Chapter I: Introduction: Cancer is a deadly disease with second highest mortality rate. Existing treatment strategies kill not only tumor cells but also non-cancerous healthy body cells leading to severe adverse side effects of cancer treatment. Among the modern therapeutic modalities, targeted chemotherapy and cancer immunotherapy (the therapeutic modality that exploit body's own immune systems) approaches are emerging out to be the most promising for combating the dreaded disease of cancer.

Targeted chemotherapy is the most widely used current treatment modality for cancer which involves selective delivery of anti-cancer chemotherapeutics (potent anti-cancer drug or siRNA) to tumor tissue. Distinguishing biomarkers over-expressed on tumor cell-surface are utilized for selective delivery of anticancer chemotherapeutics to tumor tissue with minimal harm to other healthy tissue.

Cancer Immunotherapy exploits body's own immune cells (e.g., dendritic cells, natural killer cells, T-cells, etc.) to fight against cancer. There are many types of cancer immunotherapy including monoclonal antibodies, oncolytic virus therapy, cancer vaccines, etc. Dendritic cell (DC) based genetic immunization (DNA vaccination) has shown lot of promises because of their unique potential to simultaneously induce both humoral and cellular immune responses against tumors. In DC-based DNA vaccination, dendritic cells (body's most promising antigen presenting cells) are transfected with plasmid DNA encoding distinguishing tumor associated antigen. DCs process the expressed tumor antigen and present the processed antigenic fragments on their cell surfaces with the help of major histocompatibility complexes MHC-I and MHC-II. MHC bound antigenic fragments on DC-surface are recognized by T cells and the activated T-cells thereafter get triggered to start rapid proliferation. The activated T-cells recognize the tumor cells expressing the particular antigen and induce intense humoral & cellular immune responses against the tumor cells. However, either only targeted chemotherapy or only DC-based Immunotherapy hardly results in long-lasting overall survivability of tumor bearing mice.

Liposomes, the spherical molecular assembly of amphiphiles (often having size within the range 100-200 nm) with an aqueous interior enclosed by lipid bi-layer, have long been used for targeted delivery of bioactives selectively to destined tissue under in vivo settings. In targeted chemotherapy, targeting of liposomally entrapped chemotherapeutics to tumor cells are achieved using liposomes made from amphiphilic molecule covalently connected with a tumor targeting ligands in their exo-surface regions. The liposomes covalently grafted with such a tumor-targeting ligand can target specific receptors over expressed in cancerous cells. Such tumor targeting liposomes can encapsulate both hydrophilic drug/siRNA (encapsulate inside aqueous compartment) & hydrophobic drug (encapsulate in hydrophobic lipid bi-layer) and deliver their therapeutic payloads/cargo (cytotoxic genes/siRNAs, small molecule anti-cancer drugs, etc.) to the cancerous tissues without affecting normal tissue (**Figure 1**). However efficient and selective targeting of potent cancer therapeutics to tumor cells/tissues remains a difficult challenge.

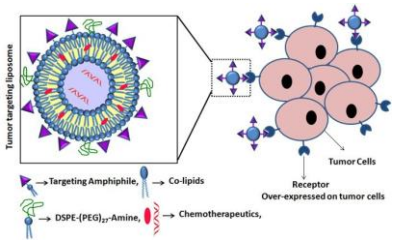

Figure 1: Cartoon for Tumor Targeting Liposomes.

In the present thesis, I report on the development and bio-activity evaluation of two new liposomal drug delivery systems for delivering potent cancer therapeutics to neuroblastoma and glioblastoma in mouse tumor models.

Chapter II: **Development of CDC20siRNA and Paclitaxel co-loaded new liposomal nanocarrier for combating neuroblastoma**

Despite significant recent progresses in neuroblastoma treatment, accomplishing markedly enhanced overall survivability remains an arduous task mostly due to tumor relapse and/or drug-resistant tumors. CDC20, a key cell cycle regulator protein required to complete mitosis, is over expressed in many cancer cells and therapeutic strategies based on selective delivery of CDC20siRNA to tumor & tumor vasculatures hold promise for combating cancer. Prior reports demonstrate that human neuroblastoma cells IMR-32 express functional GABAa receptor on their surface and nipecotic acid is a GABA related compound widely used as an inhibitor of both glial and neuronal GABA uptake. **Chapter II** of my doctoral thesis reports on the development of a novel GABAa receptor targeting circulation stable liposomal system prepared from nipecotic acid-derived cationic amphiphile (NACA) for combating neuroblastoma (**Figure 2**). I have demonstrated that liposomes of NACA can efficiently encapsulate siRNAs inside its inner hydrophilic core and protects them from attack by RNase. Findings in the cellular uptake study demonstrated that liposome of NACA delivers siRNA to human neuroblastoma IMR-32 cells via GABAa receptor. CDC20siRNA-loaded liposome of NACA silenced CDC20 gene expression in IMR-32 cell. Prior studies reported that silencing of CDC20 gene sensitizes cancer cells to paclitaxel (PTX). I have exploited this prior findings in my doctoral thesis work. Importantly, I have demonstrated that administration of NACA-liposomes containing co-encapsulated CDC20siRNA & PTX induces significant tumor growth inhibition in athymic nude mice bearing xenografted human neuroblastoma (**Figure 2**, taken from **S. Bhunia**, R.Vegesna and A. Chaudhuri, manuscript accepeted in **Nanoscale, 2016**, DOI: 10.1039/C6NR07532K, Accepted manuscript on line published on December 6, 2016).

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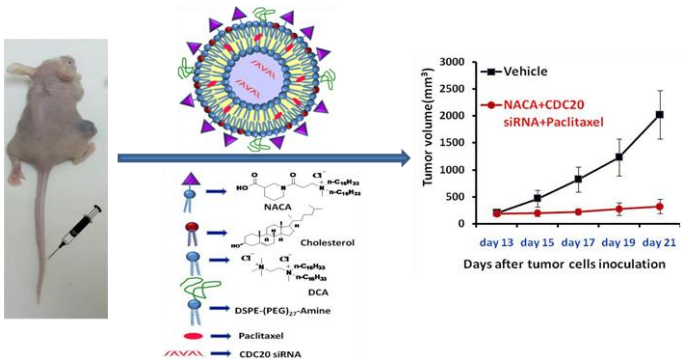

Figure 2: Intravenous administration of CDC20 siRNA and paclitaxel (PTX) co-encapsulated within liposomes of NACA significantly inhibits growth of human neuroblastoma xenografted in athymic nude mice.

Chapter III: Combating orthotopically established mouse glioblastoma using targeted Chemotherapy in combination with in vivo dendritic cell targeted cancer immunotherapy

Despite remarkable advances in glioblastoma treatments during the past decade, accomplishing significantly enhanced overall survivability (OS) remains a formidable challenge. STAT3, a key signal transduction protein, is activated in numerous cancer cells including glioblastoma. Inhibition of STAT3 leads to apoptosis in tumor cells and enhanced DC-maturation (through up regulated expressions of MHC class II, co-stimulatory molecules CD80, CD86, etc.). Major obstacle for systemic chemotherapy of glioblastoma is the presence of Blood Brain barrier (BBB) which limits entry of chemotherapeutics to brain tumor cells. Recent approaches for delivering potent

chemotherapeutics selectively to brain tumors are exploiting some receptor and transporter molecule over expressed on the brain capillary endothelial cells (BCECs) of BBB. Large Amino Acid Transporter type -1 (LAT-1) is abundantly and selectively expressed not only on BCECs of BBB but also in many types of cancers including glioblastoma multiforme. **Chapter III** of the present thesis delineates development of a novel BBB-crossing liposomal drug carrier for targeting potent chemotherapeutics selectively to orthotopically established mouse glioblastoma. The liposomes were prepared using a novel amphiphilic molecule (Amphi-DOPA) containing the well known BBB-crossing L-3,4-dihydroxyphenyl alanine (L-DOPA, a potent drug for treatment of Parkinson's disease that crosses BBB via LAT-1) moiety covalently grafted in its polar head-group region and two n-hexadecyl hydrophobic tails in its non-polar region. I have demonstrated that liposomes of Amphi-DOPA containing encapsulated WP1066 (a commercially available small molecule STAT3 inhibitor) significantly inhibit growth of orthotopic glioblastoma in BL6J/C57 mice by down regulating p-STAT3 under in vivo conditions. Furthermore, I have shown that targeted chemotherapy using WP1066-loaded liposomes of Amphi-DOPA, when used in combination with *in vivo* dendritic cell targeted genetic immunization (with a recently reported *in vivo* DC-targeting liposomes from our group in complexation with survivin encoded DNA vaccine), elicits significantly enhanced overall survivability of orthotopic glioblastoma bearing mice

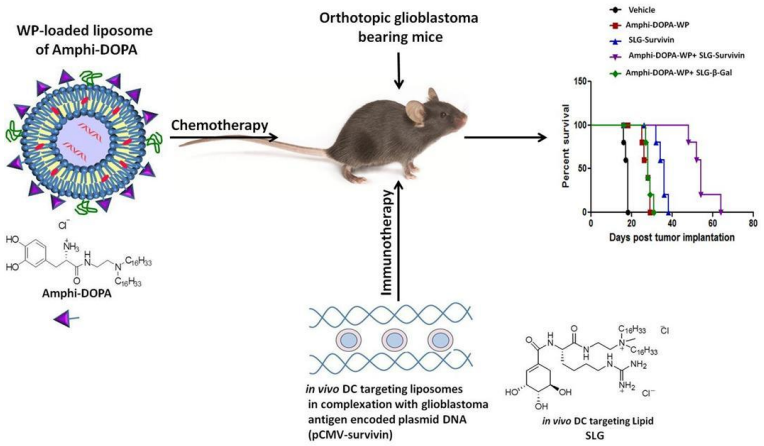

Figure 3: Targeted chemotherapy in combination with *in vivo* dendritic cell targeted DNA vaccination leads to significant enhancement of survivability in orthotopic glioblastoma bearing mice.

Conclusion: The present thesis describes design, synthesis and bio-activity evaluation of two novel amphiphilic molecules. The liposomes of the first amphiphile, upon intravenous administration, showed efficient targeting of their drug payloads selectively to neuroblastoma and causes significant tumor growth inhibition. Intravenously administered liposomes of the second amphiphile resulted into significant inhibition of orthotopically established mouse glioblastoma. The findings described in **Chapter III** of the present thesis demonstrate that targeted chemotherapy in combination with *in vivo* dendritic cell targeted DNA vaccination leads to significant survivability enhancement in orthotopic glioblastoma bearing mice.