Liposomal Annamycin Inhibition of Lung Localized Breast Cancer
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Introduction
Annamycin (ANN) is a non-carcinostatic anthracycline antibiotic with unique biological properties that have been formulated in liposomes (L-ANN) and is currently in clinical studies in ANL patients. Our earlier studies showed that ANN is not cross resistant with doxorubicin (DOX) and is a poor substrate for P-glycoprotein (Pgp) but is ATP- binding cassette (ABC) family transporter BCRP (Abcb1a) and BCRP (Abcb1b) substrate. This report investigates (i) the correlation of ANN susceptibility to a panel of ABC family transporters, (ii) the efficacy of L-ANN in a lung cancer xenograft model, and (iii) the synergistic anti-tumor effect of L-ANN in combination with a topoisomerase poison, camptothecin (CPT) in breast cancer cells. The in vivo studies were performed using L-ANN (annamycin/DOX, distribution formulation min) was administered to 20 mice by tail injection (Fig. 3A), and tumor metastasis to brain and liver was assessed by quantification of the percent survival (Fig. 3B). The median survival of treated group increased to 56 days vs 23 for vehicle (p<0.0001) (Fig. 4T).

Efficacy of L-ANN in 4T1 TNBC “lung metastasis” model
L-ANN showed remarkable inhibition of metastatic growth of lung localized 4T1 cells after two doses of the drug (Fig. 4G). Interestingly, the white group also developed a tumour phenotype, while L-ANN treated mice had smaller pulmonary nodules formation than controls (D, E and F). In addition, the L-ANN group was not found to be significant when comparing the percent survival (C). L-ANN significantly inhibited tumor growth of lung localized breast cancer cells and mice. L-ANN reduces inflammation in lung cancer bearing animals. L-ANN significantly extends survival and reduces tumor burden in mice with advanced lung localized cancers.

Discussion
Efficacy of L-ANN in CT26 colon cancer “lung metastasis” model
Female BALB/c mice were injected with 1x10^7 CT26 cells. Two days after tumor inoculation mice were randomized into three groups (vehicle treated vehicle (p<0.0001) (Fig. A)] Longitudinal analysis of organ weights. L-ANN significantly increased survival up to 12 days post inoculation (p<0.0001) (Fig. 5A) and microscopic images of excised lungs showed not significantly reduced lung metastatic burden compared with vehicle treated mice. Survival curves of vehicle treated mice (p<0.0001) (Fig. 5B). Annamycin is used in human cancer patients in combination with other chemotherapeutic agents to reduce resistance and improve clinical efficacy of L-ANN.

Summary
• ANN is a potent antitumor agent inducing DNA damage and shows nanomolar efficacy against breast cancer cells.
• ANN shows dose- and time-dependent accumulation in cells with increased synthetic lethality as compared to DOX.
• L-ANN: liposomal formulation of ANN has been developed and currently is being evaluated in clinical trials in AML, patients with chronic myelogenous leukemia (CML) and other hematological malignancies.

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Reference

Results
Annamycin shows rapid and dose-dependent accumulation inside cells, with significantly higher rate of uptake than DOX (Fig. 2A). Immunofluorescence studies of L-ANN showed significantly different subcellular distribution pattern when compared to DOX (Fig. 2B). Based on H&E (p<0.0001) staining, the L-ANN group shows significantly more accumulation in the lungs and kidneys. For ANN, exposure to ANN results in time-dependent accumulation of DNA breaks (Fig 2C and 2D) and induction of cell cycle arrest and apoptosis. The IC50 value for ANN (24h exposure) was in low nanomolar range and generally 2-3 times lower than for DOX.

Figure 3. Pharmacokinetic and organ distribution of ANN after intravenous administration
The respective concentration in plasma and organs was measured by liquid chromatography - mass spectrometry (LC-MS/MS) analysis. In comparison with DOX, ANN shows significantly higher tissue accumulation compared to plasma and has lower clearance. Furthermore, ANN has higher bioavailability than DOX, as determined by the AUC and $\text{C}_{\text{max}}$ values (Fig. 3).

Figure 4. Efficacy of L-ANN in 4T1 TNBC “lung metastasis” model
Female BALB/c mice were injected with 1x10^7 4T1-Luci cells through tail vein injection. Eight days after tail vein injection, mice were injected with L-ANN, and bioluminescence signals were monitored using a Xenogen IVIS Imaging System. The bioluminescence signal was significantly reduced in the L-ANN group compared to the vehicle group, demonstrating the efficacy of L-ANN in a lung cancer xenograft model with a peak concentration reaching 200 µg/ml.

Figure 5. Efficacy of L-ANN in CT26 colon cancer “lung metastasis” model
Female BALB/c mice were injected with 1x10^7 CT26-Luci cells. Two days after tumor inoculation mice were randomized into three groups (vehicle treated vehicle (p<0.0001) (Fig. A)] Longitudinal analysis of organ weights. L-ANN significantly increased survival up to 12 days post inoculation (p<0.0001) (Fig. 5A) and microscopic images of excised lungs showed not significantly reduced lung metastatic burden compared with vehicle treated mice. Survival curves of vehicle treated mice (p<0.0001) (Fig. 5B). Annamycin is used in human cancer patients in combination with other chemotherapeutic agents to reduce resistance and improve clinical efficacy of L-ANN.

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Figure 2. Uptake and in vitro activity of Annamycin
Annamycin shows rapid and dose-dependent accumulation inside cells, with significantly higher rate of uptake than DOX (Fig. 2A). Immunofluorescence studies of L-ANN showed significantly different subcellular distribution pattern when compared to DOX (Fig. 2B). Based on H&E (p<0.0001) staining, the L-ANN group shows significantly more accumulation in the lungs and kidneys. For ANN, exposure to ANN results in time-dependent accumulation of DNA breaks (Fig 2C and 2D) and induction of cell cycle arrest and apoptosis. The IC50 value for ANN (24h exposure) was in low nanomolar range and generally 2-3 times lower than for DOX.