Cell lines and treatment

Experiments were performed with human osteosarcoma cells (U2OS) stably expressing somatostatin receptor type 2 (U2OS+SST2) and maintained as previously described (*1*). For uptake and survival experiments, cells were treated with different activity quantities of ^{177}Lu-DOTATATE (IDB Holland). Molar activity was 53 MBq/nmol, radiometal incorporation >95% and radiochemical purity >90%.

Immunofluorescent staining and imaging of cellular morphologies

Cells were grown on quartz coverslips (Xantec bioanalytics GmbH, Düsseldorf, Germany) in 6-well plates until ~25% confluency and fixed with 2% paraformaldehyde (Sigma Aldrich) for 15 min at room temperature (RT), permeabilized for 20 min at RT in PBS containing 0.1% Triton X-100 (Sigma Aldrich) and incubated in blocking buffer (PBS, 0.1% Triton X-100, 2% bovine serum albumin (Sigma Aldrich)) for 30 min at RT. Next, cells were incubated for 90 min at RT with the primary antibody, rabbit anti-Giantin (PRB-114C BioLegend, San Diego, CA, USA, 1/1000) diluted in blocking buffer. Following incubation cells were washed with PBS 0.1% Triton X-100 and incubated with 100nM SiR-actin (SC001 Spirochrome) and the secondary antibody (goat anti-rabbit Alexa Fluor 488 1/1000) in blocking buffer for 90 min at RT. Cells were washed with PBS and incubated with 1 μg/ml propidium iodide (Sigma Aldrich) and 10 μg/ml RNase in PBS for 30 min at RT. Cell were washed with PBS and mounted in 87% glycerol pH8.6 (Sigma Aldrich). Z-stack imaging was performed using a 4Pi confocal microscope (Leica, Mannheim, Germany) and images were analyzed using Image J software (*2*).

Uptake assay

Membrane bound and internalized fractions were collected as described previously (*1*) for 2.5 MBq/ml of added activity. Samples were collected every 15 min during the 4h treatment. Gamma

counter measurements were corrected for decay and the uncertainty on estimated activity fractions in the different cell compartments was calculated as one standard deviation of 2 independent experiments, each performed in triplicate. Furthermore, the total cell number per well was measured using a CASY cell counter (OMNI Life Science).

DSBs assay

To measure DNA DSBs (*1*), cells were seeded on glass coverslips in 6-well plates 1 d before the experiment. The next day, adhered cells were incubated with 2.5 MBq/ml of ^{177}Lu-DOTATATE for 4h. Subsequently, cells were washed twice with PBS and incubated for different time points (0, 1, 2, and 3 d) in culture medium without radiotracers. Cells were fixed with 1 mL of 2% paraformaldehyde (Sigma Aldrich) for 15 min at room temperature (RT), permeabilized in PBS containing 0.1% Triton X-100 (Sigma Aldrich) by incubating twice for 10 min at RT, and incubated in blocking buffer (PBS, 0.1% Triton X-100, 2% bovine serum albumin [Sigma Aldrich]) for 30 min at RT. Next, cells were incubated for 90 min at RT with the primary antibody, anti-53BP1 (NB100-304 [Novus Biologicals]; 1/1,000) diluted in blocking buffer. After incubation, cells were washed 3 times for 5 min at RT with PBS and 0.1% Triton X-100 and incubated with the secondary antibody (goat antirabbit Alexa Fluor 594 [Life Technologies]; 1/1,000) in blocking buffer for 60 min at RT. Cells were mounted with Vectashield (Vector Laboratories) containing DAPI (49,6-diamidino-2-phenylindole). Z-stack imaging was performed using a TCS SP5 confocal microscope (Leica), and foci were counted from 30 to 40 cells per condition using Image J software (National Institutes of Health). Foci were considered legitimate when their size was between 20 and 100 squared pixels; foci smaller or bigger were considered background staining.

References

1. Nonnekens J, van Kranenburg M, Beerens CEMT, et al. Potentiation of peptide receptor radionuclide therapy by the PARP inhibitor olaparib. *Theranostics*. 2016;6:1821-1832.

2. Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: An open-source platform for biological-image analysis. *Nat Methods*. 2012;9:676-682.

3. Tamborino G, De Saint-Hubert M, Struelens L, et al. Cellular dosimetry of [177Lu]Lu-DOTA-[Tyr3]octreotate radionuclide therapy: the impact of modeling assumptions on the correlation with in vitro cytotoxicity. *EJNMMI Phys*. 2020;7:8.

Radiation	Boundary keV	N*	Yield N / Bq s	Energy MeV / (Bq s)	Mean energy MeV
X-ray	E < 1	4	1.28	2.49E-05	1.94E-05
	E > 1	56	0.09	3.51E-03	3.78E-02
Gamma		6	0.18	3.16E-02	1.75E-01
Beta		4	1.00	1.33E-01	1.33E-01
Auger e^-	E < 1	6	0.82	1.13E-04	1.38E-04
	E > 1	9	0.30	1.02E-03	3.42E-03
IC e^-	E > 1	36	0.15	1.35E-02	8.74E-02

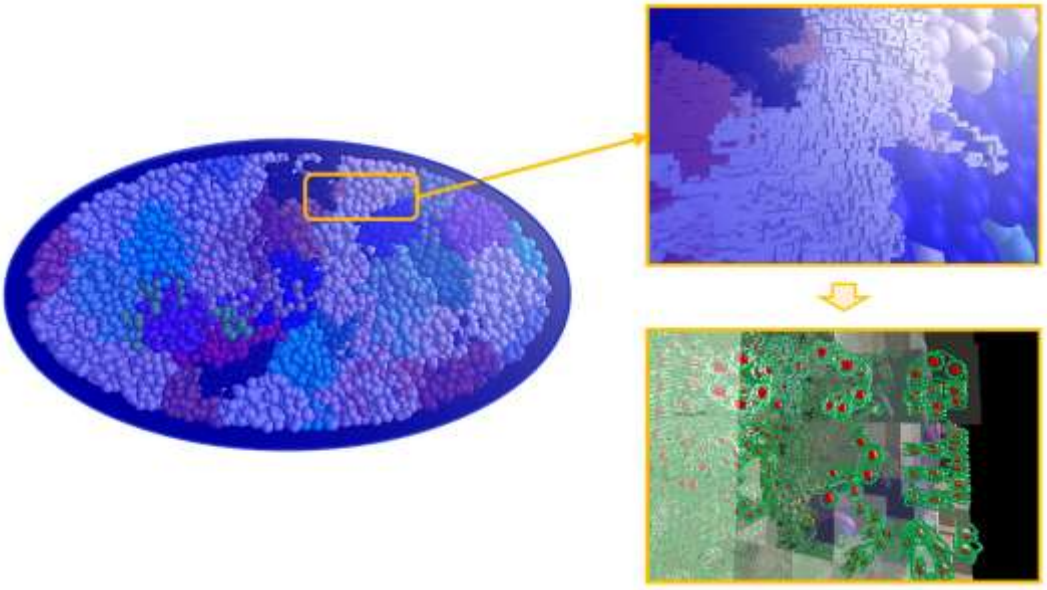
*Number of emissions

SUPPLEMENTAL TABLE 1. Summary of ^{177}Lu emissions, from ICRP-107. An energy boundary of 1 keV is used to discriminate penetrating and non-penetrating radiation contributions. Below the cut-off value of 100 eV, all energy is assumed to be absorbed at the site of emission.

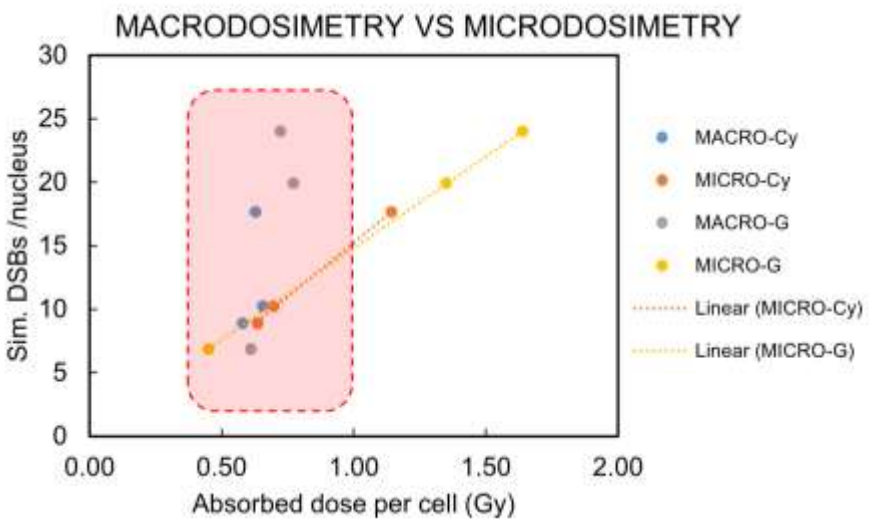
$\overline{z}$ (Gy)	Cell 1	Cell 2	Cell 3
Cy	0.96	0.51	0.45
G	1.45	0.26	1.16
Medium		0.19	

$\overline{D}$ (Gy)	Cell 1	Cell 2	Cell 3
Cy	0.29	0.32	0.24
G	0.39	0.27	0.44
Medium		0.34	

SUPPLEMENTAL TABLE 2. Microdosimetric and macrodosimetric calculations corresponding to an added activity of 2.5 MBq/ml. The S-values calculations belong to a previous study (*3*).



SUPPLEMENTAL FIGURE 1. Example of nucleus filled with genetic content. In the main image, an ellipsoidal nucleus containing chromatin regions can be seen. Different colors correspond to different chromosome territories. Two zoom levels are also reported: on the left, the voxels (i.e. cubes) filling each region can be seen. On the right, the zoomed voxel shows a detail of DNA at molecular level.



SUPPLEMENTAL FIGURE 2. Averaged absorbed dose and mean specific energy to the nucleus versus simulated number of DSBs when the internalized source is located in the Cy or in the G.