P3.031.04 Annamycin: Opening new doors for organotropic targeting of primary and metastatic lung cancer

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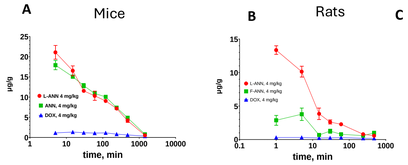
Introduction: Lung cancer and distant metastasis to lung is a major cause for cancer related deaths worldwide. Primary lung cancer or lung metastatic cancers are less responsive to doxorubicin (DOX) than other type of cancers. Even sarcomas that are typically highly sensitive to DOX are not responsive after metastasis to lungs. Annamycin (ANN) is a non-cardiotoxic analog of DOX and potent Top2a poison that exhibits consistent activity against multidrug resistant (MDR1) cancers.

Hypothesis. Suboptimal tissue-organ distribution is a significant factor limiting DOX efficacy in lung localized cancers, therefore DOX analogs with increased lung uptake will exhibit improved in vivo efficacy.

Results

1. ANN and DOX have dramatically different penetration and retention levels in lungs



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	Mice			Rats		
	L-ANN	F-ANN	DOX	L-ANN	F-ANN	DOX
$T_{1/2}$	5.4	4.4	16.4	4.6	282.8	12.4
C_{MAX} ug/ml	21.1	17.9	1.34	13.3	3.8	0.31
C_0	22.4	18.7	1.12	14.3	2.9	0.31
AUC_{last}	107.9	104.3	14.66	11.5	6.6	1.73

Figure 1. Time-dependent levels of ANN and DOX in lungs of mice and rats administered with L-ANN, F-ANN and DOX

(A) Female CD-1 mice **(n=5)** received a bolus IV injection of liposome formulation of ANN (L-ANN) or mixture of DOX and not liposome-formulated (free) ANN (F-ANN/DOX). The mice were sacrificed at different time points followed by necropsy and determination of the concentration of ANN and DOX by LC/MS in plasma and lungs. **(B)** Female and male rats **(n= 3)** received the treatment of L-ANN and F-ANN/DOX Samples were processed as described above. This study was performed and reported according to GLP standards. **(C)** Noncompartmental analysis of PK was performed using Phoenix™ (Certara).

2. Comparison of L-ANN and DOX efficacy in sarcoma lung metastasis models

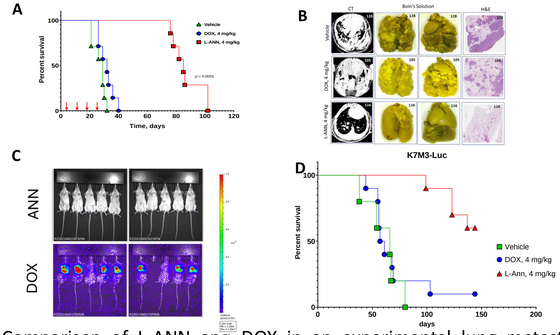
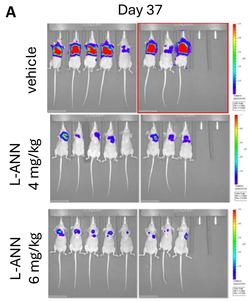
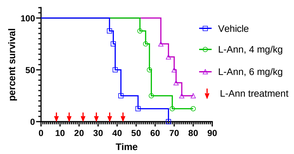


Figure 2. Comparison of L-ANN and DOX in an experimental lung metastatic model of sarcoma

(A) Survival curve **(n=7)** of mouse fibrosarcoma lung experimental metastasis model (MCA205) treated with vehicle, L-ANN or DOX, at 4 mg/kg as indicated by the arrows. **(B)** CT and ex vivo images of lung tissue extracted from MCA205 model treated with vehicle, L-ANN and DOX at day 25 of the study. **(C)** Bioluminescent image of mouse osteosarcoma model K7M3 treated with 4 mg/kg L-ANN or DOX (day 57) and corresponding Kaplan-Meyer survival plot, p<0.0001 **(D)**.

3. L-ANN treatment results in statistically significant inhibition of tumor growth and extension of survival in orthotopic lung cancer models





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Group	MS (days)*	P vs vehicle**	P vs L-ANN 4 mg/kg**
Vehicle	40.5	-	0.0087
L-ANN 4 mg/kg	57.5	0.0087	-
L-ANN 6 mg/kg	70.5	0.0005	0.0624

* Median Survival , ** p value computed using, Log-rank (Mantel-Cox) test

Figure 3. Efficacy of L-ANN in H1299 orthotopic model of lung cancer.

Female nude athymic mice received 2x10[6] of H1299-Luc cells suspended in 50% Matrigel into left lung parenchyma. On day eight, the animals were randomized into three experimental groups (n=8) and given either vehicle, 4 mg/kg L-ANN, or 6 mg/kg L-ANN each week. **(A)** Tumor progression was monitored by bioluminescent imaging on a weekly basis **(B-C)** Survival benefits were analysed using Kaplan-Meyer plot following intravenous injection of 1 x 10[5] MCA205 cells. Treatment started on day 4. Mice received four weekly injections of 4 or 6 mg/kg L-ANN. Controls received vehicle.

4. Efficacy of L-ANN in 4T1 TNBC lung metastasis model

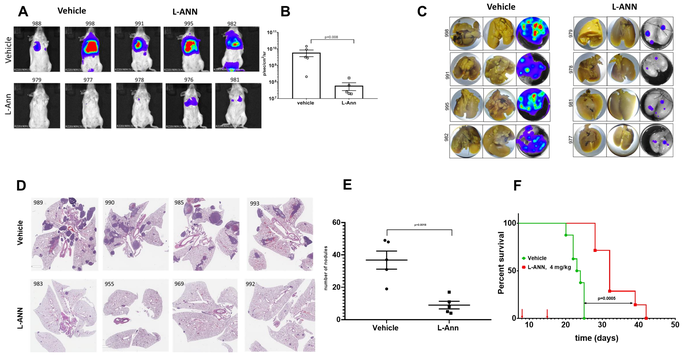

Figure 4. Efficacy of L-ANN in 4T1 TNBC lung metastasis model.

Female Balb/c mice were injected with 2 x 10[4] 4T1-Luc cells through tail vein. Eight days after tumor cell inoculation, mice were injected with L-ANN at 4 mg/kg or saline. **(A)** Bioluminescent images of mice after two doses of L-ANN. **(B)** distribution of bioluminescent signal in vehicle or L-ANN-treated mice. **(C)** Microscopic image of Bouin's solution-fixed lungs after two doses of L-ANN and vehicle along with corresponding BLI. **(D)** H&E stained lung sections with **(E)** quantification of metastatic nodules of vehicle- or L-ANN treated mice and their weight distribution (p value was calculated using Mann-Whitney test Graph Pad Prism 8.0.0 software.). **(F)** Survival curves of 4T1-luc injected mice receiving two doses of L-ANN (4 mg/kg) or vehicle on a once a week schedule. Arrows represent L-ANN injection.

5. Annamycin exhibits limited cardiotoxicity, in preclinical models when compared to DOX

A

Cell line	IC_{50} (nM) ANN	Average IC_{50} for ANN	IC_{50} (nM) DOX	Average IC_{50} for DOX	Ratio IC_{50}DOX/ IC_{50}ANN
M63.2	8.2 ± 4.3	15.8 nM	41.9 ± 7.0	54.2 nM	5.2
MCA205	17.4 ± 7.7		51.1 ± 14.0		2.9
K7M3	14.9 ± 9.8		62.7 ± 27.4		4.2
143B	22.7 ± 10.0		60.1 ± 15.0		2.6
H9c2 (Rat cardiomyocytes)	1,264.4 ± 181.2		88.8 ± 15.3		0.07

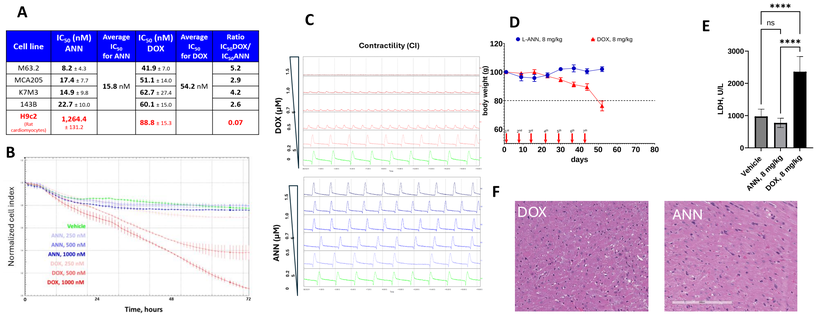

Figure 5. Analysis of viability and contractility of human cardiomyocytes exposed to ANN or DOX.

(A) The effect of ANN and DOX on viability of rat cardiomyocytes (H9C2) and selected cancer cell lines was assessed at 72 h exposure using CellTiter Glo Assay. **(B–C)** Viability and contractility of established culture of human iPSCs cardiomyocytes (iCell[2], Fiji film) exposed to increased concentrations of ANN and DOX were analyzed using xCELLigence RTCA CardioECR system (Agilent Technologies). **(D)** Female CD-1 mice (n=5) were treated with 8 mg/kg DOX or L-ANN for 7 weeks (once a week injection, marked with red arrows) for a total cumulative dose of 56 mg/kg. The DOX –treated arm showed a consistent decrease in body weight and increased morbidity following the last dose. **(E)** Serum chemistry analysis revealed significantly upregulated LDH levels which might be associated with minimal cytoplasmic vacuolation of cardiac myocytes. **(F)** Histopathology analysis revealed a few occasional cardiac myocytes with swollen or eosinophilic cytoplasmic changes. The lesions were not observed in L-ANN treated mice.

Conclusions

- Annamycin, a potent Topo 2 poison, demonstrates high uptake and retention in lung parenchyma of mice and rats when given as a free drug or in its liposome formulated (L-ANN) form.
- The therapeutic effects of DOX are diminished due to low lung DOX uptake as demonstrated in the tested in vivo models (Fig. 3). In contrast, L-ANN exhibits consistent efficacy in vivo in orthotopic and experimental lung metastatic models of sarcoma, breast, and colon cancer (not shown). This correlated with the high ANN concentration in lungs, which exceeded DOX levels by 10- to 30-fold.
- Preclinical tests clearly demonstrate a better cardiac safety profile of free ANN when compared to DOX and no cardiotoxicity of L-ANN in the in vivo models. No cardiotoxicity of L-ANN was noted in the ongoing clinical studies.
- Annamycin is currently clinically evaluated (NCT03388749; NCT05319587; NCT04887298) as a liposome formulated drug (L-ANN).
- In summary, the observed organotropic properties of ANN, its efficacy in vivo, and its promising safety profile warrant further translational studies to evaluate L-ANN in patients with primary or metastatic lung cancers, as a single agent and in combination with currently used therapeutics.

Disclosures

This work was supported by a grant (PI - Waldemar Priebe) from Moleculin Biotech, Inc. (NASDAQ:MBRX). Dr. Priebe is Chairman of the Scientific Advisory Board, inventor of Annamycin, and owns stock in Moleculin Biotech, Inc.. Dr. Fokt and Zielinski own stocks and are consultants to Moleculin Biotech, Inc. Dr. Wofram Dempke is an employee of Moleculin Biotech.