Conclusions

- It can be concluded from the above results that minicell producing bacterial strain *E.coli* PB 114 has been selected on the basis of high minicells production capacity among two other strain namely, *E.coli* K-12 x984, *E.coli* p678-54.
- New method for minicells purification was developed by combining ceftriaxone lysis and filtration. (Jivrajani et. al., 2013, journal of microbiological methods vol. 92, 2013).
- Segregation of shRNA expression vector into the minicells was confirmed.
- psNIPERDH1A1 and psNIPERDU6A2 have been validated for silencing VEGF-A mRNA in A549, LNCaP, Hela and KB cell line which was confirmed by RT-PCR.
- shRNA has been successfully packaged with a ~120-140 plasmid copy per minicells which was confirmed by plasmid DNA isolation from purified minicells.
- Folic acid was successfully conjugated on the minicells surface and amount of conjugated folic acid was quantified.
- Folic acid conjugated minicells have been successfully taken up by folate receptor overexpressing cell lines like LNCaP, HeLa and KB by receptor mediated endocytosis.
- Moreover, after effective *in vitro* delivery of shRNA (psNIPERDU6A2), it has efficiently silenced the VEGF-A mRNA.
- Immunosuppressive mice model was successfully developed in the C57 BL6 mice which was confirmed by significant decrease in total WBC and lymphocyte count.
- Tumor xenograft model was successfully developed in immunosuppressive mice using A549, LNCaP and KB cell lines. Presence of malignant tumor was confirmed by histopathology.
- Folic acid conjugated minicells have efficiently deliver shRNA specific for VEGF-A into the tumor after systemic delivery and silence the targeted gene.
- Hence, in both *in vitro* and *in vivo* delivery experiments folic acid conjugated minicells have effectively delivered shRNA in folate receptor overexpressing cancer cells which was confirmed by reduced gene expression and tumor regression.
- Minicells without folic acid conjugation(minicells<sub>psNIPERDU6A2</sub>) have failed to deliver shRNA and minicells packaged with control shRNA vector (<sup>FA</sup>minicells<sub>Scramble</sub>) could
not silence the targeted gene. Hence, active targeting of minicells with folic acid and active shRNA are required to exert the VEGF-A silencing and tumor growth regression.

- **In vivo** biodistribution study proved that $^{FA}_{\text{minicells}}_{psNIPERDU6A2}$, actively targeted to tumor cell via surface folate receptor by folic acid, followed by receptor engagement and endocytosis.

- Novel and versatile targeted delivery system was developed for shRNA. Minicells can be packaged with large number of shRNA without any alterations as compared to other drug delivery systems like liposome and other polymeric nanoparticles. Coupling of folic acid to minicells surface protein is covalent and highly stable. This enables their specific targeting of cancer cell surface folate receptor. In addition, adhesion of the $^{FA}_{\text{minicells}}_{psNIPERDU6A2}$, complex to cell-surface folate receptors appears to trigger its rapid and efficient endocytosis, with subsequent minicell degradation and liberation of $psNIPERDU6A2$ into the cytosol and nucleus, where it exerts its gene silencing effect. As a consequence, targeted minicell-mediated shRNA delivery resulted in highly significant inhibition of VEGF A and even regression of tumor growth *in vivo* in mice with human prostate and cervical cancer xenografts. $^{FA}_{\text{minicells}}_{psNIPERDU6A2}$ were found to be safe in mice. None of the injected mice showed any adverse reaction. However, further detailed study is required to confirm this.