Polymer Nanoparticles for Secreted TRAIL Therapy

Statement of Purpose Despite initial promise, TRAIL-based cancer therapeutics have yet to yield a clinically approved drug, due to delivery challenges, a lack of potency, and unacceptable off-target toxicity. To address these challenges, we have developed polymer-based nanoparticles carrying an engineered gene for a secretable TRAIL protein, which enables reprogramming of liver cancer cells into TRAIL-secreting factories.

Methods To form nanoparticles (NPs), plasmid DNA was mixed with PBAE 536 polymer **(Fig 1A)** at a 25 polymer to DNA weight ratio, and particles were allowed to form for 10 minutes. NPs were incubated with cells for 2 hours, then TRAIL sensitizers were added when necessary. Transfection efficacy was quantified using flow cytometry, secreted TRAIL protein content was measured by ELISA, and cell viability was quantified by MTS assay 48 hours post-transfection. For *in vivo* studies, NPs were injected directly into the tumor, and transfection was monitored after 6 hours using bioluminescence imaging.

Results PBAE 536 and plasmid DNA self-assemble into NPs that are approximately 150 nm in diameter as measured by DLS, with no significant change in size with different cargo plasmids**(Fig 1B)**. The NPs enable efficient and preferential delivery of eGFP DNA to HepG2 human hepatocellular carcinoma cells, with 53% transfection compared to 12% in healthy human hepatocytes and a normalized geometric mean fluorescence of 18 compared to 3.2 in hepatocytes **(Fig 1C)**. *In vivo*, PBAE 536 NPs facilitate high levels of transfection in xenograft HepG2 tumors **(Fig 1D)**.

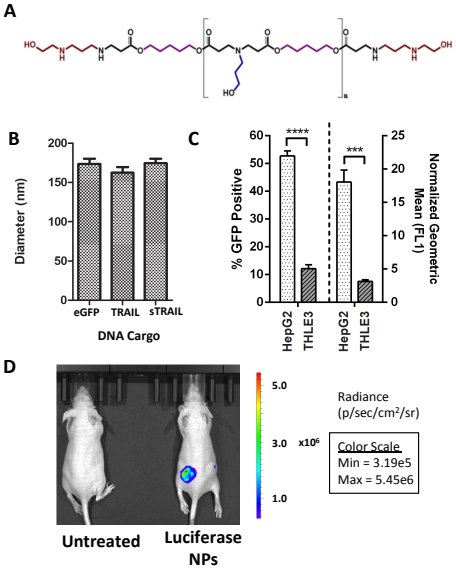

Figure 1 *PBAE 536 NP Characterization* (A) PBAE 536 (B) PBAE 536 NP sizes measured by DLS (C) eGFP NP transfection efficacy in HepG2 liver cancer cells and THLE3 hepatocytes (D) Luciferase NP transfection efficacy in xenograft tumors

To harness this DNA delivery system for an anticancer therapy, a secretable TRAIL (sTRAIL) gene was engineered by combining a secretion signal, isoleucine zipper, and TRAIL apoptosis inducing sequence. The resulting gene was inserted into a pN3 backbone under the control of a CMV promoter and the plasmid was combined with PBAE 536 to form sTRAIL NPs. Successful secretion was confirmed by transfecting HepG2 cells with sTRAIL NPs or non-secretable TRAIL NPs and quantifying TRAIL protein content in the media after 48 hours **(Fig 2A)**.

HepG2 cells were relatively insensitive to TRAIL transfection alone, but combination treatment with TRAIL-sensitizing drugs vorinostat, sodium butyrate, MS-275, and temozolomide (TMZ) increased the sensitivity to TRAIL therapy and enabled a potent cytotoxic effect. Further, sTRAIL NP therapy generally had an enhanced therapeutic effect over non-secretable TRAIL when used in combination with these sensitizers **(Fig 2B)**. TRAIL transfection did not significantly decrease the viability of healthy human hepatocytes, and cancer specificity was maintained when sensitizers were added **(Fig 2C).**

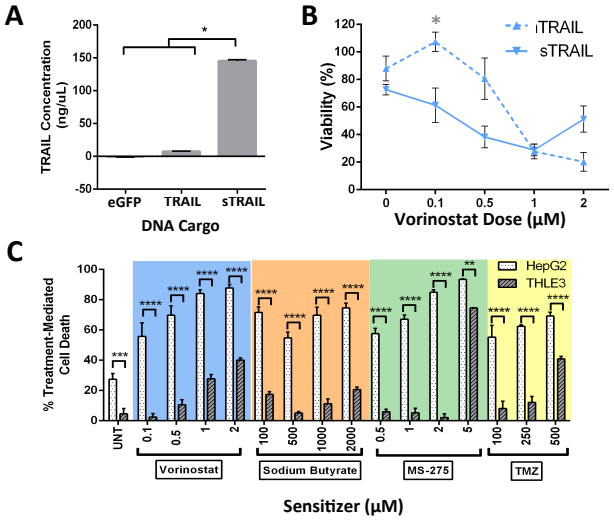

Figure 2 *Therapeutic gene delivery using PBAE 536 NPs* (A) TRAIL secretion by NP transfected HepG2 cells (B) HepG2 viability after vorinostat sensitization and transfection with secretable or non-secretable TRAIL NPs (C) Treatment-mediated cell death in sTRAIL-transfected HepG2s and healthy hepatocytes (THLE3)

Conclusions Therapeutic sTRAIL NPs demonstrate a high degree of cell killing in hepatocellular carcinoma cells when used in conjunction with small molecule sensitizers. The resulting therapeutic retains cancer-specificity and has minimal off–target toxicity to healthy hepatocytes. Therefore, by combining a cancer-specific gene delivery NP with a potent and cancer-specific therapeutic gene, this strategy addresses many of the hurdles associated with TRAIL therapy and offers a new potential approach to liver cancer treatment.