Investigating the Chromatin Damaging Properties of Anthracyclines in Ewing Sarcoma

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Background

- Ewing sarcoma (EwS) is defined by the tumor initiating fusion, most common EWS::FLI1 (~85%) which causes global transcriptional dysregulation[1,2]
- Dose escalation of anthracyclines is associated with improved cancer free survival[3] but limited by toxicity and cannot incorporate in relapsed regimens.
- Mechanism of action of anthracyclines like Doxorubicin (Doxo) : (1) DNA damage- Topo II poison and (2) chromatin damage- histone eviction[4,5]
- Aclarubicin (Acla) and Dimethyl-Doxorubicin (DiMe-Doxo) are anthracyclines that ONLY induce chromatin damage
  - Acla is widely used in China for treatment of elderly adult acute myeloid leukemia patients
- Toxicity is secondary to DNA damage

Given epigenetic dysregulation of EwS we propose that Acla and DiMe-Doxo will have efficacy in EwS preclinical models given their primary chromatin damaging properties. As Acla and DiMe-Doxo lack genotoxic damage, we will also test the safety of delivering these agents after an initial course of Doxo to mimic delivery in the relapsed setting.

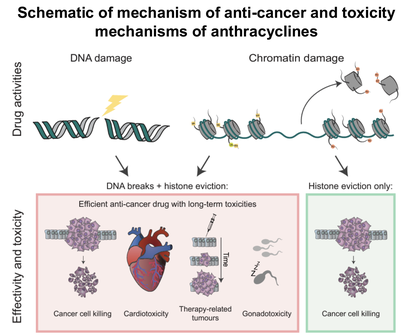


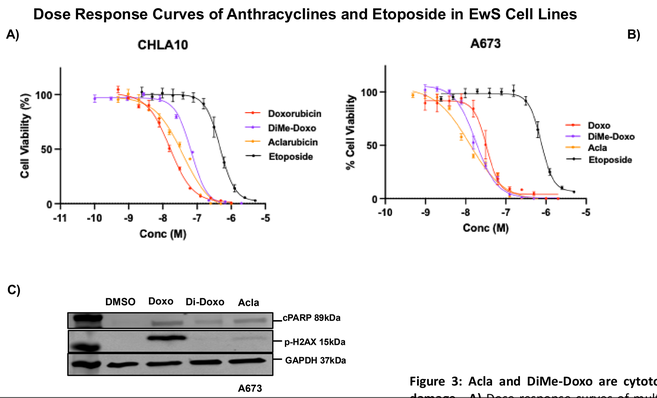
Figure 1. Anthracyclines have two mechanisms of action: DNA damage via topoisomerase II poisoning and chromatin damage through histone eviction. It is proposed that the anti-cancer effects are due to both genotoxic and chromatin damaging effects. However, toxicity is due to genotoxic effects.

Genotoxic and Chromatin Damaging Properties of Therapeutic Agents

	Doxorubicin	DiMe-Doxo	Aclarubicin	Etoposide
DNA Damage	+	-	-	+
Chromatin Damage	+	+	+	-

Figure 2: Table of anthracyclines- Doxorubicin, DiMe-Doxo, and Aclarubicin effects on DNA damage +/- chromatin damage. Additionally shown is Etoposide which is Topo II inhibitor without any inducing any chromatin damage.

Acla and DiMe-Doxo are cytotoxic *in vitro* and do not induce DNA damage



	Doxorubicin	DiMe-Doxo	Aclarubicin	Etoposide
A673	33.67 nM	17.93 nM	12.6 nM	762.7 nM
CHLA10	16.06 nM	67.5 nM	38.8 nM	473.9 nM
TC32	28.2 nM	24.6 nM		
PDX305	44.51 nM	32.97 nM		

Figure 3: Acla and DiMe-Doxo are cytotoxic in EwS *in vitro* models without inducing DNA damage. A) Dose response curves of multiple EwS cell and PDX lines exposed to Doxo, DiMe-

DiMe-Doxo activity in preclinical EwS models

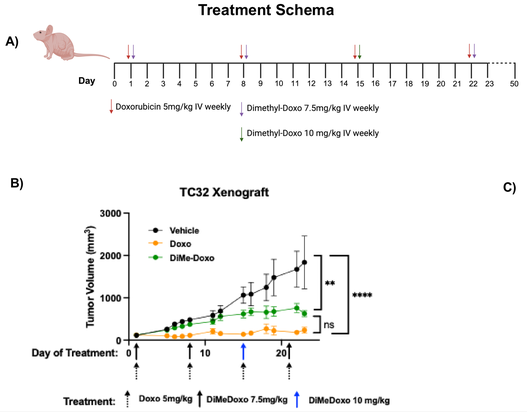


Figure 4: DiMe-Doxo has preclinical efficacy in EwS *in vivo* models. A) Schema of *in vivo* experiment. Athymic nude mice were injected with $1x10^6$ TC32 cells. Animals were randomized and enrolled on treatment when tumors reach $100m^3$. **B)** Average tumor volume of individual treatment groups. Doxo and DiMe-Doxo significantly slow tumor growth as compared to control. Statistical significance determined using one-way ANOVA at Day 23 of therapy when >50% of vehicle mice were still alive. **C)** % change in weight from Day 1 of treatment. Athymic nude mice tolerate Doxo and DiMe-Doxo without significant toxicity

Conclusions/Future Directions

Non-cardiotoxic anthracyclines are effective in Ewing sarcoma

- *In vitro* and *in vivo* activity non-cardiotoxic anthracyclines
- Non-cardiotoxic anthracyclines do not induce DNA damage in Ewing sarcoma tumor cells
- *Future Directions:* Complete PDX *in vivo* model to validate therapeutic efficacy of DiMe-Doxo

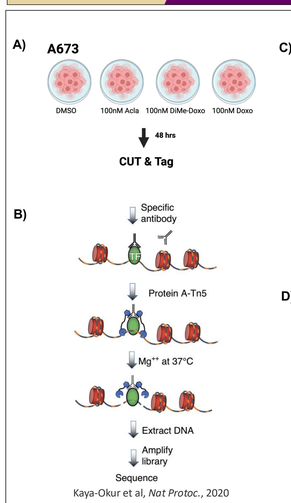
Anthracyclines disrupt histones at active promoters and enhancers

- Potential mechanism of therapeutic efficacy in EwS
- Preferential disruption of histone marks H3K4me3 and H3K27ac
- *Future Directions*: Perform transcriptional analysis (RNA-seq) to investigate if changes to the chromatin landscape following anthracycline therapy result gene expression changes

Aclarubicin is safe to deliver following Doxorubicin

- Minimal toxicity is observed when Aclarubicin is delivered after an initial course of Doxorubicin
- As anticipated second course of Doxorubicin is highly toxic following initial Doxorubicin
- *Future Directions*:
  - Test if a course of DiMe-Doxo is safe to deliver after an initial course of Doxorubicin

Anthracyclines induce changes to the EwS chromatin landscape



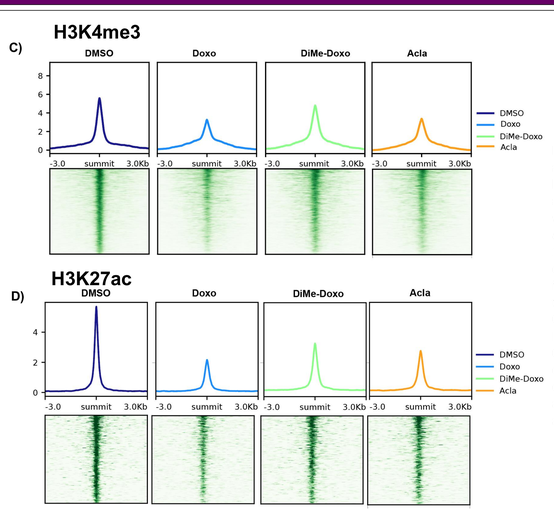


Figure 5: Treatment with anthracyclines disrupts histones at active promoters and enhancers. A) A673 cells were exposed to DMSO, 100nM Acla, 100nM DiMe-Doxo, or 100nM Doxo for 48hrs and collected for histone profiling following anthracycline treatment. **B)** Schematic of CUT&Tag
Heatmap of consensus peaks of **C)** H3K4me3 and **D)** H3K27ac in control cells (DMSO), with loss of occupancy of both H3K4me3 and H3K27ac following anthracycline treatment.

Acla delivery is safe after initial Doxo exposure

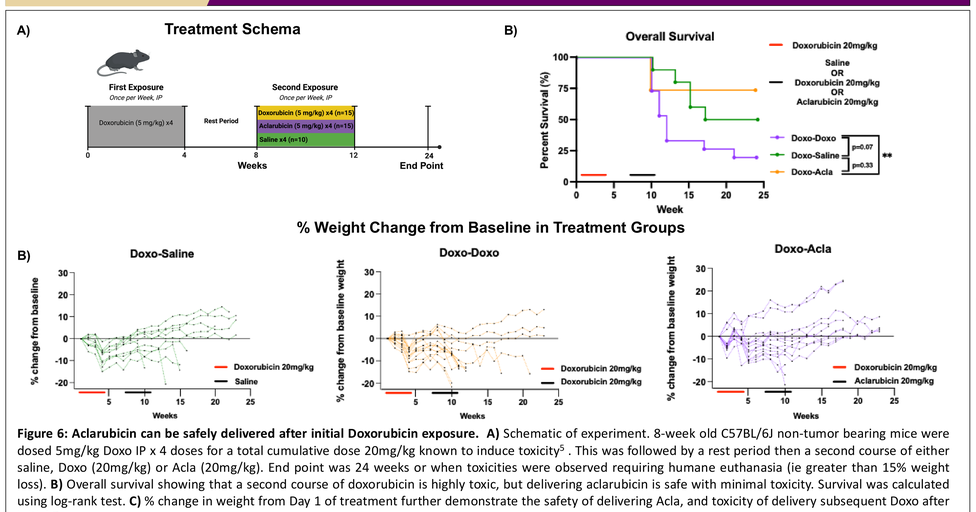


Figure 6: Aclarubicin can be safely delivered after initial Doxorubicin exposure. A) Schematic of experiment. 8-week old C57BL/6J non-tumor bearing mice were dosed 5mg/kg Doxo IP x 4 doses for a total cumulative dose 20mg/kg known to induce toxicity[5] . This was followed by a rest period then a second course of either saline, Doxo (20mg/kg) or Acla (20mg/kg). End point was 24 weeks or when toxicities were observed requiring humane euthanasia (ie greater than 15% weight loss). **B)** Overall survival showing that a second course of doxorubicin is highly toxic, but delivering aclarubicin is safe with minimal toxicity. Survival was calculated using log-rank test. **C)** % change in weight from Day 1 of treatment further demonstrate the safety of delivering Acla, and toxicity of delivery subsequent Doxo after initial Doxo course

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