Chapter 5

Summary and Future Prospective

5 Summary and Future prospective

5.1 Summary

Cancer is the most prevalent cause of death on a global scale. Among all cancer types, lung cancer is regarded as the most deadly and extremely metastatic. GMC is a potent anticancer medication used to treat lung cancer. However, GMC has been reported to undergo quick metabolism and shorter half-life with minimal selectivity towards cancerous tissue, therefore extra dose and frequent dosing regimens lead to increased toxicity. For achieving effective and specific administration of drugs into cells promising approaches are required based on multiple targeting moieties or ligands that attach specifically to the surface of cells. Significant targeting can be achieved by both single receptor and multiple receptor based targeting and functionalization of nanoparticles with the specific ligands. This helps in addressing the varied biology and complexities associated with lung cancer that sometimes challenge the therapeutic efficacy of single receptor based targeting of nanotherapeutics. In order to achieve higher targeting, we developed glycan receptor targeted as well as glycan and EGFR dual receptor targeted nanoparticles for selective and site specific delivery of anticancer drug to lung cancer cells.

Biopolymeric nanoparticles of CSN encapsulated with anticancer drug GMC conjugated with overexpressed receptor specific targeting ligands Cxmab and Neu5AC were prepared by ionic gelation technique. The Neu5AC were successfully conjugated with CSN using classical carbodiimide chemistry. The monoclonal antibody Cxmab was conjugated to the CSN and Neu5Ac was conjugated CSN-NPs via electrostatic interaction. Box-Behnken design was applied for response surface optimization of CSN-NPs and; also for the amount of Cxmab that can be efficiently conjugated to CSN-NPs. The design yielded appropriate particle size, PDI, zeta potential and entrapment efficiency of the CSN-NPs alongwith efficiently high amount of conjugation of Cxmab to CSN-NPs. EGFR and glycan receptor targeted GMC loaded CSN-NPs exhibited acceptable and homogeneous size distribution. Furthermore, the nanoparticles had an optimal surface charge, which is crucial for ensuring their stability during extended period of storage. Zeta potential of targeted CSN-NPs reduced because Neu5Ac and Cxmab have negative charges that interact with the cationic charge on the

surface of CSN. Targeted and non-targeted CSN-NPs exhibited good entrapment efficiency. Morphological studies by SEM, TEM and AFM demonstrated uniform, spherical and smooth surface particles. Solid state characterization studies by FTIR, DSC and XRD showed the presence of GMC in the targeted and non-targeted nanoparticles. In addition, XPS analyses were carried out to determine the surface chemical makeup of NPs. The XPS results suggested the conjugation of Neu5Ac and Cxmab on the surface of CSN-NPs based on greater nitrogen content on the surface of targeted NPs compared to non-targeted NPs. Drug release profile of the targeted and non-targeted CSN-NPs showed faster release at cancerous microenvironment pH 5.5 than physiological pH 7.4 that confirmed the prolonged and pH dependent release of the nanoformulations. The dual receptor targeted CSN-NPs showed higher drug release at pH 5.5 due to tissue specific receptor mediated endocytosis. *In vitro* cellular uptake studies of coumarin-6 loaded formulations on A-549 cells were performed by confocal microscopy. The dual receptor targeted NPs exhibited higher green fluorescence intensity which confirmed greater cellular uptake compared to single receptor targeted, non-targeted NPs and GMC control. *In vitro* cytotoxicity was performed on A-549 lung cancer cell lines, which confirmed a reduction in viable cells and showed that the percentage of inhibition of cells was significantly greater when treated with dual receptor targeted NPs compared to single receptor targeted, non-targeted NPs and pure drug solution. *In vitro* wound healing assays on A-549 cells were employed extensively to determine the efficacy of the different treated nanoformulations on cellular movements, which is a critical stage for the spread of cancer. The dual receptor targeted NPs resulted in less shrinkage of scratched wound and prevented the higher migration of cells up to 72 h than single receptor targeted, non-targeted NPs. *In vitro* apoptosis studies was performed using Annexin-V/PI staining on A-549 cell. The dual receptor targeted NPs showed higher apoptosis than single receptor targeted, non-targeted NPs and pure GMC, which was noticeable from higher number of apoptotic bodies in Annexin-V/PI stained cells. Further, the *in vivo* pharmacokinetic study of the formulations were conducted on Wistar rat. The various pharmacokinetic parameters were calculated by non-compartmental model using kinetica software and plasma drug concentration versus time graph plotted by GraphPad Software. The dual receptor targeted NPs exhibited higher bioavailability, extended circulation and higher retention as well as sustained release of GMC than single receptor

targeted, non-targeted NPs and pure GMC. The tissue bioditribution, *in vivo* and *ex vivo* fluorescent bio-imaging and histopathological examination on the vital organs such as lungs, liver, kidney and heart of swiss albino mice was evaluated for glycan receptor CSN-NPs in comparison with non-targeted and GMC control. The result showed that glycan receptor targeted CSN-NPs showed the better lung targeting ability and proved to be less toxic upon intravenous administration than GMC loaded CSN-NPs and pure GMC. Further, the *in vivo* efficacy studies of the GMC loaded targeted and non-targeted CSN-NPs were conducted on B[a]P induced swiss albino mice in which the parameters like histopathological observations of cancerous lesions, safety (survival) and number of cancerous cells were evaluated. The dual receptor targeted NPs exhibited few small nodules, significant less number of lung cancerous cells and higher survival rates by decreasing *in vivo* toxic effects of GMC in healthy tissues and showed better therapeutic efficacy than single receptor targeted, non-targeted NPs and pure GMC. Therefore, dual receptor targeted nanomedicine can increase the effectiveness of anticancer drugs with the probability of being associated with less severe side effects.

5.2 Future research perspectives

The present study pertains to the design as well as the development of glycan and EGFR receptor targeted nanoparticles, which should take into consideration their safety, bio-compatibility, and stability, along with their reproducibility after *in vivo* application. The *in vitro* and *in vivo* findings demonstrated highly assuring results. However, the present research suggests that the produced prototype formulation be put through more detailed examination in *in vivo* cancer induced experiments on animals in coming years in order validate the long term therapeutic outcome. Considering the positive preclinical results, glycan and EGFR receptor targeted nanoparticles require additional experimentation for successful translation into clinics and the possibility of large-scale manufacturing for lung cancer therapy.