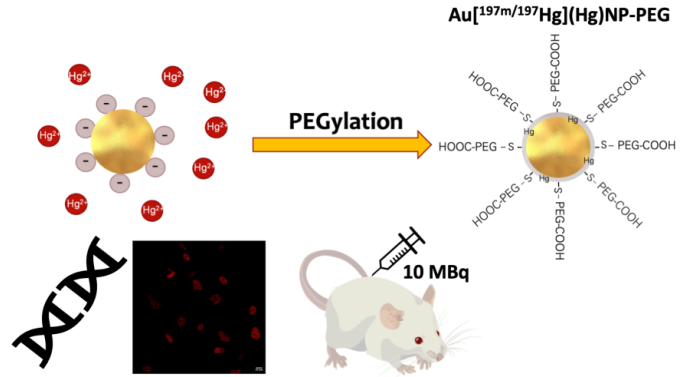
Nanobrachytherapy of Triple-Negative Breast Cancer and Glioblastoma Multiforme Using Auger Emitters



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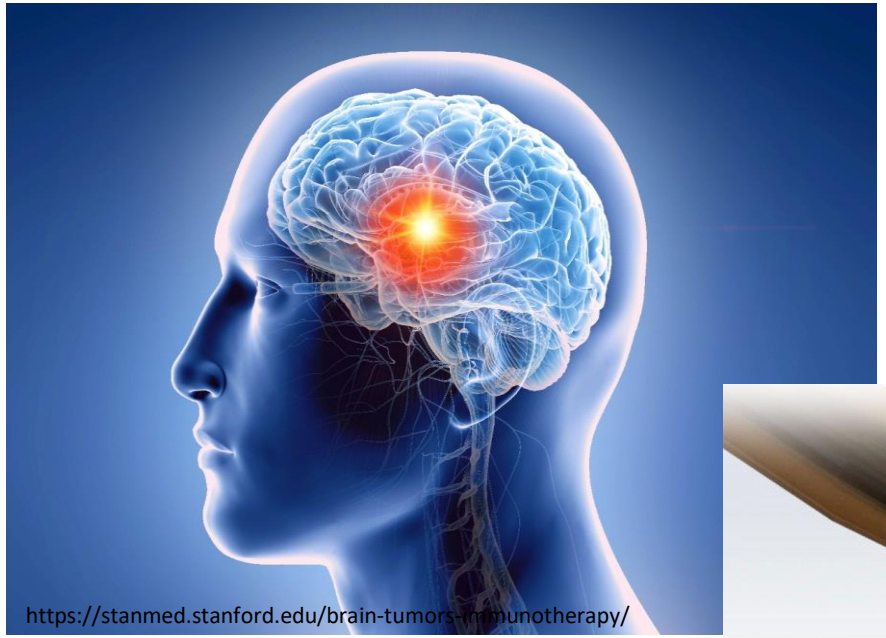
2 nd Symposium on new trends in nuclear and medical physics
24-26 September 2025

OBJECTIVES

Glioblastoma multiforme

- 50.1 % of all malignant brain tumors
- mortality of 93.1%



https://stanmed.stanford.edu/brain-tumors-immunotherapy/

Triple-negative breast cancer

- 10—15% of all breast cancers
- mortality of a metastasized form 88%

content/uploads/2021/09/DrTan-blog-breast-symptoms-

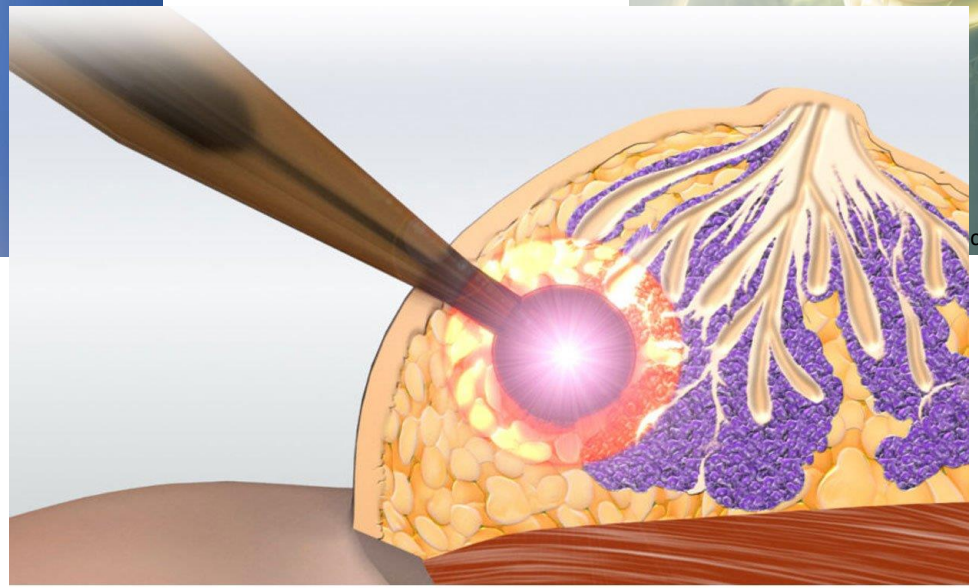
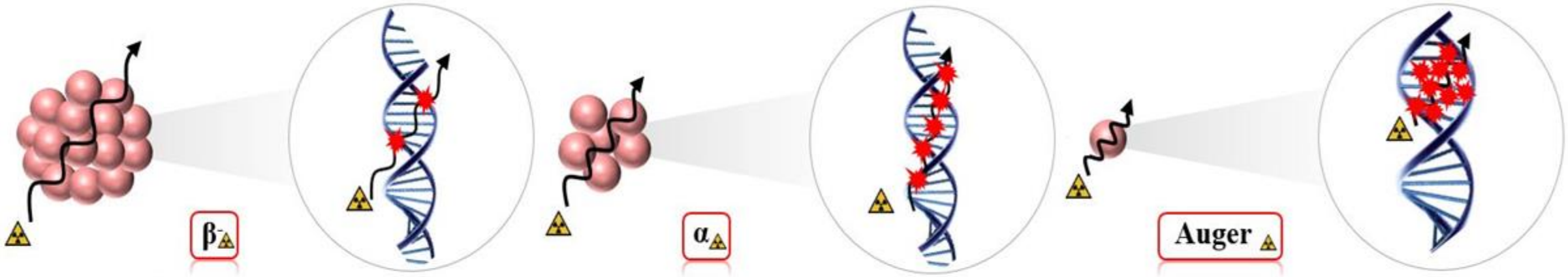


Image Source: © 2018 Radiation Oncology Associates of Northern Virginia

NANOBRACHYTHERAPY

OBJECTIVES



Range: 500-12000 µm
LET: 0,2 keV/µm

Range: 40-100 µm
LET: 50-230 keV/µm

Range: 1-20 nm
LET: 4-26 keV/µm

Property	Beta Emitters	Alpha Emitters	Auger Electron Emitters
Kinetic Energy (MeV)	0.3–2.3	2–10	0.003–0.04
Penetration Range in Tissue	0.5–12 mm	50–80 µm	1–500 nm
Linear Energy Transfer (LET)	0.2–2 keV/µm	~100 keV/µm	4–26 keV/µm
Main Mechanism	Crossfire & bystander effects	Localized, high-energy DNA damage (double-strand breaks)	Highly localized ionization near DNA
Biological Effect	Damage to both targeted and nearby cells	Irreparable DNA damage in targeted cells	Comparable to alpha particles but at nanometric scale

Andrés Núñez-Salinas et al. Pharmaceutics 2025, 17, 912

- Auger electrons are similar to α particles and produce highly damaging effects in cells.
- They are minimally toxic to surrounding non-targeted cells.
- It is possible to inject about 10-fold greater radioactivity of Auger emitters than β^- particle emitters without toxic side effects.

To obtain high therapeutic effect it is necessery to deliver the Auger electron emitter to the cell nucleus, in the vicinity of DNA.

The challenge is to ensure that the Auger electron emitter enters the cell nucleus and binds to DNA.

Table 1 Properties of Auger electron-emitting radionuclides [a]

Auger electrons (AEs)					Internal conversion (IC) electrons		
Radionuclide	Half-life	AEs/decay	Average AE energy per decay (keV)	Average energy per AE (keV)	IC electrons/decay	Average IC electron energy released per decay (keV)	Average energy per IC electron (keV)
^{125}I	57 d	23.0	12.0	0.5	0.9	7.3	7.7
^{123}I	13 h	13.7	7.2	0.5	0.2	21.0	222.6
^{67}Ga	78 h	5.0	6.6	1.3	0.3	29.7	14.1
^{99m}Tc	6 h	4.4	0.9	0.2	1.1	15.2	13.8
^{111}In	67 h	7.4	6.9	0.9	0.2	27.9	176.1
^{201}Tl	73 h	20.9	14.8	0.7	0.9	29.9	32.9
^{191}Pt	2.8 d	14	17.8	1.3	304	57.1	0.2
^{193m}Pt	4.3 d	27.4	10.9	0.4	3.0	126.8	42.4
^{195m}Pt	4.0 d	36.6	23.1	0.6	2.8	161.4	58.1
^{197}Hg	64.1 h	23.2	16.1	0.7	0.8	54.1	67.0
^{197m}Hg	23.8 h	19.4	13.5	0.7	1.6	203.5	127.0
^{119}Sb	38.2 h	23.7	8.9	0.4	0.8	17.0	20.2
^{161}Tb[b]	6.9 d	0.9[c]	5.1[c]	5.7	1.4	36.7	26.2

[a] The number of AEs and IC electrons were obtained from MIRD Radionuclide and Decay Schemes (Eckerman and Endo 2008)

[b] The number of AEs and IC electrons were obtained from the National Nuclear Data Center for ^{161}Tb (65-Terbium-161 2011)

[c] Calculation based on K and L shell Auger electrons only

^{197}Hg/^{197m}Hg: The Future Of Auger Emitters

	^{197}Hg	^{197m}Hg
$T_{1/2}$ [h]	64.14	23.8
No of Auger electrons/decay	23.2	19.4
Auger electron energy/decay [keV]	16.1	13.5
Conversion electrons/decay	0.8	1.6
Conversion electrons energy/decay [keV]	54.1	203.5
Main γ quantum [keV]	77.35 (18.7%)	133.99 (33.0%)

MERCURY PRODUCTION

- $^{197}Au(p,n)^{197/197m}Hg$
- $^{197}Au(d,2n)^{197/197m}Hg$
- $^{196}Hg(n,\gamma)^{197/197m}Hg$

MERCURY PRODUCTION

Reactor production of $^{197/197m}$Hg:

$^{196}Hg(n,\gamma)^{197/197m}Hg$

Target mass = 100 μg

Irradiation time = 7 h

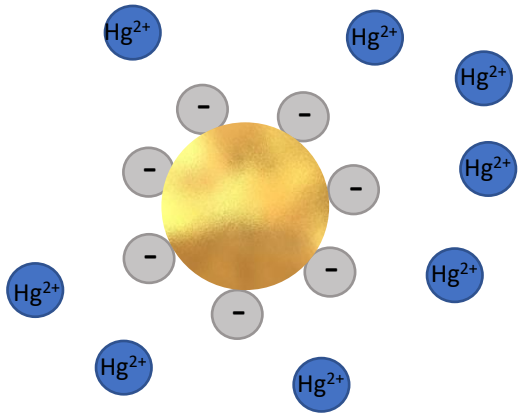
Cooling time = 1 h

Neutron flux = 10^{14} n/(cm^2 · s)

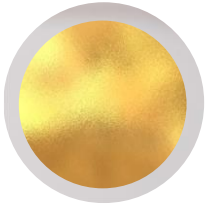
Cross sections = 3080 b; 105 b

Isotope	Activity [GBq]
Hg-197	3.57
Hg-197m	0.22

CONJUGATE'S SYNTHESIS



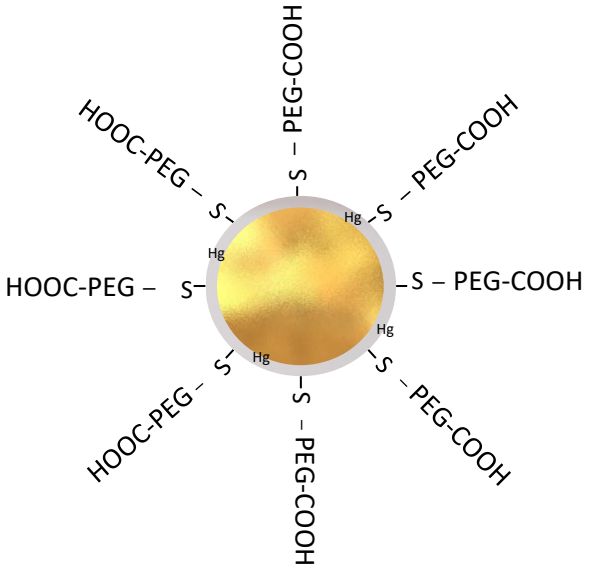
1 h



Au(Hg)NPs

PEGylation

2 h



AuNPs 5 nm

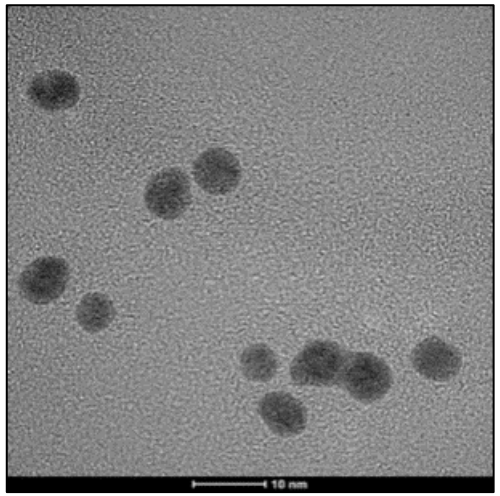
\- Sodium citrate, tannic acid

Hg2+ Mercury cation

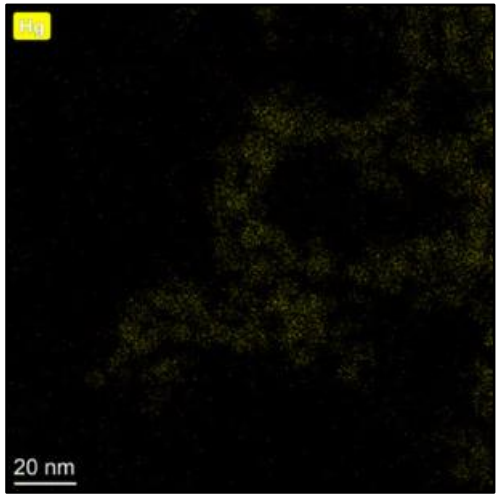
Au(Hg)NPs

PHYSICOCHEMICAL CHARACTERIZATION

TEM and HR-TEM results

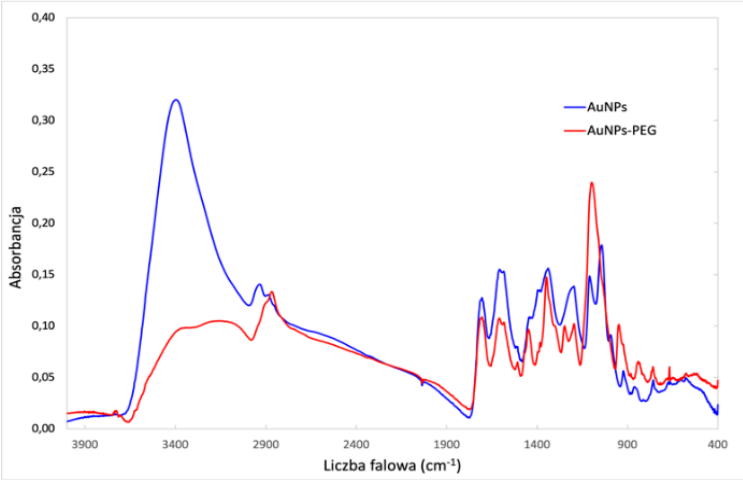


AuNPs 5 nm

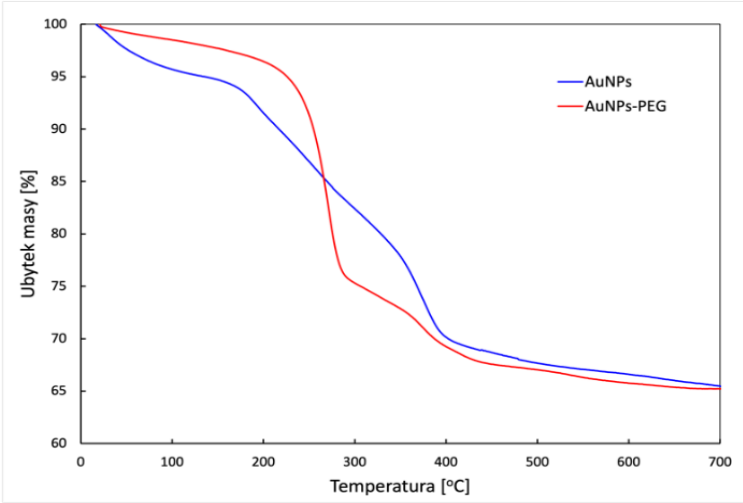


Au(Hg)NPs

FTIR results



TGA results



DLS and Zeta Potential results

Sample	Hydrodynamic diameter [nm]	Zeta Potential [mV]
AuNPs 5 nm	7.96 ± 0.22	-12.70 ± 3.29
Au(Hg)NPs	7.87 ± 0.33	-23.10 ± 0,71
Au(Hg)NPs-PEG-COOH (50:1)	13.94 ± 0.86	-24,63 ± 2,31

RADIOCHEMICAL YIELD OF $^{197m/197}$Hg CONJUGATION TO AuNPs

Radioactivity retained in AuNPs depending on the mercury mass.

Mercury mass [μg]	Retained radioactivity [%]
10	99.81±0.18
25	86.33±4.41
50	53.03±2.15
100	33.59±1.03

Radioactivity retained in AuNPs depending on the reaction time for 10 μg of Hg.

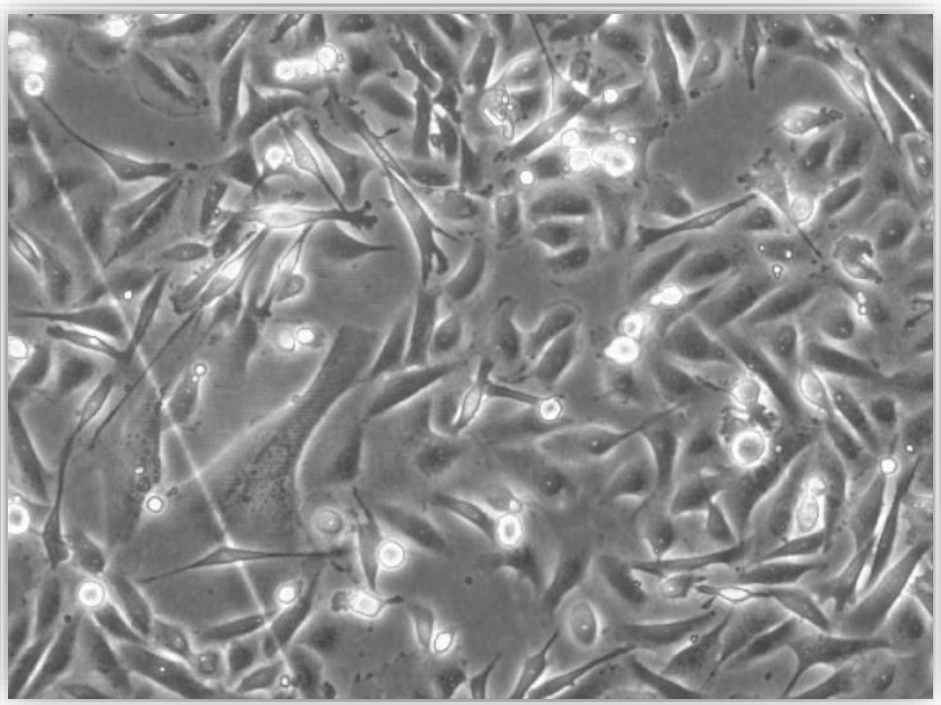
Reaction time [h]	Retained radioactivity [%]
0.25	99.80 ± 0.30
0.50	99.74 ± 0.35
1	99.87±0.22
2	99.78±0.39
3	99.98±0.04

- The highest yield (99.81 ± 0.18%) was obtained using 10 μg of Hg per 1 mL of AuNPs, corresponding to 40% theoretical surface coverage.
- The reaction time had little effect, with near-complete labeling occurring within 15 minutes. A one-hour reaction was chosen for convenience in processing multiple samples.
- The specific activity of $^{197m/197}$Hg AuNPs was **546 ± 23 MBq/ml**.

CANCER CELLS

MDA-MB-231 cell line

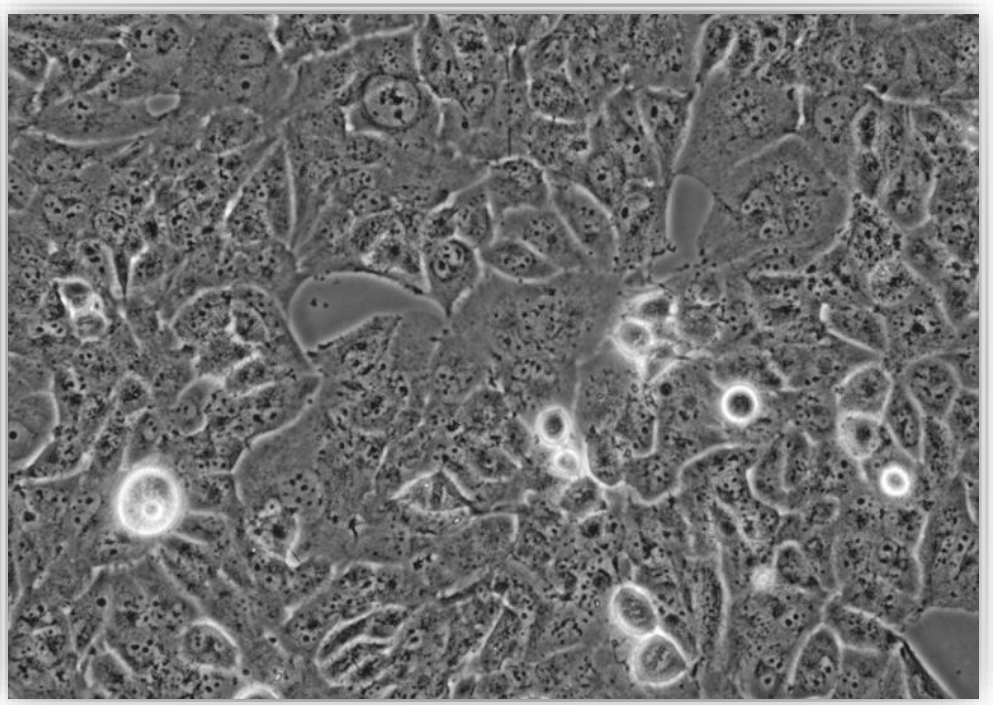
triple-negative breast cancer



https://www.culturecollections.org.uk/products/cell-cultures/ecacc-cell-line-profiles/mda-mb-231/

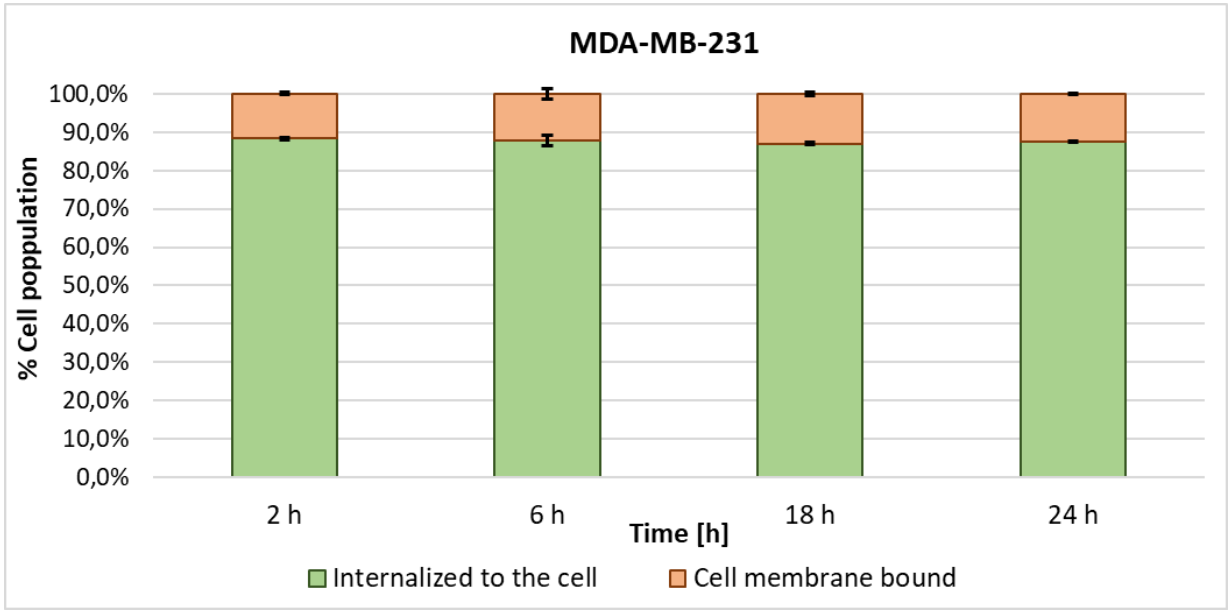
T98G cell line

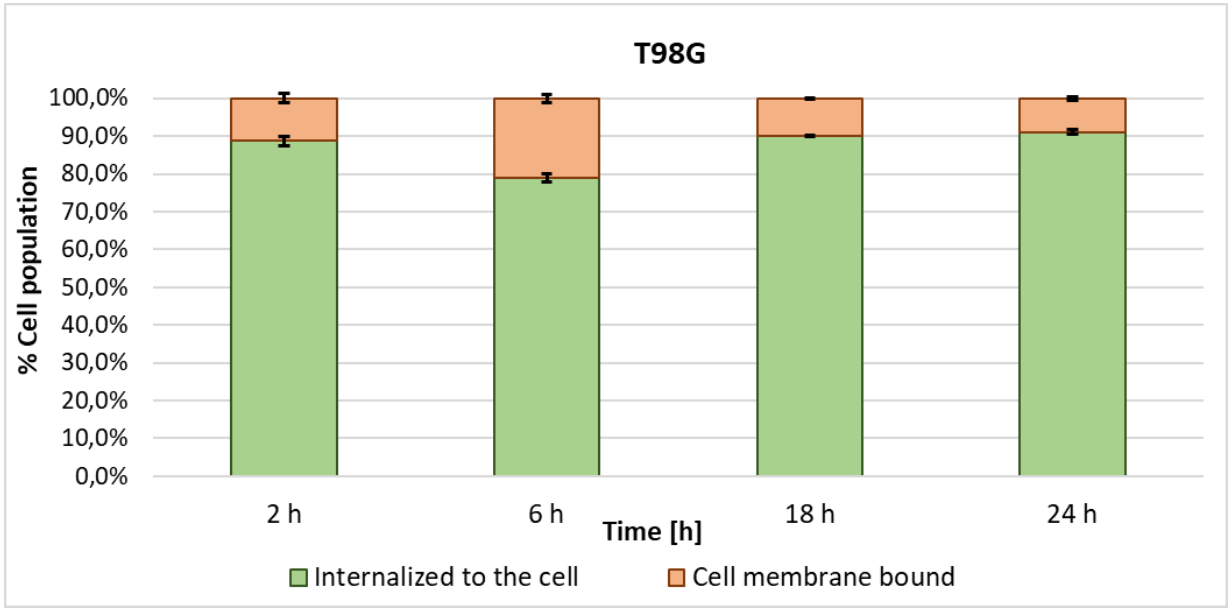
Glioblastoma



https://cellbank.brc.riken.jp/cell_bank/CellInfo/?cellNo=RCB1954

INTERNALIZATION STUDIES OF Au($^{197/197m}$Hg)NPs-PEG-COOH

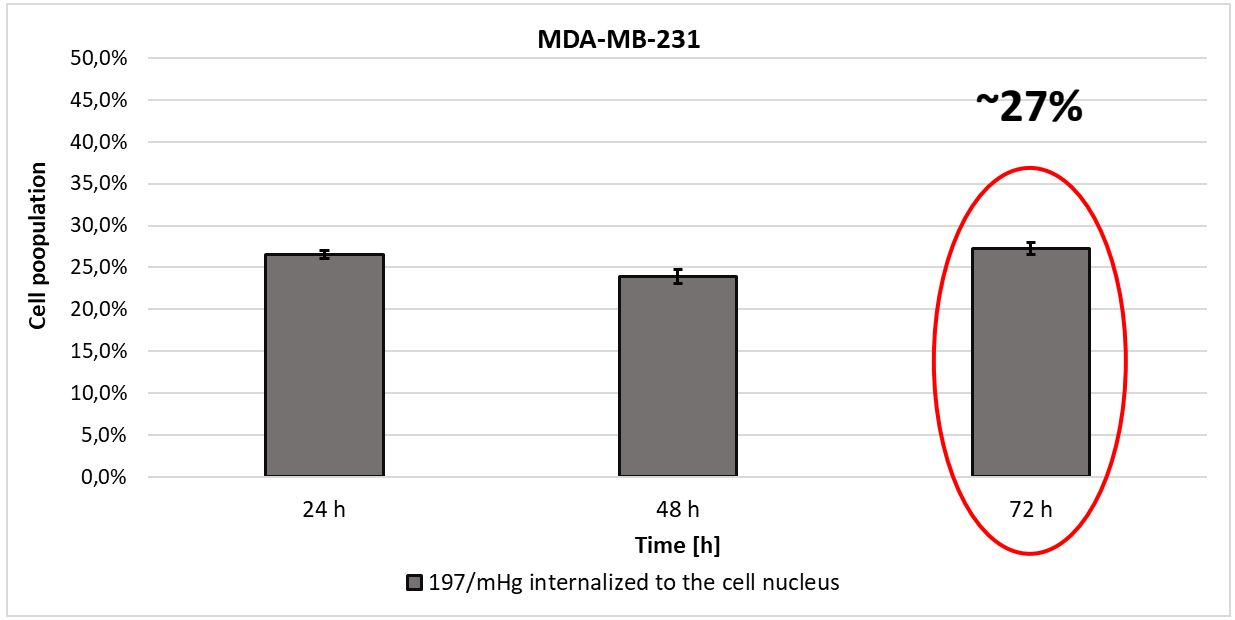


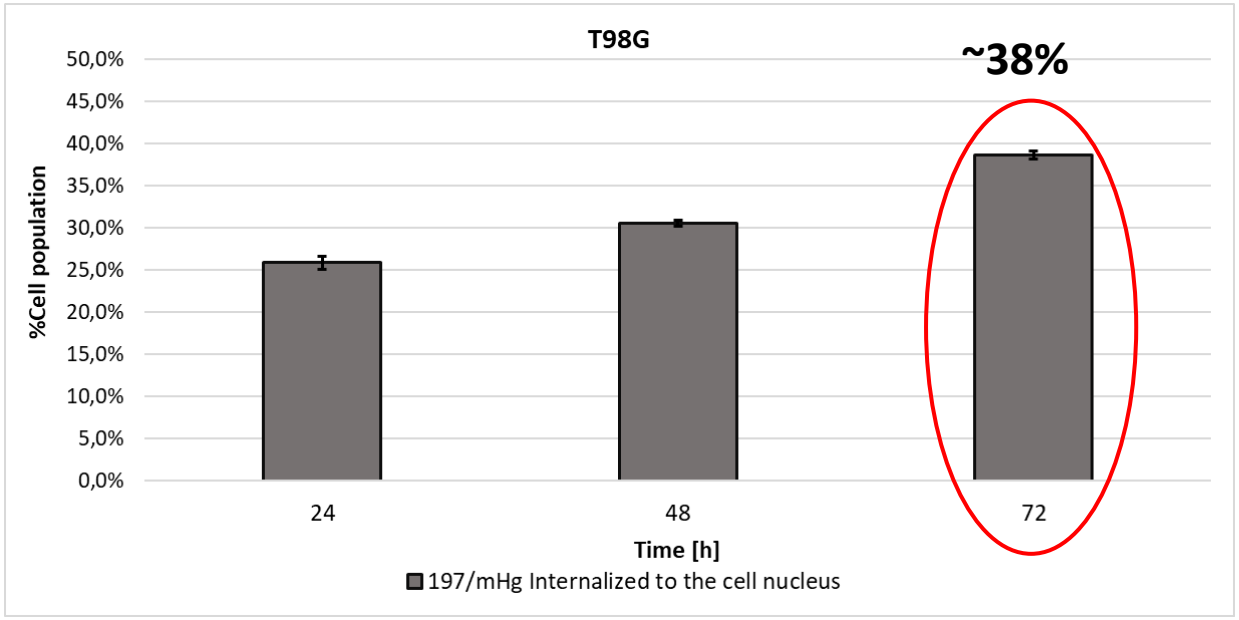


~88%

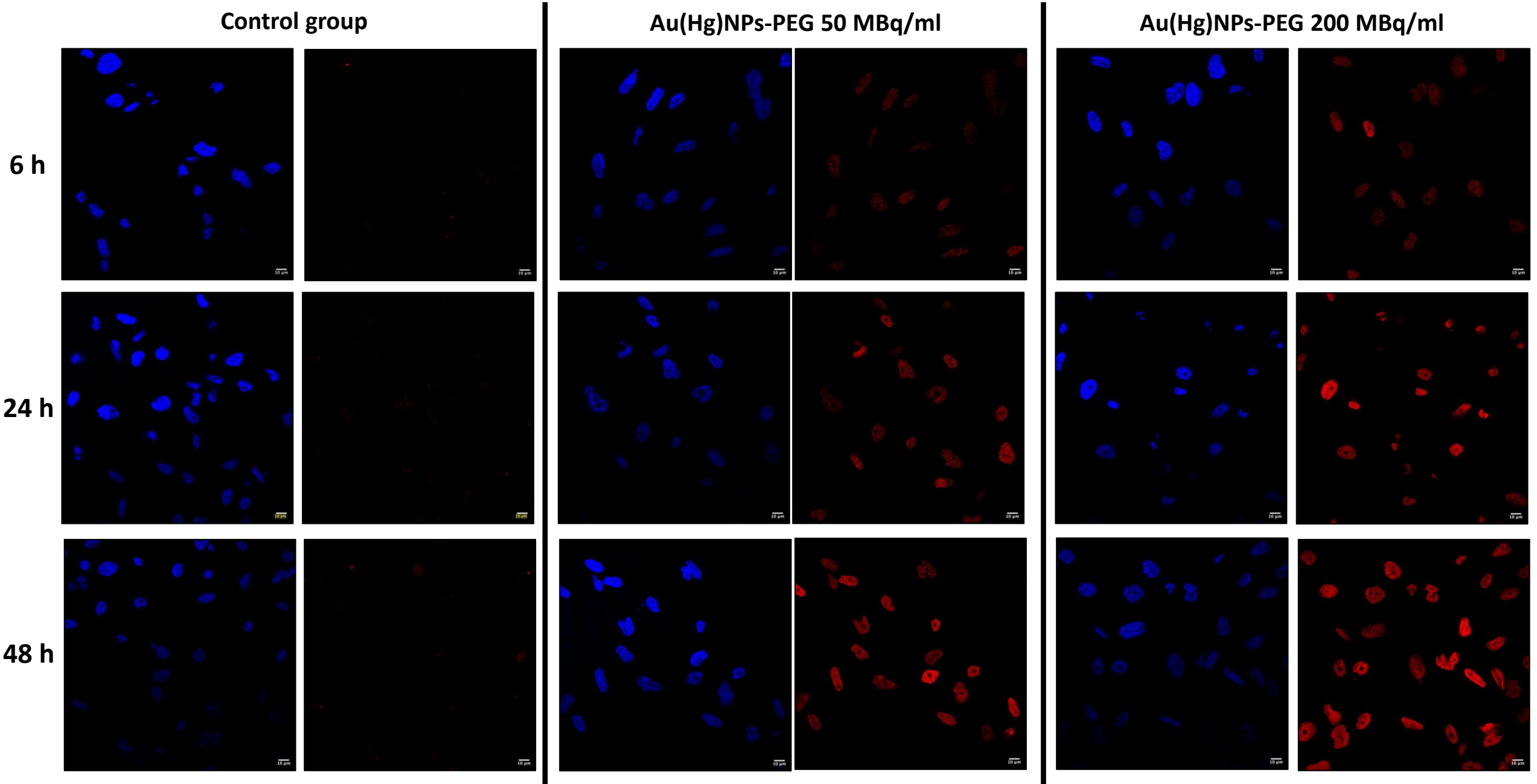
~90%

SUBCELLULAR FRACTIONATION STUDIES

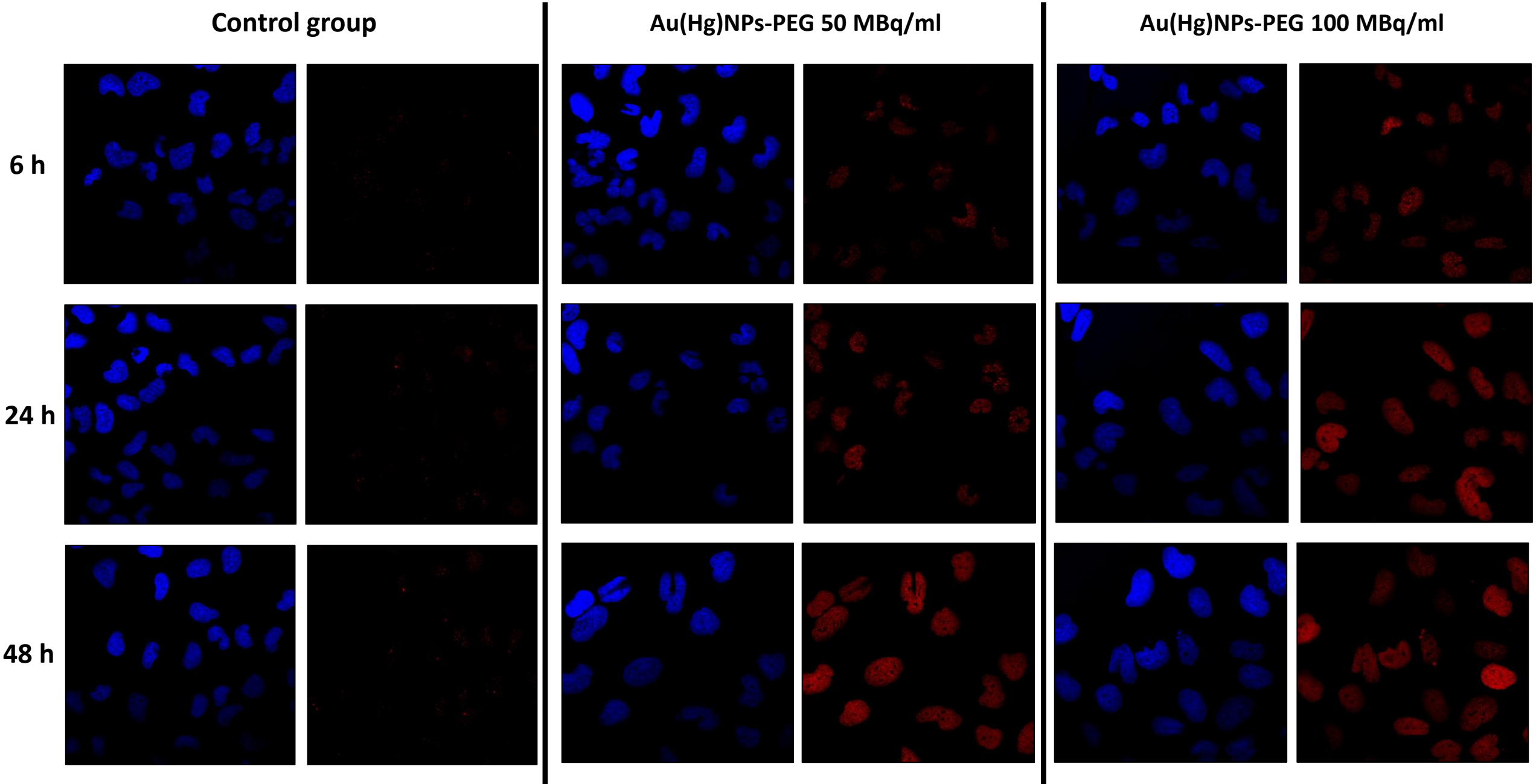




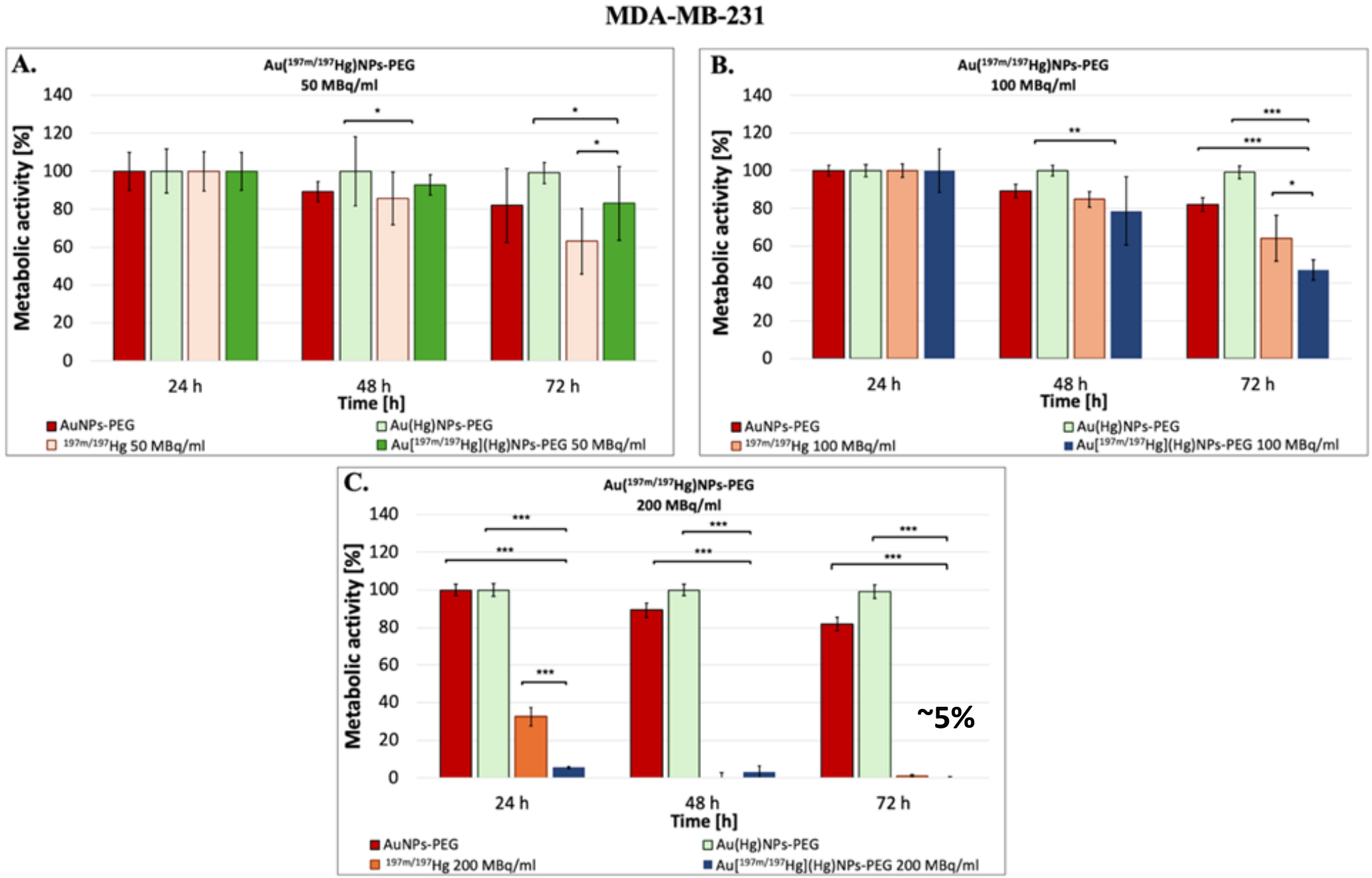
DOUBLE STRAND BRAKES – MDA-MB-231



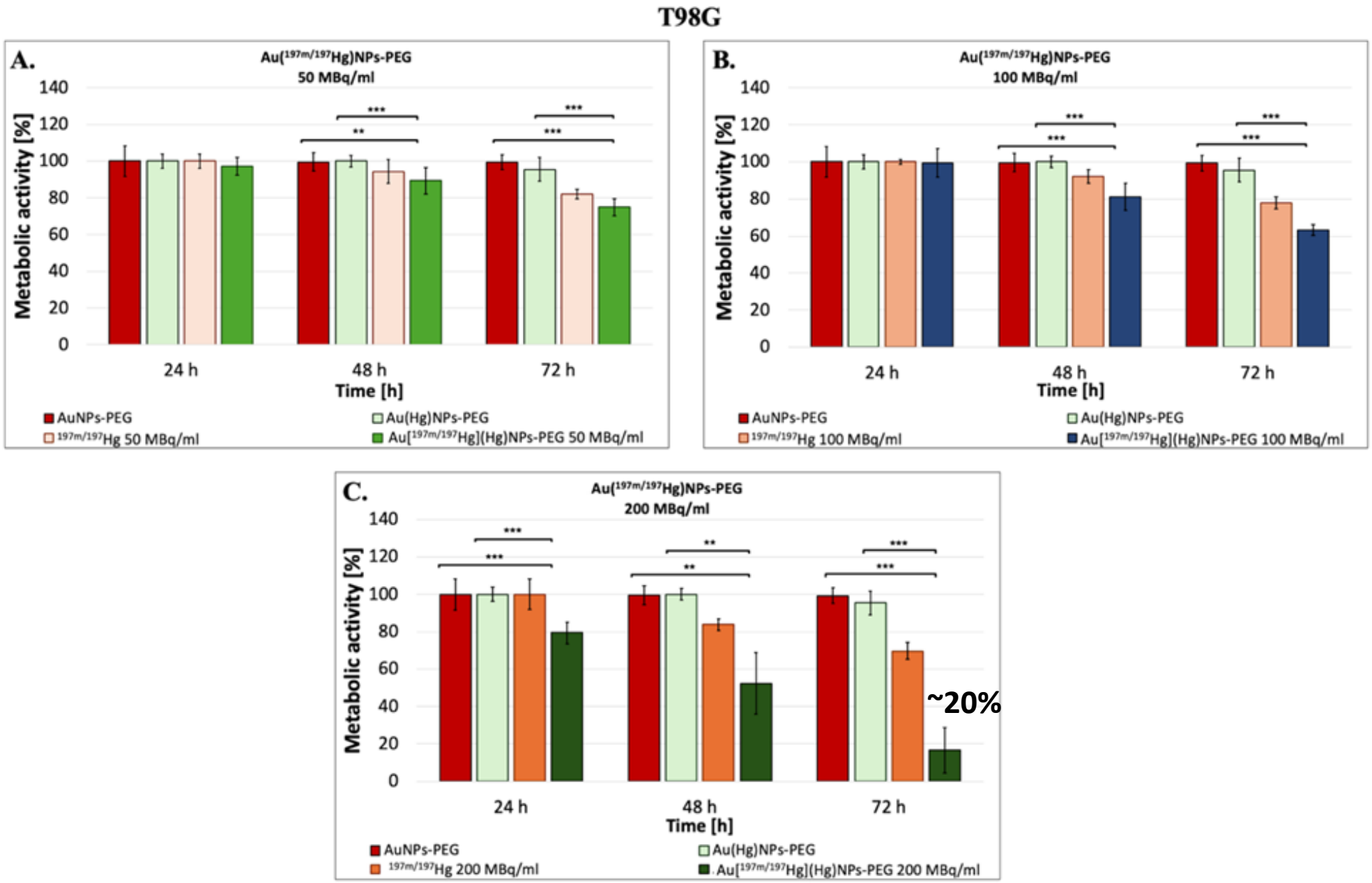
DOUBLE STRAND BRAKES – T98G



CYTOTOXICITY STUDIES – MTS ASSAY



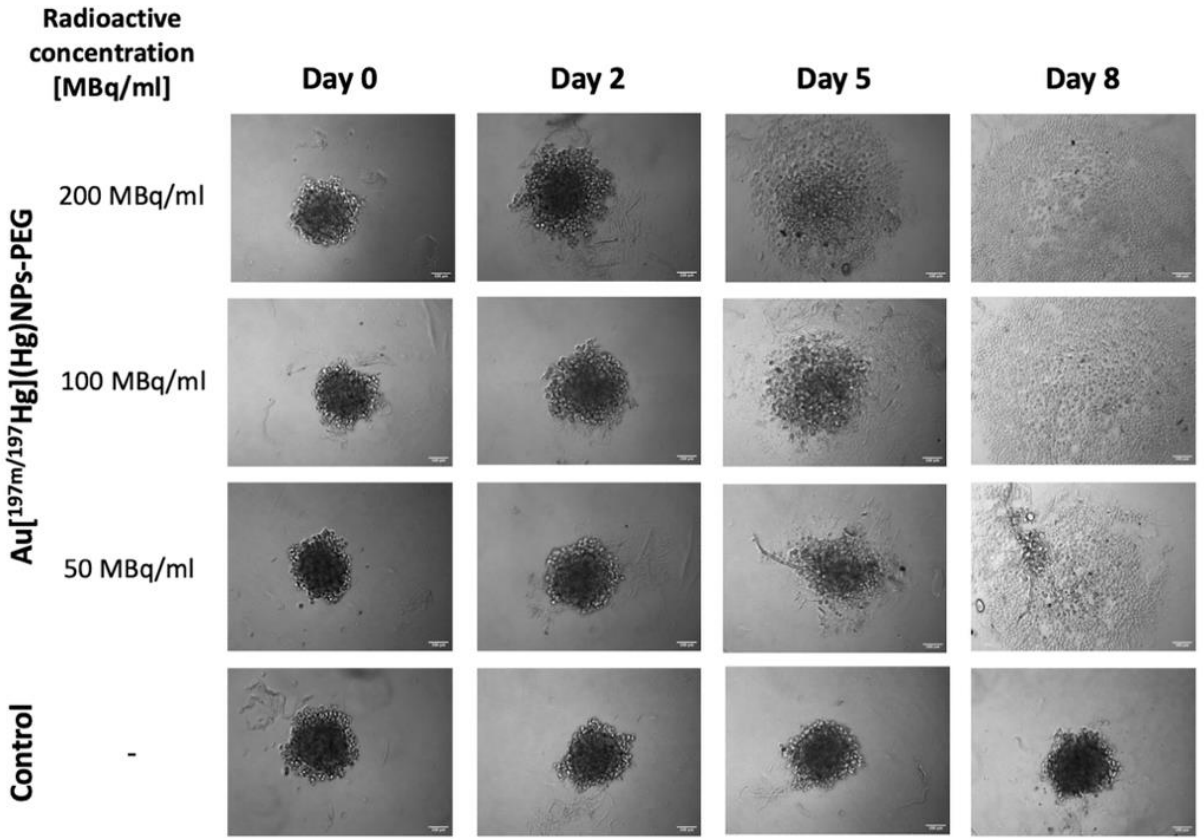
CYTOTOXICITY STUDIES – MTS ASSAY

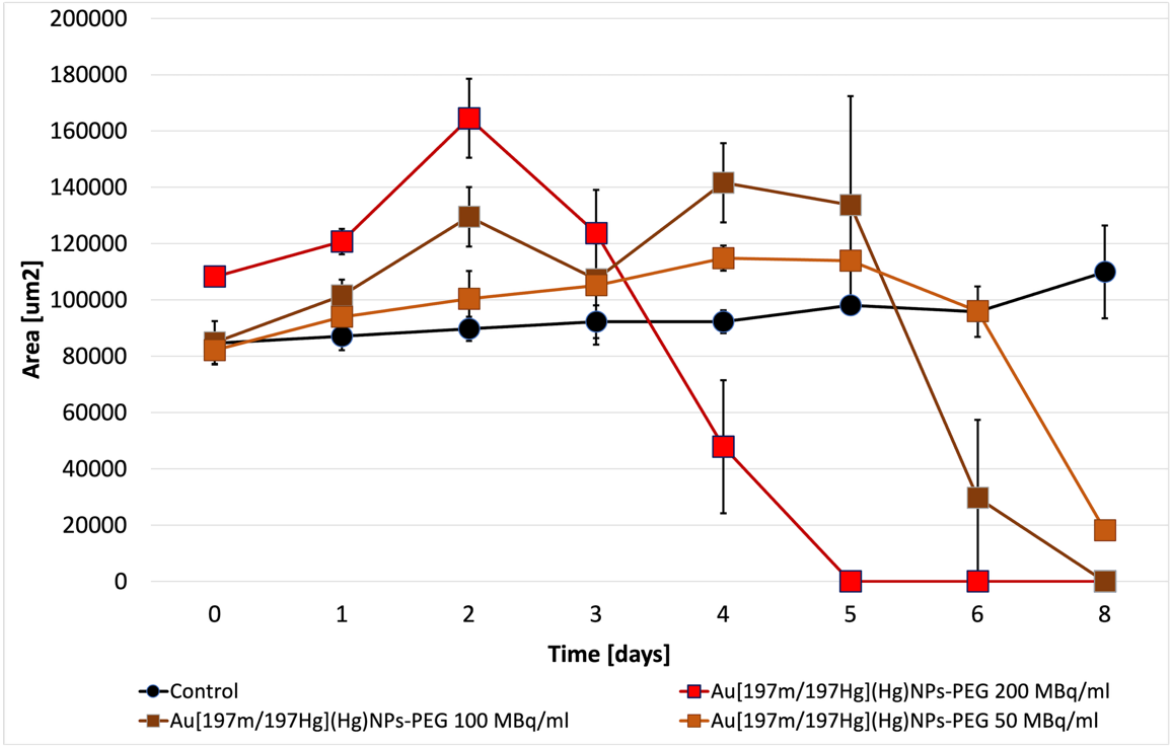


CYTOTOXICITY OF THE RADIOCONJUGATE ON MDA-MB-231 3D TUMOR MODELS

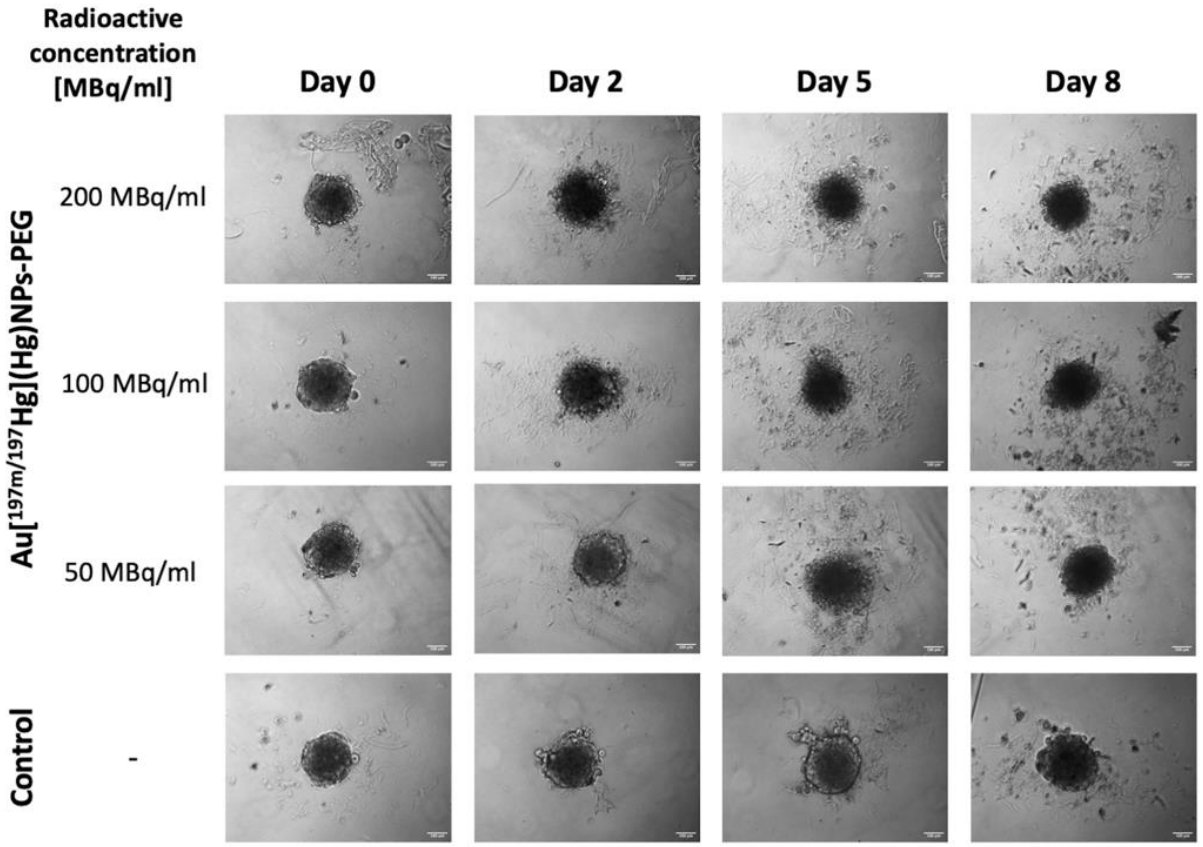
3D colonies more exactly represent tumour models than 2D cell cultures

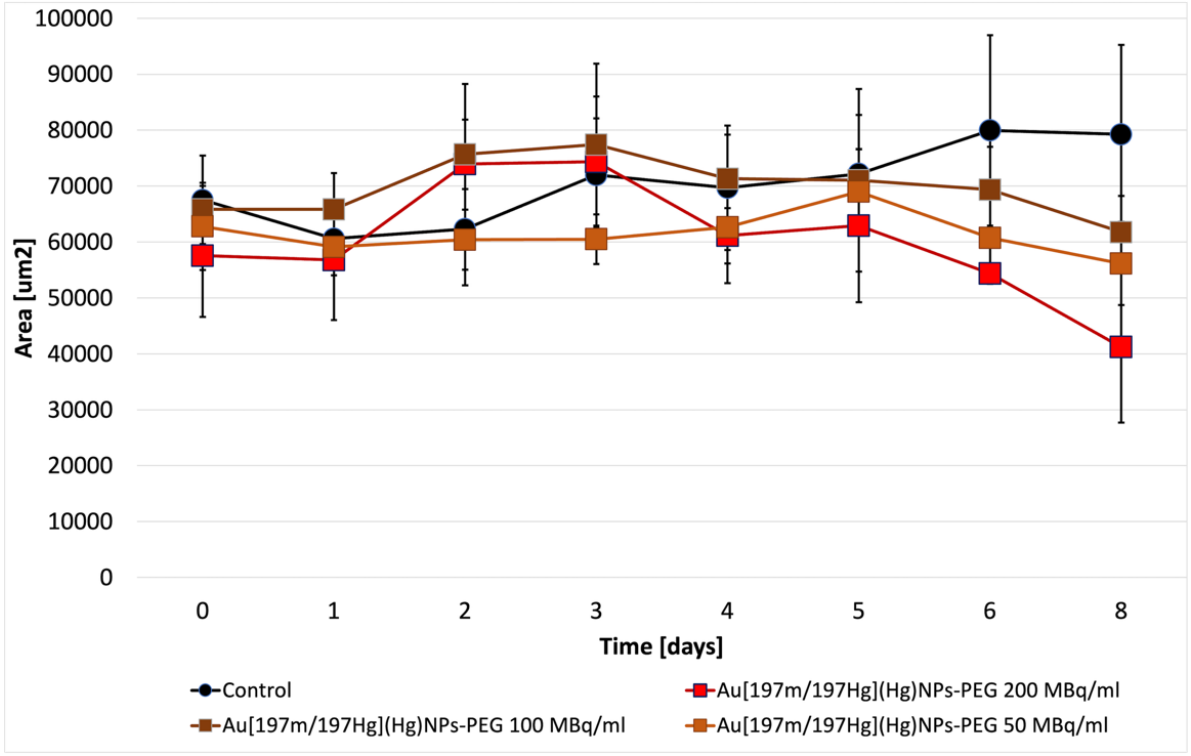
Spheroids are considered an intermediate form between cells from a monolayer culture and a spontaneously growing tumour.





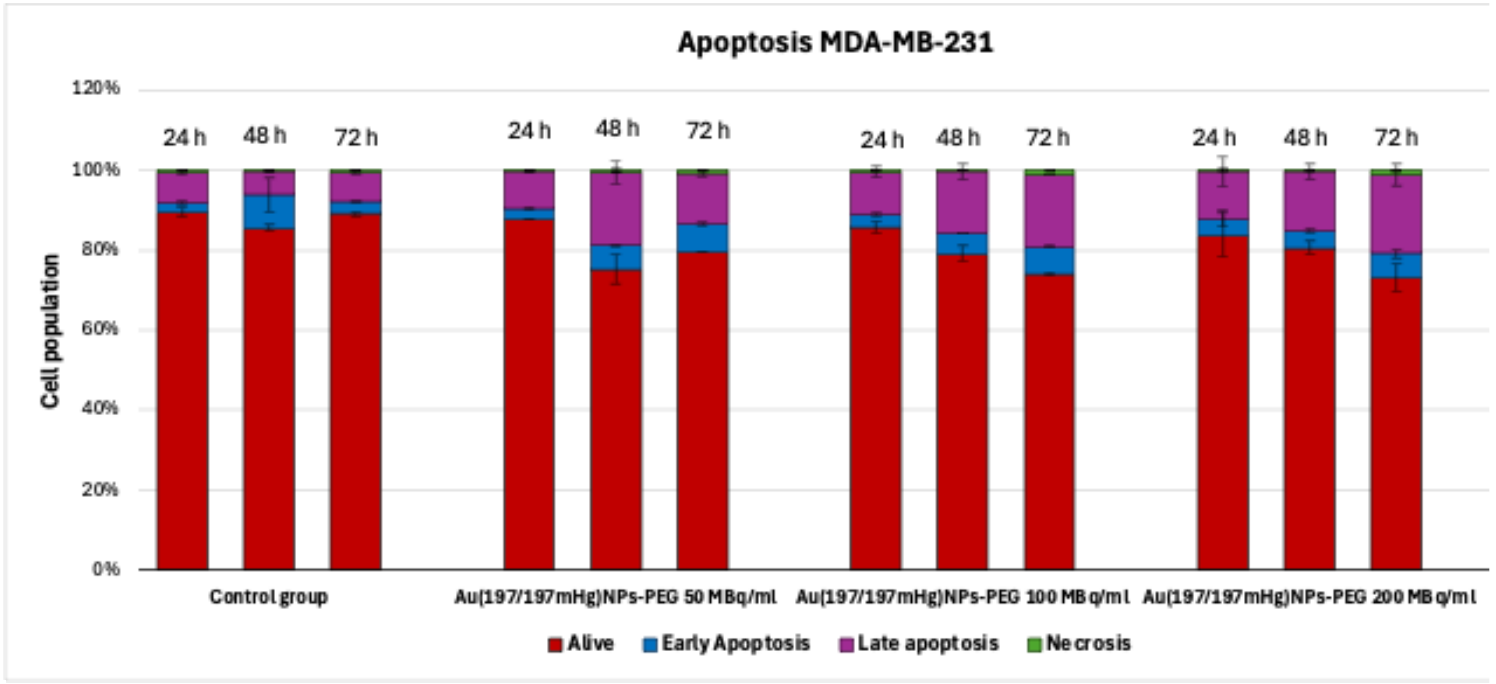
CYTOTOXICITY OF THE RADIOCONJUGATE ON T98G 3D TUMOR MODELS

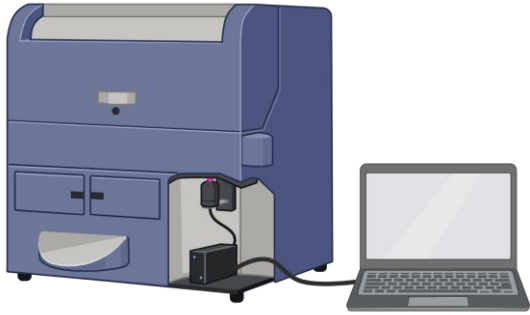




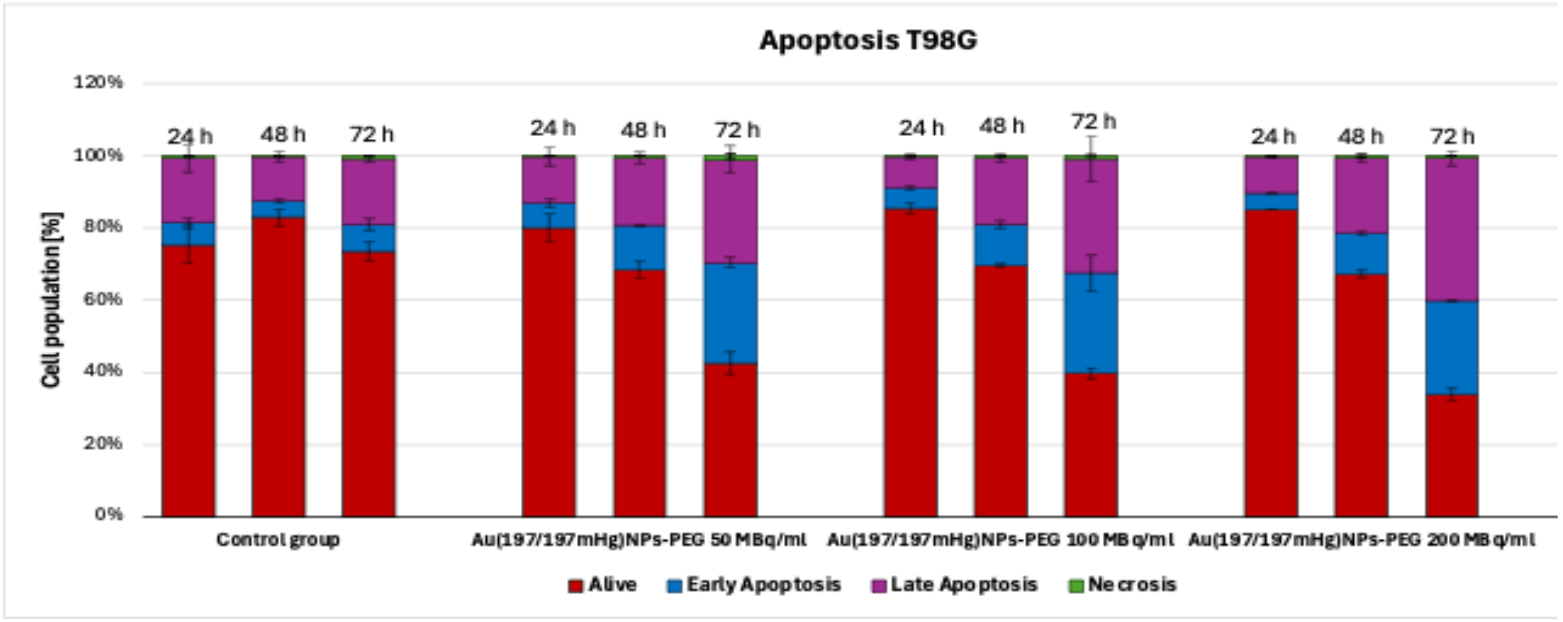
- a little disassembling of the spheroid and a reduction in size for all the tested tumors
- do not observe any significant destruction for any of the concentrations even after 4 weeks

FLOW CYTOMETRY - APOPTOSIS





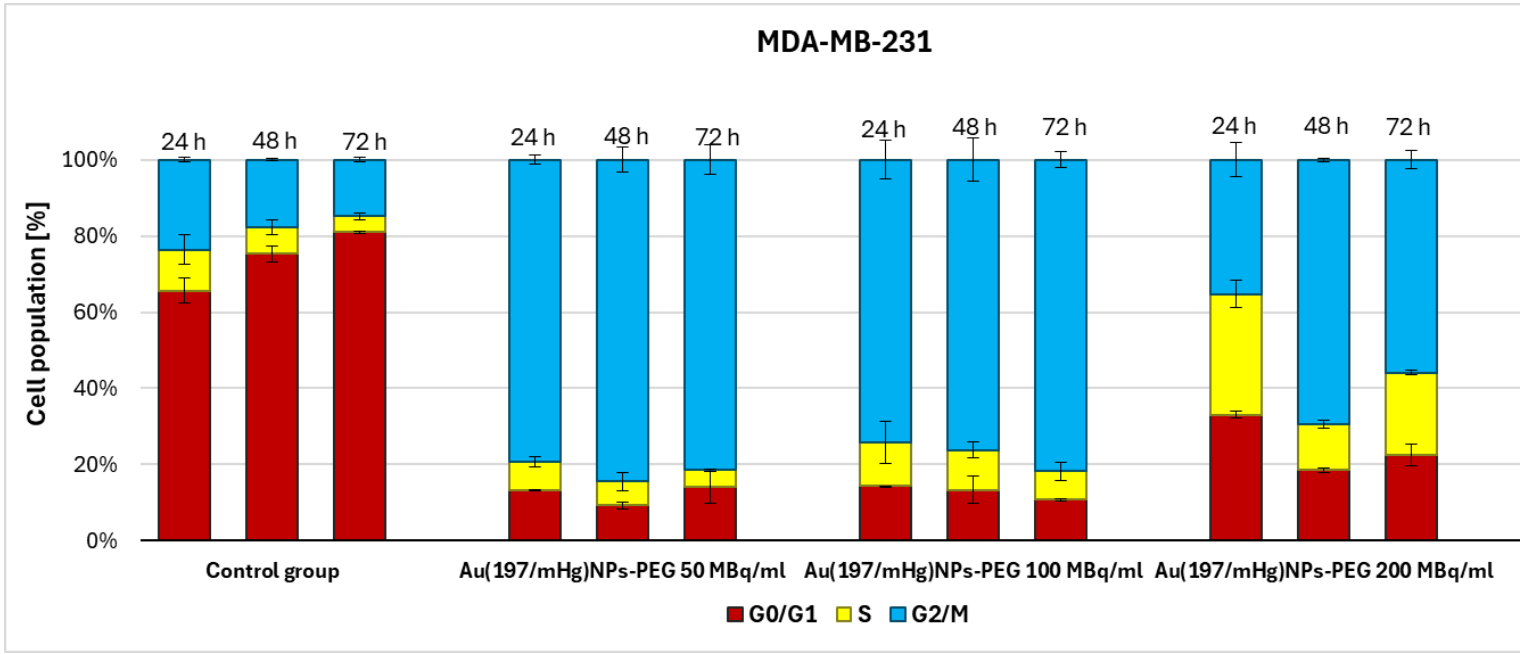
- an increase in the percentage of cells in late apoptosis was observed in both cell lines compared to the control group

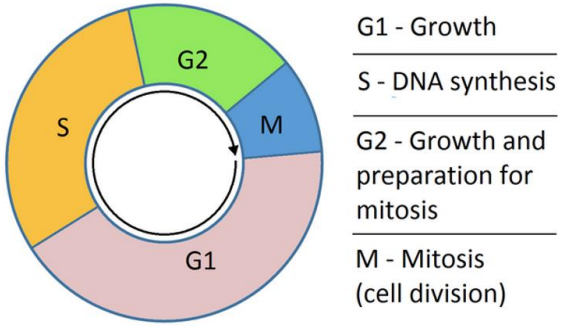


- a significantly higher proportion of cells in early apoptosis

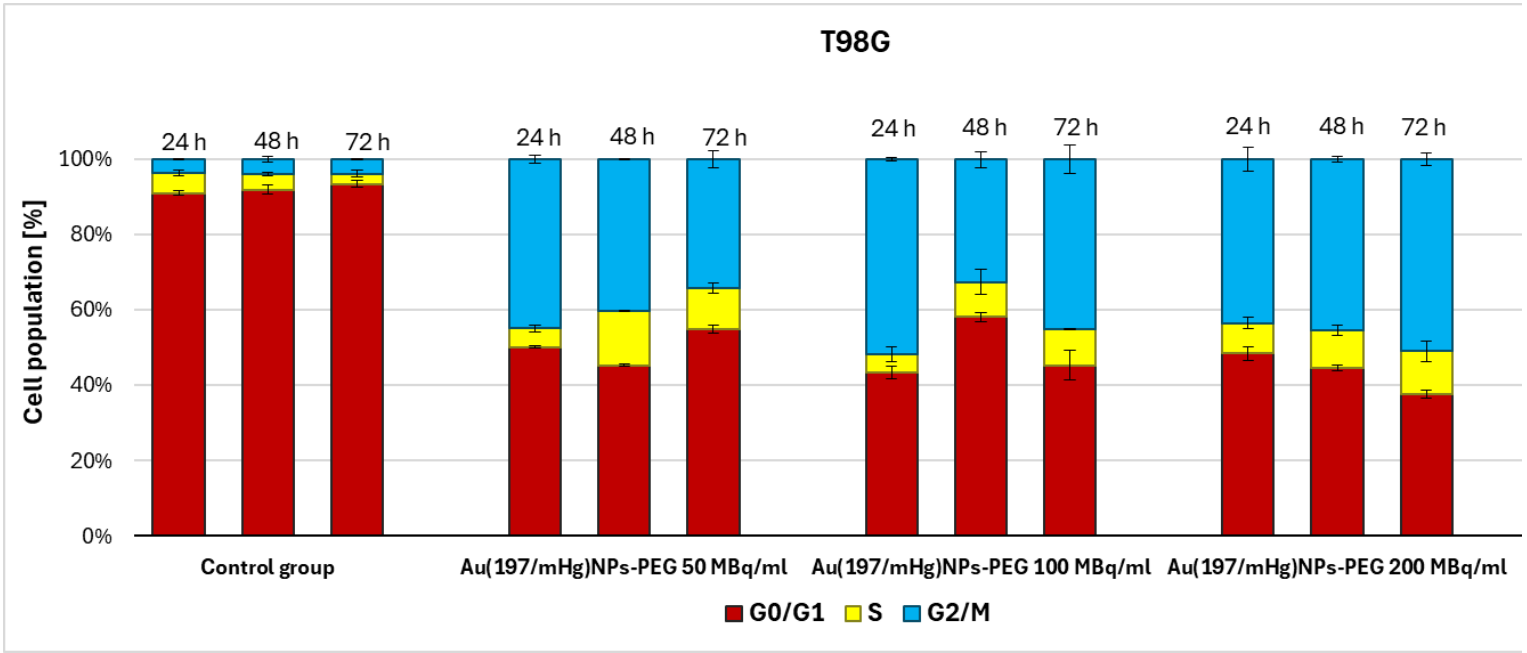
The fraction of cells in the necrotic state remained negligible and did not exceed 1.1%.

FLOW CYTOMETRY – CELL CYCLE





https://www.elucidate.org.au/content/the-cell-cycle



Ex Vivo Studies On Mice

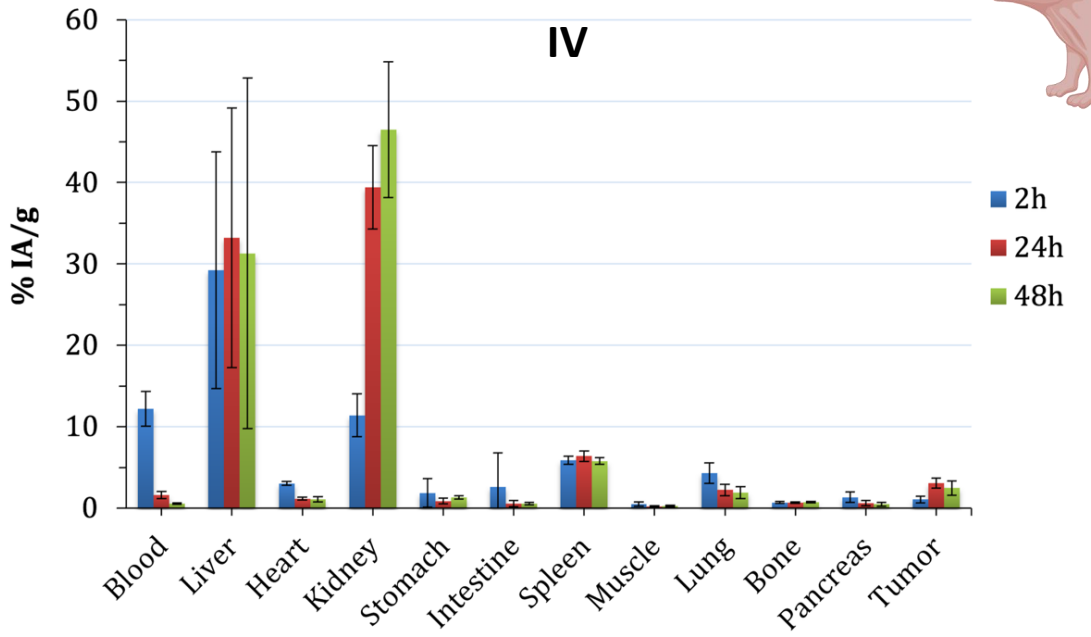
INRASTES
INSTITUTE OF NUCLEAR
& RADIOLOGICAL SCIENCES
& TECHNOLOGY, ENERGY & SAFETY

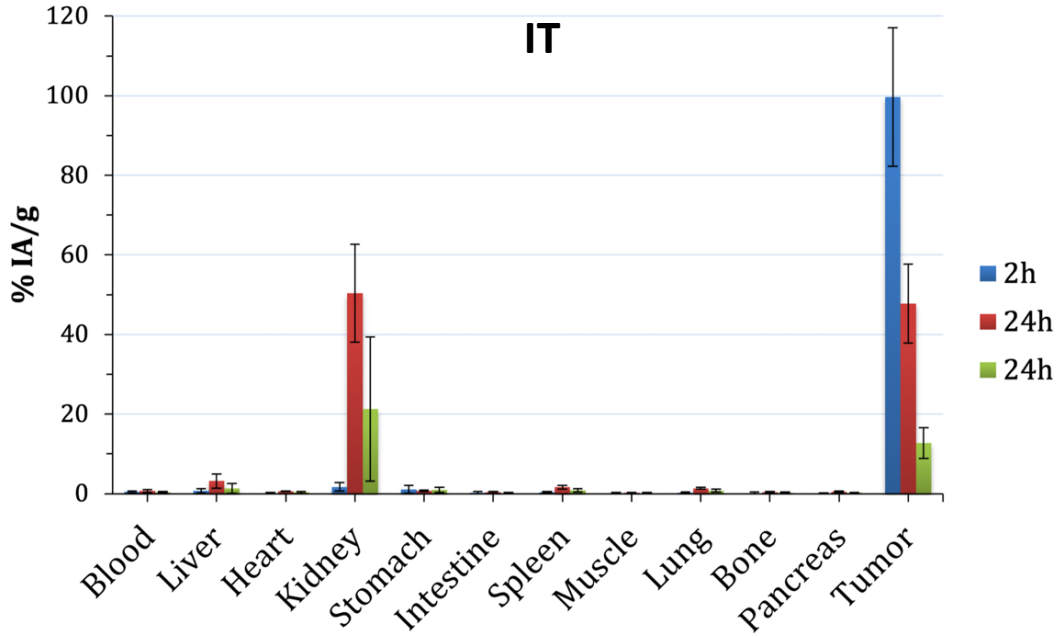
Biodistribution – 3 timepoints, 3 groups of mice with tumor from 4T1 cell line

- The first group received an intravenous injection (**IV**) (100 µL) of 3.89 ± 0.10 MBq radioconjugate.
- The second group received a direct intratumoral injection (**IT**) (50 µL) of 4.03 ± 0.08 MBq radioconjugate.

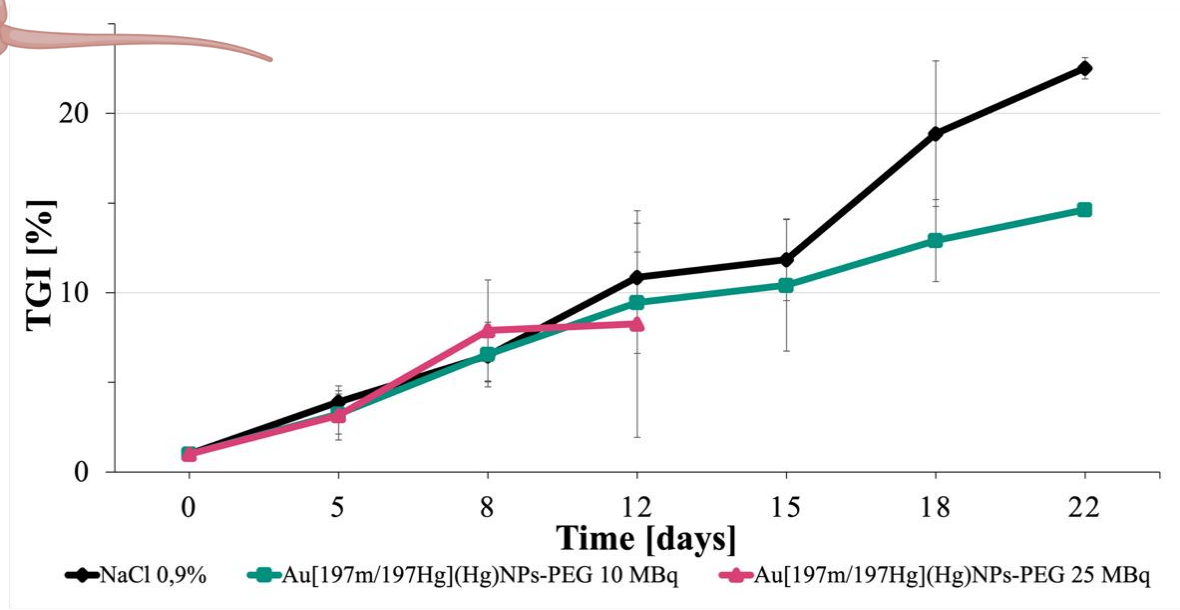
Therapeutic efficacy studies – 2 doses (25 MBq in 50 μl and 10 MBq in 50 μl) – IT injection

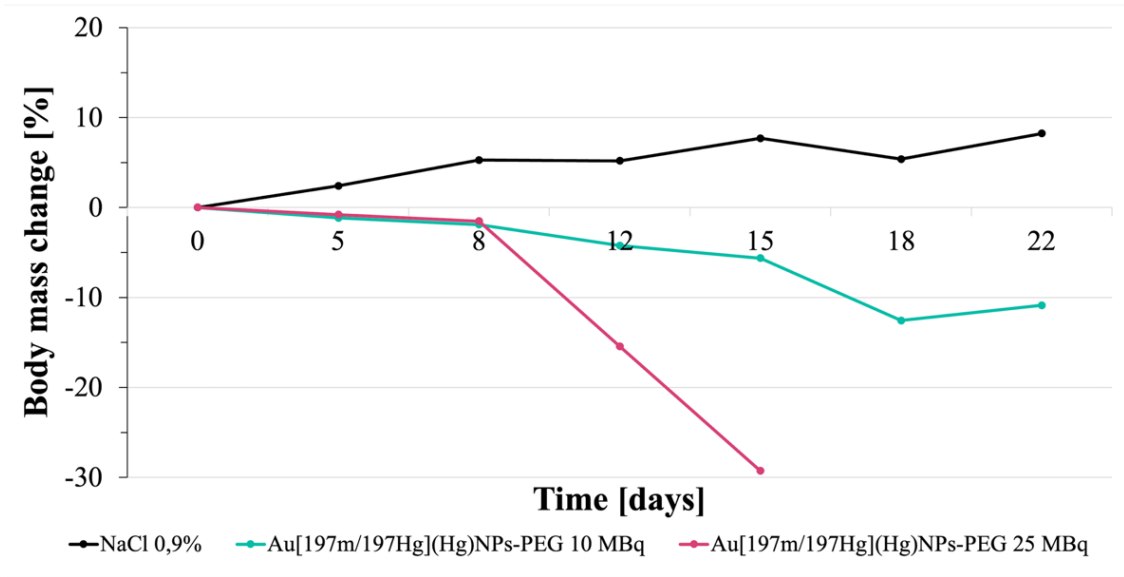
BIODISTRIBUTION RESULTS





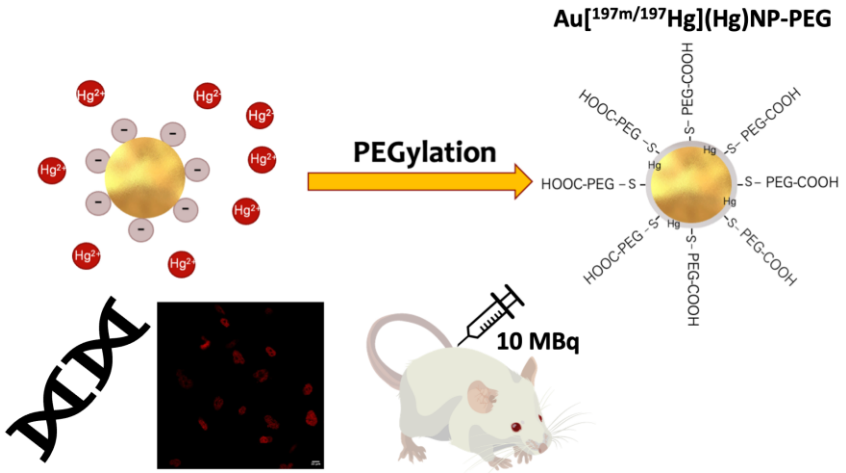
THERAPEUTIC EFFICACY RESULTS





SUMMARY

The Au[$^{197m/197}$Hg](Hg)NPs-PEG radioconjugate demonstrated promising *in vitro* and *ex vivo* anticancer activity.



- The utilized synthesis method produced a stable, monodisperse colloidal solution with high labeling efficiency.
- Cytotoxicity assessments in 2D and 3D cancer models, revealed dose and time dependent efficacy, significantly surpassing control groups.
- Advanced *in vitro* analyses showed DNA double-strand breaks induction as evidenced by γ-H2AX foci formation.
- The synthesized Au[$^{197m/197}$Hg](Hg)NPs-PEG radioconjugate predominantly induced late apoptosis and G2/M phase cell cycle arrest.
- Biodistribution studies indicated typical nanoparticle clearance pathways, with localized tumor retention upon intratumoral injection translating into therapeutic benefits, despite systemic toxicity at higher doses.

The Au[$^{197m/197}$Hg](Hg)NPs-PEG exhibits favorable physicochemical and biological properties, supporting its potential in nanobrachytherapy, however further *in vivo* dose optimization is needed to maximize its efficiency and safety.

Thank you for your attention!