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Translational Nanomedicines for the Treatment of Triple Negative Breast Cancer

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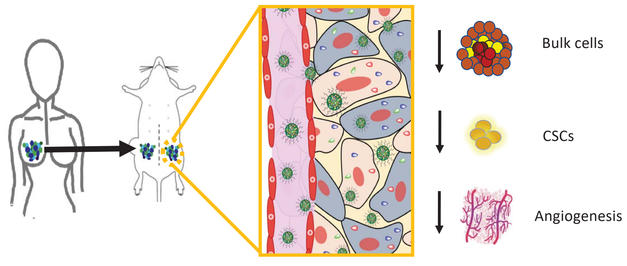
Introduction

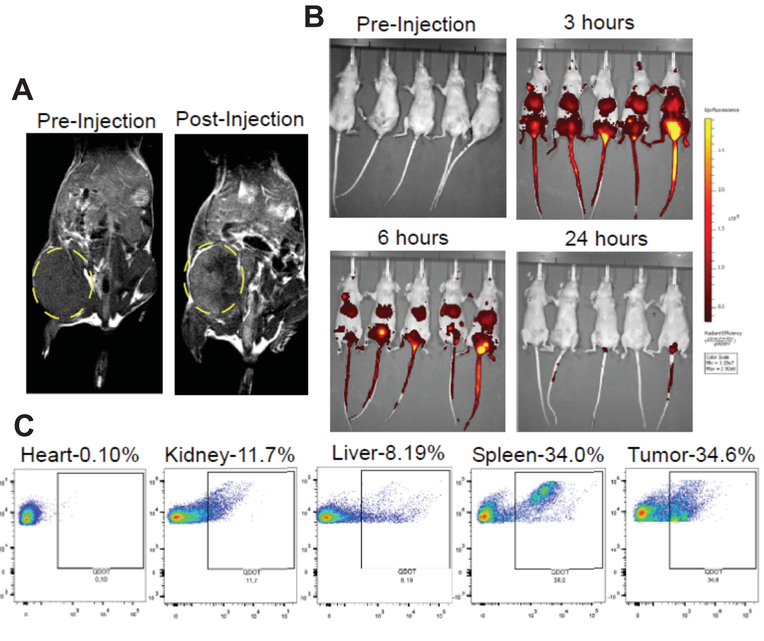
- Triple negative breast cancer (TNBC) is an aggressive subtype accounting for the majority of breast cancer related deaths.
- Due to the lack of targets in TNBC, chemotherapeutics, such as, paclitaxel, are the mainstay of treatment but have toxic side effects & contribute to the enrichment of a subpopulation of cells with tumor initiating capacity & stem like characteristics called cancer stem cells (CSCs).
- Both the Wnt & YAP pathways are implicated in tumorigenesis and CSC plasticity.
- Cancer nanomedicine has attractive features: longer blood circulation half-lives, tumor accumulation, and reduced off-target toxicity
- Nanomedicines can deliver multiple therapeutic agents to tumors in synergistic ratios
- Patient-derived xenograft (PDX) are clinically relevant animal models as they retain the heterogeneity and 3D architecture of patient tumors
- The use of PDX models could revolutionize cancer nanomedicine increasing the accuracy of preclinical research

Objectives & hypothesis

- Nanoparticles (NP) *In vivo* characterization in PDX models
- Develop NPs containing dual- and triple drug/inhibitors for the treatments of TNBC
- Determine the *in vivo* effects of drug/inhibitor-NPs on TNBC viability, CSC enrichment and tumorigenesis.



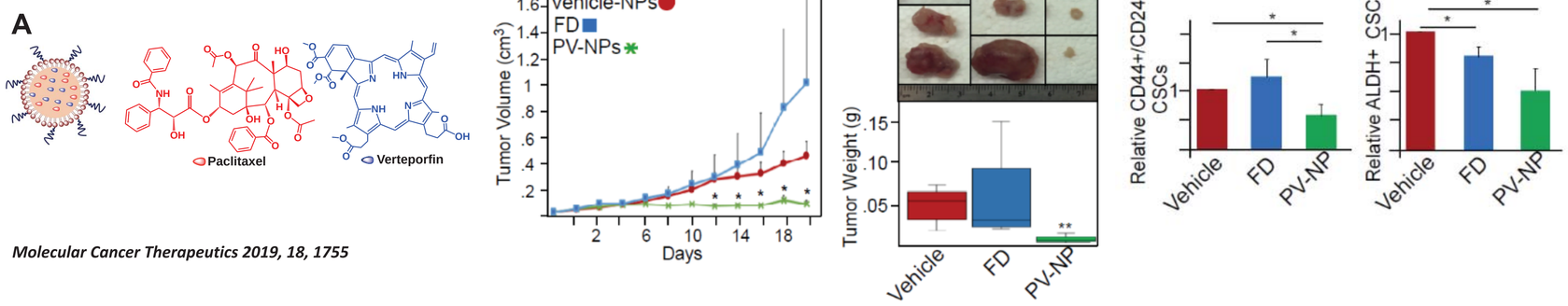
NPs accumulation PDX tumors



***In vivo* NP bio-distribution and accumulation within TNBC PDX tumors: (A)** T1-weighted MRI of PDX tumors before & 8 mins after injection of Gadovist in the same mouse. **(B)** IVIS analyses of PDX tumors (lower abdomen area) before & after injection of NPs labelled with Alexa750 at indicated time periods using excitation laser of 745 nm & emission filter of 800. **(C)** Flow cytometric analyses of cellular uptake of NPs labelled with Qdot 800 in the dissociated organs vs PDX tumors 3 hrs after tail vein injection. Flow cytometry analysis was performed using a 405 nm excitation laser and a 780/60nm filter to determine cellular uptake.

Results

Nanotherapeutic strategy 1



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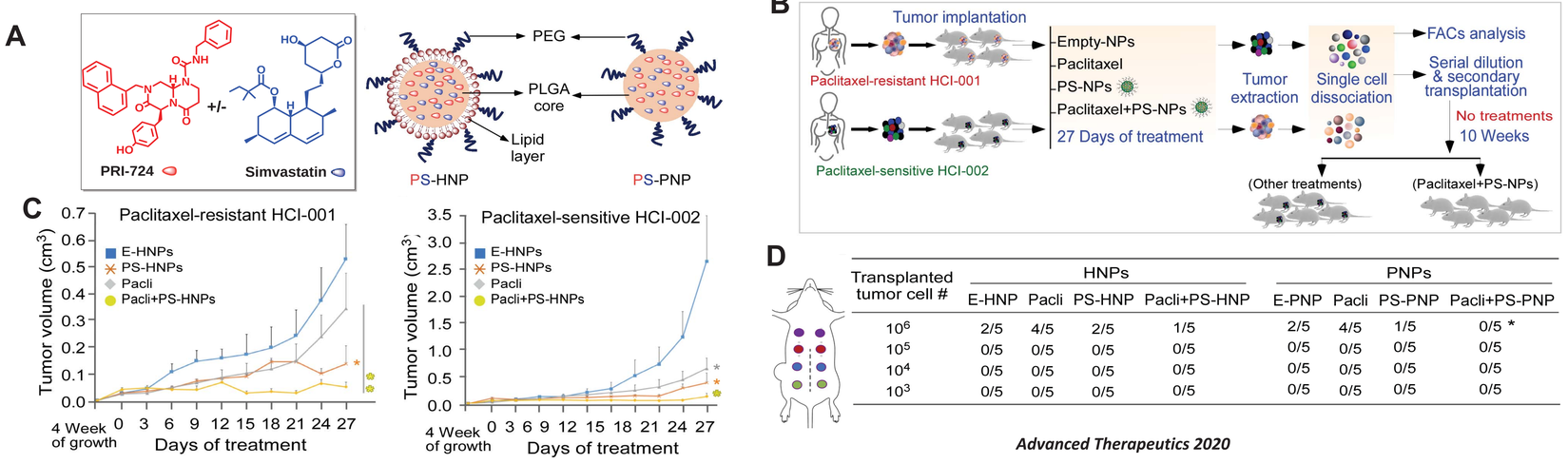
Paclitaxel and verteporfin co-loaded NPs treatment of TNBC PDX tumors: (A) Schematic diagram of PV-NPs, and drug chemical structures. (B) TNBC PDX tumors were surgically engrafted into the mammary fat pads of athymic nude mice and treated with empty NPs (Vehicle-NPs), free drugs (FD, 1 mg/kg of paclitaxel and 9 mg/kg V), or PV-NP (0.5 mg/kg of paclitaxel and 3.2 mg/kg verteporfin coloaded NPs) every other day for 20 days. Tumor volumes were determined every 2-day. (C) Representative tumor photos after treatments and tumor weights means. (D) Flow cytometric analysis of CD44+/CD24- and ALDH+ CSCs within TNBC PDX tumor after completion of *in vivo* treatment. Data represent means +/- SD; n = 5 for mice and n = 3 for flow cytometric analysis; * $p < 0.05$, ** $p < 0.01$.

Nanotherapeutic strategy 2



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Transplanted tumor cell #	HNPs				PNPs			
	E-HNP	Pacli	PS-HNP	Pacli+PS-HNP	E-PNP	Pacli	PS-PNP	Pacli+PS-PNP
10^6	2/5	4/5	2/5	1/5	2/5	4/5	1/5	0/5 *
10^5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10^4	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10^3	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

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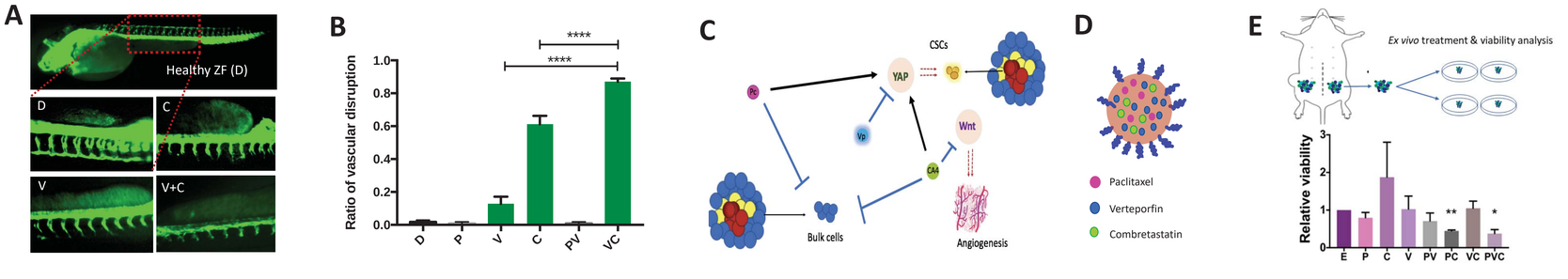
Treatment of TNBC PDX tumors with Wnt & YAP inhibitor-NPs in combination with paclitaxel: (A) Chemical structures of PRI-724 (P) and simvastatin (S) and schematic diagrams of PS-HNPs and PS-PNPs. **(B)** Study design **(C)** TNBC PDX HCI-001 and HCI-002 tumors were surgically implanted into mammary fat pads of NOD-SCID mice. When the tumors reached a diameter of 3mm, mice were injected with paclitaxel (Pacli, 10mg/kg, once a week, intraperitoneal injection), or every 3 days with empty NPs (E-HNP), NPs encapsulating 9mg/kg simvastatin & 1mg/kg PRI-724 (PS-HNP, tail vein injection) or paclitaxel & PS-HNP in combination (P+PS-HNP) for 27 days (n = 4 mice for each group). **(D)** Single cell suspension from HCI-002 PDX tumors were re-transplanted into the mammary fat pads of new nude mice in serial dilutions (10^6, 10^5, 10^4, or 10^3 cells per injection, n = 5 for each group) and tumor formation was observed for 10 weeks.

Nanotherapeutic strategy 3

Cell Death and Disease (in revision)



Development of a triple-drug nanotherapy targeting bulk cells, CSCs and angiogenesis: Using zebrafish (ZF) as model for angiogenesis, we tested the effect of the three drug; Paclitaxel (P), Combretastatin (C) and Verteporfin (V) treatment on vascular in Tg(fli:EGFP) zebrafish embryos at 56 hpf **(A)** ZF embryos treated at 8 hrs post fertilization. **(B)** Graphical representation of all treatments **(C)** triple drug treatment rationale **(D)** Schematic representation of a 3 in 1 NP system containing P, C, and V at a 1:1:2 ratio **(E)** Ex vivo viability analysis of PDX organotypic slice culture after 120- hour treatment with different NPs

Conclusion

- *In vivo* TNBC PDX studies demonstrated that the efficacy of NP-delivered P and V for inhibiting tumor growth and preventing CSCs enrichment
- Chemotherapeutic efficacy can be significantly improved by NPs containing Wnt and YAP inhibitors irrespective of tumor drug resistance.
- The development of a triple drug combination nanotherapy could co-target angiogenesis, bulk cells and CSCs in clinically translatable models

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