Liposome co-encapsulation of doxorubicin and a prodrug of mitomycin-c for pharmacological optimization of combination chemotherapy

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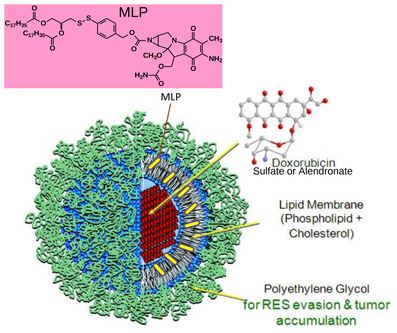
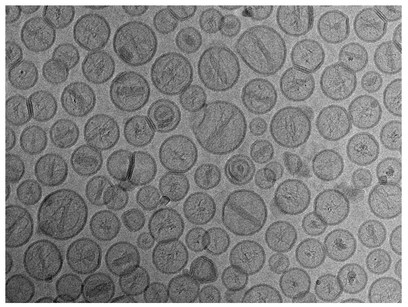
Introduction

- Combination therapies for the treatment of cancer have increased in recent years to address the multiple pathways of oncogenesis and cancer cell growth
- Nanoparticles containing co-encapsulated drugs targeting different pathways of the oncogenic scenario, and with non-overlapping toxicities are powerful tool in drug delivery (Shmeeda et al., 2016)
- Promitil is a liposomal formulation of a prodrug of mitomycin c (MLP) in Phase 2 clinical studies (Golan et al., 2015, Amitay et al., 2016)
- Combination therapy with Promitil and DOXIL is highly effective and synergistic in animal tumor models (USPTO: Gabizon et al., 10,080,807, 2018)

PROMIDOX – formulation of co-encapsulated Doxorubicin & Mitomycin Lipidated Prodrug (MLP)

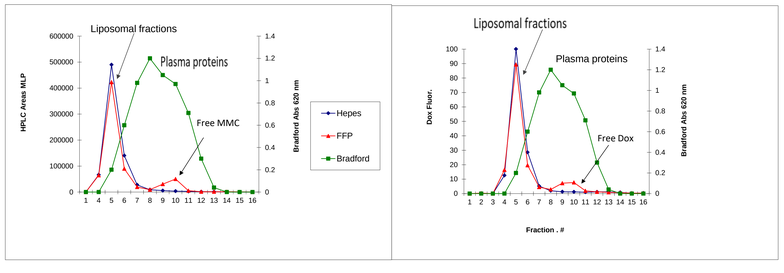


Cryo-TEM of Promidox: classical DOX precipitation in the liposome water phase



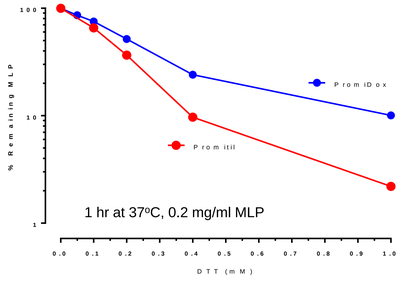
Formulation	Phospholipid µmol/ml	MLP mg/ml	DOX mgl/ml	Mean Diameter nm	PDI	Zeta potential mV
Promitil	26.7	5.0		102	0.085	- 13.1
Doxil	12.4		2.0	83	0.049	- 14.8
Promidox	23.6	3.0	2.0	113	0.081	- 15.0

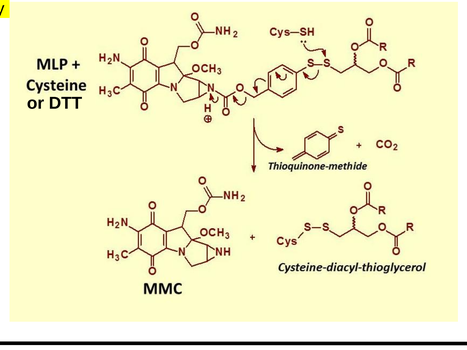
High Stability in Plasma of both agents: minimal leakage at 24 h



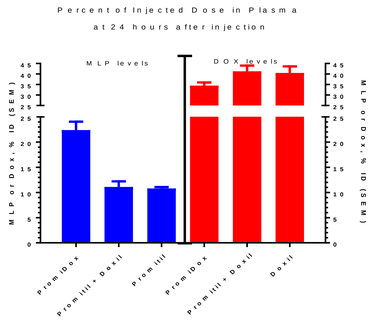
Prodrug Release Assay: Thiolytic cleavage of MLP releases active MMC
PromiDox is more resistant than Promitil to Dithiothreitol (DTT)-induced Cleavage of MLP.

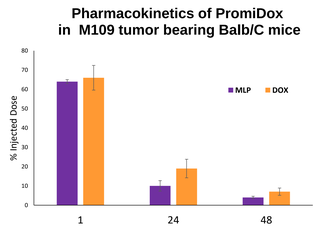
Avoiding premature release of MMC will reduce toxicity and improve delivery of intact payload to tumor

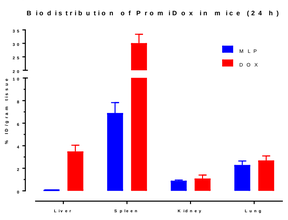


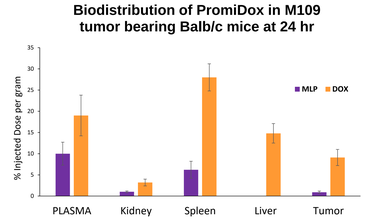


Pharmacokinetics and Biodistribution in Mice



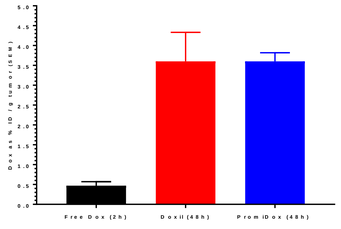






- Longer circulation time of MLP in PromiDox than in Promitil, ensuring greater tumor delivery of MLP
- Slow (Stealth) plasma clearance of both API's (MLP and Dox)
- Faster tissue clearance of MLP than of Dox, due to cleavage/activation to MMC

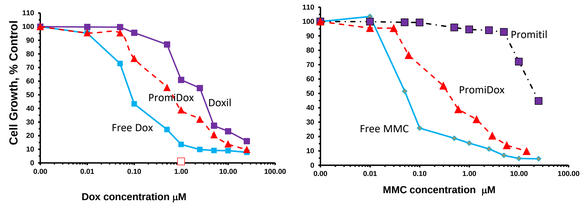
Tumor Dox levels in M109 Mouse Tumor model



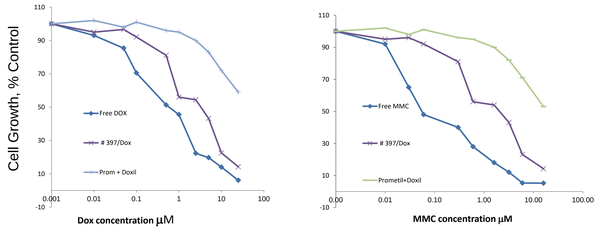
- Tumor levels of Dox after PromiDox are as high as after Doxil and 10-fold greater than after Free Dox at peak tissue levels

In Vitro Cytotoxicity: *Synergistic effect of co-encapsulated drugs*

T-24 Bladder Ca cells

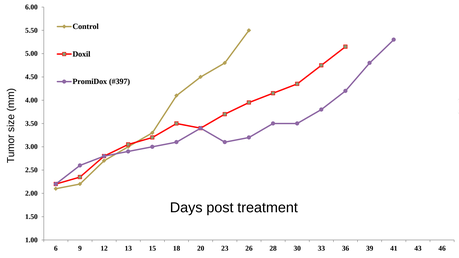


HT-29 colon Ca cells (#397/Dox=Promidox)



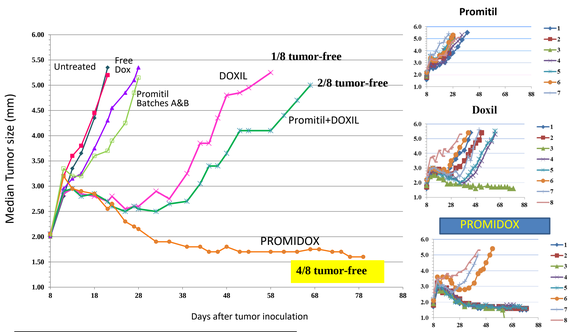
In Vivo Therapeutic Studies

M109R mouse MDR+ tumor model

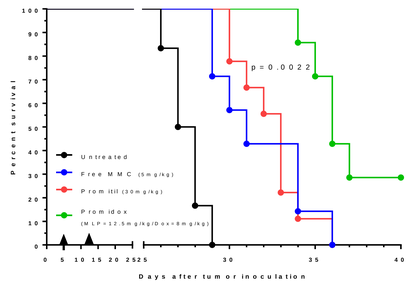


- Promidox is significantly more effective than Doxil in an MDR+ tumor model

Powerful antitumor activity of Promidox in the 4T1 tumor model (triple negative mouse breast carcinoma)



C26 Lung Metastases Model



- Greater Therapeutic efficacy of PromiDox in C26 Mouse Metastatic Tumor Model

Clinical impact:

- **Combines two valuable cancer therapy agents, MLP & Dox, with synergistic activity at well tolerated doses**
- **Circumvents MDR-1 drug resistance**
- **MLP is currently undergoing clinical testing in a liposome formulation coined PROMITIL®. PromiDox may expand the spectrum of clinical activity.**

Conclusions

- **Stable retention of Dox and MLP in Plasma Stability Assays**
- **Potent in vitro Cytotoxicity against a number of tumor cell lines (greater than Promitil+Doxil)**
- **Long circulation half-life of Dox and MLP (longer than in Promitil)**
- **Potent in vivo activity (greater than Promitil+Doxil) in mouse tumor models (M109R, 4T1, C26) at relatively low dose of MLP**
- **Unique and potent dual drug liposome formulation for combined chemotherapy of cancer with synergistic activity, broad spectrum antitumor activity and reduced toxicity**

References

1. Shmeeda H, Amitay Y, Gorin J, Tzemach D, Mak L, Stern ST, Barenholz Y, and Gabizon A: Co-encapsulation of Alendronate and Doxorubicin in Pegylated Liposomes: A novel formulation for chemo-immunotherapy of cancer. Journal of Drug Targeting, 24:878-889 2016
2. Amitay Y, Shmeeda H, Patil Y, Gorin J, Tzemach D, Mak L, Ohana P, and Gabizon A: Pharmacologic studies of a prodrug of mitomycin C in pegylated liposomes (Promitil®): High stability in plasma and rapid thiolytic prodrug activation in tissues. Pharmaceutical Research, 33:686-700, 2016
3. Golan T, Grenader T, Ohana P, Amitay Y, Shmeeda H, La-Beck NM, Tahover E, Berger R, and Gabizon AA: Pegylated liposomal mitomycin C prodrug enhances tolerance of mitomycin C: a phase 1 study in advanced solid tumor patients. Cancer Medicine, 4:1472–1483, 2015