***Supplementary Methodology: Ladjohounlou et al.***

***Ex vivo* digital autoradiography (DAR) and dosimetry**

Immediately after mouse sacriﬁce and tumor sectioning, the absolute 212Pb activity in tumor sections was determined using a previously calibrated gamma counter. By selecting the gamma-ray energy of 212Pb (238.6 keV), activities ranged from 2.87 to 45.38 Bq. The same sections were then scanned with a Beta IMAGER® dFine to establish the calibration factor (k) for the DAR images.

Then, for each mouse, the speciﬁc activity A (Bq/g) in the tumor mass, which included all nodules, was calculated from DAR images as:



where CPMtotal represents the total DAR count in the segmented image, Mvoxel is the voxel mass in grams and Nt the total number of tumor voxels. When multiple mice were available for a single data point, their speciﬁc activities were averaged. The resulting mean speciﬁc activity and standard deviation were used to build time–activity curves for each 212Pb-mAb. Time-activity curves were ﬁtted by assuming a mono-exponential decay to extract the cumulated activities for each tumor treated with 212Pb-mAbs.

Two different techniques were used for calculating the tumor absorbed dose. First, the average tumor absorbed dose was calculated by assuming a spherical shape and a homogeneous radiopharmaceutical distribution and by using the standard MIRD schema (1) and S values computed with the GATE Monte-Carlo (MC) toolkit. Then, radioactivity heterogeneity was taken into account to obtain the tumor absorbed dose distributions at the voxel level. With both approaches, only the self-absorbed tumor dose was considered because the short range of 212Pb α-particles prevents irradiation of the surrounding tissues.

The obvious issue for calculating the tumor absorbed dose from DAR images is to extrapolate the 3D absorbed dose distribution on the basis of a 2D activity distribution. This problem was overcome by performing a preliminary MC simulation (2D model) in which the tumor was modeled as bi-dimensional (XY plane) in order to assess the penetration of 212Pb emissions in the third dimension (Z). This simulation allowed demonstrating that the energy emitted in the central slice was almost completely deposited within 105 µm from the source. This piece of information was then used to build a ﬁctitious 3D model of the tumor (semi-inﬁnite model) in which the 20 µm tumor slice deﬁned from the selected DAR image was replicated 11 times along the Z axis (from -110 m to 110 m) to model a ﬁctitious 3D activity distribution. Additional details on this method are provided in (2). For each simulation, 5 million primary events allowed a voxel statistical uncertainty <1% for all considered voxels. This approach takes into account not only the absorbed dose due to the activity in the considered tumor slice, but also the absorbed dose due to the activity in contiguous slices. Although edge effects due to tumor radioactivity at the top and bottom extremities are neglected in this approximation, this was considered the most accurate approach in the absence of experimental data concerning the actual 3D activity distribution in the tumor. More details about dosimetry can be found in *"Multi-scale dosimetry for Targeted Radionuclide Therapy optimization", by Dr Marcatili S. (available at* [*https://tel.archives-ouvertes.fr/tel-01248412/document*](https://tel.archives-ouvertes.fr/tel-01248412/document)*).*

***In vitro* measurement of DNA damage**

A-431CEA and SK-OV-3MISRII donor cells were seeded on coverslips in 6-well plates. The following day, they were incubated with 0.5 MBq/mL of 212Pb-mAbs (A-431CEA cells) or 0.5MBq/mL of 213Bi-mAbs (SK-OV-3MISRII cells) for 90 min. After antibody removal, fresh medium (2 ml) was added to donor cells for 2 hours and then transferred to recipient cells. Both donor and recipient cells were fixed in 3.7% (v/v) formaldehyde for 20min, permeabilized in 0.1% (v/v) Triton X-100 for 15min, followed by incubation with 1% (v/v) PBS/BSA at 37°C for 1h. Cells were then incubated with anti-phosphorylated histone H2AX (H2AX) (Ser139, clone JBW301) mouse monoclonal IgG1 (Merck Millipore, St-Quentin-en-Yvelines, France) (1:200 dilution in 0.1% BSA/PBS), or with a rabbit polyclonal 53BP1 antibody (1:400; Novus Biologicals, Cambridge, UK) at 4°C overnight, followed by an Alexa-488-conjugated anti-mouse secondary mAb (1:200; Invitrogen; Saint Aubin, France) for H2AX, or Alexa-555-conjugated anti-rabbit mAb (1:500; Invitrogen; Saint Aubin, France) for 53BP1, at 37°C for 45min. After each step, cells were washed three times with 0.1% Tween-20/PBS for 5 min. Coverslips were mounted on microscope slides (76×26 mm, Thermo Scientific) using Moviol® andDAPI (Thermo Fisher Scientific, Waltham, MA).

Images of ɣ-H2AX/53BP1 foci were acquired with a 40× or 60× NA objective and a Leica inverted microscope. The number of H2AX and 53BP1 foci was calculated by targeted visualization of the foci in the nucleus.

**Oxidative stress arrays during alpha RIT**

A Human Cell Stress Array (Proteome Profiler Array, R&D Systems, Minneapolis, MN) was used to detect the level of oxidative stress markers in cellular extracts of SK-OV-3MISRII donor cells exposed to 0.5MBq/ml 213Bi-anti MISRII mAb and of HCT116 donor cells exposed to 4MBq/mL 125I-anti CEA mAb, according to the manufacturer’s protocol.

**Determining the contribution of targeted and off-target effects**

The contribution of off-target effects to cell death can be determined directly by considering the survival rate of recipient cells, as follows:

Contribution of off-targeted effects (%)= 100% - Survival of recipient cells (%)

The specific contribution of targeted effects to donor cell killing was calculated using a Bliss independence mathematical model (3). As donor cells are in reality at the same time donor and recipient cells (i.e., irradiated cells secrete substances toward neighboring irradiated cells), the model considers that donor cells are killed either by targeted or by off-target effects. The relative contribution of both effect types is calculated using the following formula:

Contribution of targeted effects (%)= 100% -

***In vivo* radioimmunotherapy using lowand intermediateactivities of 213Bi-labeled antibodies combined or not with MBCD**

Mice intraperitoneally xenografted with AN3CA tumor cells (8 mice/group) were treated with: i) NaCl; or ii) daily i.p. injections of 300 mg/kg MBCD from day 7 to 13 post-graft; or iii) one injection of 213Bi-anti-MISRII mAb (7.4 and 12.5MBq) at day 11; or (iv) both 213Bi-anti-MISRII mAb and MBCD. At day 14 post-graft, mice were sacrificed, tumors collected, and the tumor mass was determined.

**Western blotting of phosphorylated SAPK/JNK, p38, and NFkB in tumors**

Mice bearing intraperitoneal AN3CA tumor xenografts (3 mice/group) were treated with i) NaCl; ii) daily i.p. injections of 300 mg/kg MBCD from day 7 to 13 post-graft; iii) one injection of 213Bi-anti-MISRII mAb (3.7MBq, 7.4MBq or 37MBq) at day 11; or (iv) both 213Bi-anti-MISRII mAb and MBCD. At day 14 post-treatment, mice were sacrificed, and tumors collected. Tumors were lysed in lysis buffer containing 1M Tris (pH 7.5), 5M NaCl, 0.5M EDTA, 10% Triton-X100, 15mM MgCl2, 1M NaF, 0.1M Na3VO4, 1M PMSF and protease inhibitor cocktail (Roche, France). Proteins were then separated by SDS–PAGE and electrotransferred to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech, Uppsala, Sweden). After incubation with anti-phosphorylated SAPK/JNK, p38, and NFkB (p65) primary antibodies (Cell Signaling Technology, Danvers, MA), immune reactions were detected with horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit (Sigma-Aldrich, St Louis, MO) secondary antibodies and the ECL detection system (Amersham Biosciences, Saclay, France).

References

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