Chapter 11: Conclusion

11.0 Conclusion

Cancer as a diversified group of diseases is characterized by abnormal proliferation of cells leading to heterogeneity among the affected cells. Current chemotherapeutic treatment options entailing the usage of conventional free drug cocktail therapy targeting multiple signalling pathways have proved to be less effective due to lack of spatial and temporal simultaneous presence at the tumour site. Nanoparticulate ratio-mimetic combinatorial delivery of such agents may offer an efficient cancer cell load reduction.

The present study was aimed to investigate improvement in efficacy of clinically used PEGylated liposomal doxorubicin in NSCLC and TNBC by co-encapsulation of synergistic ratio of two drugs (doxorubicin and vincristine) in an optimized liposomal formulation. The study was divided into two parts: development of optimized dual loaded liposomal formulation; in-vitro as well as in-vivo evaluation of the biological characteristics of the co-loaded formulation.

The development of a stable liposomal formulation co-encapsulated with synergistic ratio of doxorubicin and vincristine for ensuring their temporospatial presence at the tumour site of the two solid tumors (triple negative breast cancer and non-small cell lung cancer) was intended. The synergistic ratio against the two cancers was established using in-vitro cytotoxicity studies by treatments of drug combinations (10:1 to 1: 10 weight ratios of DOX:VCR) in both the cell lines A549 and MDA MB231 using Chou-Talalay method. The best effective synergistic ratio against both carcinomas having the lowest cellular viability was determined.

Post determination of the combinatorial index in both the tumors, the most effective ratio was encapsulated in a single nanoliposome. The nanoliposome was developed using an array of OFAT studies to determine the factors responsible optimal CQA for encapsulation efficiency of both drugs, particle size, zeta potential and drug release. Causal factors including concentration of ammonium sulphate, pH of drug loading and lipid molar ratio was found to have significant effect on the tested parameters. These parameters were then evaluated using 2^3 full factorial design to understand the effect of the variation of the factors individually and together on the tested CQAs. The DOE based optimised formulation was then physico-

chemically characterized for the assessment of the stability of the carrier while drug release kinetics was evaluated simulating the blood and tumour conditions. The optimized co-loaded liposomal formulation exhibited more than 95% encapsulation of both drugs with particle size of 95.74±2.65 nm and zeta potential of -9.17±3.1 mV. The morphological evaluation using cryo-TEM showed the formation of spherical structures with the presence of characteristic gel strands inside the liposomes. The ATR-FTIR and microcalorimetry of the formulation showed the lack of any physicochemical interactions besides presenting the characteristic properties of the components which were confirmed using Small angle X-ray analysis (SAXS). Further, drug release from the combinatorial carrier at the tested conditions showed controlled release of the individual agents in a manner similar to that of the single liposomes but significantly different from the naked drugs. The tested parameters of nanocarrier indicated towards sufficient in-vitro stability at the storage conditions, stability and lack of interaction potential with blood components. The optimized liposomal formulation presented non-significant difference in physicochemical and biochemical characteristics and stability to the clinically used standard. Additionally, the newly formulated liposomal suspension was predicted to present 18M stability similar to approved product besides presenting with ease in scalability for manufacturing. The single drug and dual drug loaded liposomes were prepared using the parameters derived from DOE studies for further biological evaluation.

Next, the optimized formulation was tested for determination of the in-vitro and in-vivo biological characteristics of ratio-mimetic VCR co-loading into the clinically used pegylated liposomal DOX. The in-vitro cell viability studies of this formulation in both tumors showed significantly improved cytotoxicity potential of the drugs when co-encapsulated in a single carrier as compared to neat drugs, individual liposomal carriers and combination of individual liposomal components. The cellular uptake studies in A549 and MDA-MB 231 using confocal microscopy and flowcytometry show significantly increased uptake of the dual drug formulation as against the liposomal DOX (as well as all other formulations). This resulted in significantly increased cell cycle arrest in G2/M phase with subsequently increased apoptosis and reduced cell viability in both tumor cell lines when presented with co-loaded formulation than with the single drug liposome.

The new liposomal carrier exhibited similar acute toxicity, pharmacokinetic and tissue distribution profiles with significant increase in tumor regression as compared to currently used liposomal doxorubicin.

These results indicate towards the fact that co-encapsulation of VCR into clinically used pegylated liposomal DOX significantly improved the in-vitro and in-vivo therapeutic efficacy of the latter against NSCLC and TNBC without having a significant change in the physicochemical and biological characteristic properties of the currently approved carrier.

The results showed improved efficacy upon VCR incorporation into currently available therapeutic standard against NSCLC and TNBC. These studies indicate towards extension of therapeutic potential in NSCLC and TNBC of clinically used standard post VCR incorporation and rationale for continued investigation of therapeutic potential of such combinatorial formulation. Thus, ratio-mimetic co-encapsulation of the drugs in combinatorial dual drug loaded liposomal formulation may help in improving their spatial co-presence at the site of action in tumours as compared to the single liposomes of the agents and neat drugs leading to better therapeutic outcomes in both these solid tumors.