# Particle Encapsulation of a Prostate-Targeted Biologic for the Treatment of Liver Metastases in a Preclinical Model of Castration-Resistant Prostate Cancer

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**Background:** The liver is a common site of prostate cancer (PCa) metastasis, particularly in advanced castration-resistant disease. This is associated with a worse median overall survival compared to men with disseminated disease in other tissue sites. These facts document that secondary liver metastasis is a significant health burden in need of novel targeted therapeutic approaches.

Pharmacokinetics and biodistribution of systemically-infused particles are greatly influenced by their chemical and physical properties. Indeed, certain types of particles have been shown to selectively accumulate in the liver due to increased phagocytic uptake. This suggests that poly-lactic-co-glycolic acid (PLGA) particles may be ideal vectors for targeting liver disease.

We have previously engineered a highly potent mutant bacterial pore-forming protoxin for selective activation by prostate specific antigen (PSA). Though currently being developed clinically as a local therapy for benign prostatic hyperplasia and localized PCa, this protoxin cannot be administered systemically as a treatment for metastatic disease due to its mechanism of action, which leads to poor accumulation within the tumor microenvironment. To overcome this limitation, PLGA particles encapsulating the protoxin were developed.

**Methods:** A highly sensitive sandwich ELISA to quantify protoxin release was developed. Hemolysis and MTT assays performed to demonstrate functional pore formation and PSA-dependent toxicity of the released protoxin *in vitro*. *In vivo* efficacy demonstrated using a model of multifocal castration-resistant liver disease.

**Results:** Protoxin release from different particle formulations was quantified over 10 days. Hemolysis assays documented PSA-dependent pore formation and lytic potential of the released protoxin. Conditioned supernatant from protoxin-loaded but not blank (i.e. unloaded) PLGA particles was highly cytotoxic to PC3 and DU145 in the presence of exogenous PSA. Particle encapsulation increased the therapeutic index of the protoxin *in vivo*, and anti-tumor efficacy was demonstrated following a *single IV dose* of protoxin-loaded particles in a preclinical model of PCa liver metastasis with no obvious toxicity.

**Conclusion:** These results document robust methods to accurately quantify the release and function of a mutant PSA-activated protoxin from PLGA particles and *in vivo* efficacy in a clinically-relevant preclinical model of metastatic PCa, which are essential for future studies aimed at optimizing a systemic delivery strategy for this protoxin.

**Conflicts of Interest:** The authors declare no potential conflicts of interest exist.

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