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Blood-Brain Barrier-Penetrating MnO_2-Loaded Polymer-Lipid Nanoparticles Remodel Tumor Microenvironment and Enhance Radiation Therapy of Glioblastoma

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GLIOBLASTOMA MULTIFORME AND NEXT GEN. TREATMENT APPROACH

- **Glioblastoma multiforme (GBM)**
  - Contributes to about 12% to 15% of all primary intracranial tumors and 60% to 75% of glioma cases in North America[1].
  - Among all gliomas, GBM has the poorest overall survival, with only about 0.5% to 4.7% 5 years post-diagnosis survival and 15 months post-operative median survival[2].

- **Magnetic resonance-guided radiation therapy (MRgRT)**
  - Provides individualized, patient-centric planning and RT approach for GBM treatment.
  - Allows real-time and accurate tumor delineation for RT without extra radiation burden and reduced off-target radiation delivery.

[1]Ostrom, Q.T. *et al. Neuro. Oncol.* **21**, 1–100 (2019).
[2]Davis, M.E. *et al. Clin J Oncol Nurs.* **20(5):** S2–S8 (2016).
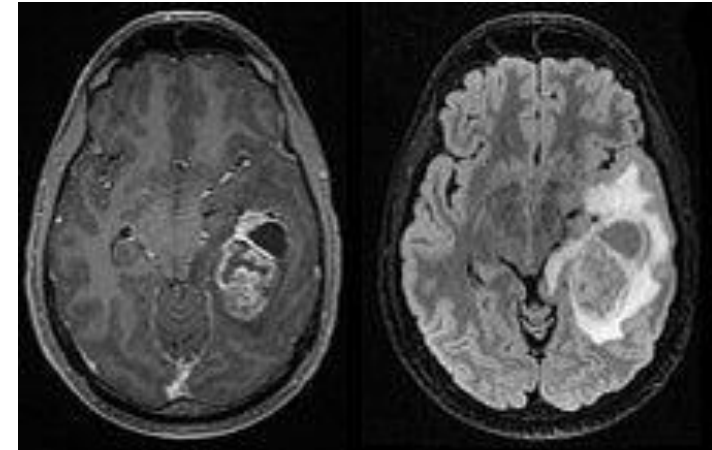

MRI of glioblastomas. (2014) UCSF.

Magnetic Resonance Guided Radiation Therapy. (2017) University Health Network.

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CHALLENGES OF MRgRT IN GBM TREATMENT

- **Challenges of MR-guided radiation therapy (MRgRT)**

  - Gadolinium-based contrast agents (GBCA) leads to **Gd toxicity** and **tissue depositions** in organs including brain.

  - Poor selectivity / **short duration of signal** enhancement.

  - **Radiation resistance** due to hypoxic tumor microenvironment (TME).

- **Terpolymeric manganese dioxide (MnO$_2$) nanoparticles (TPL-MDNPs)**

  - A **tumor-activatable** system converting H$_2$O$_2$ to O$_2$/Mn^{2+} in hypoxic TME.

  - Allows **tumor sensitization to RT** and **prolonged tumoral MRI enhancement.**

  - **Improves blood-brain barrier (BBB) and tumor penetrations** via surface functionalization and enhanced penetration and retention (EPR) effect.
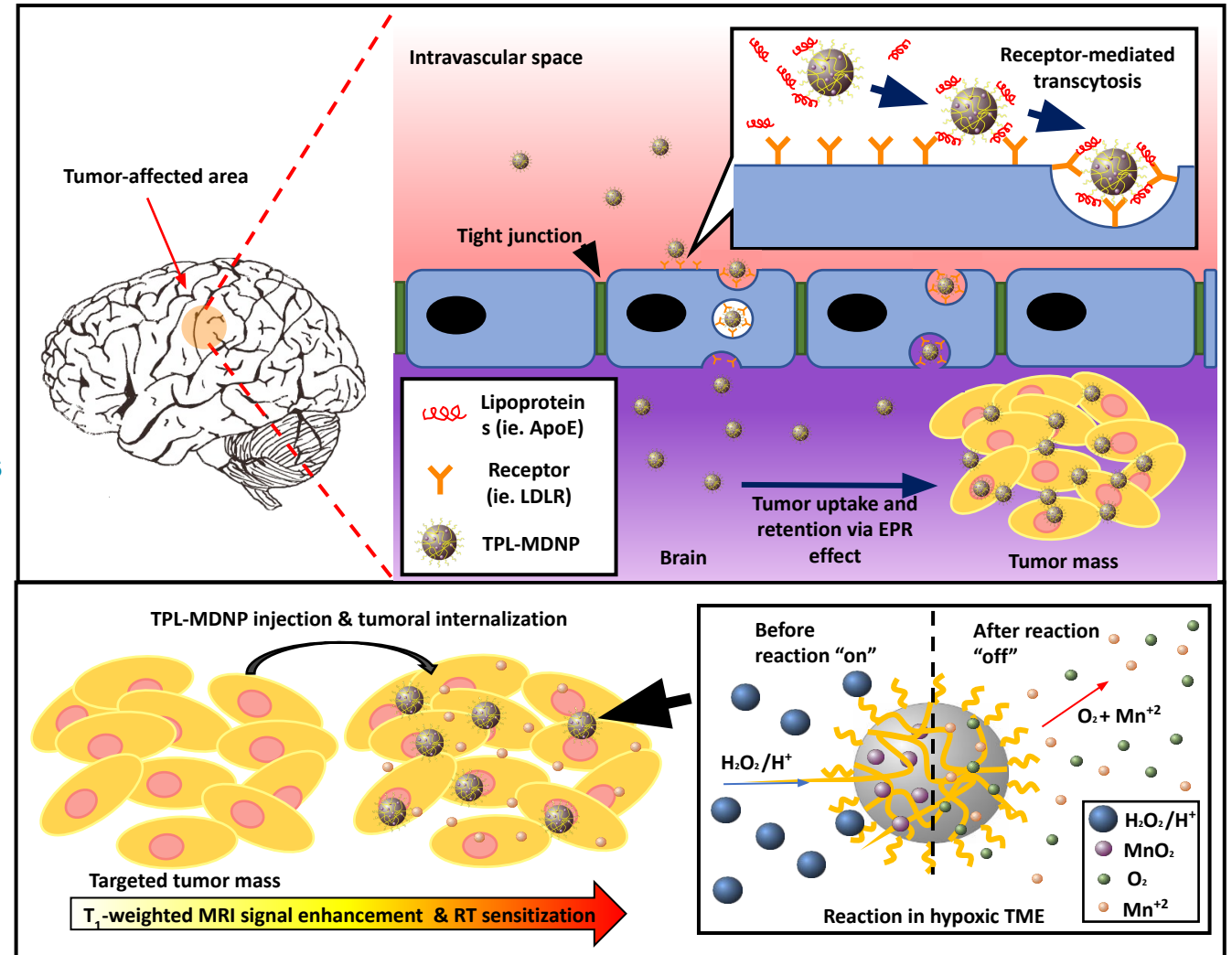
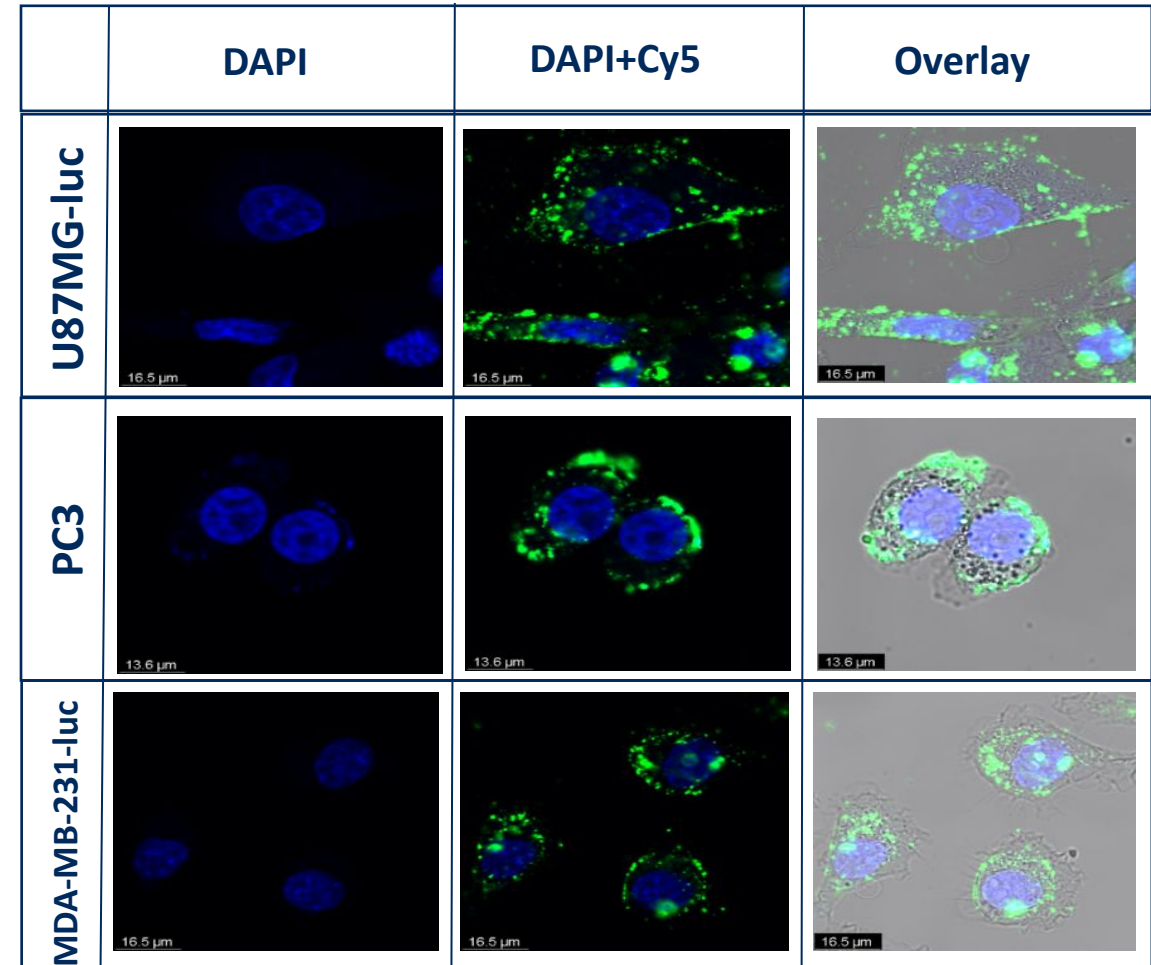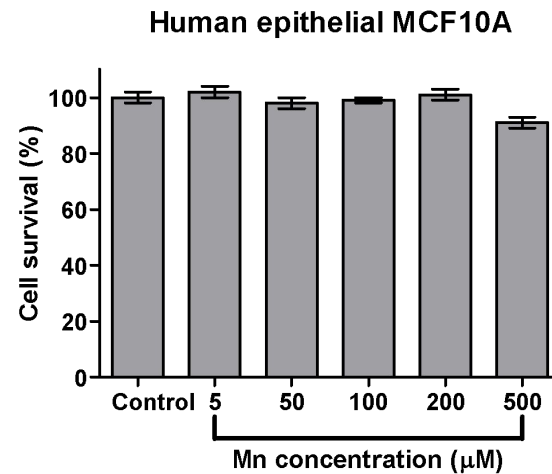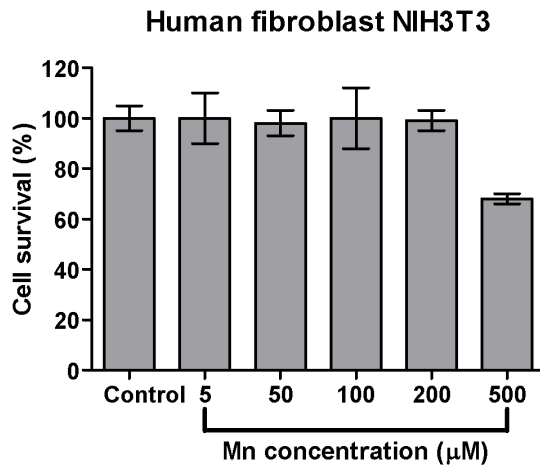# PHYSICOCHEMICAL CHARACTERIZATIONS OF TPL-MDNP

- Small nanoparticle with narrow size distribution
- Robust manufacturing process
- **Biocompatible** starting materials
- **High stability and long shelf life**
- Suitable for intravenous injection
- **High reactivity** towards ROS (H_2O_2) to generate oxygen and generate paramagnetic Mn^{2+}

$r_1 = 12.654 (mM \cdot sec)^{-1}$

$r_1 = 0.684 (mM \cdot sec)^{-1}$
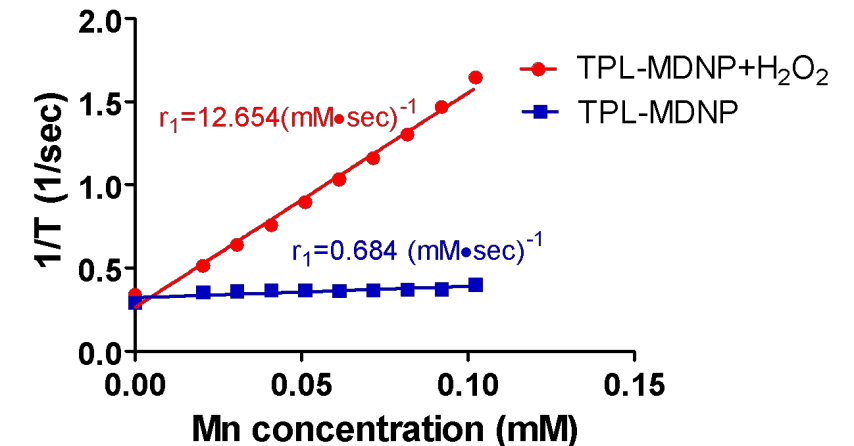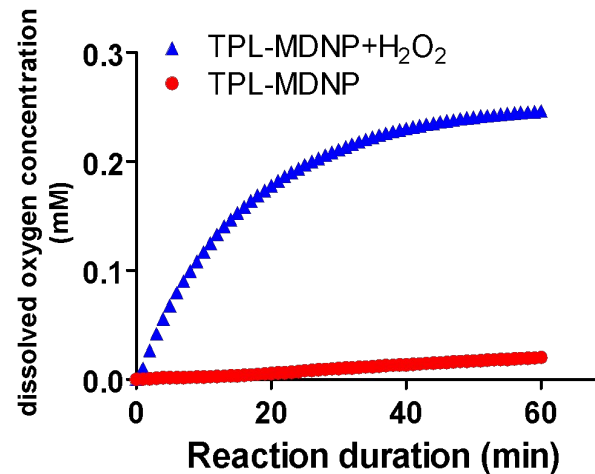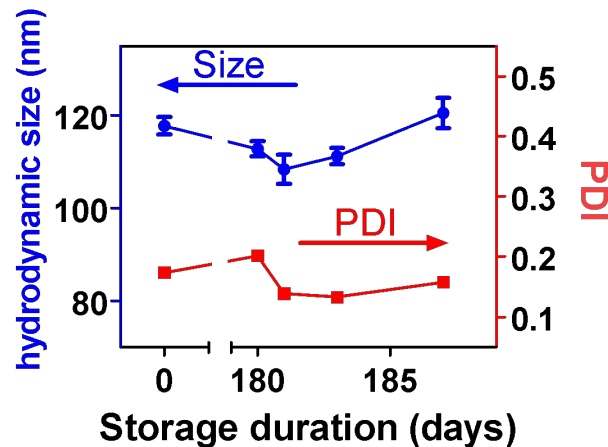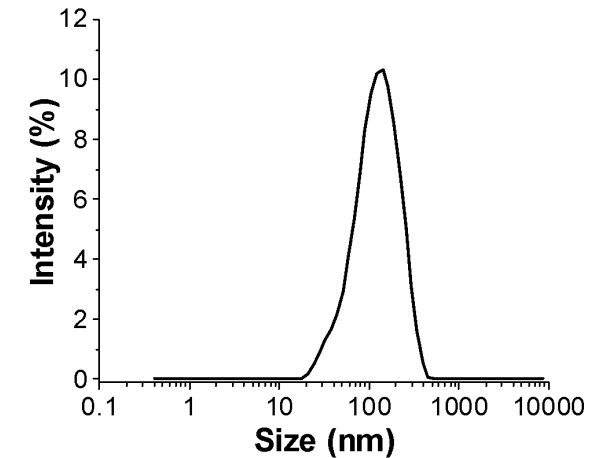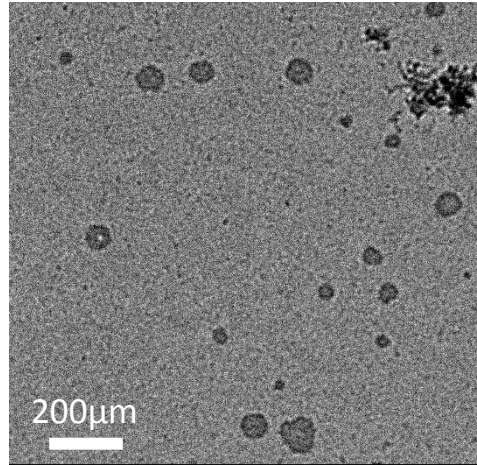
BIOCOMPATIBILITY OF TPL-MDNP CONFIRMED IN VITRO

- *In vitro* cellular uptake and cytotoxicity
 - **High cellular uptake** by various cancerous cell lines, including U87 brain cancer cells
 - **Low cellular toxicity** to non-cancerous cell lines

Human fibroblast NIH3T3

Human epithelial MCF10A

	DAPI	DAPI+Cy5	Overlay
U87MG-luc			
PC3			
MDA-MB-231-luc			

BIOCOMPATIBILITY OF TPL-MDNP CONFIRMED IN VIVO

- *In vivo* **biocompatibility and biosafety of TPL-MDNP**

  - **Low hemolysis** of i.v. formulation up to 2000µM of Mn.

  - **Excellent biocompatibility and biosafety** for single- and repeated-doses in healthy animal models.

  - **Excellent systemic clearance** based on single-dose PK analysis in healthy animal models.

  - **No tissue accumulation** in major organs.
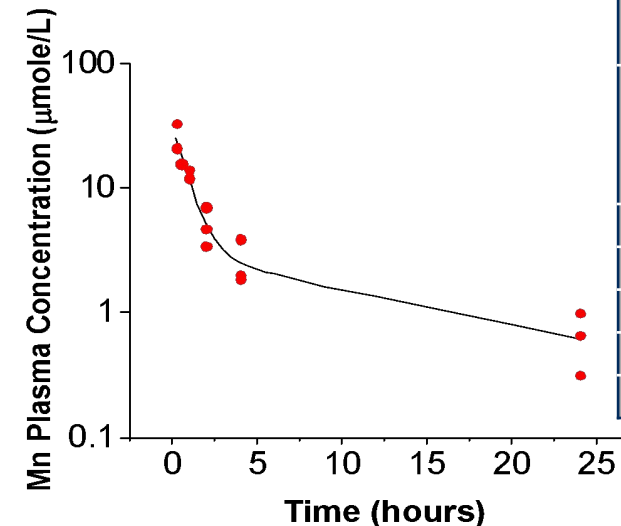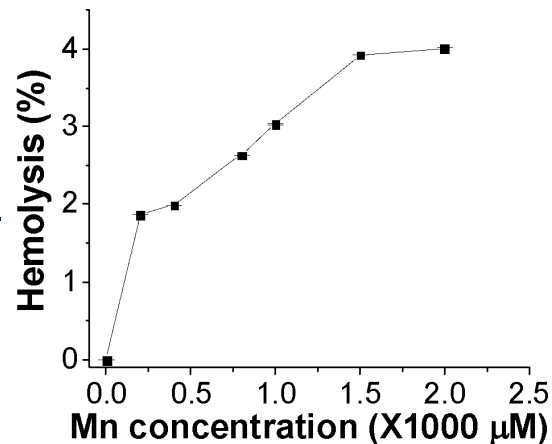
Parameters	Values
Dose (µmol/kg)	37.5
Plasma $AUC_{0-\infty}$ (µmol*hr/L) [a,b]	77.82
CL (L/hr) [c]	0.125
$t_{1/2\alpha}$ (hr) [d]	0.5642
$t_{1/2\beta}$ (hr) [d]	10.42
V_1 (L) [e]	0.295±0.03
V_{ss} (L) [e]	1.406±0.03

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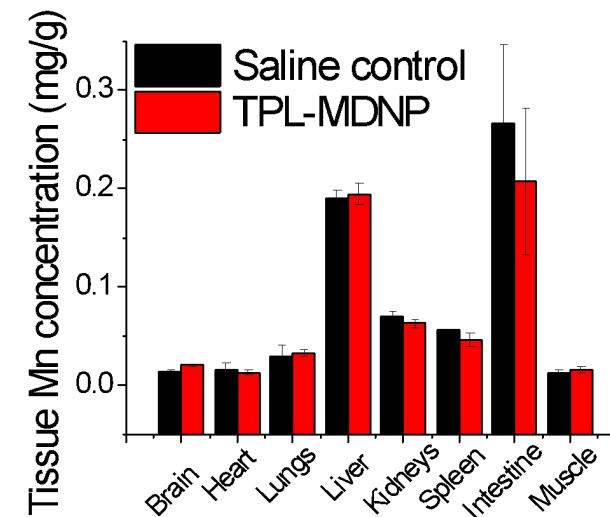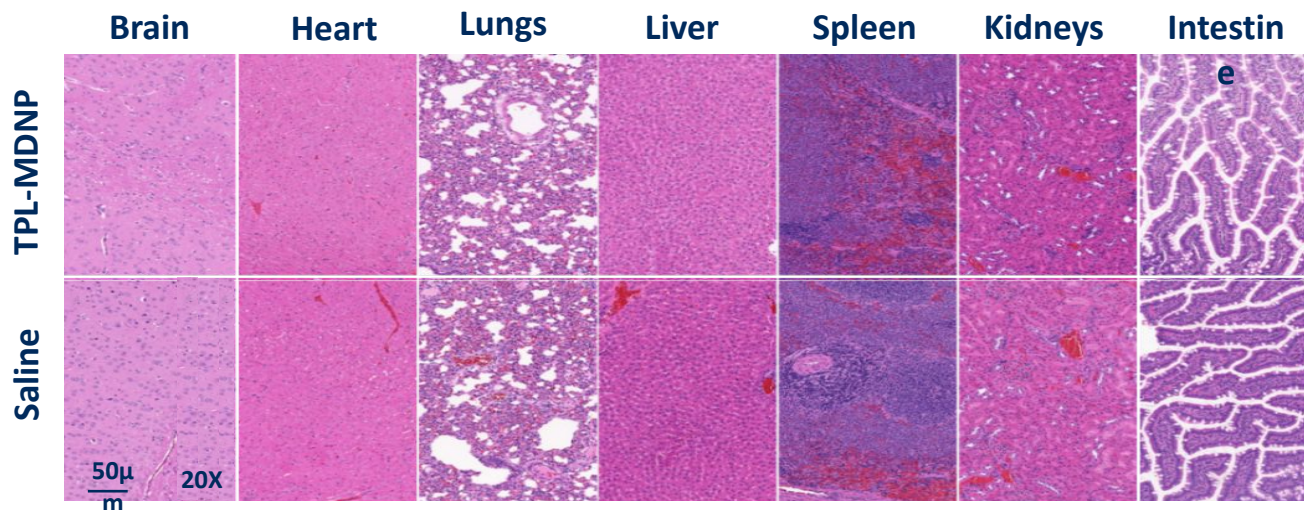
# *In vivo* MRI contrast enhancement of TPL-MDNP

- **Improved tumoral MR signal intensity and duration**
  - Ideal tumor-targeted delivery of multifunctional MDNP.
  - Strong T_1-weighted (T1W) MRI signal enhancement with **clear tumor delineation.**
  - **Improved T_1-weighted MR signal intensity** compared to GBCA.
  - **Longer MR signal enhancement duration** up to 1hr of imaging session.
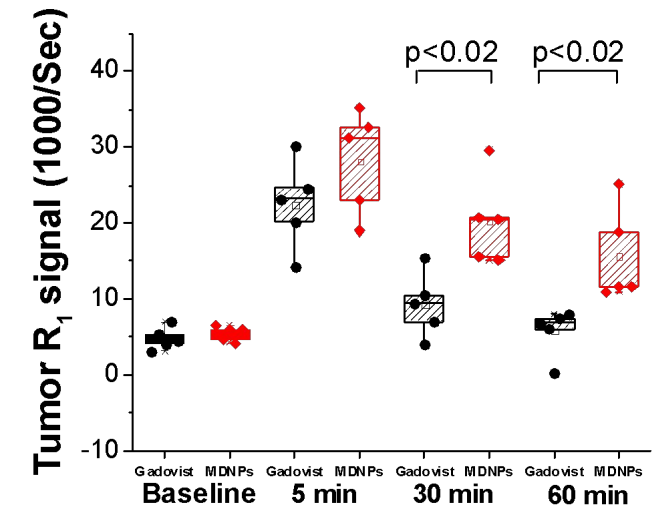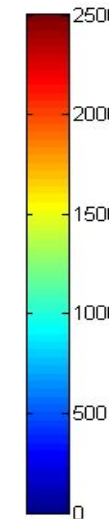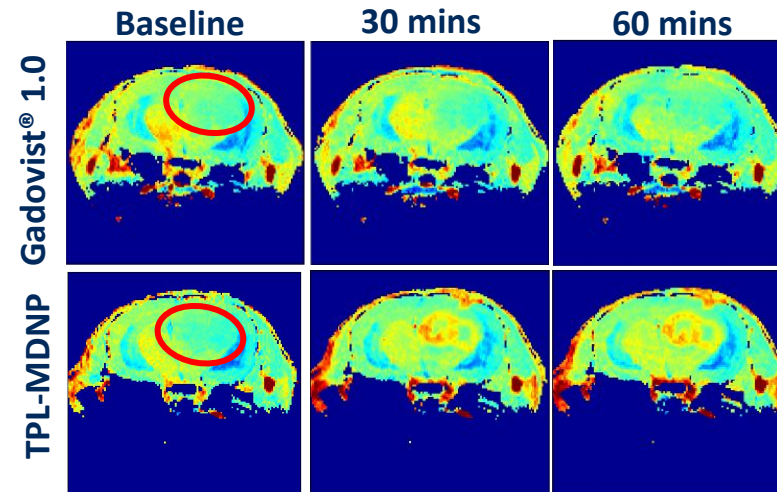
U87 GBM tumor
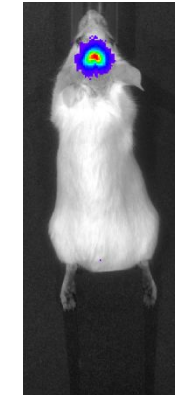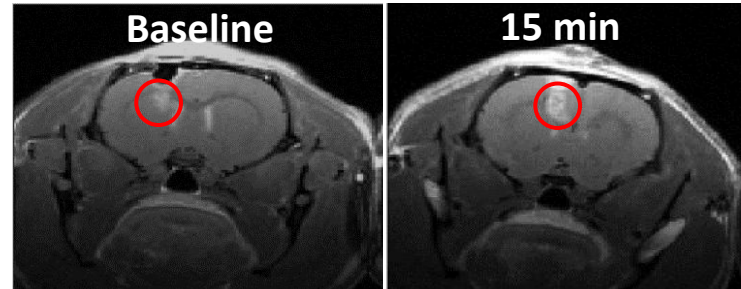
Luciferase-expressing U87MG-luc tumor mass (bioluminescence)
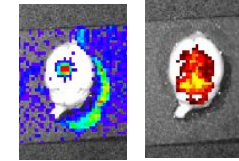
Ex vivo brain samples

ICG-labelled TPL-MDNP (fluorescence)
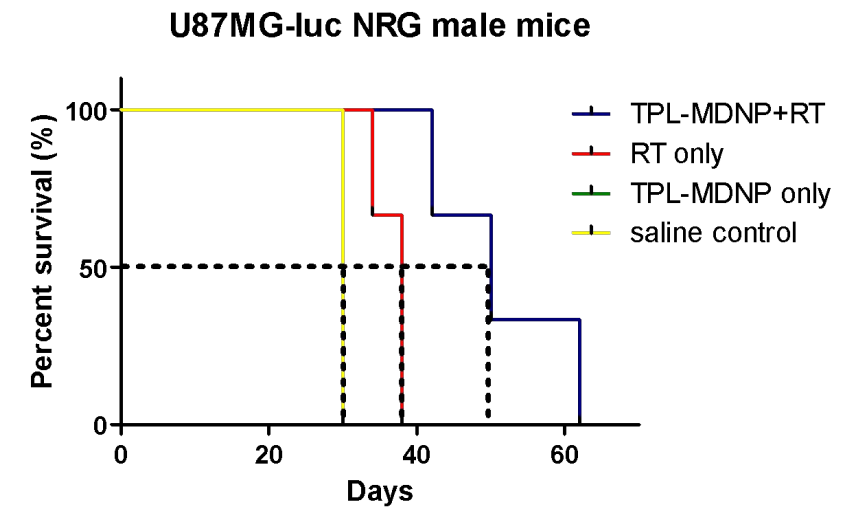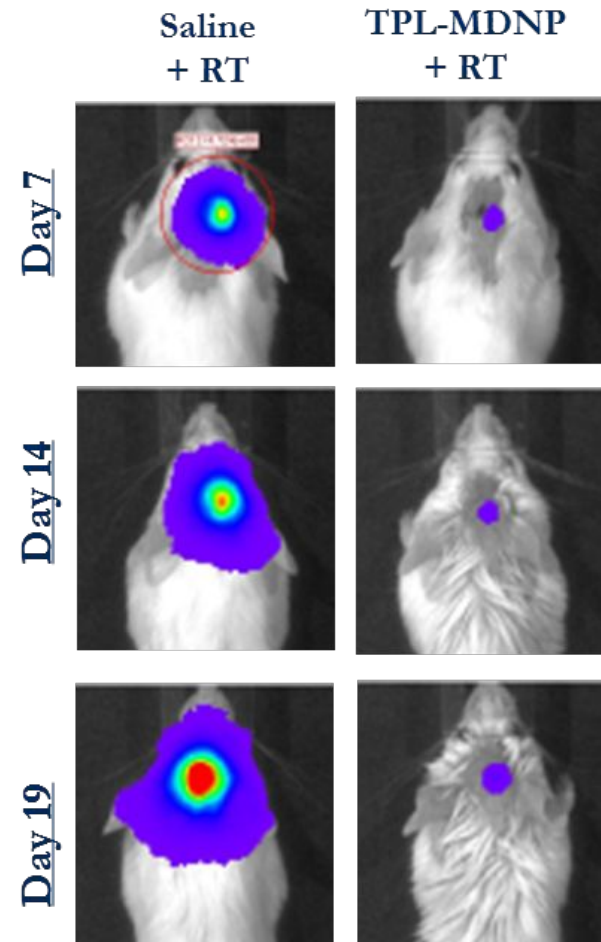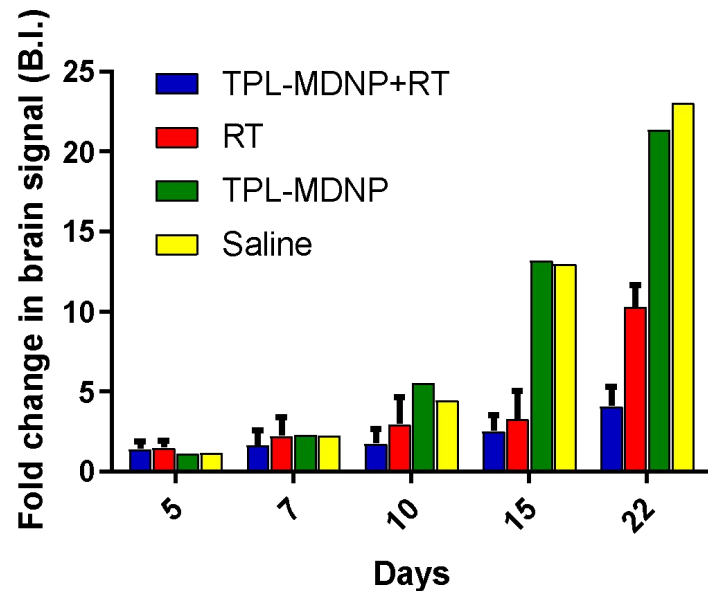

$p<0.02$ $p<0.02$

n=5, mean +/- SD

In vivo radiation therapy enhancement of TPL-MDNP in brain tumor

- **Enhances RT efficacy in brain tumor-bearing mice models**
  - Combination therapy **effectively reduces the tumor progression** by **2 folds** in comparison to RT alone.
  - **Prolongs median survival** by **2.3-folds** when compared to RT alone.

HIGHLIGHTS OF TPL-MDNP FOR THERAGNOSTIC APPLICATIONS

✔ A **pharmaceutically safe** TPL-MDNP nano-formulation exhibits **good biocompatibility** and excellent TME modulation capability.

✔ The TPL-MDNP system allows for **excellent tumor-specific uptake and retention.**

✔ The tumor-activatable TPL-MDNP **sensitizes RT** and **enhances MR signal** simultaneously.

✔ The TPL-MDNP improved tumor delineation and T1W MR signal intensity and duration in comparison to GBCA.

✔ The TPL-MDNP enhanced RT efficacy in GBM treatment in comparison to RT without radiation sensitizers and prolonged median survival in GBM-bearing animal models.

✔ This work provides an improved and safer alternative to conventional GBCA and a promising agent for the **next generation radiation technique** (i.e. MRgRT).

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Acknowledgements