Chapter 5

Summary and Conclusions

5.1. Summary

The research in the present thesis was inspired by the dose-related issues associated with clinically used anticancer medicines. All standard anticancer drugs are cytotoxic and can even damage normal cells, leading to severe non-specific cytotoxicity. The main objective of the targeted drug delivery in cancer is to deliver the anticancer drugs to the specific tumor site in the desired concentration. Nanomedicine can be employed via passive targeting (primary targeting), active targeting (secondary targeting) or both to reduce the severe non-specific toxicity of anticancer medications. It has been demonstrated that the commonly used taxane derivatives such as paclitaxel and DXL may increase the survival rate of cancer patients by reducing tumour volume. However, prolonged treatment of patients with taxanes leads to the development of drug resistance which can decrease the overall efficacy of the treatment. Therefore, USFDA authorized CZT in 2010, a semi-synthetic derivative of taxane that demonstrated a minimum level of drug resistance in cancer patients.

CS is a naturally occurring polymeric carbohydrate, having a positive surface charge, non-toxic and biodegradable. It has good mucoadhesion capabilities due to its cationic composition, which allows it to interface with the mucous membranes. Chitosan only dissolves in acidic media; however, its solubility is pH-limited. Numerous non-ionic surfactants such as TPGS, cremophor EL, and Tween-80 were used in developing nanocarriers that have enhanced the pharmacokinetics of several medications by suppressing P-glycoprotein (P-gp) efflux pumps. Among these, TPGS is an amphiphilic, naturally occurring vitamin-E derivative that has received USFDA approval and has an HLB value of 13.3. TPGS is primarily used as an emulsifier, stabilizer, and absorption or permeation enhancer. In fact, the surface modification of nanomedicine by TPGS

increases their stability by avoiding the opsonization process and hence improves the half-life of the drugs in the blood. Recently, researchers have developed tailored nanomedicine to treat lung cancer and have often used the targeting ligand of a single receptor, which has shown promising results. However, the biological response of single receptor-targeted nanomedicine is relatively constrained because of the significant heterogeneity (intra and inter-heterogeneity) in lung cancer.

As lung cancer is dense and solid, nanomedicine targeting a single receptor has little chance of penetrating the tumour. Therefore, nanomedicine targeting dual receptors can treat lung cancer more effectively. It has been reported that EGFR and folate receptors (FR) are highly overexpressed in lung cancer. Hence using both of these ligands, such as CTXmab for EGFR and folic acid for folate receptors, can improve the therapy. The dual-receptor targeted approach for treating lung cancer can overcome the dose-related issues of DXL and CZT. Therefore, the development of DXL and CZT-loaded chitosan-based nanoparticles with a dual receptor targeting approach is proposed here as a novel aspect of this research work.

The dual-receptor targeted CS and CSA based nanoparticles were prepared by solvent evaporation followed by the ionic gelation method. The prepared formulations were characterized for physicochemical parameters such as particle size analysis, scanning electron microscopy, transmission electron microscopy, and atomic force microscopy. Also, surface chemistry, *in-vitro* release, cellular uptake and cytotoxicity studies were performed. The particle size, polydispersity and surface charge were characterized, and the values were acceptable. The XPS analysis verified that CTXmab and folic acid were present on the surface of the CS-NPs. When tested *in-vitro*, the extent of DXL and CZT released from the CS-based nanoparticles were slightly higher in PBS pH 5.5 than in PBS

pH 7.4, which is attributed to higher solubilization of the chitosan in an acidic environment, implying that drug will release more in the cancerous acidic microenvironment. The cytotoxicity study was performed on A-549 and SIRC cells (control). The results demonstrated that the IC_{50} value of DXL loaded dual-receptor targeted CS-NPs was about 34 times lesser than that of DXL control. Further, it was about 38 times lesser for CZT loaded dual-receptor-targeted chitosan alginate nanoparticles than CZT control. The dual-receptor targeted nanoparticles of both drugs exhibited synergistic cytotoxicity in the lung cancer cell line, which may be due to their targeted delivery via folate as well as EGF-receptor-mediated endocytosis. The endocytosis mechanism of both receptors is exhibited through caveolae. CZT is a dimethyl derivative of DXL, bearing methoxy groups in place of hydroxyl groups at positions C-7 and C-10. These modifications confer two advantages on CZT over DXL. Firstly, CZT, a P-glycoprotein substrate, has a higher lipophilicity than docetaxel (logP 3.9 versus 3.2), resulting from the conversion of two secondary alcohols to more lipophilic ethers. This may result in increased cell penetration through passive influx, consequently leading to better activity in resistant cell lines where the permeability of the plasma membrane may be altered. Therefore, as compared to DXL-loaded dual-receptor targeted CS-NPs, CZT-loaded dual-receptor-targeted chitosan-alginate nanoparticles exhibited higher (~2-fold) cytotoxicity against A-549 cells.

The cellular uptake study of pure-C6 and C6 loaded formulations was performed against A-549 cells, and the results demonstrated that the C6 loaded dual receptor-targeted nanoparticles showed the highest area of the green channels than pure C6, C6 loaded non-targeted, FR targeted, and EGFR targeted CS-NPs.

Further, pharmacokinetic evaluation of both nanoparticle formulations was performed in Wistar rats, and all the nanoparticle formulations for both drugs have exhibited improved pharmacokinetics than their respective marketed formulations. The total numbers of nuclei on the HE-stained microscopic slides of each treatment group were counted in order to assess the anticancer efficacy of the DXL and CZT-loaded formulation on the B(a)P induced lung cancer mouse model. The dual-targeted CS nanomedicine demonstrated significant enhancement of anticancer efficacy and extended survival rate when compared to other formulations. The dual-receptor targeted nanoparticles of DXL and CZT decreased the nucleus number in B(a)P induced lung cancer mice by 56.86 % ± 7.18 and 64.68 % ± 2.21 respectively, which demonstrates the higher potency to CZT as compare to DXL. Thus, the findings of the proposed study demonstrated that dual-receptor nanomedicine of DXL and CZT might be more effective than their clinical formulation for the treatment of lung cancer with reduced side effects.

5.2. Conclusion

The objective of present thesis was to develop and characterize dual-receptor targeted CS-NPs and CSA-NPs of DXL and CZT respectively for physicochemical characteristics, *in-vitro* release, cellular uptake, cytotoxicity, *in-vivo* pharmacokinetics, *in-vivo* histopathology studies in rats, and *in-vivo* anticancer efficacy in B(a)P induced lung cancer mice model after i.v. administration.

- The particle size and polydispersity of both the formulations were within acceptable limits.
- The zeta potential of CSA-NPs was higher (+25 to +32 mV) than that of CS-NPs, indicating their higher stability.

- The morphological assessment by SEM, TEM and AFM analysis showed that CS-NPs and CSA-NPs were spherical and monodispersed.
- The XPS survey demonstrated the surface chemistry of CS-NPs and CSA-NPs, and the result supports the presence of folic acid and cetuximab on the surface of nanoparticles.
- The *in-vitro* drug release profile of DXL and CZT in chitosan and CSA-NPs showed the pH-dependent drug release with a rapid release at pH 5.5 than pH 7.4
- The C6 loaded dual-receptor targeted nanoparticles of CS and CSA showed enhanced cellular uptake as compared to pure C6, non-targeted, and single-receptor targeted nanoparticles in A-549 cells.
- The CZT-loaded dual-receptor-targeted CSA-NPs displayed 2-fold greater cytotoxicity against A-549 cells than DXL-loaded dual-receptor targeted CS-NPs.
- The pharmacokinetic evaluation of both nanoparticle formulations was performed in Wistar rats, and all the nanoparticle formulations for both drugs have exhibited improved pharmacokinetics than their respective marketed formulations.
- The *in-vivo* histopathological evaluations of dual receptor-targeted nanoparticles of both drugs have demonstrated better safety in Wistar rats as compared to their marketed formulations.
- The dual-targeted CS-NPs and CSA-NPs of DXL and CZT demonstrated significant enhancement of anticancer efficacy and extended survival rate when compared to their respective marketed formulations.
- The results obtained from *in-vivo* anticancer studies are in absolute agreement with those observed in *in-vitro* toxicity studies of both the dual-receptor targeted formulations showed significant reduction in the cell number which was measured

in terms of percent cell viability in MTT assay in A-549 cells and as reduction in cell number in the HE stained microscopic slides of lungs of respective animal groups.

5.3. Future Perspective

In future, we want to explore the dual-receptor targeted nanomedicine of other clinically used anticancer drugs in the combination of multiple targeting ligands specific to lung cancer. The focus of our future research is to develop dual-receptor targeted and multiple-receptor targeted nanomedicines, which can be beneficial to large number of patients based on the receptor expression levels in specific cancer types. We also want to integrate dual-targeting nanomedicine with theranostics to improve diagnostic outcomes since it is anticipated that dual-receptor targeted nanomedicine would accumulate selectively in cancer locations. We also want to evaluate and establish the application of these targeted nanomedicine in other types of lung cancer such as squamous cell carcinoma, large cell lung carcinoma and small cell lung carcinoma after their successful development by chemical carcinogens in mice.