Therapeutic Peptide Amphiphile Micelles as a Novel Leukemia Treatment

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Statement of Purpose: Leukemia is a cancer of the lymphocytes (*i.e.*, B and T cells), for which the two most common treatment options are systemic chemotherapy and radiotherapy. To overcome the significant deleterious side-effects associated with these approaches, peptides as an exciting alternative as they are biocompatible and can easily traffic in blood or lymphatic fluid, making them excellent candidates to treat leukemia. Previous work has been shown that the 'Plenty of SH3s' (POSH) scaffold protein plays a critical role in life and death choices in lymphocytes (Cunningham et al., 2013), for which disruption with a biomimetic POSH-derived peptide, Tat-POSH, can significantly reduce the survival of lymphocytic malignancies *in vitro* (Smith *et al.*, 2020). Some drawbacks associated with peptide therapeutics are their short circulatory half-life and minimal cellular penetration. Previous work has shown that utilization of peptide amphiphile micelles (PAMs) can improve peptidyl stability and increase cellular association (Prencipe et al., 2021).

Method: Peptide amphiphiles (PAs) may be created by conjugating a dipalmitoyllysine moiety ($Palm_2K$) to the N-terminus of the POSH(3.3A)-Tat peptide. which can then self-assemble into micelles (*i.e.*, PAMs) in water. We hypothesize that POSH(3.3A)-Tat PAMs will have higher *in vitro* cytotoxicity, relative to peptide controls, in human leukemia cell lines. To test our hypothesis, we first synthesized POSH(3.3A)-Tat peptides and PAs by solid-phase peptide synthesis, acid-cleaved them from resin, and purified products via liquid-chromatography controlled mass-spectroscopy. To confirm PA micellization, the critical micelle concentration (CMC) was determined by fluorescent sequestration of 1,6-diphenyl-1,3,5-hexatriene. Micelle structure was observed by transmission electron microscopy (TEM) and physicochemical properties, such as surface charge and particle size, were determined by Zeta potential and dynamic light scattering, respectively.

To assess the therapeutic potential of POSH(3.3A)-Tat, a broad variety of leukemia were employed including NALM-6 (B cell acute lymphoblastic leukemia - B-ALL), Mec1 (B cell chronic lymphocytic leukemia – B-CLL), Jurkat (T cell acute lymphoblastic leukemia – T-ALL), and MOLT-4 (T cell acute lymphoblastic leukemia – T-ALL) which were compared to healthy THP-1 cells (monocytes). Cells were incubated for 24 and 48 hours with PAMs and peptides at various concentrations with cytotoxicity measured by live-dead staining with 7-aminoactinomycin D (7-AAD) and flow cytometry.

Results: The percent cell viability of NALM-6 cells was plotted using the flow cytometry data (**Figure 1**). We have found that PAMs are exponentially more toxic than peptides with increasing concentrations *in vitro*.

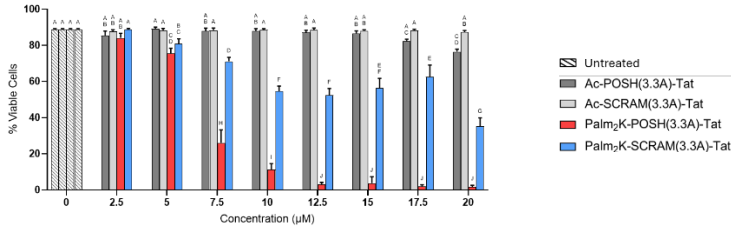

Figure 1. NALM-6 (B-ALL) viability at 24 Hour Incubation

Conclusions: Consistent with previous research done in our group, POSH(3.3A)-Tat PAMs have been shown to have cytotoxic effects on a variety of leukemia cell lines as well as healthy cells *in vitro*. Future work for this project includes extending the therapeutic window assessment for less sensitive cell lines as well as confocal microscopy imaging to observe the interactions between the PAMs and leukemia cell lines.

References: (Cunningham, CA. Eu J Im. 2013;43:3361-3371.)
(Prencipe, F. Mol. 2021;26:4049.)
(Smith, JD. Mol Sys Des & Eng. 2020;5:269-283.)