Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Information

Hybrid nano-architectures loaded with metal complexes for the co-chemotherapy of head and neck carcinoma

Melissa Santi,[1,§] Valentina Frusca,[2,§] Maria Laura Ermini,[2] Ana Katrina Mapanao,[2,6] Patrizia Sarogni,[2] Alessandra Gonnelli,[2,3] Noemi Giannini,[2,3] Agata Zamborlin,[2,4] Lorenzo Biancalana,[5] Fabio Marchetti[5] and Valerio Voliani[2,*]

[1] NEST Istituto Nanoscienze-CNR and Scuola Normale Superiore, 56127, Pisa, Italy.

[2] Center for Nanotechnology Innovation@NEST, Istituto Italiano di Tecnologia, Piazza San Silvestro 12, 56127, Pisa, Italy.

[3] Radiation Oncology Unit, Pisa University Hospital, Via Roma 67, Pisa, Italy

[4] NEST-Scuola Normale Superiore, Piazza San Silvestro 12, 56127 Pisa, Italy

[5] Department of Chemistry and Industrial Chemistry, University of Pisa, Via Moruzzi 13, 56124 Pisa, Italy.

[6] Current address: Center for Radiopharmaceutical Sciences, Paul Scherrer Institute, 5232 Villigen-PSI, Switzerland.

[§]Equal contribution

*Corresponding author: valerio.voliani@iit.it

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Figure S1

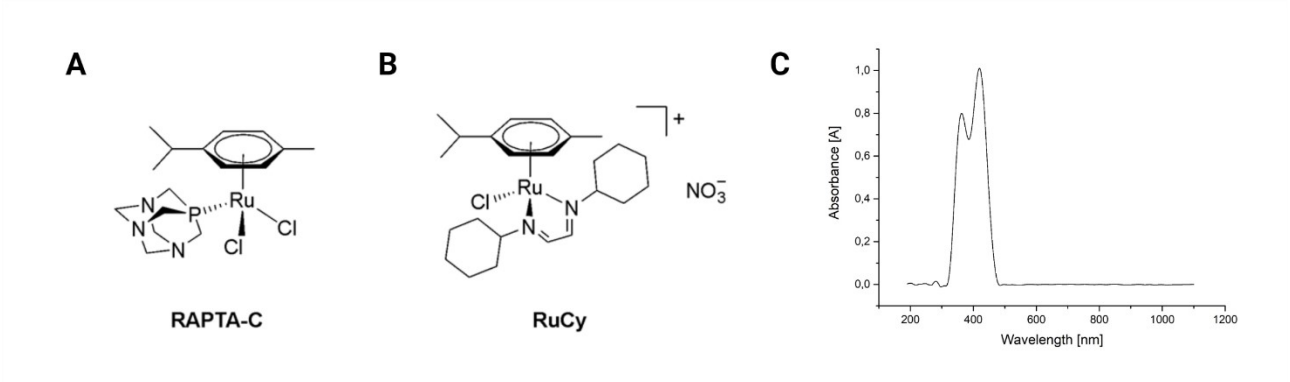

Figure S1: Chemical structures of ruthenium compounds (A) RAPTA-C and (B) RuCy. (C) UV-Vis spectrum of RuCy dissolved in milliQ® water.

Figure S2

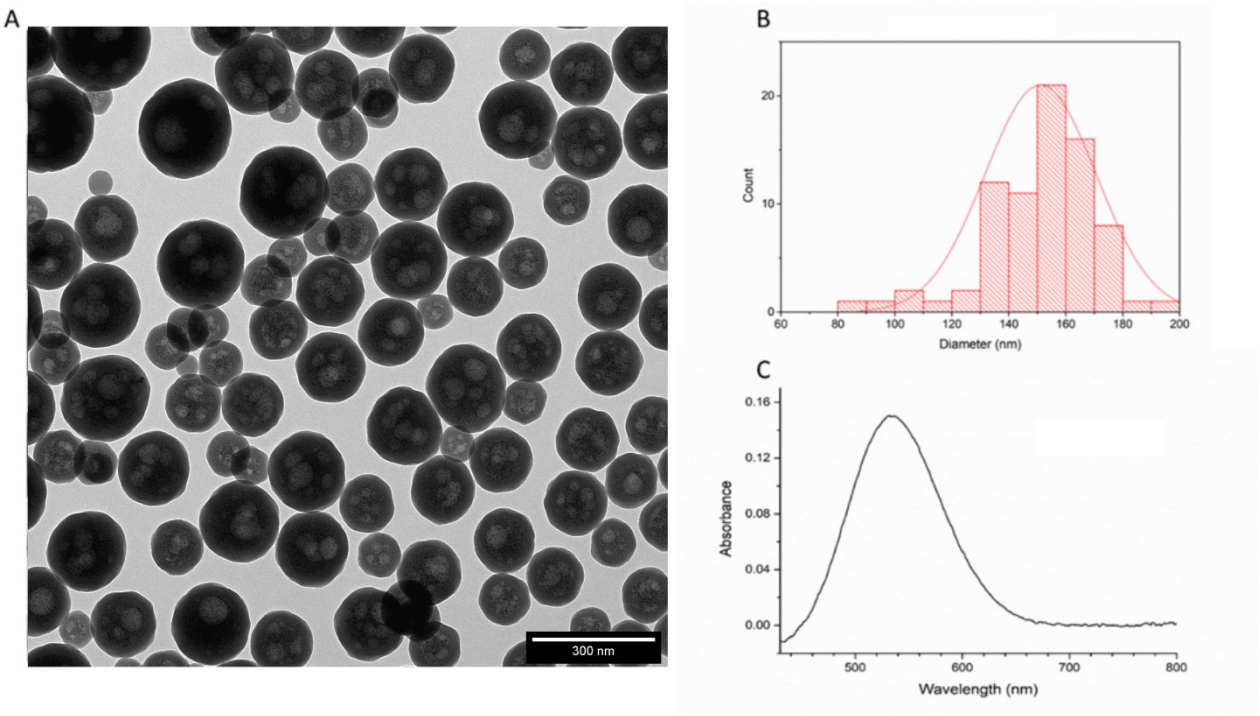

Figure S2: Physical-chemical characterization of NAs-CisPt. (A) TEM images of NAs-CisPt. Scale bar: 300 nm. (B) Size histogram of NAs-CisPt made on at least 100 NAs observed by TEM. (C) Background subtracted UV-Vis absorbance spectrum of NAs-CisPt.

Figure S3

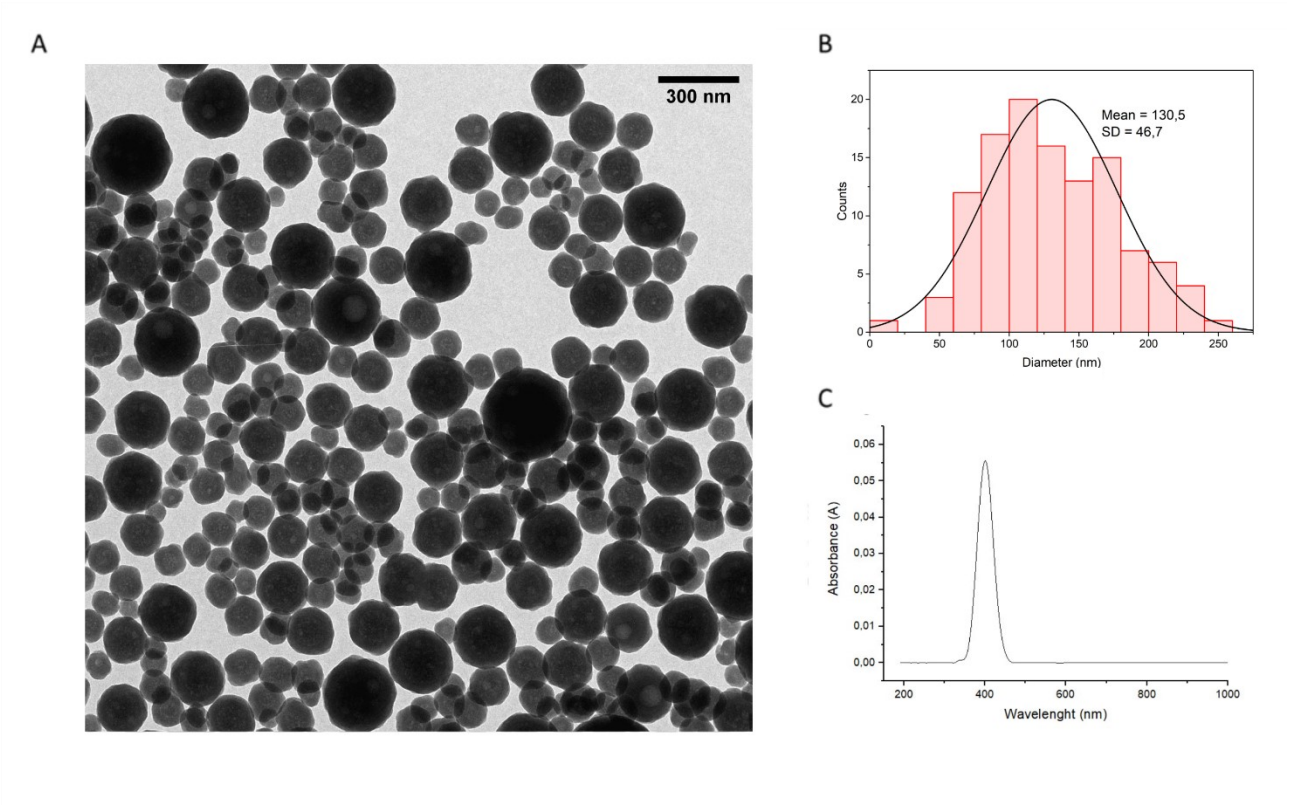

Figure S3: Physical-chemical characterization of NAs-RuCy. (A) TEM images of NAs-RuCy. Scale bar: 300 nm. (B) Size histogram of NAs-RuCy made on at least 100 NAs observed by TEM. (C) Background subtracted UV-Vis absorbance spectrum of NAs-RuCy.

Figure S4

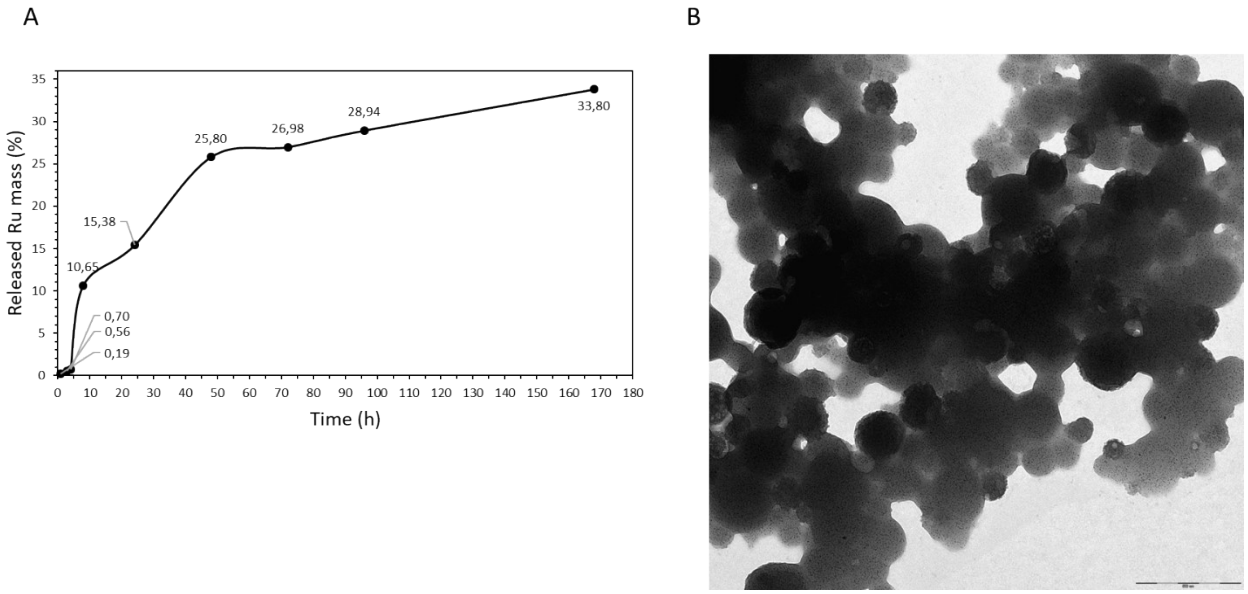

Figure S4: Release assay of RuCy from NAs-RuCy and TEM characterization after 7 days. (A) Nanoparticles were incubated in HEPES buffer for 7 days at 37°C. For each time point a buffer aliquot was withdraw and ruthenium content was analyzed by ICP-MS analysis. Percentage was calculated with respect to the amount of ruthenium placed in the dialysis membrane. (B) TEM characterization of NAs-RuCy after 7 days in HEPES buffer at 37°C.

Figure S5

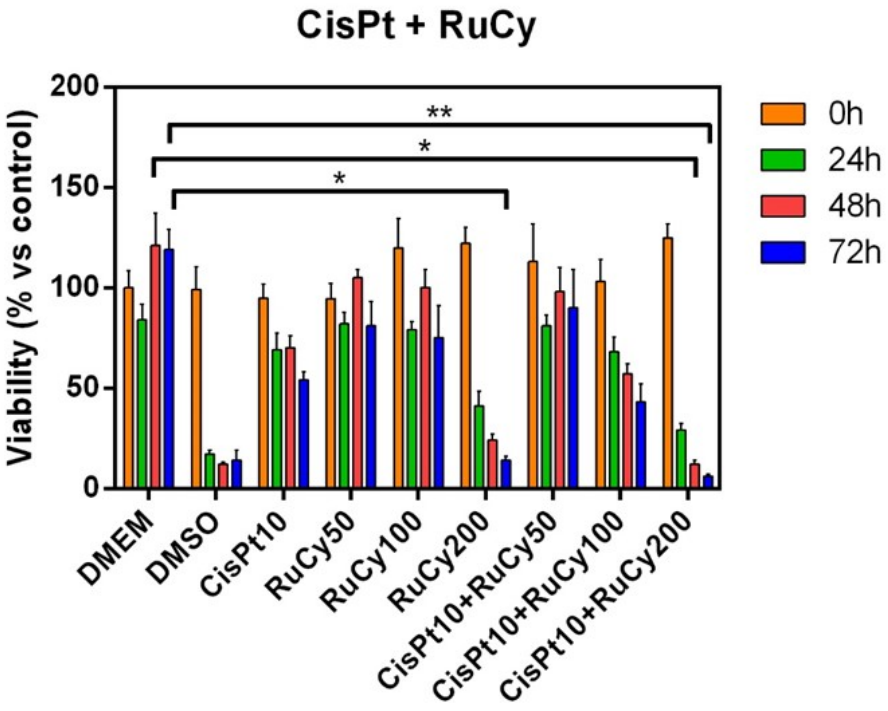

Figure S5: Viability of 2D cell cultures of SCC-25 after treatment with cisplatin (CisPt) and increasing concentrations of RuCy. Cells were incubated with free drugs alone or in combination for 2h, then washed twice with PBS and the viability was measured after 24, 48 and 72h. Viability was measured with respect to the control represented by untreated cells (DMEM). Two-way ANOVA with Turkey's multiple comparison test, *$p \leq 0,05$ and **$p \leq 0.003$.

Table S1

Table S1: Combined effect of CisPt and RuCy with respect to drugs alone.

	α 24h	**α 48h**	**α 72h**
CisPt10 + RuCy50	0,7	0,8	0,5
CisPt10 + RuCy100	0,8	1,2	0,9
CisPt10 + RuCy200	1,0	1,4	1,3

Figure S6

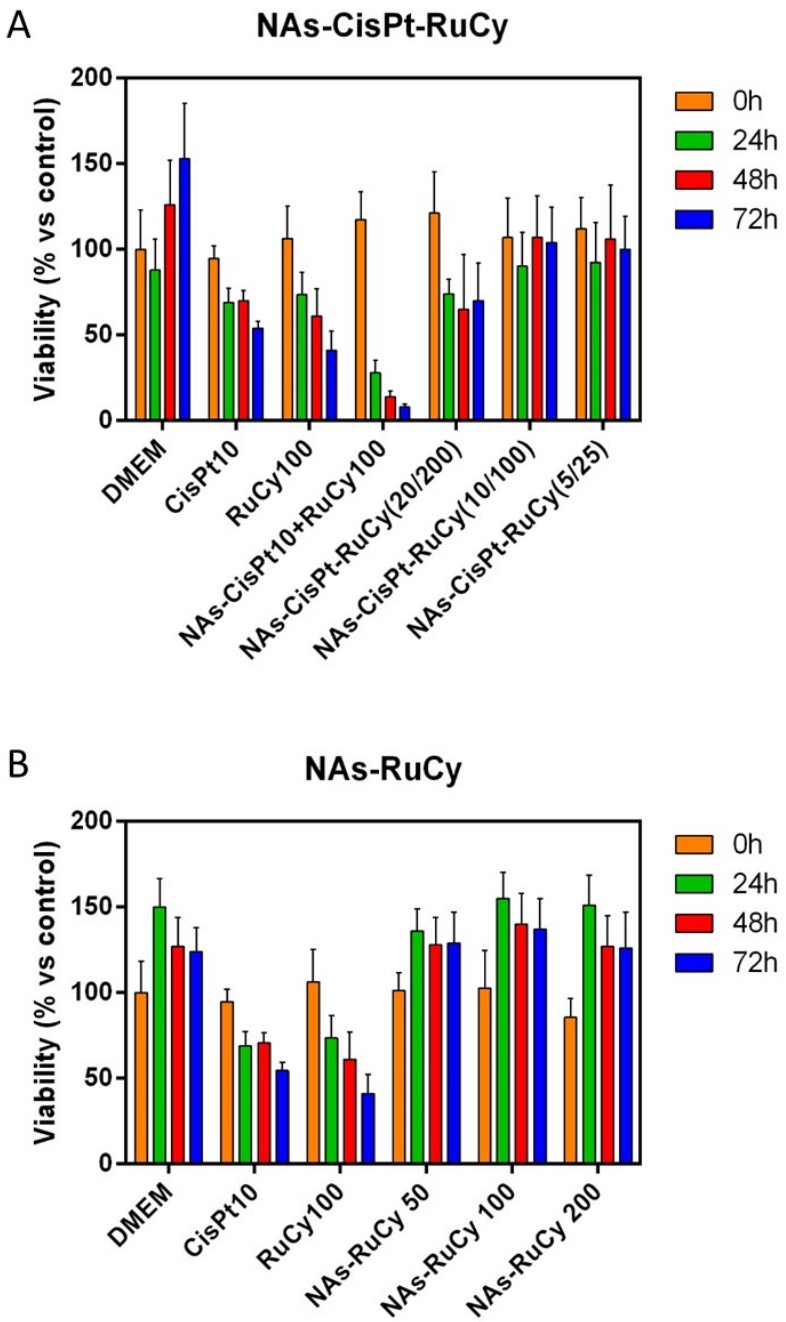

Figure S6: Viability of 2D cell cultures of SCC-25 after treatment with increasing concentration of (A) NAs-CisPt-RuCy or (B) NAs-RuCy. Cells were incubated with nanoparticles for 2h and viability was measured after 24, 48 and 72h. Viability was measured with respect to the control represented by cells not treated (DMEM). Two-way ANOVA with Turkey's multiple comparison test, *$p \leq 0{,}05$ and **$p \leq 0.003$. Error bars indicate standard deviation.

Table S2. Residual % amount of RuCy in D_2O or D_2O/DMSO-d_6 solutions (*ca*. $1.0 \cdot 10^{-2}$ M) after 6 or 24 h at room temperature (22 °C), calculated by ^1H NMR with respect to the initial spectrum and Me_2SO_2 as internal standard.

Solvent	% residual amount (6 h, 22°C)	% residual amount (24 h, 22°C)
D_2O [a]	*n.d.*	≈ 90
D_2O, 1% V/V DMSO	96	95
D_2O/DMSO-d_6 1:2 V/V	99	98

[a] Taken from the literature: L. Biancalana, L.K. Batchelor, T. Funaioli, S. Zacchini, M. Bortoluzzi, G. Pampaloni, P.J. Dyson, F. Marchetti, α-Diimines as Versatile, Derivatizable Ligands in Ruthenium(II) p-Cymene Anticancer Complexes, Inorg. Chem. 57 (2018) 6669–6685. https://doi.org/10.1021/acs.inorgchem.8b00882. The decrease of the starting material is due to the formation of the corresponding aquo complex. *n.d.* = not determined.

RuCy in D_2O 1% DMSO. ^1H NMR: δ/ppm = 8.32 (s, 2H), 6.17 (d, *J* = 6.4 Hz, 2H), 5.80 (d, *J* = 6.4 Hz, 2H), 4.45 (t, *J* = 11.6 Hz, 2H), 2.39 (d, *J* = 12.4 Hz, 2H), 2.30 (d, *J* = 11.9 Hz, 2H), 2.22 (s, 3H), 1.95 (d, *J* = 14.4 Hz, 2H), 1.88 (d, *J* = 13.5 Hz, 2H), 1.73 (qd, *J* = 12.2, 3.5 Hz, 4H), 1.58–1.45 (m, 4H), 1.36–1.18 (m, 4H). $Cl^-_{(aq)}$ detected after 6 h at 22 °C. ^{35}Cl NMR (acq. time 30 min): δ/ppm = 2.1 ($\Delta\nu_{1/2}$ = 32 Hz).

RuCy in D_2O/DMSO-d_6 1:2. ^1H NMR: δ/ppm = 8.37 (s, 2H), 6.21 (d, *J* = 6.3 Hz, 2H), 5.87 (d, *J* = 6.3 Hz, 2H), 2.75 (hept, *J* = 6.9 Hz, 1H), 2.43 (d, *J* = 12.4 Hz, 2H), 2.28 (d, *J* = 11.1 Hz, 2H), 2.24 (s, 3H), 1.96 (d, *J* = 12.9 Hz, 2H), 1.87 (d, *J* = 13.2 Hz, 2H), 1.82–1.72 (m, 4H), 1.65–1.51 (m, 4H), 1.31–1.22 (m, 4H).

Table S3. Molar conductivity ($S \cdot cm^2 \cdot mol^{-1}$) as a function of time for solutions of RuCy ($4.9 \cdot 10^{-4}$ M) or $[Bu_4N]Br$ ($1.9 \cdot 10^{-3}$ M) in water with 1% V/V DMSO or in water/DMSO 1:2 V/V at room temperature (22 °C).

Compound	**Solvent**	**Λ_m (0 h)**	**Λ_m (1 h)**	**Λ_m (3 h)**	**Λ_m (5 h)**	**Λ_m (24 h)**	**Λ_m (48 h)**
RuCy	H_2O, 1% DMSO	122	130	133	135	154	159
$[Bu_4N]Br$		133	-	-	133	135	135
RuCy	H_2O/DMSO 1:2 V/V	27	28	30	31	36	37
$[Bu_4N]Br$		26	-	-	25	26	26

Table S4

	% AD	
Metal	**NAs-CisPt + RuCy**	**RuCy**
Au	3,8±2,9	0,0±0,0
Ru	3,1±2,1	3,6±2,4
Pt	7,4±5,7	0,0±0,0

Table S4: ICP-MS quantification of gold, ruthenium and platinum in harvested tumors, treated with NAs-CisPt+RuCy and RuCy alone. Data is presented as administered dose (%) ± standard deviation (N=3 at least).

Figure S7

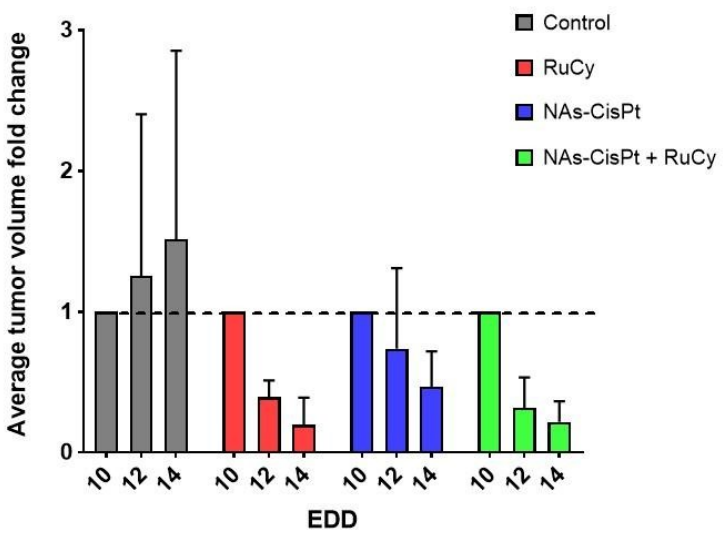

Figure S7: Average tumor volume fold change of each treatment calculated over pretreatment tumor volume at EDD10. The dashed line refers to the fold change with respect to EDD10 and it is equal to 1. Data are reported as mean ± SD of at least five samples per condition.

Figure S8

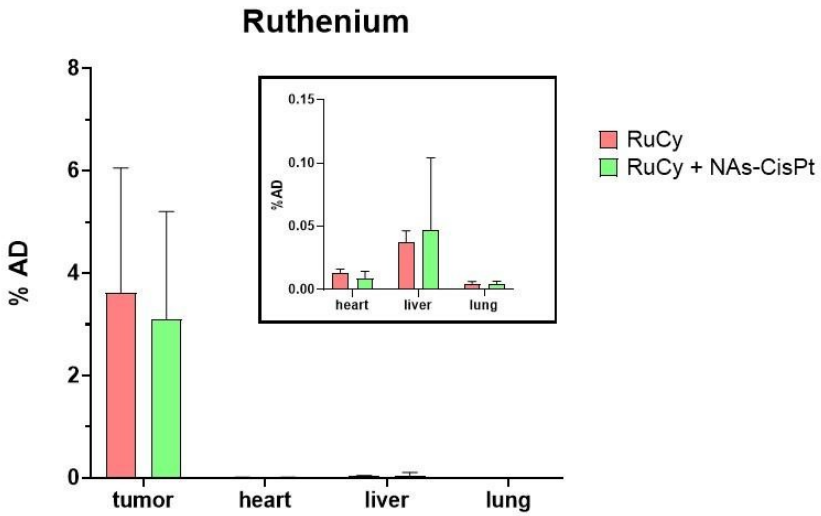

Figure S8: Average administered dose (%) of ruthenium found in harvested tumors and organs. Data are presented as mean ± SD on at least three samples per condition. Inset: zoom on %AD of organs.

Figure S9

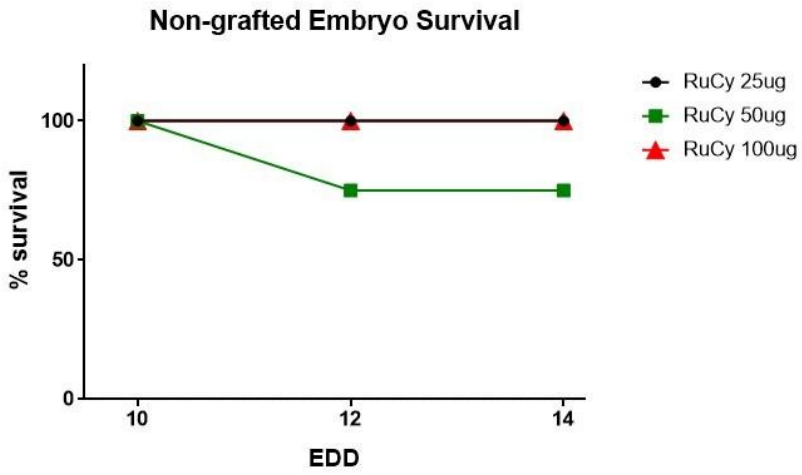

Figure S9: Embryo viability (%) in non-grafted eggs after treatment with increasing concentration of RuCy.