Chapter 5

Summary and Future Perspectives
5.1. Summary

The main objective of this research work was to develop polymer/protein nanomedicines capable of targeting the deregulated kinome, epigenome and leukemic stem cell population in AML, without causing much toxicity to healthy bone marrow cells. Extensive in vitro drug screening was carried out to identify most effective SMI that would exert its anti-leukemic activity in combination, or as single-agents. One of the key challenges of this strategy was the need of intracellular delivery of more than one drug molecules. To achieve this, we have carried out docking studies to select appropriate polymer/protein–drug combinations and developed both single carrier and multi-carrier (core-shell) nanoparticles under optimized processing conditions, and subjected to comprehensive physico-chemical characterization for their size, shape, zeta potential, drug loading and release properties. The anti-leukemic activity of these nanoparticles was assessed both in AML cell lines and patient derived cells and the toxicity was assessed using healthy bone marrow cells. Two main nanosystems that we have optimized are: (1) CD33 targeted polymer-protein core-shell nanomedicine to inhibit critically aberrant pro-survival kinases (mTOR, MAPK and STAT5) in primitive (CD34+ CD38−) AML cells (2) albumin bound vorinostat nanomedicine to address deregulated histone deacetylation patterns in AML cells. From the observed cues of stem cell targeting potential of nano-vorinostat, we have tested its specific anti-leukemic effect on CD34+ CD38− CD123+ fraction along with its hemocompatibility and synergism with another epigenetic drug, decitabine. Major conclusions drawn from the investigations carried out in this research work can be summarized as follows:

- Synthesized polymer-protein core-shell nanoparticles loaded with more than one drug molecules under optimized wet-chemical conditions. The size, shape and morphology were studied using transmission electron microscope revealing the formation of core-shell nanostructure with distinct inter-phase.

- The CD33 targeted core-shell nanomedicine exhibited targeted cellular uptake in AML cell line, KG1a compared to the unconjugated nanoparticles.
✓ Uptake of targeted nanomedicine in normal bone marrow cells was found insignificant, which implies its excellent targeting ability.

✓ Immunoblotting studies showed successful inhibition of deregulated mTOR, MAPK and STAT5 kinases by the core-shell nanosystem, leading to synergistic lethality against primitive (CD34+ CD38low) AML cell line KG1a.

✓ Compared to clinically used Ara-C/daunorubicin combination having very low efficacy to toxicity ratio (E/T) ratio (1.15), our CD33 targeted combinatorial core-shell nanomedicine exerted 8 times higher E/T ratio (8.29), suggestive of minimal toxicity to healthy bone marrow cells.

✓ Experiments of CD33-core-shell nanomedicine in patient samples showed mixed response, probably due to the heterogeneous nature of various kinase involvements in AML. Nevertheless, according to predominant molecular signatures identified in patient derived cells, this core-shell system could be used to encapsulate relevant anti-cancer agents suitable for a particular patient, enabling personalized treatment options.

✓ To target aberrant epigenome in AML, we have developed an albumin-vorinostat nanosystem by coacervation method. These NPs showed size ~100 nm and zeta potential -29.12 mV. This system exhibited enhanced HDAC inhibition leading to oxidative stress, cell cycle arrest and apoptosis in AML cell lines of varied FAB classes [CD34+ CD38 expressing KG-1a, promyelocytic HL-60 and FLT3-ITD harbouring MV4-11]. Most importantly this system registered excellent anti-leukemic activity against a heterogeneous population of 09 patient samples, including refractory and relapsed cases.

✓ Compared to clinically used Ara-C/daunorubicin combination having very low E/T ratio (1.15), 0.5 µM nano-vorinostat, which showed ~100% cytotoxicity in patient samples exerted 16 times higher E/T ratio (16.6) suggestive of excellent therapeutic index.
Nano-vorinostat also exhibited exceptional single-agent activity against the clonogenic proliferative capacity of bone marrow derived leukemic progenitors from patients (n=9) while sparing the normal bone marrow derived cells derived from healthy donors. This suggests its potential to target and stem cell population which is otherwise resistant to most of the anti-cancer drugs.

Separate experiments using flow cytometry confirmed that nano-vorinostat can ablate both CD34^{+} CD38^{-} CD123^{+} leukemic stem/progenitor and clonal blast population with almost same efficiency.

Free or nano-vorinostat presented excellent hemocompatibility with no significant myelosuppressive activity up to the tested concentration of 10 \mu M, whereas the anti-leukemic activity was observed in 0.1-2.5 \mu M range.

Decitabine treatment is a standard therapy option for elderly AML. However, many patient show refractoriness to decitabine. Low dose of nano-vorinostat treatment showed excellent synergistic lethality against refractory primary AML cells when tested with standard dose of decitabine.

Overall, our studies indicated that, by considering extermination of both primitive and blast leukemic cells, without causing any serious toxicity to healthy bone marrow cells, nano-vorinostat shows great promise as a single agent nanomedicine for clinical translation in the setting of a more patient compliant and effective epigenetic targeted therapeutic approach. In contrast, the core-shell nanomedicine capable of carrying multiple drugs is an ideal nanomedicine candidate for personalized treatment approach where the drug molecules for the nano-core and -shell need to be selected based on the molecular diagnosis of each patient.
5.2. Future perspectives

Mainly two nanoformulations were developed for targeting aberrant kinome and epigenome of AML. In the case of kinome targeted nanomedicine, its success lies with individual, molecular level identification of aberrant kinases in patients and selecting suitable drugs for loading in the core-shell system. Thus, the concept of rational design is very important for the success of kinome targeted nanomedicine approach. Hence, core-shell system is most suited for a personalized nanomedicine approaches. However, in the case of epigenetic targeted albumin-vorinostat nanomedicine, it is applicable for a wide range of AML patients, irrespective of their disease phenotype or prognosis because it is capable of targeting aberrations at the fundamental epigenomic level. Moreover, due to the pleiotropic anti-cancer effect of vorinostat, the nanoformulation could exert its activity over leukemic stem cell fraction as well. Thus, with the successful culmination of this research work, early stages of developing effective and more tolerable kinome, epigenome and LSPC targeted nanomedicines has been validated in cell lines and patient derived leukemic cells in vitro. However, to propose these nanomedicines for further clinical translation, future work should aim at establishing the above obtained results in in vivo conditions with focus on the following aspects:

- CD123 conjugation of nano-vorinostat to aid LSPC targeting in vivo.
- Assessing feasibility of targeted anti-LSPC functionality of nanomedicine in NOD/SCID mice models of AML.
- Extensive pharmacokinetics and pharmacodynamic studies in vivo.