Slow Release of An Antileukemic Artemisinin Analog from An Injectable, Biodegradable *In Situ* Gelling System

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Statement of Purpose: In recent years, targeted therapies have provided new options to manage acute leukemias. However, as monotherapies, they provide only a shallow response with minimal survival benefit. There is still a need for new agents for combination therapy regiments. Derivatives of the natural compound artemisinin (ART) have anti-leukemic efficacy and have been used widely in humans as a first line therapy for malaria with essentially no toxicity [1]. A major challenge to currently available ART derivatives used as antimalarials is their limited half-life. To enhance efficacy and improve half-life, novel dimeric ART analogs, such as ART838, have been developed [2]. ART838 has high antileukemic potency and efficacy *in vitro* and *in vivo* [3,4], but still a half-life of only 3.19 hours in mice [3]. Our lab has developed an injectable *in situ* depot-forming system (ISD) containing poly(lactide-co-glycolide) (PLGA) and poly(lactic acid) (PLA) capable of releasing hydrophobic drugs for several months after subcutaneous injection through 21-23 gauge needles [5]. Here, we demonstrate slow release of ART838 from ISDs, suggesting their use toward enhancing drug delivery for leukemia treatment.

Methods: ISDs were formed as previously described by dissolving 24 wt% PLGA and PLA in a mixture of solvents containing n-methylpyrrolidone (NMP) and triethyl citrate (TEC) [5]. ART838 was added to the formulation at 0, 1.5, 3, or 6 wt% loading content. Eighty µL depots were placed into 4 mL of PBS (pH 7.4) and incubated with shaking at 37ºC. Release media was collected at 4 h, daily during the first week, biweekly through day 28, and then every other week for up to six months. ART838 released into PBS (pH 7.4) was quantified by using mass spectrometry (Xevo TQ-S Cronos, Waters Co., Milford, MA).

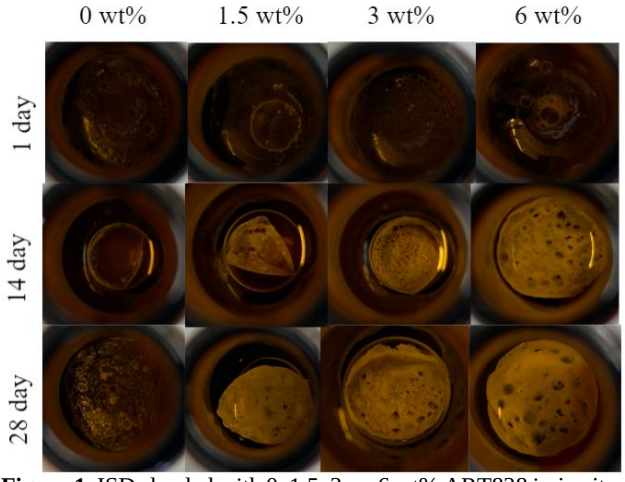

Figure 1. ISDs loaded with 0, 1.5, 3, or 6 wt% ART838 in *in vitro* release conditions at 1, 14, or 28 days after depot formation. ISDs were translucent when formed, and opacity increased with time and with increasing ART838 loading content.

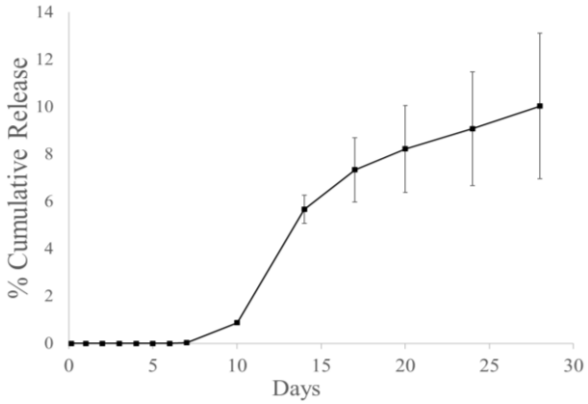

Figure 2. ART838 release loaded at 6 wt% in 80 µL depot. Data reflects mean of 2 replicates ± standard deviation. Release was initiated by day 10; initially, fast release occurred between day 10 and 14, and near zero-order release began after day 14.

Results: ART838-loaded ISDs in *in vitro* release conditions showed consistent depots across all loaded concentrations. ISD opacity increased with time and with ART838 loading content, and all loaded ISDs became opaque by day 28 (Fig. 1). Cumulative release from 6 wt%-loaded ISDs showed initiation of release by day 10 and fast release between days 10 and 14 (Fig. 2). Near zero-order release occurred from day 14 through day 28. Total released mass in the first 28 days represents 10% of loaded drug mass. The opacity and delayed release correlates well with the hydrophobic nature of ART838 and our polymer system. The diffusion of the solvents from the depot in the first week was not sufficient for ART838 to diffuse out of the ISDs due to the hydrophobic interactions between ART838 and PLGA/PLA polymers. The release of ART838 required the degradation of polymers to generate pores, enabling slow ART838 diffusion.

Conclusions: These results demonstrate that ISDs can provide sustained release of ART838 into buffer over days-weeks. Although it is conceivable that this delayed release profile might be beneficial to potentiate antitumor responses initiated by other drug agents, our future work will alter the polymeric formulations of our ISD system to tune the ART838 release curve, aiming to modulate the initiation of release while maintaining the stable release observed here. In addition, ongoing work focuses on investigating release of other dimeric ART analogs from our ISDs and analyzing cytotoxicity and bioeffects of the ART-loaded ISDs using *in vitro* cell culture models.

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