# Development of Improved Targeted Liposomal-Based Chemotherapeutics to Treat Metastatic Breast Cancer

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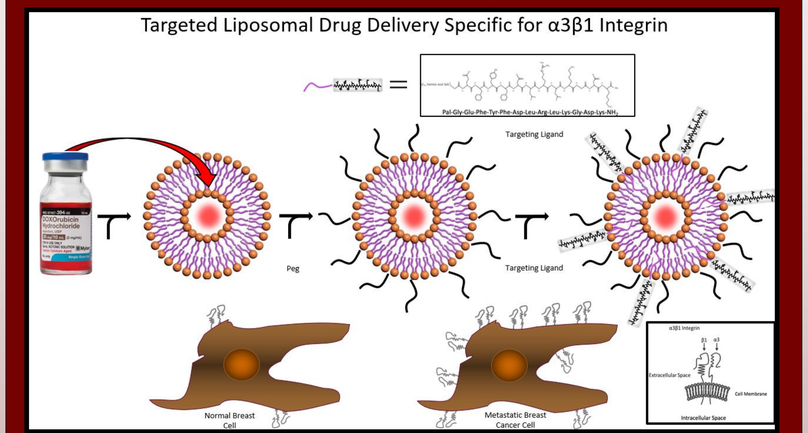
## Abstract

Worldwide, breast cancer is the most common cause of cancer death amongst women (1-2), and therefore improved chemotherapeutics are desperately needed. We have previously demonstrated selectivity of our novel targeted liposomal-based drug towards metastatic breast cancer cells. However, the necessary addition of PEG-2000 to the drug surface prior to *in vivo* use has previously shown to limit the overall drug incorporation into metastatic breast cancer cells and therefore presumably decrease the overall efficacy of the drug. Therefore, in this study we are currently working on the co-encapsulation of dual-drugs into our targeted formulation in order to recapture some of the presumed loss of overall drug efficacy attributed to the necessary pegylation prior to *in vivo* use. Specifically, we propose the co-encapsulation of the chemosenitizer drug dihydromyricetin (DMY) and the cytotoxic agent doxorubicin. DMY is used as a chemosensitizer because it is known to competitively bind sorcin (3), which is a protein known to be upregulated in metastatic breast cancer, and also known to bind to doxorubicin which limits its overall cytotoxic effect.

### Targeted Liposomal Drug Delivery Specific for α3β1 Integrin

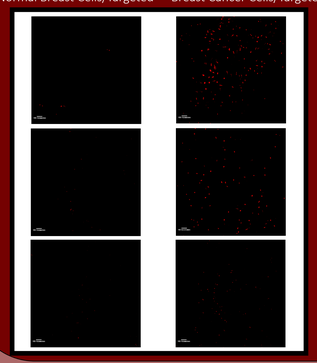



## Methods

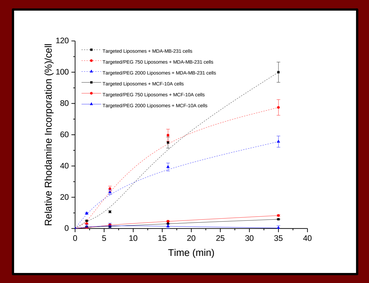
The liposomes in this study were constructed using HSPC and cholesterol, both with and without PEG 750 or 2000. The liposomes are designated “targeted” and “non-targeted”, depending on whether or not the targeting peptide is included in the formulation. These components were combined, and then a thin lipid film was developed using rotary evaporation. Rhodamine was then added, spontaneously forming multilamellar vesicles (MLVs) which were then repeatedly extruded to form small unilamellar vesicles (SUVs). These liposomes were extruded to obtain a uniform size of approximately 100 nm in diameter. The vesicles were then purified through a Sephadex G-50 medium grade resin gel column. An absorbance scan of both targeted and non-targeted liposomes was analyzed to confirm the presence of the peptide in the bilayer. To monitor stability, fluorophore leakage was measured over various time points at both 4°C and 37°C to emulate storage and *in vivo* conditions respectively. Previously, we have shown that both targeted and non-targeted formulations were in fact stable under storage conditions. With respect to the targeting peptide and α3β1 integrin, the peptide used in this study was synthesized by, and purchased from, ChinaPeptides Co., Ltd (Pudong, New Area, Shanghai China). It is designed as a single stranded peptide (SSP), which is able to mimic the α1(IV) 531-543 non-triple helical region of collagen type IV. This region is known to bind the α3β1 integrin (8), which is known to be overexpressed in metastatic breast cancer cells ( i.e. MDA-MB-231 cells) (4-7). This peptide sequence was modified to accommodate a C16 (palmitic acid) tail in order to allow for liposomal bilayer incorporation via hydrophobic interactions. It was purified using RP-HPLC, and was analyzed using ESI-MS (API 150EX Mass Spectrometer). As previously stated, α3β1 integrin is known to be overexpressed in metastatic breast cancer cells. This integrin is thought to facilitate the metastasis of cancerous cells through interactions with various extracellular matrix (ECM) components such as collagen, laminin, and fibronectin (5,8). Fluorophore accumulation into the various cell lines used in this study was monitored using fluorescence microscopy and quantified using image-J software.

## Results

Normal Breast Cells/Targeted  Breast Cancer Cells/Targeted



Relative Rhodamine Incorporation




Relative Rhodamine Incorporation from targeted no PEG, PEG 750, and PEG 2000 over 35 minutes in MCF-10A and MDA-MB-231 cells.

### Dual Drug Co-delivery of Liposomal-based Chemotherapeutics

- Dual drug co-delivery can improve the overall effectiveness of chemotherapeutics.
- This strategy can involve either two cytotoxic agents or a cytotoxic agent and a chemosensitizer.
- Chemosensitizers renders the cell more susceptible to cell death.
- Metastatic breast cancer is known to upregulate the protein sorcin.
- Sorcin has been shown to bind doxorubicin and therefore limit its cytotoxic effect on cells.
- Dihydromyricetin (DMY) can be used as a chemosensitizer as it is known to competitively bind sorcin, therby allowing for freed doxorubicin to exert its full cytotoxic effect on breast cancer cells.

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**Recently Reported Dual-Drug Co-Delivery of Liposomal-Based Chemotherapeutics to Treat Breast Cancer**

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ABSTRACT

The use of nanocarriers such as liposomes to co-encapsulate more than one anticancer drug has been shown to be a promising strategy in cancer treatment with respect to overcoming multidrug resistance (MDR). This is in part not only due to the many advantages associated with the use of liposomes, but also the effectiveness of using this dual-drug strategy to overcome potential drug resistance when compared to a single chemotherapeutic. In this review, we report promising recently reported dual-drug co-delivery of liposomal-based chemotherapeutics in order to treat breast cancer and provide some perspectives on possible future constructs.

Keywords: Breast Cancer; Nanocarriers; Liposomes; Dual-Drug; Co-Delivery; Chemotherapeutics

Abbreviations: MDR: Multidrug Resistance; EPR: Enhanced Permeability and Retention Effect; Pgp- P- Glycoprotein; TNBC: Triple Negative Breast Cancer; Res: Resveratrol; PTX: Paclitaxel; DT: Docetaxel; TQ: Thymoquinone

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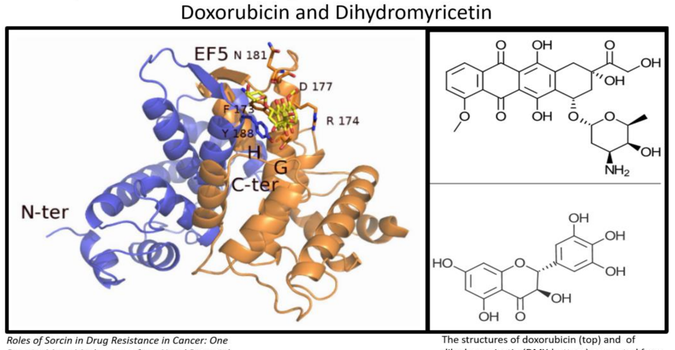
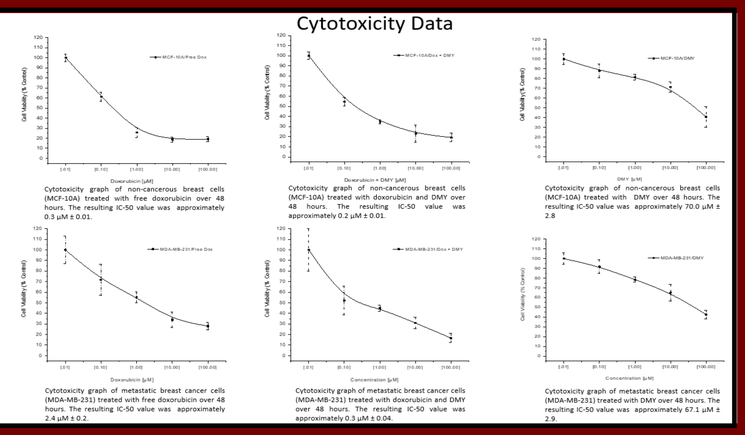
### Depiction of Sorcin Binding Doxorubicin and Structures of both Doxorubicin and Dihydromyricetin




*Roles of Sorcin in Drug Resistance in Cancer: One Protein, Many Mechanisms, for a Novel Potential Anticancer Drug Target*. Theo Battista, Annarita Fiorillo, Valerio Chiarini, Ilaria Genovese, Andrea Ilari, and Gianni Colotti, 2020.

The structures of doxorubicin (top) and of dihydromyricetin (DMY-bottom) generated from the ChemDoodle program.

### Cytotoxicity Data




Cytotoxicity graph of non-cancerous breast cells (MCF-10A) treated with free doxorubicin over 48 hours. The resulting IC-50 value was approximately 0.3 μM ± 0.01.

Cytotoxicity graph of non-cancerous breast cells (MCF-10A) treated with doxorubicin and DMY over 48 hours. The resulting IC-50 value was approximately 0.2 μM ± 0.01.

Cytotoxicity graph of non-cancerous breast cells (MCF-10A) treated with DMY over 48 hours. The resulting IC-50 value was approximately 70.0 μM ± 2.8

Cytotoxicity graph of metastatic breast cancer cells (MDA-MB-231) treated with free doxorubicin over 48 hours. The resulting IC-50 value was approximately 2.4 μM ± 0.2.

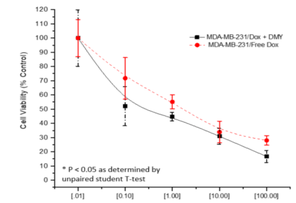
Cytotoxicity graph of metastatic breast cancer cells (MDA-MB-231) treated with doxorubicin and DMY over 48 hours. The resulting IC-50 value was approximately 0.3 μM ± 0.04.

Cytotoxicity graph of metastatic breast cancer cells (MDA-MB-231) treated with DMY over 48 hours. The resulting IC-50 value was approximately 67.1 μM ± 2.9.

The theory involving dual drug co-delivery of liposomal-based chemotherapeutics and the rationale behind using both doxorubicin and dihydromyricetin (DMY) (top), depiction of Sorcin binding doxorubicin and structures of both doxorubicin and DMY (middle), and the resulting cytotoxicity data involving free doxorubicin, doxorubicin and DMY, as well as free DMY against both non-cancerous cells (MCF-10A) and metastatic breast cancer cells (MDA-MB-231) (bottom).

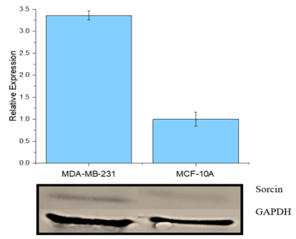
### Cytotoxicity Data and Sorcin Upregulation

| Cell Line | Free Dox | Dox + DMY | Free DMY |
|---|---|---|---|
| MCF-10A | 0.3μM ± 0.01 | 0.2μM ± 0.01 | 70.0μM ± 2.8 |
| MDA-MB-231 | 2.4μM ± 0.2 | 0.3μM ± 0.04 | 67.1μM ± 2.9 |




* P < 0.05 as determined by unpaired student T-test

Cytotoxicity graph of metastatic breast cancer cells (MDA-MB-231) treated with doxorubicin and DMY (black) and free doxorubicin (red) over 48 hours. The resulting IC-50 values were approximately 0.3 μM ± 0.04 and 2.4 μM ± 0.2 respectively. Statistical analysis of the values using an unpaired student T-test indicates a P value of less than 0.05 indicating a significant difference in the IC-50 values.




Western blot graph depicting the relative expression of sorcin in metastatic breast cancer cells (MDA-MB-231) and non cancerous breast cells (MCF-10A).

IC 50 data using free doxorubicin, doxorubicin and DMY, as well as free DMY against both non-cancerous cells (MCF-10A) and metastatic breast cancer cells (MDA-MB-231) (top), cytoxicity graph with overlaid free doxorubicin and doxorubicin plus DMY against the MDA-MB-231 cell line, as well as well as the western blot data depicting the sorcin upregulation in the MDA-MB-231 cell line compared to MCF-10A cells.

## Conclusion and Future Work

Although we have found increased fluorophore accumulation in metastatic breast cancer cells compared to non-cancerous cells, we have also observed an incremental decrease in fluorophore accumulation with increasing size of the PEG moiety, suggesting a slight steric hindrance between our peptide and the receptor. Therefore, in this study we are currently working on the co-encapsulation of dual-drugs into our targeted formulation in order to recapture some of the presumed loss of overall drug efficacy attributed to the necessary pegylation prior to in vivo use. Specifically, we propose the co-encapsulation of the chemosensitizer drug dihydromyricetin (DMY) and the cytotoxic agent doxorubicin. In this study, we have thus far demonstrated a synergistic effect using both free doxorubicin and free DMY against metastatic breast cancer cells (MDA-MB-231). Future work involves the co-encapsulation of these drugs into our unique novel targeted liposomal-based formulation for improved drug delivery and overall efficacy when used to treat metastatic breast cancer.

## Acknowledgements

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