# “Multifunctional Lyotropic Liquid Crystalline Nanoparticles for Gemcitabine and Thymoquinone Delivery in the treatment of Breast Cancer”

**Ms. Loo Yan Shan**
Dr. Intan Diana Binti Mat Azmi
Dr. Armania Binti Nurdin
Faculty of Science
**Universiti Putra Malaysia**

Breast cancer is a leading cause of cancer death in women worldwide. According to GLOBOCAN data in year 2020, an estimated 2.3 million new cases of female breast cancer and 685,000 deaths occurred, which surpassed that of lung cancer as the most commonly diagnosed cancer worldwide. About 70–80% of breast cancers are oestrogen receptor-alpha (ER-α) positive and are dependent on oestrogen for growth. Thus, for patients presenting with hormone receptor-positive (HR+) breast cancer without visceral crisis, selective oestrogen receptor modulators and degraders (SERMs/SERDs), and aromatase inhibitors (AIs) have been the recommended standard-of-care. Progression on first-line endocrine therapy in a subset of breast cancer cases and recurrence caused by invasive lesions, however, has remained a major impediment requiring optimal treatment strategies. Lyotropic liquid crystalline nanoparticles (LLCNs) are internally self-assembled (ISA)-somes formed via self-assembly of amphiphilic molecules in a mixture composed of a lipid-based fraction, stabilizer and/or surfactant, and aqueous media/dispersant. LLCNs are capable of phase transitions in response to hydration and/or externally-applied fields to form ordered mesophases. This unique modality presents tunable nanostructures for various biomedical functions. In this study, we investigated multifunctional LLCNs for gemcitabine and thymoquinone (Gem-TQ) co-delivery and targeting to tamoxifen-resistant (TamR) hormone receptor-positive (HR+) breast cancer cells by surface modification with vitamin D3-polyethylene glycol (VD-PEG). LLCNs were prepared using soy phosphatidylcholine (SPC), phytantriol (PHYT), and glycerol monostearate (MYVR), in optimized ratios containing citrem or Poloxamer 407. The series of nanoformulation exhibited composition-dependent hydrodynamic particle sizes of 200 – 500 nm, lattice parameters between 4.8 – 8.0, negative surface charge, mesophases of either hexagonal, cubic, or micellar form, high entrapment efficiency, and controlled drug release. Low cytotoxicity of SPC-citrem LLCNs were shown in MCF10A cells consistent with modulation of hemocompatibility, and were, therefore, selected for co-encapsulation prior to in vitro antitumor and cellular uptake analyses. Notably, inhibitory concentrations (IC50s) following 24 h treatment with drug-loaded SPC-citrem LLCNs (i.e., loading at a concentration ratio of 2:9 μM of Gem and TQ), were 34.7 μM (11.67 μM + 23.02 μM) and 33.5 μM (6.08 μM + 27.37 μM), while IC50s of VD-PEGylated drug-loaded SPC-citrem LLCNs were respectively, 27.03 μM (4.91 μM + 22.12 μM) and 9.2 μM (1.67 μM + 7.53 μM) in MCF7 and T47D-TamR cells (established by long-term treatment with tamoxifen). Synergistic interactions of Gem-TQ were retained for both nanoformulations. In addition, VD-PEG LLCNs upregulated the expression levels of caspase-3 and Akt1 (serine/threonine-protein kinase) in T47D-TamR cells. Cell cycle arrest at G2 phase was shown, whereby 15.25% of cell population were at G2 phase following treatment with VD-PEG LLCNs compared to 7.49% ascertained with the non-treated control cells. This study demonstrated the use of multifunctional biocompatible SPC-citrem LLCNs as a potential therapeutic nanodelivery system for Gem and TQ, with enhanced cellular uptake and anticancer activity against breast cancer cells.

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