	0.99 ± 0.08*	0.71 ± 0.04

Spleen	0.30 ± 0.022	0.30 ± 0.038	0.37 ± 0.029	0.34 ± 0.013
Intestines	14.68 ± 0.40	14.97 ± 0.56	14.31 ± 0.54	14.47 ± 0.43
Stomach	1.93 ± 0.25	2.35 ± 0.20	1.71 ± 0.14	1.52 ± 0.17
Uterus	0.96 ± 0.011	0.98 ± 0.012		
Testicles			0.63 ± 0.028*	0.51 ± 0.036

*The data were represented as mean ± SD. * indicates statistical significance when comparing the test group to the control group of the same kind and significance was considered wherever applicable as $p < 0.05$*

Table S8. Organ-to-body weight ratios of injected mice after 14 days.

Organ-to-body weight ratios (%)	**Female**		**Male**	
	Treated (n=6)	**Control (n=6)**	**Treated (n=6)**	**Control (n=6)**
Heart	0.50 ± 0.028	0.50 ± 0.022	0.50 ± 0.013	0.47 ± 0.017
Brain	1.42 ± 0.09	1.15 ± 0.10	1.39 ± 0.13	1.19 ± 0.05
Liver	4.73 ± 0.20	4.73 ± 0.22	5.00 ± 0.16	5.41 ± 0.29
Kidney	1.01 ± 0.024	0.98 ± 0.046	1.30 ± 0.07	1.16 ± 0.06
Lungs	1.41 ± 0.28*	0.67 ± 0.02	1.33 ± 0.25*	0.65 ± 0.015
Spleen	0.40 ± 0.079	0.51 ± 0.037	0.48 ± 0.051	0.48 ± 0.039
Intestines	13.44 ± 0.47	11.91 ± 0.54	11.93 ± 0.65	14.05 ± 0.83
Stomach	2.16 ± 0.25	1.37 ± 0.13	1.58 ± 0.18	1.22 ± 0.05
Uterus	0.10 ± 0.01	0.08 ± 0.008		
Testicles			0.77 ± 0.10	0.53 ± 0.03

*The data were represented as mean ± SD. * indicates statistical significance when comparing the test group to the control group of the same kind and significance was considered wherever applicable as $p < 0.05$*