Molecular Farming and Engineering of a Filamentous Platform Technology to Deliver Therapies to Breast Cancer

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Statement of Purpose: The nanometer scale capsids from (plant) viruses are currently being explored as carrier systems to delivery therapies or contrast agents to specific cells and tissues. Virus-based nanoparticles (VNPs) have symmetrical structures that are amenable to chemical and genetic engineering, and can be produced in high yields in plants. VNPs from plants are biocompatible, biodegradable, and non-infectious in mammals [1]. *Potato virus X* (PVX) is a flexible rod-shaped virus, measuring 515 x 13 nm and is comprised by 1270 identical coat proteins, each containing a solvent-exposed lysine residue available for chemical modification [2]. Recent data from our lab indicate that PVX efficiently homes to solid tumors based on the enhanced permeability and retention effect [3]. In this project, tissue-targeted PVX filaments are being developed using combination of genetic and chemical modification with i) targeting ligands specific for receptors overexpressed in breast cancer, ii) polyethylene glycol (PEG) chains to reduce nonspecific cellular uptake and improve pharmacokinetics, and iii) AlexaFluor647 for preclinical imaging. In this presentation, we will focus on the chemical modification of PVX with PEG and A647 and the evaluation of cell interactions, pharmacokinetics, and tumor homing.

Methods: PVX was produced and extracted from *Nicotiana benthamiana* plants. PEG2000 (P2), PEG5000-linear (P5L), PEG5000-branched (P5B), or PEG20k (P20) (Nanocs), and AlexaFluor647 (A647) (Invitrogen) were conjugated to solvent-exposed lysine groups of PVX using *N*-hydroxysuccinimide chemistry (Figure 1a). PVX-PEG-A647 particles were purified and characterized using SDS-PAGE, UV/Visible spectroscopy, and TEM. *In vitro* cellular uptake data were measured for two cancer cell lines and an endothelial cell line. Cell uptake was studied using flow cytometry and confocal microscopy. *In vivo* pharmacokinetic studies were evaluated in healthy Balb/c mice. Tumor homing studies were performed in female Balb/c mice with 4T1 orthotopic tumor xenografts and analyzed *ex vivo* using the Maestro imaging system. Biodistribution and tumor homing was studied over a 5-day time course.

Results: SDS-PAGE analysis and UV/vis spectroscopy indicated that approximately 300 dyes and 300 PEGs were attached to each PVX particle. TEM confirmed the structural integrity of PVX-A647-PEG. Flow cytometry data showed similar trends across various cell lines, indicating that the addition of P2 or P5L decreased cellular uptake, when compared to non-PEGylated PVX. There was no significant difference in cellular uptake between PVX-A647-P2 and PVX-A647-P5L particles (Figure 1b). Preliminary pharmacokinetic studies indicate that the addition of P5B or P20 increases circulation time of PVX-A647-PEG particles, when compared to the addition of P5L. This indicates that both molecular weight and conformation of the PEG chains impact the stealth effect. Tumor homing studies are currently being carried out using Balb/c mice with 4T1 orthotopic tumor xenografts. The specific question to be addressed is whether increased shielding and resulting circulation correlate with enhanced tumor homing and penetration into the tumor tissue.

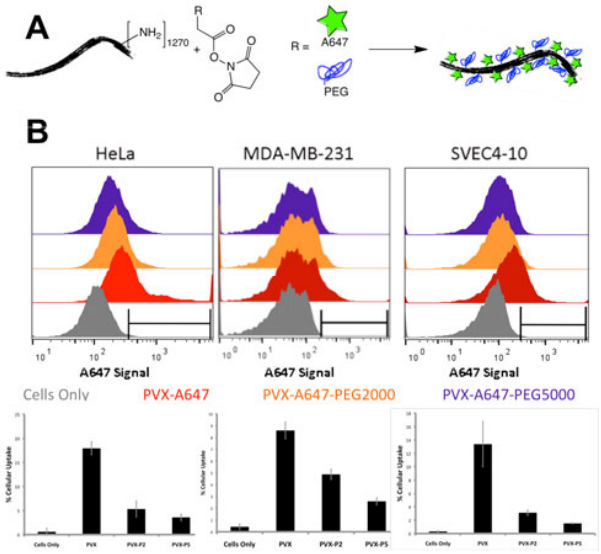

Figure 1: Conjugation and cell uptake results of PVX-PEG-A647. (A) *N*-hydroxysuccinimide reaction to attach AlexaFluor647 (green) and PEG (blue). (B) Flow cytometry in HeLa (left), MDA-MB-231 (middle), and SVEC4-10 (right) cells treated with PVX-A647-PEG.

Conclusions: We have shown that non-specific cell binding and internalization can be blocked through the addition of PEG. PEGylation also improves pharmacokinetics. Data indicate that higher molecular weight or branched PEGs increase circulation times further. In ongoing studies, we are evaluating tumor homing and biodistribution profiles. Further directions are to include targeting ligands into the design to achieve tissue-specificity. Once the design principles have been established we will delivers cargos such as chemotherapies or magnetic resonance contrast agents.

References: [1] Steinmetz NF *Nanomedicine: NBM* 2010; 6: 634-641. [2] Steinmetz NF et al *NanoLetters* 2010; 10: 305-312. [3] Shukla S et al *Molecular Pharmaceutics* 2012; DOI 10.1021/mp300240m

Acknowledgements: This work was funded by grants from NIH/NIBIB: R00 EB009105 (to NFS) and P30 EB011317 (to NFS) and from NIH/NCI: R25 CA148052 (to KLL) and P20 CA043703 (to NFS and RAK).