Exploration of Annamycin Organotropism to Target Primary and Metastatic Liver Cancers

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INTRODUCTION

Annamycin (ANN) is a novel anthracycline of our design that is clinically evaluated as L-ANN, a liposome formulated drug product. ANN and L-ANN display unique organotropism that differs from doxorubicin (DOX). In addition, ANN was shown to have increased potency against multidrug resistant cancer cell lines. Annamycin is currently the subject of multiple clinical trials including soft tissue sarcoma patients with metastasis to lungs or patients with acute myeloid leukemia.

Preclinical studies demonstrated that, in contrast to doxorubicin, Annamycin showed low or no cardiotoxicity. In clinical trials, an independent safety review of preliminary data from the first forty-two subjects revealed no evidence of cardiotoxicity. Of note, thirty-two of the forty-two subjects reviewed had received more than the lifetime maximum allowable level of anthracycline established by the FDA.

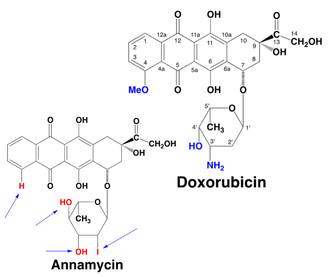

Figure 1. The chemical structures of Annamycin and Doxorubicin

OBJECTIVE

Liver is a common site for metastases of many types of cancer including PDAC and colorectal cancer. PK/PD analysis shows high levels of ANN in liver. The objective of this study was to analyze the pharmacokinetics of two formulations of ANN in the liver in comparison to DOX and to determine its tumoricidal potential in liver-localized tumor models.

METHODS

The PK and tissue-organ distribution was tested in naïve CD®IGS [Crl:CD(SD)] rats or CD-1 mice after administration of L-ANN, and free ANN (F-ANN)/DOX cocktail. The levels of ANN and DOX were measured using LC/MS. The efficacy of L-ANN was then tested in orthotopic HCC models (tumor fragment implantation and cells injection model, Hepa 1-6 Luc), a liver experimental metastasis model (CT26 Luc), and liver-implanted PDAC tumor fragments (MIA PaCa-2).

RESULTS

Pharmacokinetics and tissue-organ distribution

LC/MS analysis of plasma and extracted revealed drastically higher amount of ANN detected in the liver parenchyma when compared to DOX **(Figure 2A)**. Increased retention of ANN was also observed in lungs and pancreas (**Figure 2B**). A similar distribution pattern was also confirmed in mice (not shown).

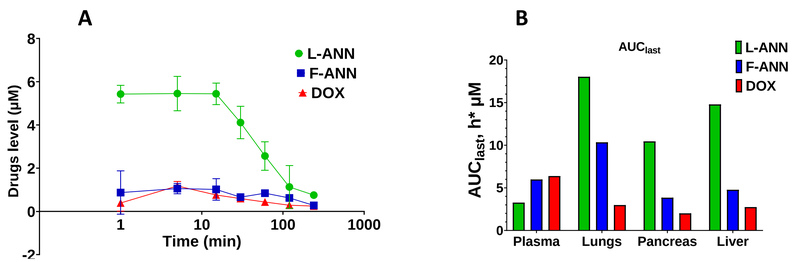

Figure 2. The pharmacokinetics and tissue-organ distribution of ANN and DOX in rats.

CD®IGS [Crl:CD(SD)] Rats (n=3-6) were administered with L-ANN, F-ANN or DOX at 2mg/kg. **(A)** At indicated timepoints, the animals were euthanized followed by necropsy and determination of ANN and DOX levels using LC/MS/MS. **(B)** AUC values were computed using non-compartmental analysis in Phoenix WinNonLin software.

Efficacy of L-ANN in orthotopic HCC Tumors

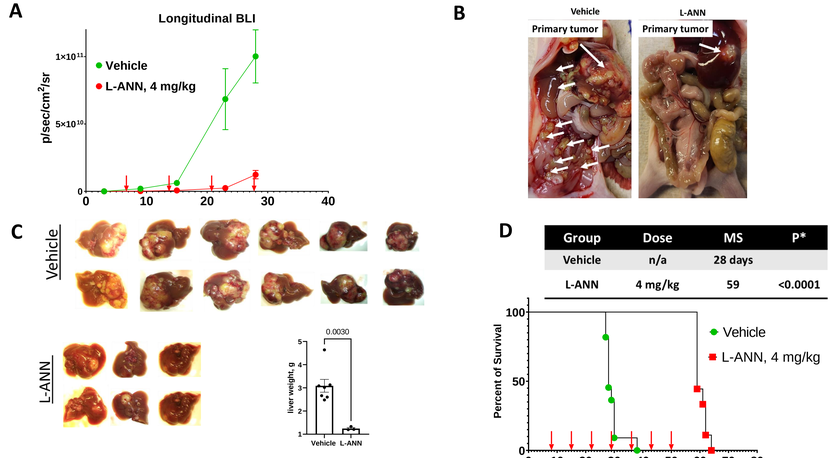

Figure 3. The efficacy of L-ANN was tested in orthotopic model of HCC.

(A) Longitudinal bioluminescence analysis of Hepa 1-6 Luc tumors surgically implanted into liver parenchyma and treated with L-ANN (arrows indicate 4 mg/kg L-ANN administration). **(B)** The extent of metastatic changes in the abdominal cavity in vehicle or L-ANN treated mice on day 29. **(C)** Comparison of the liver extracted on the day 29 from the vehicle group (moribund animals, n=6) and randomly selected L-ANN treated mice n=3). **(D)** Kaplan-Meyer survival analysis

1-2 mm fragments of Hepa 1-6 Luc were surgically implanted into liver parenchyma of female athymic mice. Tumor progression was monitored using bioluminescent imaging. On day 8 of the study the animals were randomized into two groups receiving L-ANN at 4 mg/kg and vehicle once a week for seven doses. Tumor progression was monitored by BLI. At they 29 of the study the vehicle- treated mice were euthanized due to morbidity. The livers were extracted and compared to three randomly selected animals from the L-ANN cohort.

Efficacy of L-ANN in CT26 Luc colorectal liver metastatic model

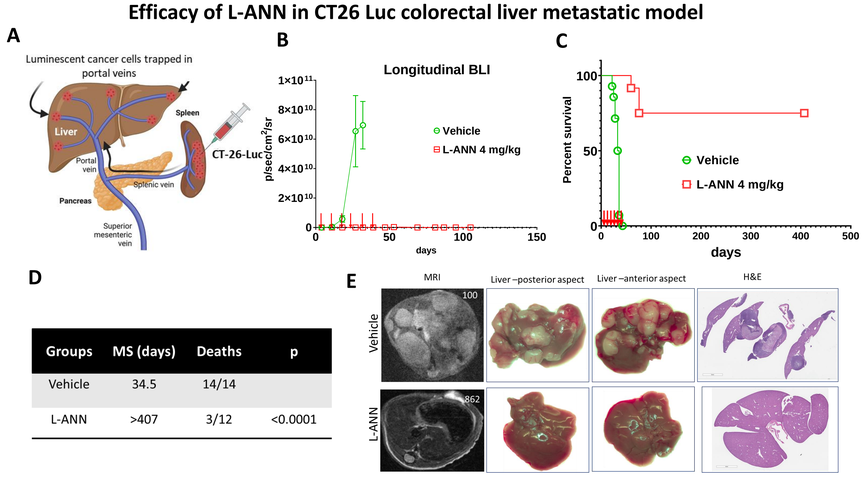

Figure 4. The efficacy of L-ANN in liver-established experimental metastatic model of colorectal cancer.

(A) Schematic representation of the experimental liver metastatic model through intrasplenic injection of the cells. **(B)** Longitudinal analysis of bioluminescence intensity in mice treated with vehicle or L-ANN. **(C)** Kaplan-Meyer survival analysis of both groups along with **(D)** survival parameter **(E).** Representative MR images, pictures of extracted liver and H&E stain of vehicle and L-ANN treated mice on day 25.

The efficacy of L-ANN was tested in orthotopic model of HCC. Briefly, 5 x 10^5 of CT26 Luc cells were injected intrasplenically into female Balb/C followed by radical splenectomy 15 min later. Treatment was initiated on day 8 (4 mg/kg, red arrows). The tumor progression was monitored using bioluminescent imaging and MRI at day 25. At day 29, the vehicle- treated mice were euthanized due to morbidity. The livers were extracted and compared to three randomly selected animals from the L-ANN cohort.

Efficacy of L-ANN in liver-implanted pancreatic tumor fragments

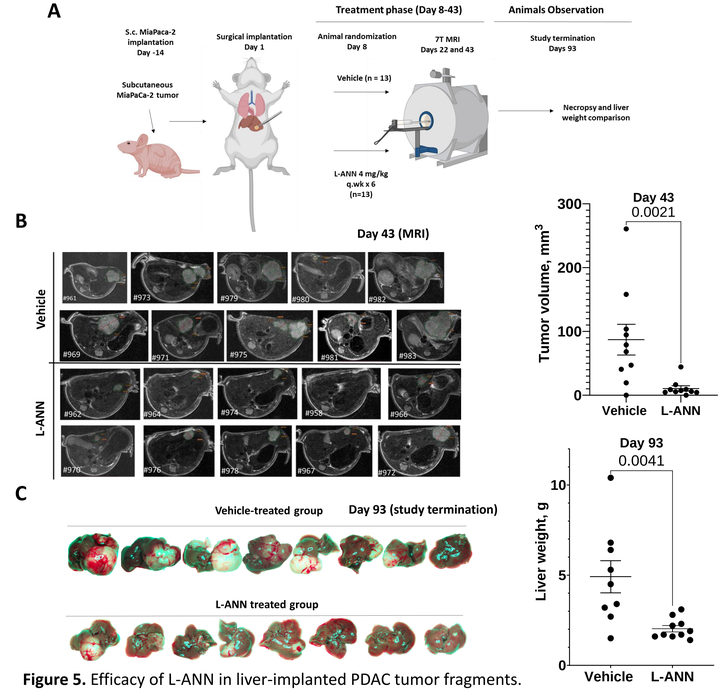

Figure 5. Efficacy of L-ANN in liver-implanted PDAC tumor fragments.

(A) Overview of the experiment design. **(B)** MRI of the abdomen of mice treated with vehicle or L-ANN performed on day 43. **(C)** Images and liver weights distribution at the study termination on day 93.

1-2 mm fragments of subcutaneously grown MIA PaCa-2 PDAC tumor fragments were implanted in the liver parenchyma of female nude athymic mice. On day 8 the animals were randomized into two experimental groups receiving vehicle and L-ANN at 4 mg/kg once a week for a total of six cycles. Tumor growth was monitored by abdominal MRI on day 22 and 43. DICOM images were analyzed and tumor volumes estimated using RadiAnt DICOM vewer. The study was terminated on day 93 and the liver weights were compared.

CONCLUSIONS

- ANN is a novel anthracycline antibiotic combining unique features of all approved anthracyclines,
- In contracts to DOX, ANN shows no cardiotoxicity in preclinical and clinical studies,
- High levels of ANN have been confirmed in liver, lungs and pancreas after systemic delivery, demonstrating unique organotropic properties of this drug,
- L-ANN shows excellent antitumor activity in seberla liver-colonized tumor models: i) orthotopic HCC models (Hepa 1-6 Luc), ii) experimental liver metastatic model of colorectal carcinoma (CT26 Luc) and iii) liver-implanted PDAC tumors (MIA PaCa-2 Luc)

ACKNOWLEDGEMENTS

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