Affinity of Plant Viral Nanoparticle Potato Virus X (PVX) Towards Malignant B cells Enables Cancer Drug Delivery

Sourabh Shukla^{1#}, Anne Jessica Roe^{2#}, Ruifu Liu², Frank A. Veliz³, Ulrich Commandeur⁴, David N. Wald^{2*} and Nicole F. Steinmetz^{1,5,6,7,8*}.

Departments of ¹NanoEngineering, ⁵Bioengineering, ⁶Radiology, ⁷Moores Cancer Center, ⁸Center for Nano-ImmunoEngineering, University of California, San Diego, La Jolla, California, 92093, United States; Departments of ²Pathology, ³Biomedical Engineering, Case Western Reserve University, Cleveland, Ohio 44106, United States; ⁴ Department of Molecular Biotechnology, RWTH-Aachen University, Aachen 52064, Germany.

*Correspondence: <u>nsteinmetz@ucsd.edu</u>, <u>dnw@case.edu</u> # <u>These authors contributed equally</u>



Figure S1. SDS gel electrophoresis shows conjugation of Cy5 fluorescent dye to the PVX coat protein.



Figure S2. Biodistribution of PVX-Cy5 in Raji⁻ and Raji⁺ male and female NSG mice. Statistical analysis was performed between tissues from Raji⁻ and Raji⁺ mice were performed using student t test (* p<0.05; ** p<0.01).



Kidney from healthy NSG mice injected with PVX-Lys-Cy5

Ovaries from healthy NSG mice injected with PVX-Lys-Cy5

Figure S3: Confocal microscopy of kidney (**A**) and ovary sections (**B**) from healthy NSG mice (Raji⁻ mice) injected with PVX-Lys-Cy5 shows no PVX accumulation in these tissues. Tissue sections were stained for CD45⁺ Raji cells and with DAPI nuclei staining (blue).



Figure S4: **A)** Fluorescent PVX particles were also prepared using maleimide chemistry targeting the cysteine residues on PVX coat protein. **B)** PVX-Cys-Cy5 particles were characterized using FPLC. **C)** Cell binding of PVX-Cys-Cy5 was evaluated with flow cytometry; statistical analysis was performed using Ordinary one-way ANOVA (Tukey's multiple comparison tests; **** p<0.0001).



Figure S5: Dynamic light scattering analyses showed comparable sized PVX and PVX-vcMMAE particles.



Figure S6: Efficacy of PVX-vcMMAE treatment compared with soluble MMAE in Raji B cell lymphoma bearing mice (n=5). Mice were engrafted with 1x10⁶ Raji-Luc B lymphoma cells and treated three times at four-day intervals with PBS, PVX, MMAE and PVX-vcMMAE, starting at day 11 post-tumor challenges. Survival curves were analyzed using the Log-Rank Mantel-Cox test using GraphPad Prism8 software.