An Integrated Approach Utilizing Nanotechnology, Chemotherapy, and Radiotherapy for Pancreatic Cancer Treatment

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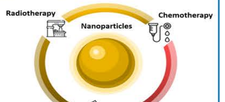
PURPOSE / HYPOTHESIS

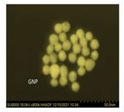
The Problem: Chemotherapy and radiotherapy (RT) suffer from normal tissue toxicity.

The Solution: Use nanoparticles as drug delivery vehicles for chemotherapy and radiosensitizers for RT.

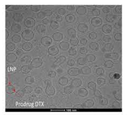
How does it work? Nanoparticle size allows for better tumor-targeting by surface conjugating and by exploiting the leaky tumor vasculatures.



Mechanism of Action



Gold Nanoparticles (GNPs)



Docetaxel Prodrug Lipid Nanoparticles (LNP$_{DTX-P}$)

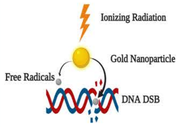
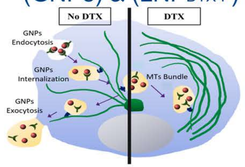


Figure 1. GNPs absorb radiation, emitting short-ranged electrons which cause the production of free radicals that damage the DNA.



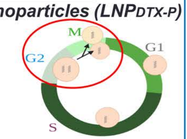


Figure 2. Docetaxel traps cells in the G2/M phase; the most radiosensitive phase of the cell cycle.

Figure 3. GNPs use the microtubules (MTs) highway network to move within the cell. Docetaxel damages MTs causing an increase in GNPs accumulation in cells.

Hypothesis: LNP$_{DTX-P}$ will result in an increase in cell synchronization in the G2/M phase resulting in a significant increase in the uptake of GNP in tumor cells. Combining this with radiotherapy will lead to a reduction in tumor size.

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EXPERIMENTAL SET-UP

- ***Radiosensitizers:*** GNPs of ~ 13 nm in diameter functionalized with PEG and RGD.
- ***Chemosensitizers:*** Free-form DTX vs LNP$_{DTX-P}$.
- ***Radiotherapy:*** A single 2 Gy dose
- ***3D Spheroids:*** MIA PaCa-2 cell co-cultured with patient-derived Cancer-Associated Fibroblasts (CAFs).
- ***Dosages:*** dosed with 7.5 μg/mL of GNPs & 100 nM of free-form DTX or an equivalent dose of LNP$_{DTX-P}$.

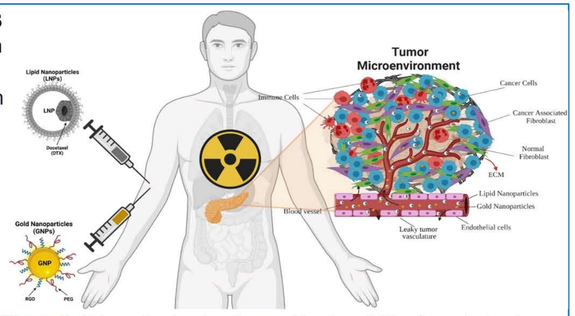

Figure 4. Schematic showing the combined modality of nanotechnology, chemotherapy, and radiotherapy for the treatment of pancreatic cancer.

IC-50 Curves in 3D Spheroids

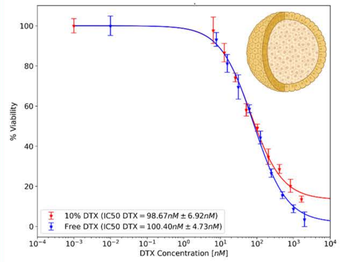

Figure 5. IC-50 curves of free-form DTX vs LNP$_{DTX-P}$ in 3D MIA PaCa-2 monoculture spheroid model.

GNPs & LNPs Characterization

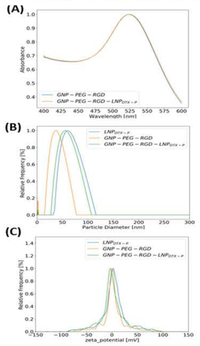

Figure 6. **(A)** UV–visible absorption spectra of GNP$_{PEG-RGD}$ and LNP$_{DTX-P}$/GNP$_{PEG-RGD}$. **(B)** The hydrodynamic diameter of GNP$_{PEG-RGD}$, LNP$_{DTX-P}$, and LNP$_{DTX-P}$/ GNP$_{PEG-RGD}$. **(C)** The ζ-potential of GNP$_{PEG-RGD}$, LNP$_{DTX-P}$, and LNP$_{DTX-P}$/ GNP$_{PEG-RGD}$.

RESULTS

- LNP$_{DTX-P}$ treated 3D co-culture spheroids exhibited more cells in the G2/M phase and 183% more GNP compared to controls.
- RT/LNP$_{DTX-P}$ treated samples showed a significant increase in DNA DSB and a decrease in tumor size compared to RT.
- GNPs/RT/ LNP$_{DTX-P}$ treated samples displayed a 39% increase in DNA DSB and a 28% reduction in tumor size compared to RT.

3D Co-culture Spheroids Size Post-Treatment

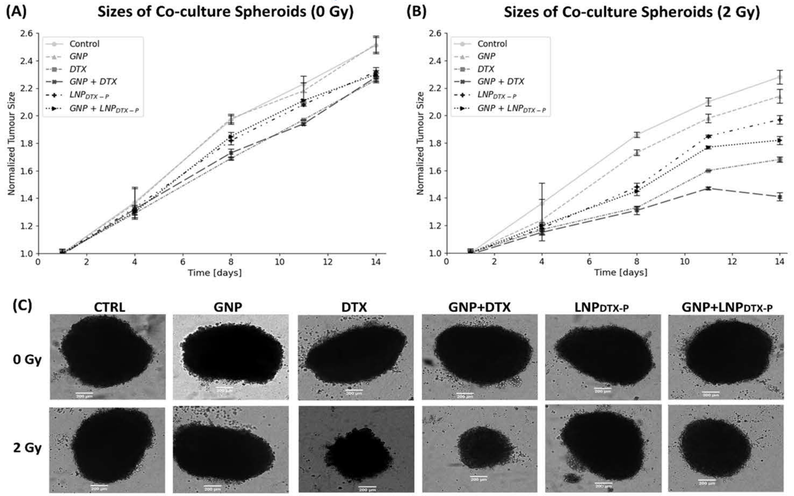

Figure 8. Co-culture spheroids sizes post-treatment with radiation/drug. **(A–B)** Normalized spheroids sizes over 14 days post-treatment: **(A)** 0 Gy, **(B)** 2 Gy. **(C)** Bright-Field images of co-culture spheroids taken 14 days post-treatment. Scale bar: 200 μm.

DNA DSB in 3D Co-culture Spheroids

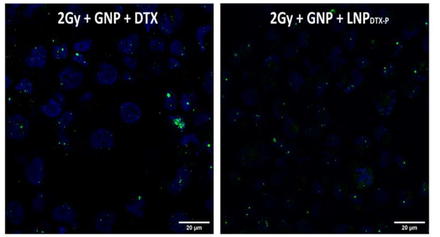

Figure 9. Confocal microscopy images of repair protein 53BP1 in the nucleus of co-culture of MIA PaCa-2 and CAF-98. Scale bar: 20 μm. The cell nuclei are stained blue, while the green dots indicate DNA DSB damage.

Cell Cycle in 3D Co-culture Spheroids

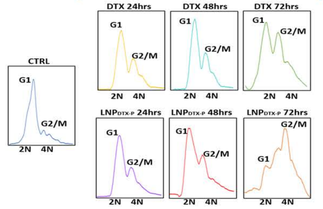

Figure 7. Cell cycle data of co-co-culture spheroids cells using DTX and LNP$_{DTX-P}$ over 3 days.

GNPs Content in 3D Co-culture Spheroids

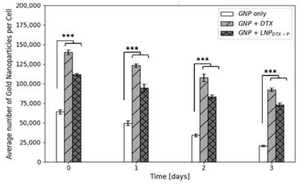

Figure 10. GNP amount per cell as measured using ICP-MS. *** indicates $p < 0.001$.

CONCLUSIONS

- Both RT/LNP$_{DTX-P}$ and RT/GNPs on their own showed enhancements over traditional RT, positioning them as potential additions to the existing RT protocol..
- While DTX demonstrated a better therapeutic outcome than LNP$_{DTX-P}$ *in vitro*, we anticipate that the effectiveness of LNPs will be more pronounced in an *in vivo.*
- The combination of GNPs and LNP$_{DTX-P}$ with RT showed a superior collaborative effect due to their radiosensitizing properties improving the therapeutic efficacy of each modality alone.