Antibody-targeting of Drug-loaded Micelles to Acute Myeloid Leukemia Cells

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Statement of Purpose: Although 70% of patients diagnosed with acute myeloid leukemia (AML) achieve complete remission, the associated five-year survivability is under 25% (1). The long-term failure of current AML therapies is largely due to the inability to eliminate a rare subset of AML cells that are responsible for disease recurrence. These cells, known as leukemia stem cells (LSCs) have many properties similar to normal hematopoietic stem cells, including cell cycle quiescence, which makes them an elusive target for conventional chemotherapeutics such as anthracyclines and nucleoside analogs (2). Parthenolide (PTL) is a naturally occurring small molecule that is known to specifically eliminate leukemia cells and LSCs *in vitro* via nuclear factor-kappa B (NF-κB) inhibition. Unfortunately, poor bioavailability has limited the efficacy of PTL *in vivo (3, 4)*. To overcome this obstacle, we have developed an LSC-specific polymer micelle delivery system to achieve targeted therapeutic delivery of PTL to LSCs. Briefly, amphiphilic diblock copolymers of poly(styrene-*alt*-maleic anhydride)-b-poly(styrene) (PSMA-b-PS) self-assemble into highly stable micelle nanoparticles with diameters of ~20 nm as shown in Fig. 1B. Additionally, these micelles have demonstrated robust PTL loading and controlled PTL release in PBS (5).

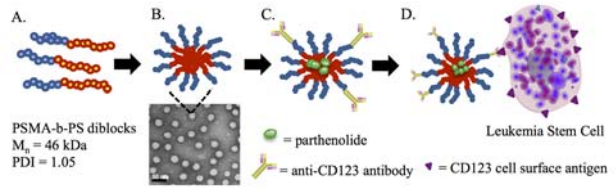

Figure 1. Antibody-targeted PTL delivery system. (A) PSMA-b-PS diblock copolymers. (B) Self-assembled micelle NPs (scale bar = 50 μm). (C) Antibody labeled PTL-loaded micelles. (D) LSC-specific delivery of PTL via CD123 targeting.

PTL-loaded PSMA-b-PS micelles have exhibited a potent dose-dependent cytotoxicity similar to free PTL when administered to MV4-11 AML cells *in vitro*. Additionally, anti-CD123 monoclonal antibodies have been bound to micelles for LSC-specific cytotoxicity.

Methods: PSMA-b-PS micelles were synthesized and loaded with PTL as described (5). To assess the effect of antibody targeting on AML cell binding/uptake, carbodiimide bioconjugations were performed to covalently bind anti-CD123 (7G3) antibodies and fluorescent probes (fluorescein) to PSMA-b-PS micelles. Fluorescently-labeled micelles (FLMs) and antibody-targeted FLMs were purified by dialysis against PBS prior to use. Antibody and fluorescein incorporation were quantified by fluoraldehyde (OPA assay) and absorbance at 495 nm, respectively. For cell binding/uptake studies, $1x10^6$ MV4-11 human AML cells were incubated in culture media with therapeutically relevant doses (~25 μg/mL) of FLMs and antibody-targeted FLMs for 30 minutes. Cells were analyzed by flow cytometry on Accuri C6 (BD) and ImageStreamX (Amnis) systems. PSMA-b-PS micelle uptake was qualitatively assessed via transmission electron microscopy. AML cells were attached to glass slides with poly-L-lysine and dosed with 25 μg/mL PSMA-b-PS micelles for 24 hrs. Cells were washed with PBS, fixed, sectioned and imaged as previously described (6).

Results: Carbodiimide bioconjugations resulted in 25% incorporation efficiency, which equated to ~3.5 mmoles antibody per mole of diblock (1.25 % w/w). As shown in Figure 2B, FLMs were uptaken by MV4-11 cells, as evidenced by a 1.5-fold increase in median fluorescence intensity (MFI) over untreated cells. When incubated for longer times (24 hrs), MFI of FLM-treated cells increased by 3-fold over untreated cells. Incorporation of anti-CD123 antibodies enhanced cell binding by 5-fold compared to non-targeted FLMs. Importantly, FLMs were endocytosed by MV4-11 cells (see Fig. 2C), where antibody-targeted FLMs were more likely surface bound which can have a large effect on PTL delivery mechanisms.

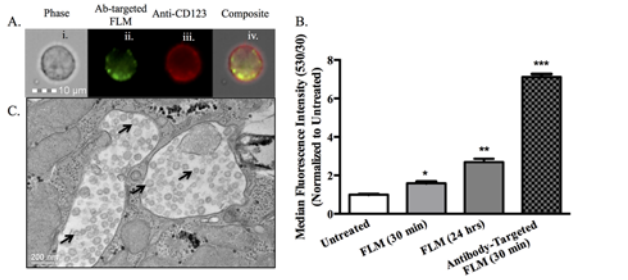

Figure 2. (A) Representative ImageStreamX panel for MV4-11 cells incubated with antibody-targeted FLMs for 30 mins, followed by costaining with anti-CD123-PE. Images show 3 channels: i. phase, ii. FITC, iii. PE, and iv. composite FITC/PE. (B) MFI of cells treated with FLMs for 30 mins and 24 hrs (grey) and anti-CD123 targeted FLMs for 30 mins (checkered) (*$p < 0.05$, **$p < 0.01$ vs. untreated by 2-way ANOVA). (C) Transmission electron micrograph of MV4-11 cells dosed with PSMA-b-PS micelles (higlighted by arrows).

Conclusions: Antibody targeting greatly enhanced the ability of PSMA-b-PS micelles to bind CD123 positive cells *in vitro*. Homing of PTL-loaded micelles has not significantly enhanced PTL cytotoxicity compared to untargeted micelles *in vitro*, but may drastically improve PTL efficacy *in vivo* due to increased bioavailability and LSC-specific delivery. Future studies will focus on PTL delivery mechanisms of targeted and non-targeted micelles, and the ability to reduce off-target drug accumulation in mixed primary cell culture systems.

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