Functional Personalized Complex Combination Nano Therapy for Osteosarcoma

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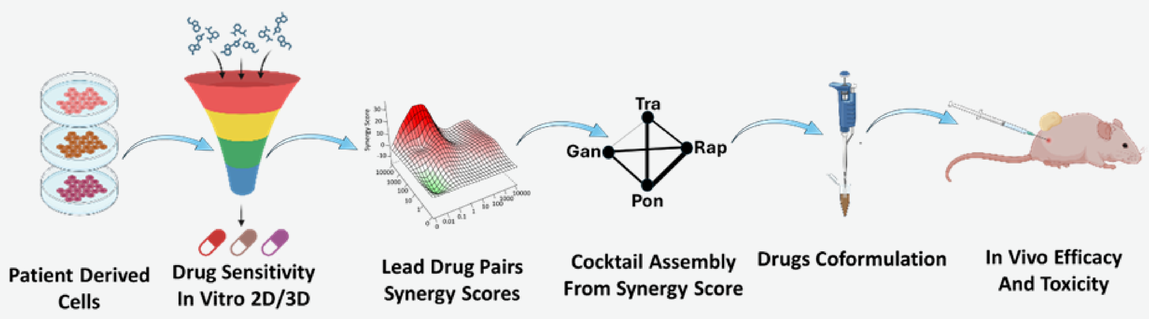
INTRODUCTION

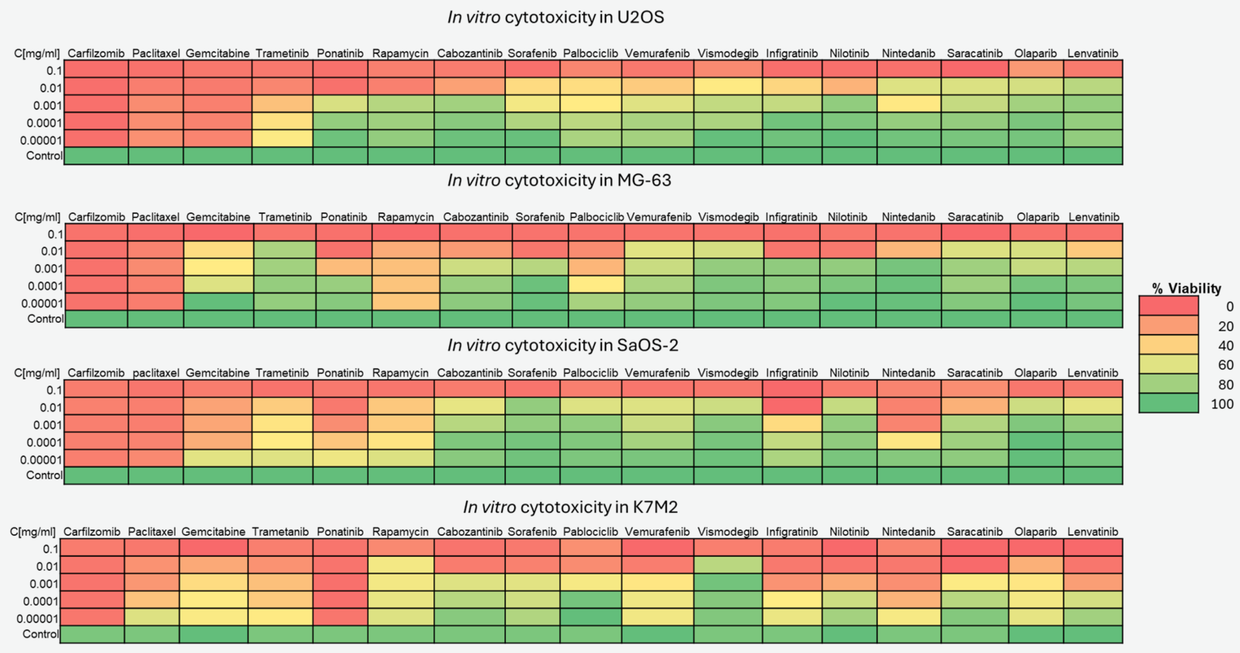
Osteosarcoma (OS) is the most common primary bone cancer, affecting predominantly teenagers and young adults. Despite advances in surgery, radiation, and chemotherapy, treatment outcomes have remained stagnant over the past 30 years, with 30-50% of patients experiencing recurrence within 2-3 years post-treatment. Current therapies are non-personalized, resulting in significant toxicity and limited long-term efficacy. Moreover, metastatic disease, particularly in the lungs, remains a leading cause of mortality in OS patients.

In this study, we aimed to overcome these limitations by developing a personalized nanomedicine approach. Our strategy leverages functional drug combinations encapsulated in Polydopamine-coated nanoparticles (NPs) for targeted delivery, aiming to enhance anti-tumor efficacy, reduce toxicity, and overcome drug resistance. By integrating both *in vitro* and *in vivo* models, we demonstrate the potential of personalized nanomedicine to transform the treatment landscape of osteosarcoma.



RESULTS

In vitro



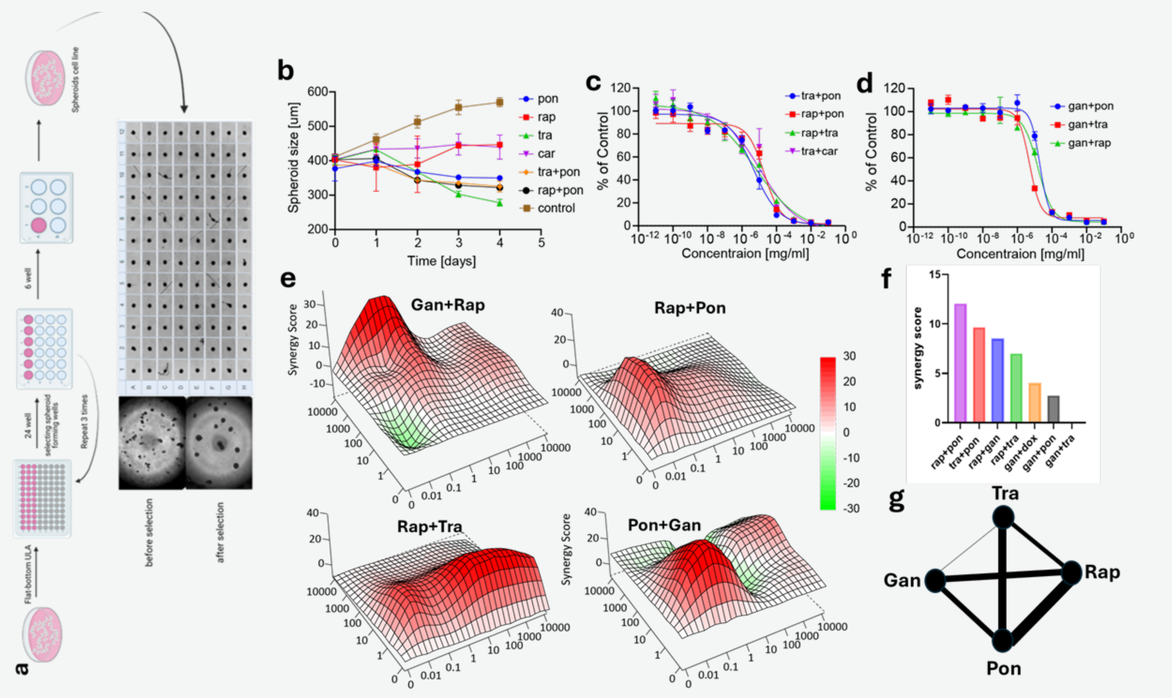


Figure 1: Free Drug Screening on Osteosarcoma Cell Lines MTT viability assay of 17 drugs at 6 concentrations applied on: a. U2OS b. MG-63 c. SaOS-2 and d. K7M2 cell lines, incubated for 72hr.

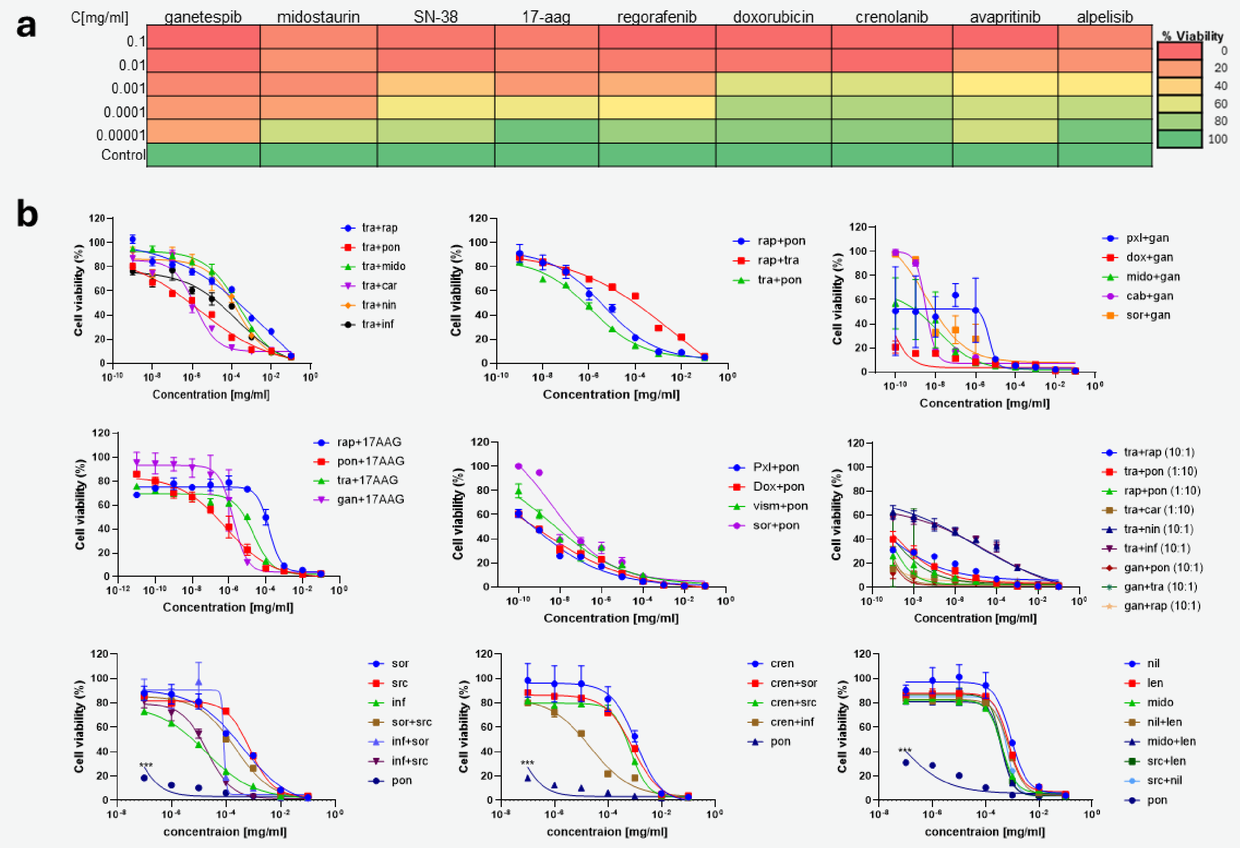
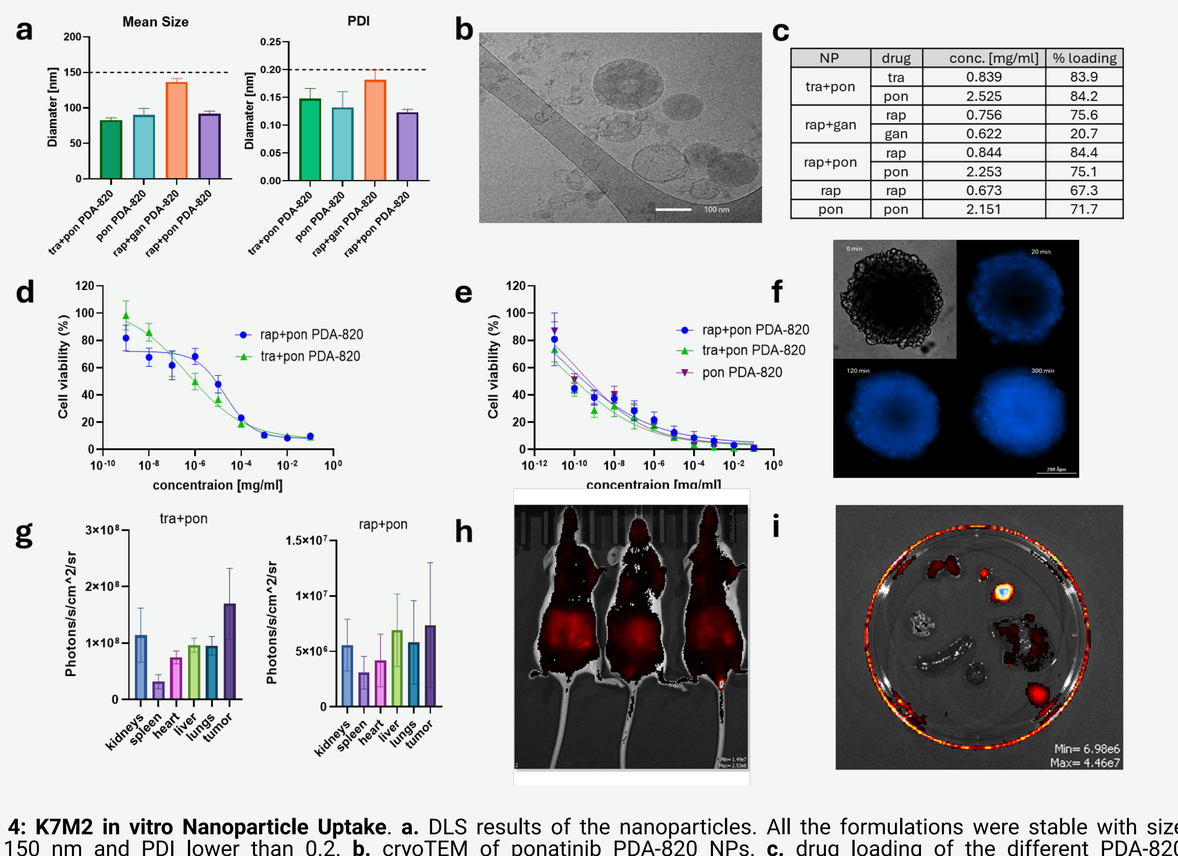


Figure 2: K7M2 *in vitro* 2D Drug Screening. a. CTG viability assay of kinase inhibitors at 6 concentrations, incubated for 72hr. **b.** CTG viability assay of single and combinations of kinase inhibitors. n=2. ***$p < 0.001$ by unpaired *t*-test. Error bars indicate mean ± SD



NP	drug	conc. [mg/ml]	% loading
tra+pon	tra	0.839	83.9
	pon	2.525	84.2
rap+gan	rap	0.756	75.6
	gan	0.622	20.7
rap+pon	rap	0.844	84.4
	pon	2.253	75.1
rap	rap	0.673	67.3
pon	pon	2.151	71.7

Figure 3: K7M2 in vitro 3D Spheroids. a. Creation of spheroid cell lines. **b.** Anti-tumor efficacy as measured with diameter change over time after incubation with 1×10^{-4} mg/ml of different kinase inhibitors. **c.** CTG viability assay of ponatinib and trametinib drug combinations. **d.** CTG viability assay of ganetespib drug combinations. **e.** ZIP synergy maps of leading combinations. **f.** Synergy score of drug combinations. **g.** Network representation of the 4 main drugs based on their respective synergy score. n=2. Error bars indicate mean ± SD

Figure 4: K7M2 in vitro Nanoparticle Uptake. **a.** DLS results of the nanoparticles. All the formulations were stable with size under 150 nm and PDI lower than 0.2. **b.** cryoTEM of ponatinib PDA-820 NPs. **c.** drug loading of the different PDA-820 nanoparticles. **d.** Cell viability assay in 2D, nanoparticles formulations using PDA-820 **e.** Cell viability assay in 3D, nanoparticles formulations using PDA-820 **f.** Time-dependent ponatinib nanoparticles uptake into a spheroid at various time points. Images taken using Lionheart microscope. Error bars indicate mean ± SD. g. Biodistribution experiment of trametinib+ponatinib and rapamycin+ponatinib nanoparticles 24h after IP injections to K7M2 tumor xenografts model as measured with IVIS (λ_{ex} = 745 nm, λ_{em} = 840). h. biodistribution whole body at 0hr, i. biodistribution example of organs on a plate 24hr after injection

Complex Combinations

Figure 5: K7M2 complex combinations in vivo studies. a. High complexity combination therapy treatment plan **b.** *in vivo* efficacy measured by % of tumor volume from day of randomization. ****$p < 0.0001$ by unpaired *t*-test. **c.** Survival curve of mice in subcutaneous xenografts model of K7M2 cells, p=0.0006 according to Mantel-Cox test analysis.**d.** Histologic examination of tissue samples. Hematoxylin and eosin (H&E) staining, original magnification X 20.Representative images of H&E staining in different tissues of mice treated tri-weekly with nanoparticles compared with standard of care and non treated. Error bars indicate mean ± SD

Data Driven vs Hypothesis Driven

Drug	osteosarcoma	U2OS	MG63	SaOS	K7M2
cabozantinib	46	2	1	0	0
carfilzomib	7	2	1	0	0
gemcitabine	191	19	14	2	2
lenvatinib	29	1	1	0	0
nilotinib	3	2	2	0	0
nintedanib	2	1	0	0	0
olaparib	32	133	2	0	2
paclitaxel	211	50	15	1	0
palbociclib	26	9	1	0	0
ponatinib	6	4	2	0	3
rapamycin	184	80	20	0	5
saracatinib	9	1	0	0	1
sorafenib	139	7	10	0	0
trametinib	10	4	2	0	0
vemurafenib	1	0	0	0	0
vismodegib	9	1	0	0	0

Figure 6: SPIKE search results of published data of the drugs and osteosarcoma cell lines

Figure 7: further drug efficacy and drug combinations efficacy on K7M2 cell line compared to the experimental data.

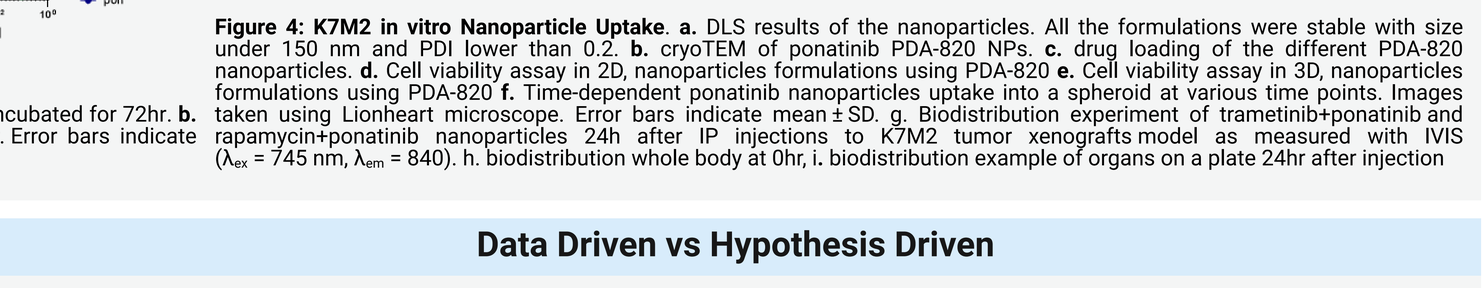


Figure 8: Predictability evaluation of drug efficacy using state of the art LLMs Published data of drug efficacity vs. LLMs predictions in a. KRAS driven cancers b. osteosarcoma cell lines. average performance for the 3 AI models on all cell line KRAS mutation cell lines and Osteosarcoma cell lines.

CONCLUSIONS

We successfully developed a personalized nanomedicine platform for OS, combining potent drugs into polydopamine-coated NPs. Four key drugs—Ponatinib, Trametinib, Rapamycin, and Ganetespib—were identified as highly effective in both 2D and 3D models. Drug combinations exhibited additive and synergistic effects, providing enhanced therapeutic potential.

Our nanoparticle formulations were stable, with high drug loading efficiency (>80%) and sizes under 150 nm.
In vivo safety studies confirmed that the polydopamine NPs were non-toxic, while efficacy studies demonstrated that alternating drug regimens outperformed concurrent administration and standard-of-care treatments, leading to improved tumor suppression.

This study highlights the promise of personalized drug delivery systems to significantly improve treatment outcomes in osteosarcoma, offering a pathway toward more effective, less toxic cancer therapies.

REFERENCES

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