Chapter 2:
Literature review

2.0 Introduction

2.1 Introduction

Cancer as a broad cluster of disorders may be defined as the abnormal uncontrolled growth of the cells with inherent ability to spread to other tissues of the human body facilitated majorly through the components of haematic systems. Amongst all cancers affecting the human system, American Cancer Society (ACS) estimates indicate lung cancer and breast cancer to be amongst the most plausible causes of newer cases and deaths for the year 2020 (1). The basal subtype of breast cancer, Triple negative breast cancer (TNBC) is characterized by the lack of expression of hormonal receptors (estrogen receptor and progesterone receptor) and tyrosine-protein receptor kinase human epidermal growth factor (HER2). This aggressive form is often associated with poor prognosis and lack of effective therapeutic treatments than the other subtypes while accounting for approximately 15% of the newly diagnosed breast cancer (BC) patients (2). When compared to others forms of BC, TNBC has often been associated with low overall survival rates, median progression free survival (PFS: 3-4 months) and high recurrence within the first three years of diagnosis. The enhanced possibility of delayed detection coupled with reduced survival rates and metastatic potential through lymphatic system has resulted in lung cancer being associated with a quarter of the cancer associated deaths (3). The most common type of lung cancer, non-small cell lung cancer (NSCLC) comprises of adenocarcinoma, large-cell carcinoma and squamous-cell carcinoma while having etiological presence in constituents of respiratory tract and presenting a low median survival time of 8.0 months (4). The low rates of early stage tumor detection, metastatic potential along with chemoresistance have reported for reduced rates of curability of these aggressive forms of cancers (5). While TNBC has been reported to have tumor cross-talk resulting in the development of secondary NSCLC, metastasis of NSCLC has been associated with bone, brain cancer, hepatocellular carcinoma and adrenocarcinoma (6, 7). Clinical research associated with NSCLC and TNBC indicate that late detection has often been associated with dysregulated autophagy, tumor heterogenicity, genetic mutations, alterations of intended drug targets resulting in lack of desirable therapeutic outcomes (8, 9). Importantly, stage of the neoplasm has been the major determinant of the type of the treatment option being used for these two cancers including surgery, radiation, conventional chemotherapy, targeted and immune-

therapeutics. The current chemotherapeutic treatment options for these diseases involve the usage of a cocktail of drugs with the approach of having multiple agents acting through multiple mechanistic pathways on the tumor cells (10). The treatment of such of cancers entail usage of multiagent-mechanistic treatment approach using free drug cocktail treatment options (10). These drugs act through different cellular pathways acting on multiple targets during different cell cycle phases ensuring a more efficient reduction in tumor cell survival (Figure 5) (10). While anthracycline and taxane based neo-adjuvant/adjuvant chemotherapeutic combination regimens have often been used for treatment of TNBC, the systemic delivery of lipophilic platin and taxane based cocktail of drugs have been one of the mainstays of NSCLC treatment regimen. Immune check point inhibitors along with drugs targeted against specific genes as well as biomarkers of NSCLC have also been used in combination with the conventional chemotherapeutic agents. Although, this conventional approach has provided some benefits in the therapy, it is saddled with the un-coordinated pharmacodynamic (PD) and pharmacokinetic (PK) profiles of these individual drugs being used. Consequently, the current therapeutic regimens have exhibited limited therapeutic efficacy, increased drug resistance and increased toxicity profile presenting low overall survival rates along with poor quality of life (11).

Additionally, the problem of lack of desired efficacy and the co-presence of all chemotherapeutic agents indicated for the chemotherapy in the desired synergistic ratio cannot be ascertained using the conventional combination therapy (13). Importantly, a rationalistic approach to selection from available naïve and controlled release formulations need to be considered while designing the therapeutic regimen. The evaluation of clinical risk-to-benefit ratio needs to be adopted for developing such effective therapies intended for eliciting the favourable response in TNBC and NSCLC (14). Further research activities indicate that strategies to improve the efficacy of TNBC and NSCLC treatment would encompass chemotherapy, gene therapy, immunotherapy, targeted therapy along with nanocarrier based delivery approaches (15, 16) . The current chapter intends to identify the clinical unmet need in these cancers and evaluation of potential benefits offered by the tested nano-formulation approaches. The effect of administration of single drug as well as combinatorial lipid and polymer-based nanocarriers against both neoplasms delivering multiple chemotherapeutics simultaneously to desired site of action has been mentioned. Finally, need of identification of

newer specific biomarkers, potential formulation design and challenges along with suitable opportunities for effective treatment of both cancers has been mentioned.

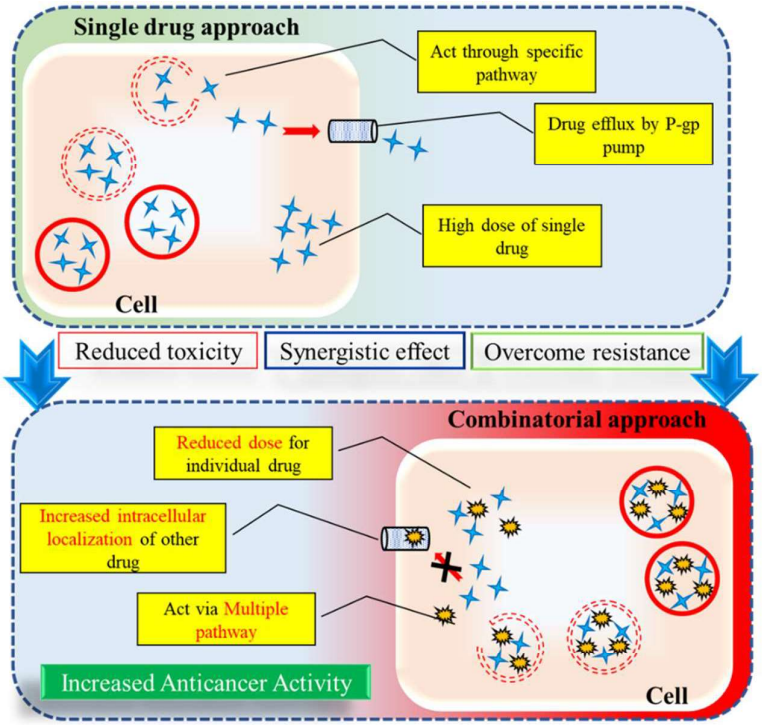

Figure 5: Advantages of Combinatorial approach over Single drug regimens (The figure has been reproduced with permission (12))

2.2 Current treatment regimens and options

Based on the stage of the carcinoma and degree of metastasis, the current therapeutic regimens may be subdivided into localized treatments (surgery, radiation) and systemic treatments (chemotherapy, immunotherapy, gene therapy, targeted therapy). Importantly, use of multidisciplinary approach to cancer treatments have presented a significant improvement in quality of lifestyle of cancer patient (17). Further, the treatment modalities of NSCLC and TNBC require integration of the conventional therapies with personalised medicine to target the specific biomarkers (18). The various treatment approaches for both these neoplasms have been indicated in Table 2. The classic treatment approach for localized therapy for both these neoplasms have been surgery and/or radiation therapy or combination. The prognostic effects

of mastectomy (TNBC) and pneumonectomy (NSCLC) have been identified by various researchers as being less invasive while preserving the aesthetics of the organs (19). External beam radiation therapy (EBRT) and radiofrequency ablation (RFA) have been used as components of radiotherapy for these cancers (20). The systemic treatments for both NSCLC and TNBC include the use of conventional chemotherapies along with immunotherapy and targeted therapy against the specific biomarker and oncogenes (Table 2).

Metastatic tumours have reported to have poor response rates and chemo-resistance generates the declining in treatment response (21). Apart from traditional surgery and radiation therapy, neoadjuvant chemotherapy have often been advised for TNBC (22). Amongst the widely used neoadjuvant therapy, anthracycline–cyclophosphamide (AC-scheme) chemotherapy has reported to be highly effective (23). In presence of BRCA mutations, the AC therapy, has presented an improvement in pathological complete response rate (pCR) with reduction in the relapse (24). Platins as components of neoadjuvant therapies have further enhanced the prognosis with higher pCR rates as compared with AC (25). The biomarker based molecular therapies commonly used including poly (ADP-ribose) polymerase (PARP) inhibitors, vascular endothelial growth factor (VEGF) inhibitors and epidermal growth factor receptor (EGFR) inhibitors have shown higher efficacy in reducing side effects (26). Importantly, VEGF and EGFR targeted therapeutics have shown better efficacies then PARP inhibitors and have often been combined with chemotherapeutic agents (27). The advent of adjuvant therapy has been based on genomic, proteomic profiles and clinical histopathological staging conditions of the patient post-surgery to prevent the occurrence of metastasis. Clinically, the incorporation of neoadjuvant therapy has presented with improved pCR rates and quality of life as compared to adjuvant chemotherapy (25). Taxane based therapies with capecitabine or ixabepilone have been approved for use in advanced and metastasised stages. However, the use of these agents has been associated with systemic and peripheral toxicity. Recently approved immunotherapy and gene therapy-based therapeutics such as ipilimumab (anti-CTLA-4), pembrolizumab (anti-PD-1) and atezolizumab (anti PD-L1) have been included in first line therapy with taxane based regimens.

The lack of diagnosis of NSCLC at early stages has been one of the potential reasons for its low overall survival time. Platin and taxane based chemotherapeutic regimens have often been used for the treatment of squamous and non-squamous NSCLC (28). Similar to TNBC,

specificity and selectivity to neoplasms have proved to be detrimental to effective treatment of NSCLC. Incorporation of monoclonal antibody-based therapies ipilimumab, pembrolizumab, nivolumab or atezolizumab in chemotherapy have been reported to have improved the treatment outcomes with increase overall survival and reduction in metastasis. Importantly, the use of immunotherapeutics have often been associated with the inherent immune-mediated toxicities such as pneumonitis, hepatitis, endocrinopathies, life-threatening opportunistic infections as well as embryo-fetal Toxicity (29).

Despite the advances in the immunotherapy and gene therapeutic options in both these cancers, the lack of specificity and selectivity towards the tumor cells along with toxicity necessitates the development of nanocarrier based passive and active targeted drug delivery systems for better effective management of the tumors.

Triple Negative Blood Cancer (TNBC)					
Local Treatment		**Systemic treatments**			
***Radiation**	***Surgery**	**Chemotherapy**	***Immunotherapy/ **Targeted therapy**	**$Gene therapy**	**References**
External beam radiation therapy (EBRT) • Whole breast radiation • Hypofractionated radiation therapy • Accelerated partial breast irradiation (APBI) • Intraoperative radiation therapy (IORT) • 3D-conformal radiotherapy (3D-CRT) • Intensity-modulated radiotherapy (IMRT) **<u>Brachytherapy</u> (internal radiation)** Intracavitary brachytherapy	1. Breast-conserving surgery (Syn : lumpectomy, quadrantectomy, partial mastectomy, or segmental mastectomy) or Mastectomy 2. Sentinel lymph node biopsy (SLNB) or axillary lymph node dissection (ALND) 3. Breast reconstruction 4. To relieve symptoms of advanced cancer	**1. After surgery (adjuvant chemotherapy)** **2. Before surgery (neoadjuvant chemotherapy)** PARP inhibitors Carboplatin, Cisplatin Other chemotherapeutics mTOR inhibitors Growth-factor inhibitors c.PD1/PD-L1 inhibitors Other immune checkpoint inhibitors d. mTOR inhibitors EMT-targeted therapy CSC-targeted therapy AXL inhibitor. d. PI3K inhibitors Antiangiogenic therapy Src antagonist e. Antiandrogen blockade CDK4/6 inhibitors Immune checkpoint inhibitors Examples: Anthracyclines, such as doxorubicin (Adriamycin) and epirubicin (Ellence), Taxanes, such as paclitaxel (Taxol) and docetaxel (Taxotere), 5-fluorouracil (5-FU) or capecitabine, Cyclophosphamide, Carboplatin	**Advanced therapeutic strategies** Passive, Active and Immunotherapy **Poly ADP-ribose polymerase (PARP) enzyme** inhibitors Avastin, Ibrance, Kisqali, Lynparza, Piqray, Trodelvy, Talzenna, Verzenio Eg.: **Anitbody-drug conjugate:** Sacituzumab govitecan	Metastasis suppressor genes (MSGs) and Metastasis promoter genes (MPGs). 1.Epidermal growth factor receptor and their inhibitors 2.Inhibitors of multiple receptors of EGFR 3.HER2 inhibitors: 4. Dual inhibitors of EGFR and HER2 5. Inhibition of the urokinase-type plasminogen activator system (uPA 6. Matrix metalloproteinases inhibitors 7. Histone acetyl transferases (HATs) and histone deacetylases (HDACs) insulin-like growth factor and insulin-like growth factor inhibitors (IGF-IR)	(29) (30)

Interstitial brachytherapy		**3. For advanced breast cancer:** Taxanes, such as paclitaxel, docetaxel, and albumin-bound paclitaxel, Anthracyclines (Doxorubicin, pegylated liposomal doxorubicin, and Epirubicin), Platinum agents (cisplatin, carboplatin), Vinorelbine, Capecitabine, Gemcitabine, Ixabepilone, Eribulin **4. Dose-dense chemotherapy:** Doxorubicin (Adriamycin) and cyclophosphamide, followed by weekly paclitaxel. **5. Nanocarriers:** Liposomal doxorubicin (Doxil™), albumin-bound paclitaxel or Nab-Paclitaxel (Abraxane™).		8.Vascular endothelial growth factor(VEGF) and five glycoproteins VEGFA, VEGFB, VEGFD, and placental growth factor.	
Non-Small Cell Lung Cancer (NSCLC)						
Local Treatment		**Systemic treatments**				
Radiation	**Surgery**	**Chemotherapy**	**Targeted therapy**	**Immunotherapy**	**Palliative Procedures**	**References**
1.Radiofrequency ablation (RFA) **2. External beam radiation therapy** a. Stereotactic body radiation therapy (SBRT) also known as stereotactic ablative radiotherapy (SABR)	**Early stage of cancer** ➢ Pneumonectomy ➢ Lobectomy ➢ Segmentectomy or wedge resection ➢ Sleeve resection	**Neoadjuvant and Adjuvant Therapy:** For locally advanced NSCLC: For metastatic (stage IV) NSCLC: **Chemotherapy Regimens Used with Radiation Therapy (RT):** • Carboplatin + Paclitaxel	**1.Angiogenesis inhibitors**: Bevacizumab Ramucirumab **2.Epidermal growth factor receptor (EGFR) inhibitors:** Erlotinib, Afatinib, Gefitinib, Dacomitinib **3.EGFR inhibitors that target cells with the T790M mutation** Osimertinib	**Immune checkpoint inhibitors:** **a. PD-1/PD-L1 inhibitors:** Nivolumab and pembrolizumab. b. Atezolizumab c. Durvalumab **CTLA-4 inhibitor:**	**Treating fluid build-up in the area around the lung (Pleural effusion):** 1.Thoracentesis 2.Pleurodesis: Chemical a. pleurodesis b. Surgical pleurodesis 3.Catheter placement	**(16)**

b. Three-dimensional conformal radiation therapy (3D-CRT) c. Stereotactic radiosurgery (SRS) **3.Brachytherapy (internal radiation therapy)**		• Carboplatin +Pemetrexed (nonsquamous) • Cisplatin + Etoposide • Cisplatin + Pemetrexed (non-squamous) • Cisplatin + Vinblastine • Consolidation Therapy • Durvalumab Patient sensitive to Cisplatin are administered with Carboplatin and its combination therapy.	**4.Drugs that target cells with ALK gene changes:** Crizotinib, Ceritinib, Alectinib, Brigatinib, Lorlatinib. 5.**Drugs that target cells with ROS1 gene changes:** Crizotinib, Ceritinib, Lorlatinib, Entrectinib. **6.Drugs that target cells with BRAF gene changes:** Dabrafenib, Trametinib (MEK inhibitor**).** **7.Drugs that target cells with RET gene changes:** Selpercatinib **8.Drugs that target cells with *MET* gene changes:** Capmatinib **9. Drugs that target cells with NTRK gene changes:** Larotrectinib, entrectinib **10. Nanocarriers:** Liposomal doxorubicin (Doxil™), albumin-bound paclitaxel or Nab-Paclitaxel (Abraxane™).	Ipilimumab	**Treating fluid buildup around the heart (Pericardial effusion):** 1.Pericardiocentesis 2.Creating a pericardial window **Treating an airway blocked by a tumor:** Photodynamic therapy (PDT) Laser therapy Stent placement	

Note: Information related to therapeutic regimen of TNBC and NSCLC has been accessed from United States Food and Drug administration- Drugs@FDA: FDA Approved Drug Product (https://www.accessdata.fda.gov/scripts/cder/daf/); Information has been accessed from the American Cancer society for NSCLC (https://www.cancer.org/cancer/lung-cancer/treating-non-small-cell/chemotherapy.html) and TNBC (https://www.cancer.org/cancer/breast-cancer/treatment/treatment-of-triple-negative.html) (Accessed on 5 March 2021).

Table 2: Conventional and novel treatment strategies for TNBC and NSCLC

2.3 Treatment issues of current therapeutics in NSCLC and TNBC

Drug resistance is one of the major reasons for the failure of treatment regimens. Identification of appropriate host and the epigenetic factors are central to the development of effective therapies against these cancers. Host changes would encompass the lack of drug bioavailability, effective carrier for transfer and temporospatial presence at the tumor site, tumor angiogenesis, interactions with interstitium as well as multi-drug resistance (MDR) (31). Genetic aberrations leading to faulty generation/accumulation of important proteins, includes resistance to chemotherapies prior (intrinsic) and post (acquired) administration of the drugs. Acquired resistance has often been associated with most of the anticancer therapies and have led to remission and relapse of the carcinomas leading to reduced survival times (32).

2.3.1 Treatment issues of current therapeutics in NSCLC

NSCLC accounts for 85% of total lung cancer cases detected and has presented very low 5-year survival rate (17.8%) amongst all cancers (33). Based on the physiological site of the tumor, this carcinoma may be broadly categorised as adenocarcinoma, squamous cell carcinoma, large cell carcinoma, adenosquamous carcinoma and sarcomatoid carcinoma subtypes of cancer. The ineffective treatment of NSCLC has been associated with lack of early detection as well as intrinsic properties of these carcinomas to develop in the bronchial squamous epithelium and alveolar epithelium while spreading to the adjoint lymph nodes of the lungs (34). Importantly, determination of effective treatment needs to be known based on identification of cancer stage, the tumor size, molecular pathophysiology, epidemiology, extent of lymphatic spread and its metastatic potential (35). NSCLC have been known to metastasize to brain and bones tissues among other organs (36). Current efforts towards improving of the overall survival of NSCLC patients have been oriented towards early diagnosis coupled with development of therapies against molecular targets or having personalized medicine. However, the availability of the effective treatment options has been riddled with development of chemotherapeutic resistance (37). Another issue associated with the failure of the NSCLC therapy is the tumor heterogeneity associated with these tumors (38). Point mutations as well as dysregulated chromosomal aberrations have led to the development of drug resistant cancer

stem-cells (CSC) leading to formation of multiple types of neoplastic cells (39). These heterogenous population of tumor cells when acted upon by a specific chemotherapeutic agent present resistance to structurally similar drugs, often referred to as MDR. This phenotypic response to exposure of tumor cells to chemotherapeutic agents often has been mediated through P-glycoprotein efflux transporter (P-gp), multidrug resistance protein (MRP) and lung-resistance protein (LRP) (40). Importantly, the cellular location and density of these ABC transporter proteins (ATP-binding cassette) often play an important role in determination of the pharmacokinetic-pharmacodynamic profile of the drugs along with resistance profile. While P-gp and MRP has been associated with reduced accumulation of neutral and charged therapeutic agents, the vault protein LRP has been found to effect redistribution of active agents from inside the cells (41).

The upregulation of three protein families (Pgp, LRP, MRP) have been implicated intrinsically for the failure of the chemotherapeutic treatments in NSCLC patients. Downregulation and mutation of the transporter proteins associated with the non-specific pinocytosis of the therapeutic agents have also been implicated for the drug resistance. Additionally, deregulated topoisomerase and glutathione (GSH) enzymes expression; p53 and NOTCH genetic aberrations as well as enhanced anti-apoptotic bcl-2 gene expression have been associated with NSCLC treatment failures (42, 43). Further, enhanced tumoral cell migration, invasion and generation of extracellular matrix components along with reduced apoptosis mediated through epithelial-mesenchymal transition (EMT) has been correlated with the chemoresistance to tyrosine kinase inhibitors (TKI) (44). Platinum based drug resistance in NSCLC have been associated with upregulation of efflux transporters, intracellular GSH with reduction in Na+K+ATPase and CTR1 gene expression. Mutated expression of tubulin protein, histone deacetylase (HDAC6) and suppression of mitotic chromosomal checkpoint have resulted in poor overall survival of patients receiving taxane based therapies (45). Gemcitabine resistance has been mediated to neoplasms lacking the drug transporter hENT-1 (46). Similarly, the overexpression of vault protein RLIP76 leading to reduced tumoral cell accumulation has been associated with the MDR to anthracyclines, taxanes and vinca alkaloids (47).

The upregulation of oncogenes responsible for activation of tyrosine kinases has been implicated in the NSCLC. The mutation of EGFR (constitutive to the normal cellular

proliferation and angiogenesis) at exon19/exon21 has been associated with the NSCLC initiation and progression (48). Consequently, TKI targeted against EGFR such as gefitinib and erlotinib have been widely used in NSCLC treatment. However, prolonged usage of TKIs and tumor heterogeneity has been associated with intrinsic drug resistant exon 20-T790M mutations leading to relapse of the disease (49). Additionally, suppression of the pro-apoptotic protein BIM and cross resistance mediated through other gene mutations have also been associated with treatment failures. NSCLC treatment failures are further associated with re-arrangements of ALK (anaplastic lymphoma kinase) gene, ROS proto-oncogene (ROS1) and RET (rearranged during transfection) which are associated with downstream cellular migration, proliferation and apoptosis resistance (50-52). Mutations of the protooncogene BRAF (constitutively activates the downstream activities of mitogen-activated protein kinase (MAPK) pathway) leads to unregulated cellular growth and has been found in 7-10% NSCLC patients. RAS independent V600D/E/K/R mutations and RAS dependent non-V600 mutations leads to BRAF activation, MAPK/ERK upregulation and subsequent tumor growth. The drug resistance to BRAF inhibitors have been attributed to CDK4 (cyclin dependent kinase 4) mutation, negative feedback on neurofibromin 1(NF1) and phosphatase-tensin homolog (PTEN) as well as MAPK alterations (53). NTRK gene (Neurotrophic Receptor Kinase) family encoding for the controlled activity of TRK, has been found to undergo splicing, codon deletion, fusion and overexpression in patients with NSCLC. Such re-arrangements lead to overexpression of TRKs resulting in increased oncogenic activity while on-target and off-target mutations associated with upregulation of other pathways lead to treatment failures (54). HER2 (human epidermal growth factor receptor) mutations at exon 20 have been implicated in NSCLC as it increases the cellular levels of downstream proteins in MAPK pathway. HER2 amplifications have been indicated for the EGFR independent and dependent drug resistance (55). Similarly, the amplifications of receptor tyrosine kinase, MET at exon 14 and fibroblast growth factor receptor (FGFR) has been observed to potentiate the EGFR mediated drug resistance and metastasis progression (56, 57). KRAS (Kirsten rat-sarcoma) oncogene mutation has been found to be the most common (at G12C and G12V) and targeted genetic alteration in advanced as well as metastatic NSCLC. The treatment efficacy of patients diagnosed with KRAS mutations have been very low owing to the heterogeneity amongst the tumor cells undergoing such alterations. Viable approaches to modulation for such mutations included the

targeting the receptor, its cellular association or reduction in expression of downstream proteins. Interestingly, neoplastic cells with KRAS mutations have shown improved sensitivity to taxanes in combination with MEK inhibitors (58). The downstream signal transducer protein MEK has been associated with nuclear cellular programming and MAPK pathway checkpoint. The MEK mutations K57N/Q56P have been found to increase the tumor cell sensitivity to chemotherapeutics and improve the cellular death outcomes(59). Importantly, many of these genetic alterations have been found to be mutually inclusive and drug treatment failures have often have been associated with multiple such oncogenetic factors (60). Additionally, the downstream amplifications of the MAPK/JAK/STAT pathway afforded by these genetic mutations have led to increased EMT, angiogenesis and tumor survival (61).

2.3.2 Treatment issues of current therapeutics in TNBC

TNBC has been highlighted by the reduced expression/amplification of three biomarker proteins ER, HER2 as well as PR and has been detected in 15-20% of breast cancer patients (62). Similar to NSCLC, early detection of TNBC presents a major challenge to treatment opportunities. Physiologically, the aggressive neoplasm has shown characteristic geographic invasion, infiltration, necrosis in breast cells surrounded by lymphocytic stroma with high rates of metastasis and relapse. Historically, this carcinoma has presented heterogenous morphology, genomic mutations and altered signalling/cross-talk among the tumor cells (63). Morphologically, the disease has been classified as MES (mesenchymal), MSC (mesenchymal stem-cell like), immunomodulatory, LAR (lumen-androgen receptor), BL1 (basal cell-like-1), BL2 (basal cell-like 2) and CLT (claudin-low tumor) subtypes. Chemotherapeutic TNBC treatment options are dependent on the type of the physiology presented these heterogenous tumors (64). While antimitotic and DNA damaging agents have been found to be effective against BL1 tumors, tyrosine kinase inhibitors and agents targeted against downstream activities of genetic alteration were effective against BL2 tumors (65). Mesenchymal subtype of TNBC have shown increased levels of EMT and are treated using inhibitors against sarcoma kinases (SRC), PI3K (phosphoinositide-3-kinase) as well as mTOR (mammalian-target of rapamycin). TGF (transforming-growth factor) and receptor proteins have been implicated in invasiveness and migration of these subtypes (66). However, the major clinical challenge for pathological complete reponse (pCR) has been the heterogeneity exhibited amongst these

tumors and the lack of suitable oncogentic targets against the same. Although, the use of neo-adjuvant traditional chemotherapeutic agents has been associated with higher pCR, the treatment efficiencies have been affected by the emergence of MDR, genetic alterations and immune-resistance (67). Abnormal expression of genes responsible for p53 protein (associated with tumor suppression-TP53) as well as breast cancer gene BRCA1/2 (important for DNA repair) have been implicated in TNBC with the altered chemosensitivity to platinum and taxane based therapies (68). Surface overexpression of CD73 protein has been found to affect the anthracycline efficacy (69). Post neo-adjuvant chemotherapy, the treatment failures and relapse of disease have been found to be affected by altered expression of genes such as proto-oncogene Myc, p53, PIK3, PTEN, retinoblastoma (RB1), cyclin-dependent CDK4/CDKN2A, janus kinase-2 (JAK2), BRCA1/2, EGFR and anti-apoptotic MCL1 (70).

It is important to understand the biomarkers associated with the heterogeneity as well as chemoresistance of TNBC and the plausible ways of effective targeting. BRCA signalling pathway has been associated with the DNA repair in the normal cellular functioning and this gene has been noted for its mutation in TNBC while being responsible for resistance to DNA damaging chemotherapeutic and metastasis. Drugs mediated inhibition of PARP1 (constituent of downstream-signalling leading to DNA damage) may help in reducing the tumor load and drug-resistance reversal (for taxanes and anthracyclines) (71). Further, the overexpression of receptor proteins like EGFR and VEGFR have been implicated in the growth, invasion, spread and the angiogenesis of TNBC (72). Interestingly, drugs targeted against these surface proteins have been able to only improve the PFS with no effect on pCR and OS. Similarly, the inhibition of LAR protein improves tumor regression and prevents TNBC metastasis (73). Immune-resistance in TNBC has been proved to be conferred by upregulation of transmembrane protein PD1 (programmed cell-death 1) and its ligand (PDL1) by the malignant cells. Overexpression of these components have been known for inhibition of apoptosis and conversion of $T_{effector}$-to-T_{reg} cells as well as increased cellular death of $T_{effector}$ cells. Consequently, drugs targeted against PD-1, PDL1 and CTLA-4 showing modest statistically significant changes in PFS, have been included in the combinatorial therapeutic regimens in TNBC (74). Additionally, treatment failures of conventional anticancer agents, MDR, disease relapse, proliferation and metastasis in TNBC has been attributed to the presence of quiescent as well as proliferative breast CSC (BCSC) (75). The heterogenous regulation with genetic/epigentic alterations has been shown

to generate niche colonies of BCSC with surface overexpression of CD44/CD24, STAT3 and ALDH1. These proteins and BCSC have been shown to be responsible for the treatment failure of BL1/BL2 subtype TNBC (76). Further, the TNBC MDR has been affected by presence of multiple tumor microenvironment (TME) factors such as CAF (cancer-associated fibroblasts), TAM (tumor-associated macrophages), mesenchymal stromal cells (MSC) and extracellular matrix (ECM) (77). BCSC secretion of cytokines like IL6/IL8 and CXCL12/CXCL7 has potentiated the MDR. CAF signalling has been found to activate STAT1/NOTCH3 and immune cells leading to reduced drug uptake in neoplasms. MSC immunomodulation through PI3K/Akt and proto-oncogene Src enhances the MDR of TNBC to platins and taxanes. Overexpression of Tenascin C in ECM has been shown to upregulate the Wnt/NOTCH mediated signal transduction leading to stabilization of BCSC and improved MDR (78). Similar to NSCLC, MDR in TNBC has been further upregulated by the overexpression of genes encoding the efflux proteins like Pgp/MDR1 (ABCB1), MRP1 and breast cancer-resistant protein (BCRP). These three protein families have been implicated with the MDR of anthracyclines and DNA-damaging agents (79). Notably, research in TNBC MDR suggests the presence of interrelationship between ABC efflux proteins, induction of EMT and stabilization of quiescent BCSC. EMT mediated through TWIST/SNAIL/FOXC2 has been shown to improve the immune-resistance, stemness, quiescence and drug resistance in TNBC (80). Further, the tumor reprogramming of these neoplasms with subsequent proliferation, metastais as well as MDR have been modulated by the altered transcription of genes of cellular HIF-1 (hypoxia-inducible factor), ROS and ALDH (81).

The mutation/downregulation of apoptosis regulatory genes such as p53, SNAIL have resulted in EMT resulting in ZEB1/CHK1 mediated reduced efficiency of DNA damaging agents against the BCSC and treatment failures. Consequently, it is important to identify and target BCSC specific molecular proteins to improve the therapeutic efficacy of the current chemotherapeutic regimens (82). Targeting of CSC signalling pathways associated with MDR TNBC such as Hedgehog, Src tyrosine kinase, NOTCH and Wnt are being explored clinically to improve ensure improved regression and removal of stem cell-niche (83). Further, modulation of oncogenetic/epigenetic targets such as ERK, CDK4, GAS6, IGF-1R, BMP4/ BMP7, KRAS and TGF-2 associated with BCSC quiescence, metabolism, proliferation and resistance have been explored for reduction of chemoresistance as well as improved BCSC cell

death (84). The cross talk between upregulated autophagic proteins (BECLIN1, ATG4), EMT, TME as well as cellular chromosomal stability has further enhanced the survival of BCSC cells and their inhibitors are being explored clinically for better efficacy (85).

A paradigm shift in landscape of NSCLC and TNBC treatment has been observed with the identification of novel disease targets and chemotherapeutics having single/multitargeting capabilities. Although, these newer agents have been added to conventional combination chemotherapy, the unmet need for effective controlled delivery of these agents to tumor sites having temporospatial presence and reduced toxicity profiles has remained unsolved (86). These necessitate the need of delivery of therapeutic agents through nanocarrier systems to lacunae of action (26).

2.4 Nanocarrier alternatives to conventional therapy

Nanocarrier based drug delivery has been synonymous with the treatment of cancer and has been widely explored for the amelioration of the various unmet clinical needs associated with each of the neoplasms. The effective translation of the conventional combination drug therapy having enhanced PK-PD profiles with spatial and temporal presence at tumor site may be achieved using nanocarrier based delivery of the agents (87). Importantly, the controlled delivery of the drugs during transit to the tumor cells and within the tumor cells needs to be ascertained for desirable therapeutic outcomes post systemic administration. The use of nanocarriers for the delivery of anticancer agents may invariably exhibit increased the circulation half-life along with bioavailability leading to more effective presentation of the drugs at the intended sites of action. PEGylation based surface modulation of these nanocarriers has presented the controlled delivery of therapeutics with stealth mediated passive targeting opportunities to the tumor cells with reduced systemic exposure to the normal tissues. Various biodegradable as well as biocompatible lipid and polymer based passively targeted nanoparticulate formulations have been tested preclinically and clinically as part of the combination therapies. Based on the controlled release, improved safety and efficacy profiles, a plethora of nanosized drug delivery vehicles have replaced the naïve drugs as a part of the established conventional therapeutic against various solid tumors (88). Clinically, these nano-

formulations have been tested for delivery of chemotherapeutics providing altered PK-PD profiles resulting in EPR (enhanced permeation and retention) mediated specific controlled drug delivery to the desired loci of action with reduced dose and toxicity profiles (89). Pegylated liposomal Doxorubicin (Doxil™, Lipodox™), and nanoparticulate albumin-based paclitaxel therapeutic (Abraxane™) have been clinically used as a component of established combination chemotherapies against TNBC and NSCLC in place of the conventional drug solutions. The various polymeric and lipidic nano-formulation approaches against TNBC and NSCLC are presented in Figure 6. Additionally, stimuli sensitized (thermal/magnetic) and surface engineering of the nanocarriers for active targeting to overexpressed surface receptors or proteins specifically associated with these tumor may help in site specific drug delivery (90) (91, 92). Such approaches may help in site specific actions against the pulmonary epithelium (NSCLC) and epithelial-mesenchymal transition (TNBC) which has been associated with the poor clinical outcomes in these diseases (93). Tailored fabrication of nanocarriers to exploit the conditions of tumor microenvironment such as pH, redox potential, proteins, internalization mechamisms among others may be utilized for improving the therapeutic outcomes with reduced toxicity. Importantly, as opposed to the use of naïve drugs. nanocarrier based delivery of therapeutics may help in the suppression of the NSCLC and TNBC associated oncogenes (94).

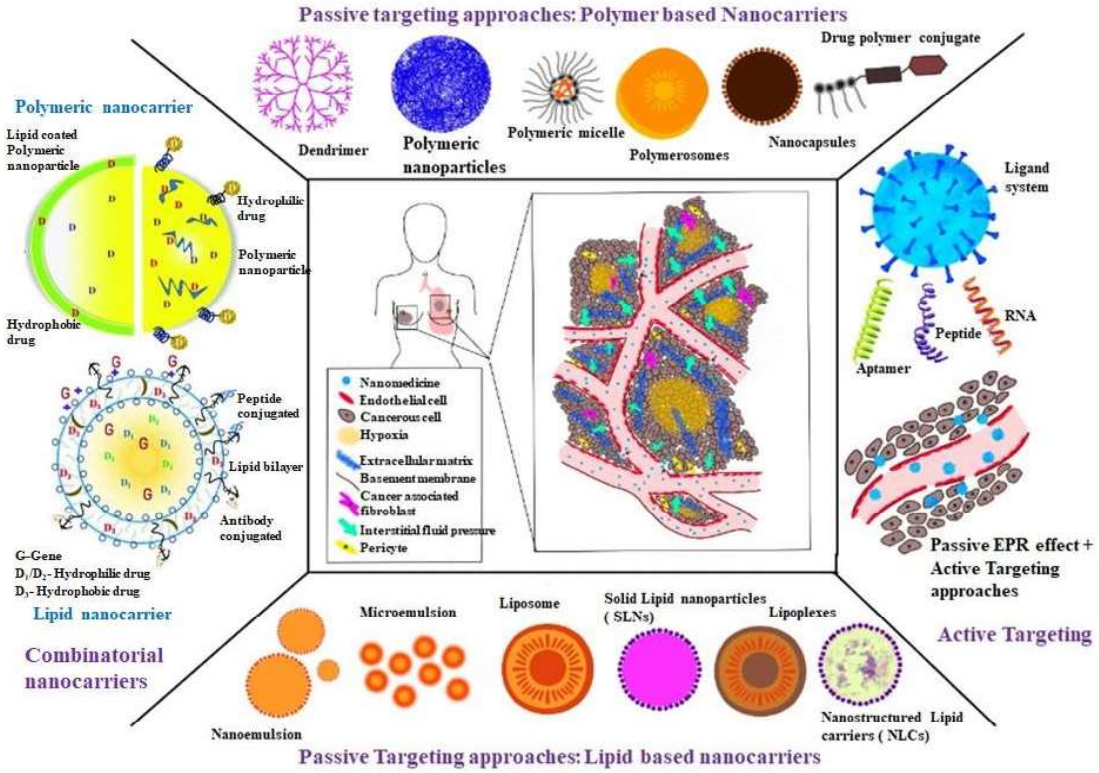

Figure 6: The lipid and polymer based nanocarrier approaches against NSCLC and TNBC (Adapted with permission from (12)).

2.5 Conventional nanocarriers

Conventional nanocarriers may be referred to as polymeric and lipidic constructs intended for controlled delivery of hydrophilic, hydrophobic or amphiphilic drugs with particle size tuned to less than 200 nm and surface properties modulated according to the physiological conditions at the intended site. These physicochemical modifications enable the attainment of the desired PK-PD profile of therapeutic agents targeted to tumor sites besides evading the components of reticulo-endothelial system (RES) post systemic administration. Such targeting to tumor cells has often being achieved using passive or active means (95). Passive targeting of nanocarriers mediated through the diffusion mediated cellular transport have been primarily affected by the physiological conditions of the tumor (96). The growth of neoplasms has been reported to be associated with deficient lymphatic drainage and enhanced vasculature leading to enhanced

permeation and retention (EPR) of the nanocarriers into the tumor cells (97). Neovascularization of newer cancer cells may lead to differences of angiogenic regulators and matrix metalloproteinases generating tumor vessels that are enlarged and expands the gap junctions in endothelial cells and deficiency in lymphatic drainage (98). Cancerous tissues have been categorised by rapid cell proliferation, enhanced levels of pro-angiogenic growth factors, active neovascularisation and excessive deposition of ECM. Hypoperfusion along with insufficient levels of oxygen and nutrients during neovascularisation stage leads to alteration of TME and may lead to metastasis (99). Traditionally, prototype nanocarrier systems like liposomes, polymeric nanoparticles among others have often being developed to utilize the EPR for improving the risk to benefit ratio of efficacy to toxicity. However, significant clinical intrasubject and inter-subject EPR variability has been observed due to heterogeneity in TMEs leading to reduced efficacy (98). Further, the pre-clinical to clinical translational potential of the EPR mediated passive targeting of nanocarriers are affected physiological conditions such as tumor-stromal ratio, degree of perfusion, stromal architecture (dysplastic as observed in both TNBC and NSCLC), vascular architecture, efficiency of lymphatic drainage, level of vasogenic permeability affecting factors (eg. cytokines, VEGF among others) and vascular obstructions offered by resident fibroblasts/pericytes (95). It is important to note that clinical effectiveness of EPR mediated passive uptake have often being affected by the combination of the aforementioned factors and such uptake has been debated widely in case of both these cancers (100). The lack of therapeutic effectiveness of passive targeted nanocarriers are further affected by the pathophysiology of the lung tissue and inherent stripping of such drug delivery prior to reaching their intended sites of application (101). Active targeting of the nanocarriers via receptor mediated-transport can be achieved by functionalization/decoration with specific targeting moieties i.e. receptor ligands, monoclonal antibodies, Fab' fragments, peptide/aptamer functionalization, etc. for tumor cell thus facilitates site-specific delivery of the cytotoxic agent within the tumor microenvironment, decreases the undesirable side effects and distribution to the non-target tissues (102, 103). These targeting moieties have a greater affinity for these specific targets (cancer cells, TME) and accumulate there. Basically, this active targeting approach has specific requirements such as higher level of overexpressed receptors in the TME compared to normal tissue, targeting can be easily accessible by nanocarriers (target surface receptors), expression levels of overexpressed receptors should be

linked with the malignant behaviors (drug resistance or aggressiveness) and efficient uptake/endocytosis of nanocarriers into the cancer cells, etc. (104, 105).

2.5.1 Passive targeted nanocarriers in NSCLC and TNBC

Taxanes, platins and anthracycline based combination regimens have often been identified as first line of chemotherapy in NSCLC and TNBC. However, the delivery of these agents in form of drug solutions have been associated with high toxicity and poor quality of life for the patients (106). Lipid-based nanocarriers with their intrinsic capabilities of high loading capacity, thermal stability, reduced adverse effects, drug resistance and increased drug accumulation at tumor site (107). The alleviation of the toxicities has often been achieved using nanocarrier based passive targeted delivery of these potent therapeutics. The high affinity of doxorubicin towards cardiac phospholipid cardiolipin has been associated with specific accumulation in mitochondria, disruption of components of electron transport chain and subsequent irreversible cardiomyopathy (108). Surface coating of lipid and polymer surface with polyethylene glycol (PEG) has resulted in the increased blood circulation time while presenting reduced uptake by the components of the reticulo-endothelial system. These second-generation stealth nanocarriers present suitable opportunities for delivery of various therapeutic agents to desired targets with the modulation of the surface charge, hydrodynamic diameter and the fixed aqueous layer thickness for EPR mediated uptake and reduced toxicity (109). PEGylated liposomal Doxorubicin (Doxil™, Lipodox™) has presented controlled delivery of the drug, reduced toxicity and similar efficacy to the drug solution (Adriamycin™) when tested clinically in NSCLC and TNBC. Importantly, the nanocarrier has replaced the drug solution in the established drug regimens and has been widely tested in clinical studies as a part of combination therapy. However, the use of PEGylated nanocarriers has been associated clinically with palmar-plantar erythrodysesthesia (PPE), complement activation related pseudoallergy (CARPA) and accelerated blood clearence (ABC) (110, 111). The non-Pegylated liposome, Myocet liposomal™ has been approved for use along with cyclophosphamide or vinorelbine in metastatic breast cancer forms including TNBC (96). The carrier free paclitaxel solution (Taxol™) containing Cremophor EL has been approved for use in combination therapy against TNBC and NSCLC. However, this microtubule stabilizing agent which inhibits the growth of neoplastic endothelium, in the solution form has been

associated with non-linear pharmacokinetics, severe neurotoxicity, hyperlipidemia and hypersensitivity reactions (112). Lipid based paclitaxel nano-formulations such as LEP-ETU™ (cationic nanosome of 150 nm size), EndoTAG™-1 (vascular targeting cationic lipid encapsulated paclitaxel), Lipusu™ and stealth liposomal paclitaxel have been tested in-vivo and clinically (113, 114). These lipidic nanocarriers have presented reduced drug resistance, side effects with significantly improved efficacy against both these diseases as compared to the conventional paclitaxel solution (115, 116). Topotecan solution in chemotherapeutic combinations for both these cancers have presented hematological toxicities when used at maximal tolerated dose (117). Metronomic chemotherapy in low doses and surface engineering with PEGylation of liposomal topotecan have exhibited significantly improved properties of tumor uptake, efficacy with desired pharmacokinetics and reduced toxicity when tested in-vitro as well in-vivo (118, 119).

Further, delivery of chemotherapeutic agents using lipidic carriers have been often associated with improved efficacy at reduced drug concentrations. Liposomal delivery of Cisplatin (Lipoplatin™) has exhibited improved clinical characteristics of partial response (PR), reduced progressive disease (PD) and adverse effects as compared to cisplatin (120). Clinically used liposomal irinotecan (Onivyde™) presented 4.9-fold increase in median survival time at a 5-fold reduced concentration as compared to free irinotecan in orthotropic model of advanced metastatic in female SCID mice (121). Similarly liposomal delivery of vinorelbine (Alocrest™), has been evaluated preclinically and clinically comparative efficacy against NSCLC and TNBC (122). Additionally, stimuli sensitive lipid carriers have been tested for the passive targeting of therapeutics to NSCLC and TNBC tumor micrometastasis (123). Themosensitive long circulating liposome (ThermoDOX™) has been investigated for the delivery of Doxorubicin to tumors in response to heat (>40˚C) mediated through radiofrequency thermal ablation (RFA) have exhibited encouraging results in preclinical setup (124). Despite being associated with improved overall survival in the clinical set up, the thermosensitive liposomes have presented promising yet variable results associated with the metastasis and clinical study design (125).

Natural, semi-synthetic or synthetic polymers have often been used for the development of polymeric nanoparticles (nanocapsules or nanospheres) or drug-polymer conjugates (126).

Similar to the lipidic nanocarriers, the polymeric nanoformulations have presented exciting opportunities for controlled delivery of chemotherapeutic agents associated with improved plasma circulation, reduction of RES induced opsonization and dose induced side effects (127). Poly(lactic-co-glycolic acid) (PLGA) based nanoconstructs have been widely evaluated for the controlled delivery of paclitaxel, cisplatin, doxorubicin, campothecins in both NSCLC and TNBC. These formulations have presented increased passive targeting mediated improved efficacy as compared to the free drug solutions in case of TNBC and NSCLC (128). The toxicity associated with solvent based paclitaxel formulation, has been further circumvented by the development of the drug albumin-bound nanoparticles (Abraxane™) (102). This polymeric formulation utilizes the reversible human albumin binding properties of the drug before administration and during systemic transit leading to the transport across cellular endothelium and high concentration in tumor. This preferential transcytosis into tumor cells is mediated by glycoprotein-60 with increased tumoral accumulation due to specific albumin binding to surface SPARC (Secreted Protein Acidic Rich in Cysteine) protein and helps in reduction of paclitaxel resistance (129). This paclitaxel nanocarrier has been clinically used as a part of various combination therapies against NSCLC as well as TNBC and has presented significant improvement in terms of PK-PD profile, response rates, progression free survival, overall survival with reduced reversible toxicity profiles as compared to the free drug (130). Poly-ion complex (PIC) micelle may be designed to incorporate negatively charged molecule by electrostatic interaction with positively charged block copolymers and may be used for reducing the dose dependent toxicity. Genexol™, the polymeric micelle of monomethoxy poly (ethylene glycol)-block-poly(D,L-lactide) (mPEG-PDLLA) loaded with paclitaxel exhibited much lower toxicity with very high maximal tolerated dose. The polymeric formulation when tested pre-clinically showed significantly improved efficacy and reduced toxicity in NSCLC and TNBC as compared to Taxol™. This paclitaxel formulation has been approved for use in NSCLC and being tested clinically for TNBC (131). Further, biodegradable and biocompatible polyester-polyether block copolymers may be used for the controlled delivery of the hydrophilic as well as hydrophobic drugs with improved efficacy, reduction of drug resistance and toxicity (132). PEGylated polycaprolactone copolymer-based delivery of campothecin have provided improved stability of the drug in the plasma and presented improved efficacy as compared to naïve drug in NSCLC (133). Triblock co-polymer PCL-PEG-PCL based

paclitaxel formulation presented for the chrono-modulated circadian release of the drug with significantly lower toxicity and improved tumor regression than free drug injection when tested in A549 based Balb/c mice (Figure 7) (134).

Further, nanoconstructs using polymer-drug conjugates have been tested for overcoming drug resistance and improved efficacy in NSCLC and TNBC (135). These composites present intrinsic ability to form self-assembled nanocarriers while presenting the opportunities for tumor microenvironment-based drug release. The pH, hypoxia and enzyme dependent degradation of high molecular weight polymeric chains to low molecular weight components inside the tumor cells resulted in the drug release with reduced systemic side effects (136). Paclitaxel conjugated with N-(2-hydroxypropyl methyl) acrylamide presented significantly improved drug uptake and residence in the tumor cells while presenting improved tumor cell killing and induction of apoptosis when tested in 4T1 murine tumor model (137).

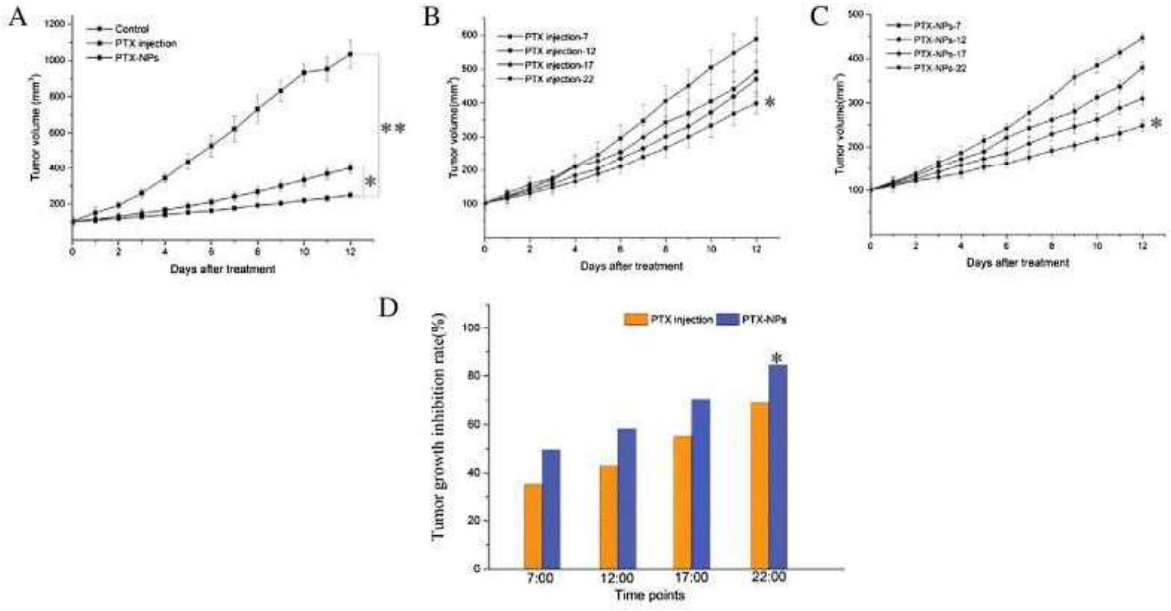

Figure 7: Antitumor effect of Paclitaxel injection and nanoparticles in A549 tumor bearing mice. (A) tumor volume of various treatment groups (control, injection and nanoparticles at 15HALO); time dependent comparison of tumor volume of injection (B) and nanoparticle (C);

Inhibition of tumor growth (%) of injection and nanoparticles (level of significance: *P<0.05, **P<0.01) (The figures have been reproduced with permission (134)).

N-(2-hydroxypropyl methyl) acrylamide conjugated doxorubicin exhibited increased apoptosis and significantly improved tumoricidal activity in 4T1 TNBC mice model on intravenous delivery (138). Similarly, doxorubicin conjugated with Polyethylene glycol-Beta-cyclodextrin, poly(oligoethylene glycol acrylate) and N-(1,3-dihydroxypropan-2-yl) methacrylamide have presented improved circulation time, enhanced tumor accumulation, improved anti-angiogenic profile, reduction in tumor growth and metastasis in 4T1 TNBC animal models as compared to the free drug solution (139-141). Systemic administration of the PEGylated Poly (β-L-malic acid)-Trastuzumab conjugate presented enhanced tumor inhibition as compared to free trastuzumab in-vivo in TNBC model (142).

In addition to the delivery of the drug substances, lipid and polymeric nanocarriers have been tested for the delivery of the active drug metabolites. Lipidic and polymeric nanocarriers encapsulated with highly toxic and active metabolite of irinotecan, 7-ethyl-10-hydroxy-camptothecin (SN-38) have been tested in-vivo against TNBC. Serum albumin loaded nanoparticles and palmitate conjugated SN-38 prodrug loaded PEGylated liposomes have exhibited improved half-life, enhanced cytotoxic potential with modulated PK-PD profile, reduced haemolysis and toxicity as compared to free SN-38 as well as irinotecan (143-145).

The clinical status of the various tested lipid and polymer based nanocarriers are highlighted in Table 3. Passive targeted nanocarriers have presented promising in-vivo and clinical results by replacing the carrier free drug solutions in the combination chemotherapy. However, one of the major issues with treatment failures of these two aggressive tumors are the need of specificity of the targets associated with the tumor heterogeneity and drug resistance (146). The specific targeting of the overexpressed surface proteins on these tumor cells may be achieved using the active targeting of the surface engineered nanocarriers (147).

Nanocarrier	Active Ingredients	Brand / Name	Company / Applicant	Clinical Phase	Clinical trial identifier	References
Triple Negative breast cancer						
Liposome	Doxorubicin	Doxil/Caelyx™	Janssen Research & Development, LLC	III	NCT00103506	(122)
	Doxorubicin	Myocet™	Sopherion Therapeutics, Inc.	II	NCT00294996	
	Doxorubicin	Thermodox™	Celsion Corporation	III	NCT00826085	
	Doxorubicin	Lipo-Dox™	TTY Biopharm	II	NCT00465673	
	Doxorubicin	Herceptin™	Merrimack Pharmaceuticals	II	NCT02213744	
	Paclitaxel	LEP-ETU™	INSYS Therapeutics Inc.	I	NCT00080418	
	Vinorelbine	Alocrest™	Taiwan Liposome Company	I/II	NCT02925000	
Lipid charged complexes	Paclitaxel	EndoTAG™-1	MediGene	II	NCT00448305	(129)
Polymeric nanoparticles	Paclitaxel	Abraxane™	Abraxis life sciences	Approved	NCT00609791	(88)
	Docetaxel	ABI-008 (nab-Docetaxel)	Celgene	I	NCT00531271	(126)
Polymeric-lipidic nanoparticle	Paclitaxel	PICN™. or SPARC1210	Sun Pharma Advanced Research Company Limited	II	NCT02136927	(129)
Polymeric Micelles	Cynviloq™ and Abraxane®;	TRIBECA™ Cynviloq (IG-001 or Genexol-PM™;	Sorrento Therapeutics, Inc.	II	NCT02064829	(126)
	Paclitaxel + NK105	NK105	Nippon Kayaku Co., Ltd.	III	NCT01644890	
Polymeric Drug conjugate	Paclitaxel poliglumex	Xyotax™ (CT-2103)	Dana-Farber Cancer Institute	II	NCT00148707	
	Paclitaxel–Angiopep2 conjugate	ANG1005	Angiochem Inc	II	NCT02048059	

Nanocarrier	Active Ingredients	Brand / Name	Company / Applicant	Clinical Phase	Clinical trial identifier	References
Non-Small Cell Lung Cancer						
Liposomes	Paclitaxel	Lipisu™	Nanjing Luye Sike Pharmaceutical Co., Ltd.	IV	NCT02996214	(96)
	Irinotecan	Onivyde™	Ipsen	II	NCT03088813	
	Doxorubicin	Doxil/Caelyx™	Janssen Research & Development, LLC	II	NCT01051362	
	Cisplatin	Lipolatin™	Alliance for Clinical Trials in Oncology	II	NCT01091454	
	Vinorelbine	Alocrest™	Taiwan Liposome Company	I/II	NCT02925000	
	Lurtotecan	OSI-211	Astellas Pharma Inc	II	NCT00046787	
Liposomal Inhalation	Amikacin	Arikayce™	Insmed Incorporated	II	NCT03905642 NCT03905642	(13)
Nanoparticles	Docetaxel	BIND-014,	BIND Therapeutics	I	NCT01300533	
	Camptothecin	CRLX101	National Institutes of Health Clinical Center (CC) (National Cancer Institute (NCI))	II	NCT02769962	
	Albumin-bound Paclitaxel	Abraxane™ (ABI-007)	Abraxis Bioscience, Astrazeneca, Abraxis Bioscience, Celgene, Celgene	Approved	NCT00540514	(88)
Polymeric Micellar system	Paclitaxel	Genexol® -PM	Gachon University Gil Medical Center	II	NCT01770795	(129)
Polymer drug conjugate	Camptothecin	XMT-1001	Mersana Therapeutics	I	NCT00455052	(96)
	Paclitaxel poliglumex (Paclitaxel and poly-L-glutamic acid)	Xyotax (CT-2103)	Dana-Farber Cancer Institute	II	NCT00148707	
	Paclitaxel–Angiopep2 conjugate	ANG1005	Angiochem Inc	II	NCT02048059	

Table 3: Clinical status of tested lipid and polymeric nanocarriers in TNBC and NSCLC

2.5.2 Active targeting nanocarriers in TNBC and NSCLC

The etiology of TNBC and NSCLC indicates the presence of various overexpressed/mutated surface and genetic biomarkers which may be targeted for the efficient delivery of the therapeutics. Ligand conjugated nanocarriers targeted against the surface overexpressed receptors on TNBC and NSCLC have been explored for the receptor-mediated site-specific endocytosis of the chemotherapeutic agents. TNBC has been characterized as HER2 negative, progesterone receptor-negative, and estrogen receptor-negative aggressive type of breast cancer with reduced responsiveness to chemotherapeutics (30). Several novel neoplasm specific receptor targets have been identified for active targeting of nanocarriers in TNBC such as EGFR, VEGF receptor, Folate receptor, BRCA1/2 mutation, FGFR, Androgen receptor, CD-44, and C-X-C chemokine receptor type 4 (CXCR4) amongst others (148, 149). EBP-1 (EGFR-binding peptide 1; sequence: KDKEFVVWEYGC), having high affinity and specificity for EGFR receptor was conjugated to poly(amidoamine)-doxorubicin dendrimer and was further conjugated to cell-penetrating peptide. This multifunctional nanocarrier demonstrated a significantly enhanced anti-proliferation effect in human breast cancer cell line (MDA-MB-231). Furthermore, superior anti-tumor efficacy and prolonged survival time were observed in BALB/c xenografts (bearing MDA-MB-231 cells) with this functionalized nanocarrier (150). Fibronectin-mimicking peptide PR_b coated PEGylated liposomal doxorubicin were targeted against surface overexpressed integrin α5β1 on MDA-MB 231 cells. The formulation resulted in significantly improved cellular uptake, tumor regression and reduced toxicity in animal model as compared to free drug and non-targeted liposome (151).

Interestingly, the folate receptor is also known to be 50-86 % expressed in metastatic TNBC patients. Recently, Hassan and co-workers have formulated folate targeted human serum albumin NPs loaded with Artemether via the desolvation method (152). Furthermore, folate conjugated NPs demonstrated higher in-vitro cytotoxicity and enhanced cellular uptake compared to non-targeted NPs in MDA-MB-231 breast cancer cells. Besides, the novel PARP poly(ADP-ribose) polymerase inhibitors such as Olaparib, Talazoparib, Veliparib, etc. represents a promising treatment approach in TNBC with BRCA mutations (30). Corsi and co-workers have formulated nano-liposomes loaded with Talazoparib via nanoprecipitation by using NanoAssemblr Benchtop (153). The developed nanocarriers have demonstrated a

significant increase in the survival rate of BRCA-deficient mice along with enhanced anti-tumor effect and cellular uptake. Similarly, CD44 receptors are also overexpressed in the TNBC for example, 80-90% TNBC cell lines are CD44 +ve, offer alternative targeting approach in the TNBC (154). Hyaluronic acid (HA) has high affinity for CD44 receptors and had been widely investigated polymer for CD44 targeting (155-158). Recently, Qin and co-workers have formulated Doxorubicin loaded hybrid micelles functionalized with HA for CD44 receptor targeting in TNBC cell lines (159). These HA modified hybrid micelles demonstrated multiple anti-metastatic effects via the downregulation of MMP-9 and inhibition of platelet adhesion. Apart from these receptors, Wu and co-workers have formulated novel dual-targeting liposomes having active recognition of both overexpressed $\alpha_v\beta_3$ and $GLUT_5$ targets. (160). They functionalized liposomes by using fructose and RGD peptide molecules for specific targeting to $GLUT_5$ and $\alpha_v\beta_3$ respectively. Notably, more than 90 % fructose is transported by these hexose transporters; more specifically by $GLUT_5$ transporters whereas $\alpha_v\beta_3$ integrin receptors can easily recognize RGD peptides (161, 162). Additionally, these dual-targeted liposomes exhibited enhanced anti-proliferation effect along with increased cellular uptake and anti-tumor efficacy.

The cell cycle transcription regulator Forkhead Box M1 (FoxM1) controlling the cellular transitions of G_1S / G_2M have been found to be overexpressed in metastatic TNBC and NSCLC besides being contributing to the neoplastic progression (163). FOXM1 targeted siRNA lipid nanoparticles when tested in MDA-MB 231 xenograft model in nude mice exhibited the downregulation of transcriptional expression and subsequent tumor load regression. In-vitro mechanistic evaluation indicated the reduced neoplastic reduction was associated with downstream inhibition of cyclin D1 and Src (Y416)/Erk activation (164). Further, targeting of the overexpressed cyclin and casein dependent kinases CDK11/ CK2 implicated with enhanced cellular proliferation, metastasis, suppression of apoptosis, downstream transcription and protein synthesis in TNBC have been tested (165). Tenfigben coated polyamine micellar formulation co-encapsulated with siRNAs targeting CDK11/ CK2 was tested in TNBC tumor model in mice. The protein coating was targeted against the stromal cells associated with TNBC neoplasm and the formulation presented significant reduction of primary tumors (166). Overexpression of oncoprotein Myc has been associated with metastasis and drug resistance in

TNBC (167). PEGylated PLA cationic lipid hybrid nanoparticles loaded with siRNA against CDK-1 exhibited improved apoptosis, significantly reduced cellular viability, tumor load, and decreased Myc expression in TNBC animal model (168). Alterations in the normal physiological EMT have been implicated in the drug resistance, neoplastic-microenvironment remodelling, increased metastasis and immunogenicity of the neoplasms in TNBC (169). Modulators of EMT such as TWIST, Zeb as well as Snail have been targeted for improved efficacy in TNBC. Arginine coated amphiphilic dendrimer loaded with siRNA were targeted against TWIST in SUM1315 cell line. This targeted delivery resulted in significant reduction in the TWIST expression, N-cadherin/vimentin EMT markers and neoplastic invasive migration. The delivery of this formulation resulted in preferential uptake in the tumor cells as compared to normal cells (170). EMT mediated remodelling of microenvironment associated with β3 integrin and downstream TGF-β (transforming growth factor) have been targeted in TNBC (171). Prolonged in-vitro gene silencing of β3 integrin and reduced cellular proliferation was achieved using lipidic siRNA carrier ECO (172). Further, active targeting and enhanced tumor uptake was achieved by surface functionalization with RGD peptide and PEGylation of the lipid carrier in MDA-MB 231 tumors in nude mice. Such presentation significantly decreased the expression of β3 integrin, tumor regression, angiogenesis and metastasis as compared to control group (173).

Drug delivery targeted against neoplastic surface overexpressed proteins such as EGFR, mTOR, VEGFR, FGFR1, HGF, ALK, BRAF, PI3K, DDR2, PLK-1, RET and ROS rearrangements, *P53* gene and KRAS mutations have been explored for improvements in therapeutic efficacy in NSCLC. Among these targets, EGFR, and KRAS mutations are most frequently observed and have been investigated to improve the anti-tumor and cytotoxic effect of targeted nanocarriers in NSCLC. Multifunctional nanocomplexes prepared by co-incorporating erlotinib, survivin shRNA expressing plasmid and Cy7 (near-infrared heptamethine cyanine dye) demonstrated dual stimuli responsiveness (near-infrared and pH) on in-vivo testing. Such treatment showed synergistic photosensitization and downregulation of survivin expressions together with improved anti-tumor efficacy of nanocomplexes against Erlotinib sensitive/resistant EGFR-mutated PC-9 cells (174). Similarly, Moataz and co-workers formulated dual-targeted nanoparticles loaded with cetuximab and siRNA for specific

targeting to PLK-1 and EGFR (175). Notably, PLK-1 plays an important role in mitotic regulation and is found to be overexpressed in a variety of cancers including NSCLC. The formulation showed substantial inhibition of overexpressed PLK-1, which may lead to G2/M cell cycle arrest along with apoptotic death of cells and specifically targets EGFR receptors. Moreover, the inhibition of PLK-1 also results in improved radiation sensitivity in A-549 flank tumors. Surface engineering with cell penetrating peptides present suitable opportunities for enhanced active transport of therapeutics across the cell membranes in NSCLC and TNBC. These have been evaluated for improving the efficacy and tumoral uptake of liposomal doxorubicin against NSCLC. Octa-arginine modification of pegylated carrier improved accumulation in A549 cells, increased levels of caspase3/7, apoptosis and subsequent tumor regression in A549 xenograft model in nude mice when compared with the non-targeted nanoliposome (Figure 8) (90). As discussed earlier, KRAS mutations have been found to be overexpressed in NSCLC (approximately 27%) at codons 12 and 13. KRAS as a member of the RAS family that encodes a GTPase, the regulator of fundamental cell processes (176). Merkel and co-workers have demonstrated an active targeting approach by formulating bovine serum albumin NPs encapsulating siRNA having target specificity towards KRAS G12S mutation. These NPs showed 85% entrapment efficiency of siRNA and 13.4 % loading efficiency by NPs having particle size and zeta potential of 132 nm and -20.5 mV respectively (177). Furthermore, the NPs showed a significant reduction in migratory ability (53% with *siKRAS G12S* and 47% with *siKRAS*) compared to control cells along with target-specific knockdown of KRAS by NPs. Apart from these, recently Zhong and co-workers have formulated novel $\alpha_3\beta_1$ integrin receptor-targeted Docetaxel loaded polymersomes functionalized with cyclic cNGQGEQc peptide for receptor-specific targeting (Figure 9) (178). These polymersomes carry 8.1 wt % Docetaxel and have an average diameter of 93 nm. Moreover, these polymersomes have demonstrated increased in-vitro cytotoxicity and high tolerability (8 times higher than free drug) along with significant tumor accumulation (14 times) and anti-tumor efficacy in A-549 xenografts. The various tested active targeted approaches against TNBC and NSCLC are presented in Table 4.

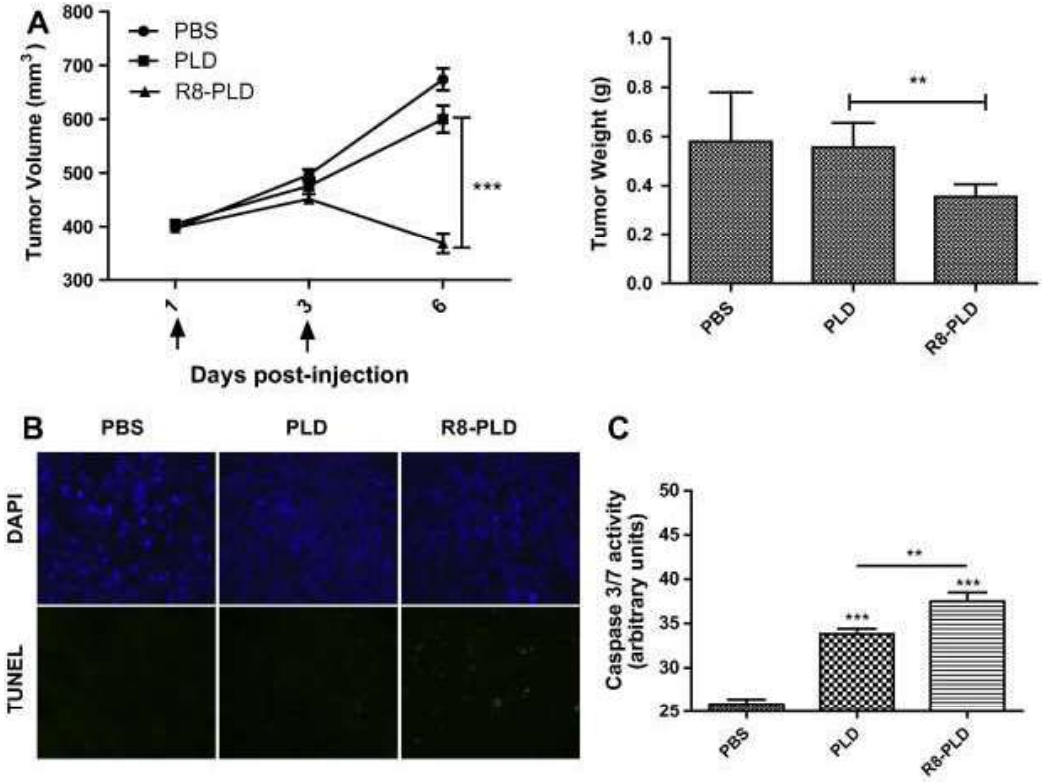

Figure 8: Comparative evaluation of in-vivo efficacy of octa-arginine modified pegylated liposomal doxorubicin against unmodified liposome: (A) tumor volume and tumor weight, (B) apoptosis assay, (C) Caspase 3/7 assay as indicator of apoptosis. (The figures have been reproduced with permission (90))

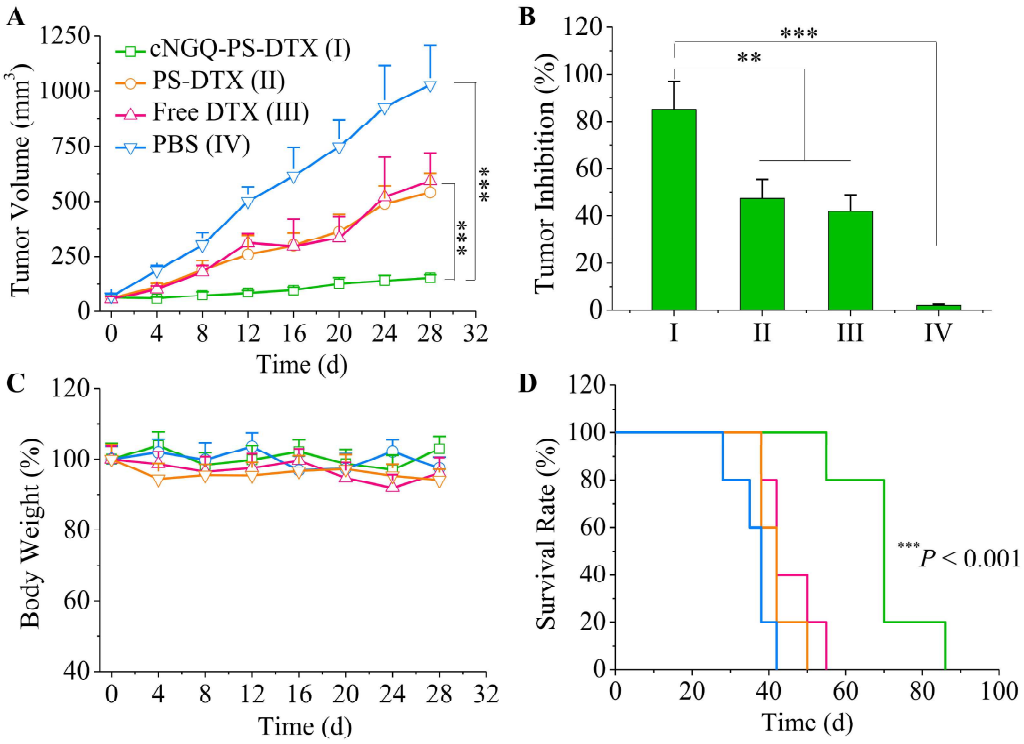

Figure 9: Evaluation of the anti-tumor effect of cyclic peptide conjugated polymerosome loaded docetaxel in A549 tumor in mice (dose 10 mg/Kg administered on days: 0,4,8,12). (A) change in tumor volume with time (B) Mean values of tumor inhibition of the treatments (C) Changes in bodyweight (D) Survival curves with treatments (Kaplan−Meier curves) (Adapted with permission from (178). Copyright (2021) American Chemical Society).

Target	Therapeutic agent	Nanocarrier	Intrinsic characteristics	Key findings	References
Triple-Negative Breast Cancer (TNBC)					
EGFR	Paclitaxel	Cetuximab conjugated TPGS coated Paclitaxel-piperine liposomes	Prepared by solvent injection method, size of ~ 218 nm, % EE of ~ 73%, conjugation efficiency of 75%	Superior uptake and in-vitro cytotoxic effect in MDA-MB-231 cells, higher IC_{50} values of liposomes compared to free paclitaxel	(179)
	pDNA	Low Molecular Weight polyethyleneimine conjugated guar gum	Spherical shaped complexes	Biocompatible, higher in-vivo transfection efficiency due to their high buffer capacity	(180)
	Aminoflavone	Unimolecular Micelle NPs loaded with Aminoflavone and were conjugated with GE11 (12 amino acid EGFR targeting peptide)	Prepared by ring-opening polymerization-esterification and dialysis method, size of 58 nm, the zeta potential of -9.64 mV	pH-responsive drug release, higher plasma levels (~72 fold) and superior anti-tumor efficacy of targeted NPs in MDA-MB-468 cells xenografts	(181)
Folate	Doxorubicin and Astragaloside IV	Liposomes co-modified by the folate acid & octa-arginine polypeptide	Prepared by ethanol-injection method, size of 109 nm, %EE of ~ 98% for both drugs	Significantly inhibited the proliferation of doxorubicin-resistant MDA-MB-231 cells in-vitro, excellent anti-tumor efficacy in-vivo	(182)
	Curcumin	Nanoscale metal-organic framework conjugated with folic acid NPs	Size of 117 nm, the zeta potential of -10.9 mV	Targeted NPs demonstrated increased survival rate with a significant reduction in tumor volume in BALB/c mice compared to non-targeted NPs	(183)
	Orlistat (as repurposed drug)	Folate conjugated micellar NPs of 2-hydroxyethyl acrylate	Synthesized by RAFT polymerization, the enhanced water solubility of a drug (~70-80 µg/ml), size of 75 nm, the zeta potential of -16.8 mV, % drug loading of 70-80%	Significant apoptosis in MDA-MB-231 cells with nanomolar concentrations via PARP and caspase-3 inhibition, reduced tumor volume in xenograft mice	(184)
VEGFR	siRNA	poly[*bis*(ε-Lys-PEI) Glut-PEG] nanocomplex	Size of 267 nm, Zeta potential of ~ +20 mV, substitution efficiency of primary amine was 57.7%	siRNA nanocomplex demonstrated negligible cytotoxicity, higher cellular uptake and transfection efficiency in MDA-MB-231/HeLa cells, increased in-vivo tumor penetration and efficacy	(185)

Target	Therapeutic agent	Nanocarrier	Intrinsic characteristics	Key findings	References
	Paclitaxel	DEAE-Dextran coated Paclitaxel NPs	Prepared by a solvent evaporation method, size of ~ 150 nm, %EE of ~ 65%, % drug loading is ~ 25%	Maximum cellular internalization at 1 hr in MDA-MB-231 cells, superior anti-tumor efficacy in xenograft mice, dual VEGF and NOTCH 1 inhibition	(186)
BRCA1 mutation	Talazoparib	Solid lipid NPs	Prepared by hot homogenization method, size of 218 nm, the zeta potential of -28.5 mV	Significant anti-tumor effect in-vitro, G2/M cell cycle arrest & PARP cleavage, can overcome homologous recombination-mediated resistance	(187)
	Olaparib	H-Ferritin conjugated NPs	Prepared by disassembly/reassembly method & drug complexation with Cu(II), size of 10-15 nm, the zeta potential of -24.8 mV	Dual targeted drug delivery i.e. BRCA muted cells and transferrin receptor, 1000-fold higher anti-cancer activity compared to free drug	(188)
	siRNA	EF2-kinase targeted cobalt-ferrite NPs	Prepared by EDS/NHS chemistry and coprecipitation method, size of 160 nm, the zeta potential of -39.5 mV	Significant anti-tumor efficacy and accumulation at the targeted site, increased cellular uptake of NPs	(189)
CD44	Co-delivery of Embelin & TRAIL plasmid	HA-coated pH-sensitive amphiphilic polymeric NPs	Prepared by a solvent evaporation method, size of 84.69 nm, the zeta potential of 34.3 mV, % EE of 58%, % drug loading of 7.24 %	Improved cytotoxic and pro-apoptotic efficacy, promotion of caspase3/7 activity, inhibition of expressions of apoptosis-related proteins	(190)
	Lapatinib (targets EGFR receptor)	Dual targeted (CD44 and EGFR) HA-TS-TPP NPs	Size of 207 nm, the zeta potential of -24.2 mV, % EE of 83.6 %	Enhanced anti-proliferation and tumor suppression effect, increased apoptotic efficacy & mitochondrial destabilizing activity	(191)
	Codelivery of Gemcitabine (GEM) & Docetaxel (DTX)	HA modified cationic liposome nanocomplexes	Size of ~ 200 nm, the zeta potential of -31.1 mV, high drug loading efficiency (9.3 % for GEM & 3.1 % for PTX)	Superior cytotoxic and synergistic effect against MDA-MB-231 cells, increased anti-tumor efficacy in mice xenografted with MDA-MB-231 cells	(192)
RAGE	Di-allyl-disulfide (DADS)	Solid lipid NPs with anti-RAGE antibody	Prepared by solvent diffusion method, size of 148.18 nm, the zeta potential of 3.7 mV,	Downregulating anti-apoptotic proteins (Bcl2 & survivin) & upregulating pro-apoptotic caspase-9, enhanced cellular uptake in MDA-MB-231 cells	(193)

Target	Therapeutic agent	Nanocarrier	Intrinsic characteristics	Key findings	References
			% EE of 69.76 %, % drug loading is 34.72 %	due to surface modification with anti-RAGE antibody	
HIF-1α	Echinomycin	Liposomes	Prepared by thin-film hydration method, size of 98 nm, the zeta potential of -30 mV	Inhibits HIF-1α transcriptional activity in metastasized MDA-MB-231 cells	(194)
Non-Small Cell Lung Cancer (NSCLC)					
EGFR	Afatinib	Transferrin conjugated redox-sensitive lipid-polymer hybrid nanoparticles (LPNs)	Prepared by nanoprecipitation method, size of 103.5 nm, the zeta potential of -21.2 mV, % EE of 90%, % drug loading of 7%	Significant increase in the release of drug in the presence of glutathione, improved pharmacokinetic profile and in-vivo tumor efficacy of LPNs in comparison with free drug	(195)
	Doxorubicin (DOX)	Cetuximab (Cet) conjugated dextran-coated Fe_3O_4 magnetic NPs	Size of 144.5 nm, % loading of 41.5 µg (DOX) and 36.24 µg (Cet) in 1 mg of dextran-coated Fe_3O_4 NPs	No concentration-dependent cytotoxic effects of NPs, enhanced in-vitro cytotoxicity and cellular internalization in A-549 cells	(196)
	Osimertinib (AZD9291)	Chitooligosaccharides-modified PLGA NPs	Prepared by emulsification and solvent evaporation method, size of 176 nm, the zeta potential of 18.65 mV, % EE of 95.22 %	IC_{50} decreased to 46% in H-1975 cells, inhibition of expression of immune checkpoint PDL1 (via NF - κB pathway) and EGFR along with downregulation of Bcl-2, promotes apoptosis by regulating EGFR, Bak, caspase -9, PARP, Bax, & Bcl -2 proteins	(197)
	Docetaxel	Cetuximab conjugated PLGA NPs	Prepared by a solvent evaporation method, size of 128.4 nm, the zeta potential of -31.0 mV, % conjugation efficiency of 39.77 %	Sustained release of drug from NPs (25% drug release at pH 5.5 after 48 hrs), enhanced anti-proliferative and cytotoxic effects of NPs in A-549 cells and A-549 xenografts	(198)
KRAS	Afatinib	PLGA NPs	Prepared by solvent emulsification evaporation method, size of 180 nm, the zeta potential of -23.1 mV, % EE of 34.4 %, MMAD: 4.7 µm; FPF: 77.8 %	Demonstrated sustained release of the drug (57% after 48 h), significant cytotoxic potential against KRAS mutated H460 cells compared to free drug	(199)

Target	Therapeutic agent	Nanocarrier	Intrinsic characteristics	Key findings	References
VEGFR	shRNA	GE-11 (EGFR specific binding ligand) or pH-responsive (GALA fusogenic peptide) modified self-assembled CD-PEI and/or Ad-PAMAM NPs	Prepared by self-assembly method, size of 120 nm, the zeta potential of 1.6 mV	Enhanced cellular internalization and endosomal escape of NPs, downregulates the intratumoral VEGF protein levels and significant anti-tumor efficacy in A-549 xenografts, the substantial increase in receptor gene expression & effective targeted gene silencing	(200)
p-PI3K	miRNA-29b and genistein	Mucin-1-aptamer conjugated hybrid NPs	Size of 598 nm, zeta potential of 4.2 mV, % EE of 99 % for both, % loading capacity of 8.6 % (miRNA-29b) and 51.8 % (genistein)	Active targeting to A-549 cells by aptamer functionalization, downregulates p-PI3K, pAKT, MCL-1 & DNMT3B, superior anti-proliferative and apoptotic effects in-vitro	(201)
ROS & EphA2	Cantharidin	YSA peptide modified TiOx NPs	Size of 150 nm, the zeta potential of -21.77 mV, % EE of 92.33 %, % loading efficiency of 23.75 %	Improved cytotoxic effect and specific recognition to EphA2 overexpressing A-549 cells by NPs, efficient generation of ROS (when X-ray irradiated),	(202)
CD 44	Afatinib and chlorin e6 (Ce6) derivative	Enzyme and ROS responsive HA modified NPs	Size of 230 nm, the zeta potential of -35.5 mV	ROS generation produced by Ce6 triggers rapid oxidation of thioether linker to facilitate Afatinib release in the cytoplasm, synergistic apoptotic activity by Afatinib and ROS generation	(203)
	MicroRNA-125b	HA-PEI NPs	Prepared by self-assembly method, size of 112 nm, the zeta potential of -27 mV, % EE of 99 %	6-fold increase in M1/M2 macrophage ratio along with a 300-fold increase in M1 marker (iNOS) and M2 marker (Arg-1) ratio, the substantial decrease in CD206 antibodies by NPs in KRAS/*p53* double mutated mouse model	(204)
	Doxorubicin and Celecoxib	HA decorated glycol chitosan pH-sensitive NPs	Size of 152 nm, the zeta potential of -25.2 mV, % loading efficiency of 7.15 % for Dox and 6.73 % for Celecoxib	pH-responsive drug release profile, significant tumor suppression in A-549 xenografts, reductions in Ki-67 & COX-2 expressions in the tumor	(205)

Target	Therapeutic agent	Nanocarrier	Intrinsic characteristics	Key findings	References
				microenvironment, decreased levels of MMP-2 and NF-kB	
p53	miR-660 (CCL660)	Cationic lipid NPs	Size of 121 nm, zeta potential of -17.1 mV, % EE of 85-90%	Inhibited the tumor growth (47%) by inhibiting the MDM2-*p53* axis, restores *p53* function and its downstream effectors (p21), no significant acute/chronic toxicological effects of NPs in-vivo	(206)

Table 4: Active targeting strategies for lipid and polymer based nanocarriers in TNBC and NSCLC

2.5.3 Ongoing clinical studies of conventional nanocarriers in NSCLC and TNBC

The current therapeutic regimen for the treatment of triple negative breast cancer and non-small cell lung cancer includes the usage of nanocarriers such albumin bound paclitaxel (Abraxane™) and pegylated liposomal doxorubicin (Doxil™). Traditionally, these nanocarriers have been evaluated against established treatment regimens replacing the drug solutions with the nanocarrier based drug delivery based on improved efficacy and reduced toxicity associated with carrier free treatments (207). Abraxane™ (Celgene Corp. USA) replaced Taxol™ (Bristol-Myers Squibb, USA) for treatment of advanced NSCLC in the clinical combination of paclitaxel and carboplatin based on the improved overall response, progression free survival (10% improvement in both), patient compliance and reduced neuropathy (208). Similarly, pegylated liposomal doxorubin (Doxil®) was approved as monotherapy for metastatic breast cancer due to reduced toxicity profile of the nanocarrier in place of drug solution (209). Holistically, newer clinical trials for the improvement in the treatment efficacy of NSCLC and TNBC have been based on the incorporation of newer agents in the established nanocarrier based-regimens (Table 5) (13). The anti-programmed death ligand-1 (PD-L1) antibody, Atezolizumab (Tecentriq™, Roche, Switzerland) was approved as a treatment option for both NSCLC and TNBC in nab-paclitaxel based regimens in patients with expression of PD-L1 (210, 211). The median overall survival and median progression free survival increased 4-fold over the drug treatment regimens without it. However, the use of Atezolizumab in NSCLC and TNBC treatment therapy has been associated with severe immune sensitivity against one's own system leading to life threatening conditions (212). Such manifestations require a careful evaluation of the risk-to-benefit ratio when considering the combination therapy for the treatment of these cancers. Currently, nab-paclitaxel as an interventional treatment is being evaluated with various other therapeutic agents with 149 and 93 clinical trials in NSCLC and TNBC respectively being registered at United states national library of medicine (www.clincial trial.gov). Similarly, pegylated liposomal doxorubicin is being tested as combinatorial regimen in 13 and 54 clinical trials in NSCLC and TNBC respectively. Some of the nanocarrier based clinical evaluation of therapeutic regimens have been presented in Table 5.

Chemotherapies activating multiple signalling pathways can lead to different cell death outcomes. However, a detailed understanding of how the pathways cooperate and interfere is essential for the design of rationally-based chemotherapeutic combinations. A paradigm shift in the cancer treatment occurred with the approval of Vyxeos (Duanorubucin and cytarabine liposome injection, Jazz Pharmaceuticals, USA) for treatment of acute myeloid leukemia (AML). This combinatorial nanocarrier translated the benefits of synergistic combination therapy of cytarabine and daunorubicin into more efficient therapeutic outcomes with reduced toxicity (213). The clinical translation of in-vivo efficacies of ratio-metric potent drug combinations like irinotecan-cisplatin in NSCLC may elicit similar responses in the solid tumors (214).

Clinical trial identifier (Year of initiation)	Therapeutic intervention	Type of cancer	Objective	Intended Primary outcomes & study results (year of completion)	Type of clinical trials	Phase
NCT04301739 (2020)	HLX10, nab-paclitaxel, carboplatin, doxorubicin or epirubicin and cyclophosphamide	TNBC	Pathological Complete Response (pCR) rate	Evaluation of efficacy of neo-adjuvant therapy in absence of invasive cells post-surgery. Study is active and early results are expected in 2022 (ongoing).	Randomized, Double blind	III
NCT04418154 (2020)	Epirubicin hydrochloride, Cyclophosphamide, Albumin bound paclitaxel, Toripalimab	TNBC	Pathologic complete response	Evaluation of efficacy of neo-adjuvant therapy in absence of invasive cells post-surgery. Study is active and early results are expected in 2022 (ongoing).	Open Label, single arm study	II
NCT04033354 (2019)	HLX10 carboplatin and nab-paclitaxel	NSCLC	Tumor assessment, progression free survival	Efficacy and safety evaluation. Study is active and early results are expected in 2022 (ongoing).	Double blind, randomized	III
NCT03799679 (2019)	Nab-paclitaxel, Cylcophosphamide, Epirubicin	TNBC	Pathologic complete response	Evalution of safety and efficacy of neo-adjuvant therapy. Study is active and recruitment is ongoing (ongoing).	Open Label, single arm study	IV
NCT03671044 (2018)	Nanosomal Docetaxel Lipid Suspension Taxotere	TNBC	Interventional study with patients till progression in disease with/ without toxicity is observed	Complete response and Progression free survival to be monitored in test arms as compared to Taxotere. Recruiting ongoing for this	Open label, randomized multicentre	III

Clinical trial identifier (Year of initiation)	Therapeutic intervention	Type of cancer	Objective	Intended Primary outcomes & study results (year of completion)	Type of clinical trials	Phase
				study and early results are expected in 2022 (ongoing).		
NCT03719326 (2018)	AB928, IPI- 549, Pegylated liposomal doxorubicin, nanoparticle albumin-bound paclitaxel	Advanced metastatic TNBC	dose-escalation, and dose-expansion study to evaluate the safety, tolerability, pharmacokinetic (PK), pharmacodynamic (PD), and clinical activity of combined drugs.	Incidence of Adverse Events (1 year), Incidence of dose-limiting toxicities (28 days). Study is active and recruitment is ongoing (ongoing).	Non-randomized, Open label	I/Ib
NCT03289819 (2017)	Pembrolizumab, nab-paclitaxel, Epirubicin, Cyclophosphamide	TNBC	Pathological Complete Response (pCR) rate (120 days)	Combination Treatment regimen. Study ongoing and results are awaited. (ongoing).	Open label, multicentre	II
NCT03076372 (2017)	Liposomal formulation of a docetaxel prodrug	NSCLC	Assessment of Liposomal docetaxel formulaton as a monotherapy until a maximum tolerated dose (MTD) is established	Maximum tolerated dose (MTD) of docetaxel liposomes as monotherapy administered once every 3 weeks in patients with metastatic solid tumors (18 months). Recruiting ongoing for this study (ongoing).	Open label	I
NCT03164993 (2017)	Atezolizumab, Pegylated liposomal doxorubicin, Cyclophosphamide	Metastatic TNBC	Evaluating the safety and efficacy of Atezolizumab when combined with immunogenic chemotherapy	Assessment of toxicity, Progression-free survival (PFS) for 18 months. Recruiting ongoing for this study and early	Randomized, double-blind	II

Clinical trial identifier (Year of initiation)	Therapeutic intervention	Type of cancer	Objective	Intended Primary outcomes & study results (year of completion)	Type of clinical trials	Phase
				results are expected in 2023 (ongoing).		
NCT02425891 (2015)	Atezolizumab, Nab-paclitaxel	TNBC	Determination of efficacy in patients with untreated TNBC	Progression free survival, overall survival. The drug combination was approved as line therapy having presented delayed disease progression (2020).	Randomized, Double blind	III
NCT02366143 (2015)	Atezolizumab, Bevacizumab, Carboplatin, nab-Paclitaxel	NSCLC	Determination of efficacy in patients with untreated NSCLC	Progression free survival, overall survival. The combination of the four drugs presented a favourable tolerated manageable outcome in patients as compared to that receiving monotherapy of monoclonal antibodies (2020).	Randomized, open label	III
NCT02456857 (2015)	Pegylated liposomal doxorubicin, bevacizumab, and everolimus	Localized TNBC	Efficacy of combination in TNBC tumors predicted insensitive to standard chemotherapy	Determine excellent clinical response rates. Recruiting ongoing for this study and early results are expected in 2022 (ongoing).	Open Label	II
NCT02315196 (2014)	Pegylated Liposomal	Stage II-III TNBC	To find alternative treatment for TNBC	To determine the rate of pathologic complete response.	Open label	II

Clinical trial identifier (Year of initiation)	Therapeutic intervention	Type of cancer	Objective	Intended Primary outcomes & study results (year of completion)	Type of clinical trials	Phase
	Doxorubicin Hydrochloride, epirubicin hydrochloride, carboplatin			Recruiting ongoing for this study and early results are expected in 2022 (ongoing).		
NCT01770353 (2013)	Nanoliposomal irinotecan,	TNBC	To study biodistribution of nanoliposomal Irinotecan and to determine the feasibility of using Ferumoxytol as a tumor imaging agent.	Tumour Levels of Irinotecan at Cycle 1 Day 4. Appreciable tumor regression was not observed and antitumor activity was observed in patients having high dose pre-treatment history (2019).	Non-randomized, Open label	I
NCT01525966 (2012)	nab-paclitaxel, carboplatin	TNBC	Pathologic complete response	Evaluation of efficacy of neo-adjuvant therapy in absence of invasive cells post-surgery. The study did not attain the desired PCR and may have attained desired PCR with prolonged neo-adjuvant therapy. The study is active and not recruiting due to lack of appropriate patient population (ongoing)	Open Label, single arm study	II
NCT01051362 (2006)	Pegylated liposomal Doxorubicin; carboplatin	NSCLC	Overall response rate	Efficacy and toxicity evaluation. Median time to disease progression (9.5 weeks) with	Open label, single arm	II

Clinical trial identifier (Year of initiation)	Therapeutic intervention	Type of cancer	Objective	Intended Primary outcomes & study results (year of completion)	Type of clinical trials	Phase
				18.6 weeks of median survival (2010).		
NCT01145430 (2010)	Pegylated Liposomal Doxorubicin Hydrochloride, Veliparib	TNBC	To determine dose and adverse events of combinatorial approach	To determine the recommended Phase II dose of ABT-888 (veliparib) given in combination with pegylated liposomal doxorubicin. The results indicated safety of the nanocarrier drug combination and toxicity studies were planned as a part of Phase II studies. (2017).	Open label	I
NCT00540514 (2007)	nab-paclitaxel, Paclitaxel, Carboplatin	NSCLC	Determination of efficacy of first line therapy with replacement of paclitaxel in solution with nab-paclitaxel	Complete response/ Partial response, progression free survival. The response rate was found to be better in case of nab-Paclitaxel+ carboplatin arm (2019)	Open label, randomized	III

Note: The clinical trials for the lipid and polymer based nanocarriers under clinical trials from United States National library of medicine, ClinicalTrials.gov; website: https://clinicaltrials.gov/. (Accessed on 5 March, 2021)

Table 5: Some Ongoing clinical trials of NSCLC and TNBC with lipid and polymer-based nanoparticles.

2.6 Combinatorial nanocarriers

2.6.1 Synergism and MTD for combination therapy

Current chemotherapeutic regimens of NSCLC and TNBC are based on dosage of MTD based drug concentrations assuming the hypothesis of maximum therapeutic efficacy being achieved from maximum dosing of individual drugs. However, this hypothesis does not consider the combined cumulative toxicities which may be presented by the usage of multiple drugs along with the temporospatial presence of the agents at therapeutically effective concentrations at the tumor loci (Figure 5) (215). Considering the heterogenic nature of these neoplasms, these treatments present lesser than perfect options with moderate improvements in efficacy and additive toxicities. Thus, selection of appropriate chemotherapeutic regimens involves rationale based integrative approach (216). Such integrative approaches would include the determination of property (synergism, additive and synergism) and corresponding dose of treatment for the effective treatment of the disease. This approach is based on the determination of the combinatorial index between the drugs being used for the treatment of the disease based on Chou-Talalay method (217). The method determines the combinatorial index (CI) which presents a correlation between multiple drugs when acting on the tumor cells as discrete entities and in combination. The CI values quantitatively summarises the effects of the drugs as synergistic, additive and antagonistic while encompassing the Henderson-Hasselberg, Scatchard as well as Micahelis-Menton equations (217).

2.6.2 Need of combinatorial nanocarriers

Chemotherapeutic treatment for aggressive solid tumors like NSCLC and TNBC currently includes non-personalized conventional combination of drugs often acting on multiple cellular targets leading to improved tumor cell death. The components of such therapies are based on metastatic, drug resistance and aggressive potential of both carcinomas (95). The current trend of regulatory drug approvals has focussed on novel therapeutic agents providing improved control against the barriers to effective management of these diseases. The heterogenicity of these tumors have shifted the treatment focus to use of multiple agents targeting non-overlapping mechanisms to effect improved therapeutic effectiveness against disease progression and relapse (72, 218). The design of effective combination therapies would require

rationalistic choice of agents acting on disease specific pharmacologic targets being added to the conventional agents to ensure optimal effect on tumor dynamics (219). However, absence of antagonistic mechanisms leading to enhanced tumor growth and drug resistance with additive toxicities need to be ascertained prior to adoption of such combinatorial regimens (220). Currently, most of the neo-adjuvant and adjuvant therapeutic regimens for NSCLC and TNBC include the usage of nanoarriers such as Abraxane™ and Doxil™ in combination with naïve agents at their maximum tolerated dose (MTD) levels (148, 221). Since NSCLC and TNBC conventional therapies are based on specific cell cycle agents, the presentation of drugs to cell cycle transition phases along with presence of efflux pumps shall be detrimental to the efficacy of such therapies (106). Further, the non-synchronised pharmacokinetic and pharmacodynamic profiles of individual agents, result in varied distribution and elimination leading to non-uniformity in co-delivery at the intended sites. Consequently, novel combinatorial nanotechnology platforms need to be adopted for synchronized controlled delivery of therapeutic agents with improved bioavailability (222). Nanocarrier based combinatorial approaches for multiple agents would include delivery using combination of single nanocarriers or having the drugs co-loaded in a single nanocarrier. The earlier nanocarrier combination would require either sequential systemic administration or in a mixture depending on the components used. Although, this approach enables the clinician with patient dosing flexibilities, the simultaneous co-presence of both the carriers at the tumor site cannot be ascertained (223). This approach diminishes the nanocarrier based therapeutic opportunity of optimum synergism of drug combination while presenting with increased immunological potential (89). Thus, presentation of potential combinations (drug-drug, drug-gene) co-encapsulated in a nanocarrier may ensure controlled synergistic co-delivery of these combinations to tumor cells with improved efficacy as compared to combination of single component nanocarriers as well as free drug cocktail (Figure 10) (12).

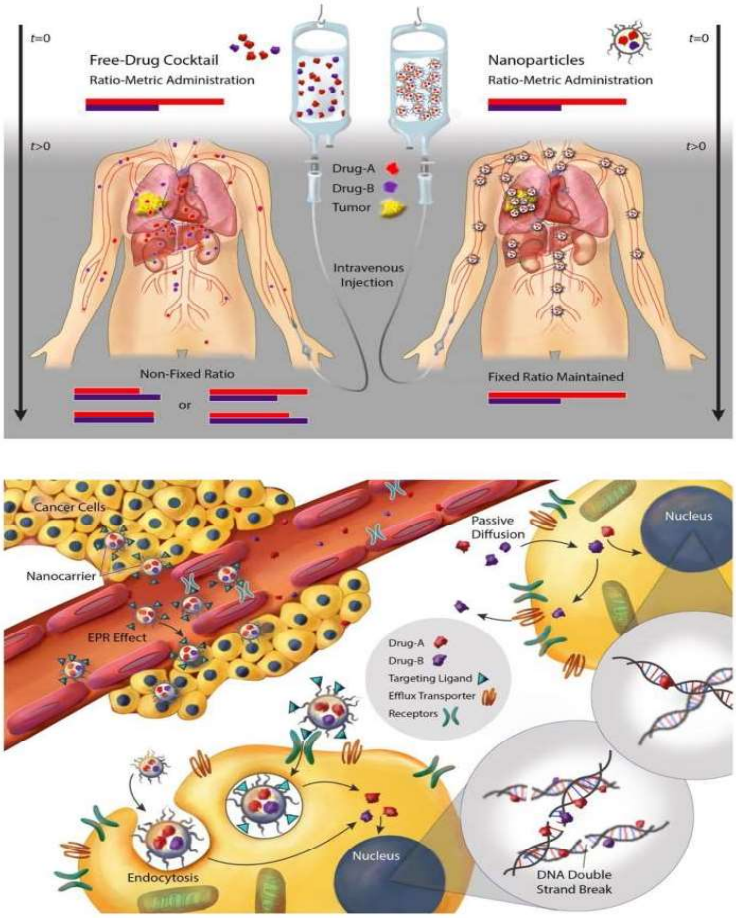

Figure 10: The need and potential advantages of combinatorial nanocarriers over single nanocarriers as well as free drug cocktail for chemotherapy in clinic. The use of ratiomimetic dose of synergistic combination of drugs through nanocarriers results in co-ordinated PK-PD profiles of the agents to site of action with reduced toxicity (A). Combinatorial nanocarriers on intravenous administration presents synergistic temporospatial presence of agents at tumor site resulting in improved chances of effective treatment (B). (The figures have been reproduced with permission (215)).

2.6.3 Dual Drug Combination nanocarriers

Delivery of the combinatorial nanocarriers encompassing multiple agents present suitable opportunities for the delivery of therapeutics targeting multiple pathways to provide more efficient tumor cell killing. Importantly, the combination of such chemotherapeutic agents may be achieved by locking the combination in suitable nanocarriers for passive or active targeted

delivery. These combinations may be comprised of two therapeutic agents or adjuvants improving the therapeutic efficacy of the active chemotherapeutic.

One rationalistic approach for efficient delivery of active drug-drug combinations involves the determination of the synergism between two agents for improving the antitumor efficacy and providing a better control over the disease conditions. Ratiometric evaluation of the potent drug combination of irinotecan and cisplatin was carried out in-vitro in cell lines of TNBC and NSCLC. The combination presented potential synergistic ratios in both carcinomas. The synergistic molar ratio of irinotecan:cisplatin (7:1) was co-loaded in negatively charged low cholesterol liposomes using the COMBIPLEX™ platform and evaluated at maximum tolerated dose in human NSCLC cell line H460 xenograft model in nude CD-1 mice in comparison with the free drug cocktail (Q4DX schedule). The results indicated significant improvement in tumor regression and disease progression with the ratio being maintained for atleast 24 hours in plasma (214).

Reports suggest significant role of microtubule destabilizing agents as potential mitotic cell cycle arresting agents indicated in both TNBC and NSCLC potentiating the action of non-cell cycle specific anthracyclines. The drug combination of Doxorubicin hydrochloride (DOX) and Vincristine sulphate (VCR) acting on multiple targets during different cell cycle phases present improved chances of reduction in tumor cell load and have been indicated in combination together in low doses for these cancers (224). While DOX arrests the cell cycle majorly at G2/M phases, VCR has been reported as mitotic inhibitor of the cell cycle. Additionally, patients suffering from TNBC have shown clinically favourable secondary prognostic factor for NSCLC development. The synergistic co-delivery of vincristine with doxorubicin in PEGylated liposome, improved the efficacy of the clinical standard liposomal doxorubicin when tested against A549 and MDA-MB 231 models in female nude athymic mice (Figure 11). The prepared nanocarrier presented improved cellular uptake, apoptotic potential while having physico-chemical and biochemical properties, tissue distribution and pharmacokinetic profiles similar to the approved liposomal formulation (224, 225).

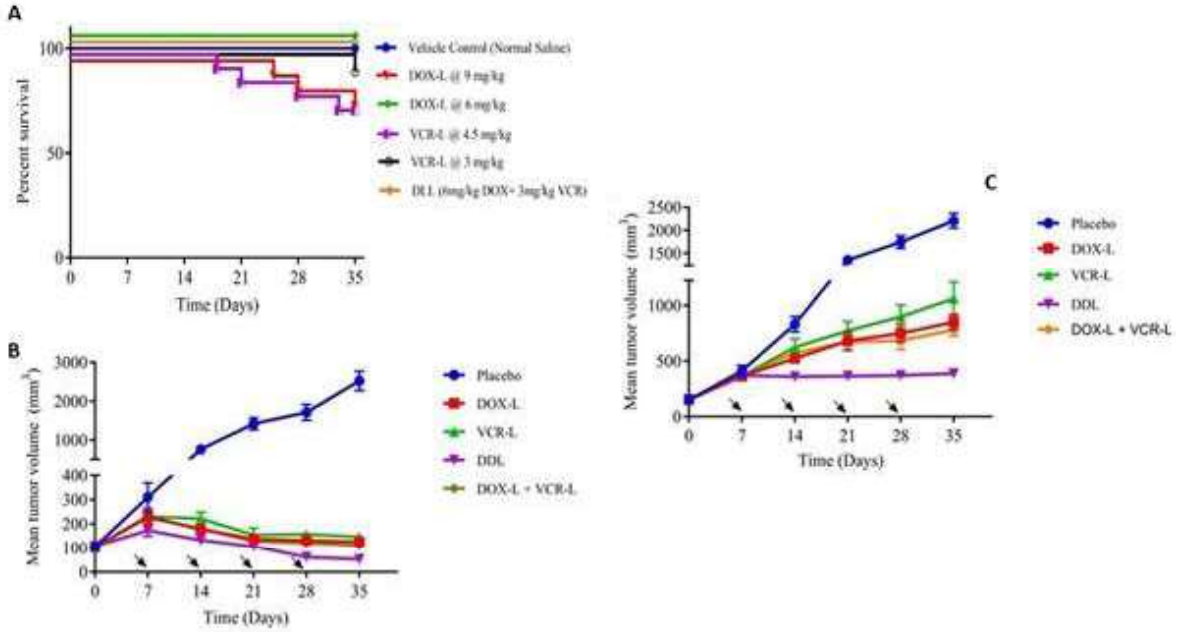

Figure 11: Synergistic combination of Doxorubicin and Vincristine co-loaded in single liposome: (A) Acute toxicity at various concentrations (B) Tumor regression against MDA-MB 231 (TNBC); Tumor regression study against A549 (NSCLC). (The figures have been reproduced with permission (225)).

Radiotherapy with combination chemotherapy indicated for advanced NSCLC has often been associated with localized recurrence and reduced overall survival. The co-delivery of cisplatin and paclitaxel with radiotherapy was attempted using PEGylated PLGA nanoparticles for improving the therapeutic efficacy with reduced toxicity to normal tissues. The dual drug nanocarrier with radiotherapy presented significantly improved tumor regression as compared to free drug combination, combination of single drug nanocarriers when tested in H460 tumor model in female athymic mice (226). Active targeting of the overexpressed integrin α3 on tumor cells of TNBC may be achieved by surface engineering nanocarriers with the peptides to ensure enhanced cellular uptake. The synergistic active codelivery of doxorubicin and rapamycin was attempted using cyclic octapeptide conjugated liposomes. The formulation presented significantly enhanced tumor cell uptake, regression with preferential distribution to the neoplastic cells as compared to the drug solutions and non-targeted liposomal delivery. Presentation of rapamycin in such targeted nanocarrier enhanced the inhibition of HIF-1α

protein. The overexpression of this protein has been associated with increased angiogenesis, drug resistance, neoplastic growth and delayed prognosis (227).

The active therapeutic agents have often been combined with adjuvant drugs for overcoming the drug resistance in NSCLC and TNBC. The delivery of these drug combinations may be facilitated using passive or active targeted nanocarriers and have been explored for improvement in the efficacy of established therapeutic regimens. Polymeric metformin was co-encapsulated with cisplatin polyglutamic acid complex in self-assembled cationic DOTAP liposome. This nanocarrier presented significantly enhanced tumor accumulation, apoptosis and regression without nephrotoxicity as compared to free cisplatin when tested in H460 tumor model in female nude athymic mice. Mechanistically the synergistic drug combination was found to have inhibited mTOR(mammalian target rapamycin) and AMPKα (activated protein kinase α) which have been associated with the cisplatin toxicity in NSCLC (228). Similarly, gemcitabine hyaluronate complex co-loaded with cisplatin-chitosan conjugate in layer-by-layer nanoparticles were prepared. This self-assembling nanocarrier significantly improved cytotoxicity in-vitro as well as anti-neoplastic effect in-vivo in H460 BALB/c mice model as compared to free drug and single drug nanocarrier (229).

Tumor specific targeting may be achieved by surface modulation of the nanocarriers using ligands against single or multiple surface overexpressed proteins on the surface of these neoplastic cells (147). The co-delivery of docetaxel and curcumin using folate targeted PLGA nanoparticles showed improved cytotoxicity in A549 cell line with enhanced anti-tumor efficacy in C57BL/6 mice bearing S180 cell line as compared to untargeted nanoparticles (230). The ratio-metric delivery of docetaxel and curcumin was achieved using lipidic nanocarriers surfaced engineered to target folate receptors in NSCLC. This formulation presently significantly improved relative bioavailability and tumor regression as compared to Taxotere®. The presentation of such combination targeted to the overexpressed folate receptors in NSCLC exhibited improved apoptotic, tumor regression and anti-angiogenic potential with reduced toxicity as compared to the marketed docetaxel formulation (231). Doxorubicin co-loaded with astragaloside in folate and octa-arginine (R8) surface modified liposome showed improved cellular uptake and tumor targeting. The use of R8 penetrating peptide and folate receptor provided targeted tumor cell uptake while the use of astragaloside

reduced the doxorubicin resistance leading to significanly improved the tumor regression in MDA-MB 231 model in nude mice when compared to the free drug cocktail (182).

Additionally, delivery of synergistic drug/ prodrug combination targeted to tumor microenvironment may provide site specific delivery of the therapeutic agents while modulating the conditions associated with the migration and nutrition of tumor cells. Glutathione and pH sensitive delivery of conjugated prodrug of cisplatin and doxorubicin loaded in glyceryl monostearate with soya lecithin nanocarrier presented strong synergistic antitumor efficiency (approximately 80%of tumor reduction as compared to free drug combination) in A549 model in female BABL/c mice model (232). R8 surface modified liposomes co-loaded with epirubicin and dihydroartemisinin were prepared for targeting vasculogenic mimicry (VM) associated with NSCLC neoplasms. R8 engineered liposomes improved selective accumulation, in-vitro cytotoxicity, destruction of VM channels and reduction in cellular migration related to A549 cells. This may be mechanistically associated with the down regulation of transforming growth factor-β1, matrix metalloproteinase-2, CD144 and hypoxia-inducible factor 1α. The nanocarrier exhibited significantly improved tumor regression and reduced metastasis as compared to single drug liposomes and non-surface modified dual liposomes in A549 model in BALB/c mice (233). Similar results were obtained when RPV peptide modified epirubicin and dioscin co-loaded liposomes were tested in A549 model in male BALB/c mice in comparison with free drugs, single liposomes and RPV free dual liposome (figure 12) (234).

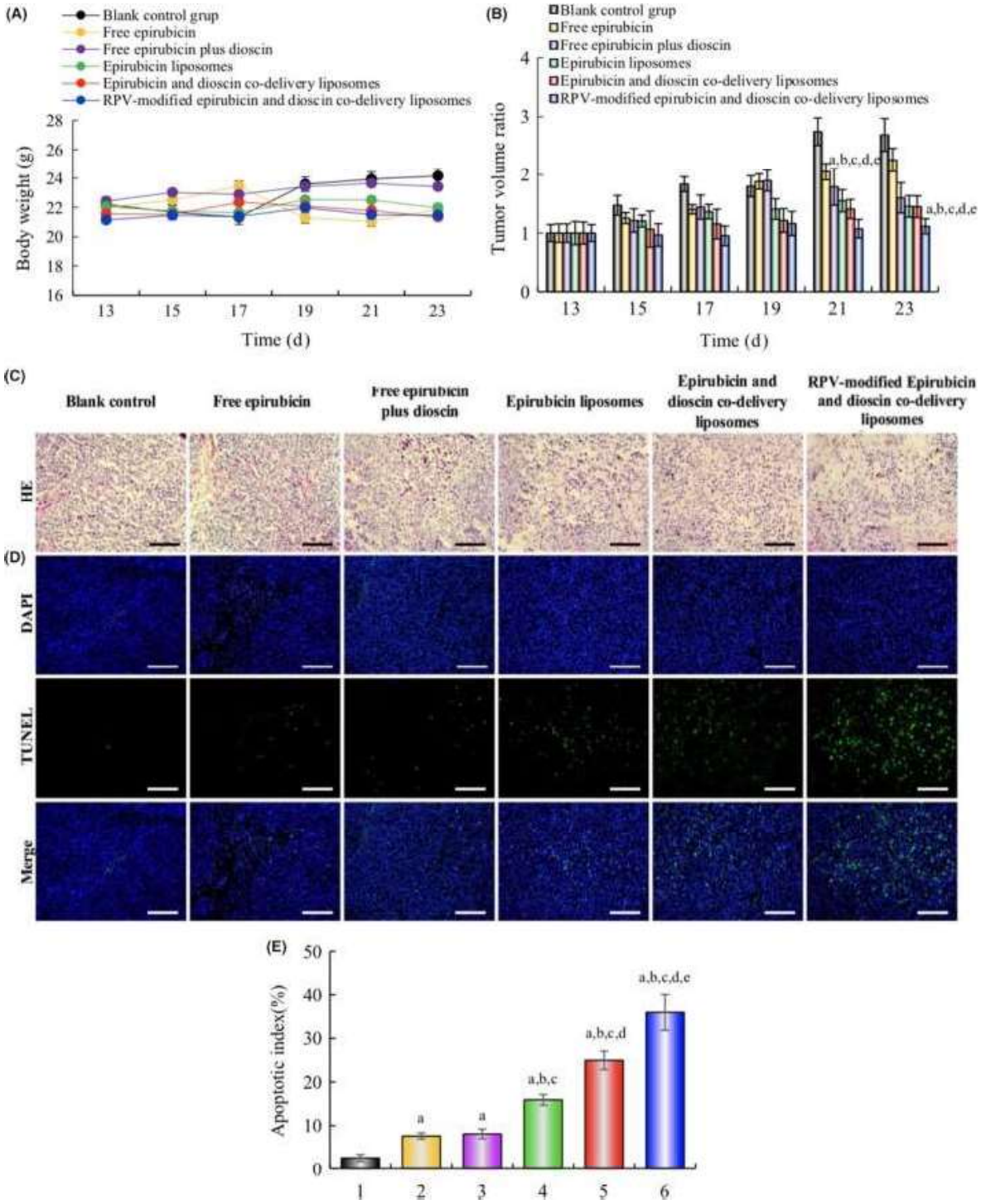

Figure 12: Antitumor efficacy of active targeted RPV peptide modified epirubicin- dioscin liposome in NSCLC (A) changes in bodyweight (B) Changes in tumor volume (C) Pathological images of neoplastic tissue (H&E staining) (D) Apoptosis pictographs of tumor cells by

TUNEL assay (E) Quantitative estimation of apoptotic index (1- control, 2- free drug epirubicin, 3- free drug epirubicin+dioscin, 4-liposomal epirubicin, 5- dual (epirubicin+dioscin) liposome, 6- RPV modified dual liposome. Data has been represented as mean±SD (n=8). [Adopted with permission from (Open access under Creative Commons Attribution-NonCommercial-NoDerivs License) (234)]

The use of lipidic-polymeric hybrid nanocarriers provide the combined advantages of both the delivery systems for improved delivery of the agents to the targeted sites, improved therapeutic efficacy with minimised toxicity and resistance to therapeutic agents. Active targeting for co-delivery of drugs to surface EGFR overexpressed in NSCLC may present suitable opportunities for improvement in tumor specific drug delivery and accumulation (235). PEGylated hybrid nanocarrier of polylactic acid prepared for the synergistic co-delivery of cisplatin and doxorubicin showed improved tumor regression and reduced systemic toxicity when tested in A549 model in male C57BL/6 mice when compared to free drug combination (236). Active co-delivery of docetaxel and reservatrol through PEGylated stearic acid hybrid nanocarrier to surface EGFR overexpressed and ROS sensitive NSCLC tumor model was attempted. The nanocarrier showed improved uptake and cytotoxicity when tested in-vitro in HCC827 and NCIH2135 cell lines while showing significantly improved synergistic antitumor efficacy in HCC827 model in BALB/c mice and reduced systemic toxicity when compared to free drug as well as non-targeted hybrid carrier (237).

The systemic toxicity associated with combination chemotherapy may also be reduced by the use of drug polymer conjugates. The temporospatial co- delivery of the doxorubicin and gemcitabine using hyaluronic acid conjugates exhibited improved tumor regression of the 4T1 orthotropic TNBC model when compared to free drugs and single conjugated drugs (238). The combination chemotherapy may be further enhanced using co-delivery of genes along with drugs. Consequently, presentation of suitable drug combinations in nanocarriers effective against both these carcinomas may prove to be beneficial in increasing the efficacy, overall survival rates and improving quality of life of the patients while reducing the toxicity and associated drug resistance. Further effective combinatorial nanodelivery may further be achieved by combining drugs with genetic components.

2.6.4 Drug Gene Combination nanocarriers

The tumor heterogenicity associated with TNBC and NSCLC have often been associated with the failure in the effective management of these carcinomas. Combinatorial approach of delivering gene therapeutics with chemotherapeutics may present suitable opportunities in efficient treatment of both cancers (239). Numerous genetic targets have been identified as potential targets for gene therapeutics with siRNA, miRNA and dsRNA have been studied extensively for the gene therapy in various disease including triple negative breast cancer and non-small cell lung cancer. RNA based carrier delivery has been studied extensively nowadays due to clear advantage over DNA based carriers. First of all, target location of the RNA delivery has been only cytoplasm rather than nuclease in DNA delivery system which makes RNA very efficient for the targeting. Even higher levels of protein transcription have been mediated through RNA compare to DNA which broads its scope (240). Most importantly incorporation of RNA molecule in to cells has been obsoleting the host DNA mutation hence gene amplification or suppression with RNA is safer compare to DNA (241). Currently, amino lipid loaded paclitaxel nanoparticles have been developed for the entrapment of the P53 gene mRNA. The developed nanoparticles have possessed mRNA loading efficiency of 88.7 % along with enhanced loading of paclitaxel ~94% compared to registered paclitaxel advanced formulations. Improved cytotoxicity has been noticed for the developed combinatorial nanoparticles in MTT essay. Even results of In-vivo tumor inhibition study has been suggested less tumor volume in miRNA loaded nanoparticles compare to paclitaxel nanoparticles (242). Similarly, lipid nanoparticles have developed for the successful entrapment of siRNA targeting polo-like kinase 1(PLK 1) gene which is responsible for the neoplastic proliferation in cells. Surface of the nanoparticles have been decorated with antibody against EGF. Biodistribution study using radio-isotope loaded nanoparticles along with fluorescent probe loaded siRNA have shown improved intracellular siRNA concentration in MB-231 carcinoma bearing mice. Expression of PLK-1 is suppressed after treatment with developed formulation and it is confirmed with RT-PCR (243). Another immunotherapeutic agent anti-CLTA4 has been combined with mRNA for the downregulation of the MUC1 tumor antigen gene in the dendritic cells. The developed nano vaccines are able to delivered combinatorial therapeutic to the dendritic cells and activates the production of tumor specific T-cells. The cytotoxicity studies

suggested higher cellular toxicity of the TNBC 4 T1 cell in cell lines. Even in-vivo tumor inhibition study in mice has suggested higher tumor inhibition in combinatorial therapy compare to monoclonal antibody treatment alone. Hence above two study confirms the potential of the RNA and immunotherapy approach as outstanding combination treatment for the TNBC (244). Role of siRNA in combination with photodynamic agent was evaluated using multifunctional porphyrin grafted cationic lipids along with siRNA HIF (hypoxia inducible factor)-1α. HIF-1α induced proteins are responsible for the energy metabolism, angiogenesis and apoptosis of tumor cells. The amphiphilic nature of lipids it can be self-assembles as microbubbles and cationic groups of lipids are responsible for High siRNA loading due to electrostatic interaction. Distribution of the microbubble can be easily monitored using ultrasound imaging with range of 3-12 MHz and it could be self-converted in to nanocarriers which can accumulate siRNA and porphyrin at tumor cells with external effect of ultrasound (due to cavitation). The developed microbubbles have been evaluated for in-vivo tumor suppression which shows dramatically reduction in tumor volume after treatment. In-vitro efficacy of formulation for siRNA delivery has been confirmed by RT-PCR which shows down-regulation of HIF levels in cell line (245).

Targeted co-delivery of cisplatin and human antigen R siRNA was achieved using polyamidoamine dendrimers surface decorated with folic acid. This nanoconstruct exhibited significantly improved cytotoxicity in H1229 NSCLC cells overexpressing folate receptors as compared with the individual treatments. Additionally, these nanocarriers exhibited significantly reduced toxicity against the lung fibroblast MRC9 cells (246).

Similarly, drug-gene combinatorial approach has been proved beneficial for the reversal of the drug resistance in non-small cell lung cancer. The increase in the chemo sensitization of A549 cells in the presence of the siRNA targeted for ribonucleotide reductase subunit 1 was attempted. Level of the ribonucleotide reductase has been fallen significantly when siRNA has been delivered through the nanoconstructs. And cell line studies suggest that preexposure of A549 cells with siRNA nanoconstructs has been shown to increase chemo-sensitization of the gemcitabine up to 5 folds (247). The cisplatin efflux promoting ABCC3 gene was silenced using lipid and poly(lactic acid-polyethylene glycol) co-polymer nanocomposite which delivered ABCC3-siRNA along with cisplatin. These composite hybrid carriers showed

improved cisplatin loading as well as in-vitro uptake, cytotoxicity in A549 cell line and disruption of the cell cycle at G_2-M phase. The in-vitro gene knockdown studies showed improved silencing of the gene while presenting significantly improved tumor suppression in A549 xenograft tumor in BALB/c nude mice. This hybrid formulation presented significantly improvement in efficacy and reduction in cisplatin resistance as compared to currently marketed formulations (248).

Co-delivery of therapeutic agents with the antisense siRNA targeted against antiapoptosis protein Bcl-2 may improve the apoptosis potential of the drug in the drug resistant NSCLC. Doxorubicin and Bcl-2-siRNA were co-loaded in pegylated PLGA complexed with poly l-lysine (PEAL polymer) nanoparticles and surface engineered with epidermal growth factor ligand for evaluation against DOX resistant NSCLC. These polymeric nanoparticles presented improved tumor cell uptake, controlled release profile of the agents, reduced toxicity and significant change in apoptosis as compared to single component nanocarriers and non-targeted nanocarriers. Further, the nanocarrier presented enhanced DOX accumulation in neoplasms, tumor regression with reduction in the expression of Bcl-2 when tested in H1299 xenograft model in BALB/c nu/nu mice (249).

Layer-by-layer nanoparticles may be used for the co-delivery of siRNA along with chemotherapy agents for the knockdown of genes associated with drug resistance. Such preparations improve the loading capacity of the siRNA while providing enhanced stability in in-vitro and in-vivo settings. The nanocarriers were prepared by kneading poly-l-arginine layers with MDR1(multidrug resistance protein-1) targeted si-RNA on the Doxorubicin liposome with entire assembly being surface pegylated and coated with hyaluronic acid for targeting CD44 overexpressed on TNBC cells. When tested against MDA-MB-468 xenograft model in NCR nude mice, the nanocarrier presented 80% reduction in expression of targeted gene, 4-fold increase in doxorubicin uptake as well as 8-fold reduction in the neoplastic volume with non-significant toxicity profile as compared to the naïve agents and drug combination with scramble siRNA (250). Further, co-delivery with siRNA targeted against IKBKE (inhibitory κB kinases ε) oncogene responsible for enhanced neoplastic migration, invasiveness and aggressive growth of TNBC may improve the efficacy of the chemotherapeutics. Self-assembling nanocarrier made of cholesterol peptide micelles when loaded with cabazitaxel,

IKBKE siRNA and surface modified with hyaluronic acid presented improved cellular uptake and tumor accumulation in CD44 overexpressed TNBC cells. This nanocarrier showed improved silencing of the IKBKE oncogene, reduced angiogenesis, invasiveness and enhanced anti-tumor activity in orthotropic model of MDA-MB 231 in nude mice as compared to the naïve drugs (251).

In non-small cell lung cancer cells mutation of the KRAS (Kirsten ras sarcoma viral oncogene homolog) mutation along with lost p53 function has been found prominent. Hence combining chemotherapeutics with gene targeting of both can be proved beneficial for the reversal of the mutation with increased therapeutic efficacy of drug. Liposomal formulation of cisplatin has been developed including multiple layers of polyelectrolytes which possess siRNA for KRAS and miRNA for p53 gene by group of researchers to evaluate on orthotropic lung cancer model. They developed formulation have shown enhance cytotoxicity in A549 cell lines as well as on KP cell lines. Even prolonged survival of the mice has been noticed with the combination treatment. In-vivo studies are provide the source for the future clinical trials on the combination treatments (252). Triple combinatorial approach combining gene delivery along with chemotherapy and phototherapy by formulating nanohydrogels was further attempted. Three strains of miRNA including let-7a, microRNA 34a and microRNA 145 were incorporated with chemotherapeutic agent doxorubicin along with photosensitive agent 5,10,15,20-tetrakis (1-methylpyridinium-4-yl) porphyrin to delivery drug at non-small cell lung cancer cells. The results indicated that microRNAs integrated with DOX and TMPyP4 played significant synergistic role (as presented by in-vivo studies) in reversal of multi-drug resistance in non-small cell lung cancer cells. Even the simultaneous delivery of the three different miRNAs are able to silence pump as well as non-pump mediated drug resistance in chemoresistance cells and significantly inhibit target cells proliferation (253). A chitosan-based delivery system has been established using shRNA plasmid for surviving, erlotinib (EGFR-TKI) and heptamethine cyanine dye (photothermal agent) for the treatment of EGFR mutated non-small cell lung cancer. The obtained nanocomplex have shown high DNA binding efficiency along with increased heptamethine cyanine delivery to EGFR mutated NSCLC cell due to targeting properties of erlotinib. The results of *in-vivo* and *in-vitro* proved reduced expression of survivin and signifies the synergistic photothermal effect with erlotinib for anticancer effect in

erlotinib sensitive or resistant EGFR mutated NSCLC. Hence, the downregulation of survivin along with the photothermal therapy acts synergistically with erlotinib which helped in reversal of erlotinib resistance (174). The effect of co-delivery of the gene therapeutics with chemotherapy drugs in TNBC may be further improved by construction of nanocarriers responsive to phototherapy. Laser irradiation of thermosensitive PLGA-polymethacrylate co-polymer based nanocarrier encompassing paclitaxel and survivin siRNA resulted in highly significant tumor regression at reduced drug concentrations in MDA-MB 231 xenograft model in BALB/c nude mice as compared to the free drugs and single nanocarriers (254).

These studies indicate that drug-drug and drug-gene combination therapy when delivered using effective nanocarrier system were able to overcome multi-drug resistance and improved the sensitivity of chemotherapeutic agents.

2.7 Clinical challenges and formulation approaches

2.7.1 Preclinical-to-clinical translation

Despite all the advances in the nanocarrier based therapies in NSCLC and TNBC, the translation from pre-clinical to effective clinical outcomes have presented limited success. Consequently, it is important to explore potential factors which may be responsible for treatment failures in clinical set up despite exhibiting promise in pre-clinical models (255). it is important to map the potential factors affecting the efficient transport of the nanocarriers post injection through blood to the tumor (256). The formation of protein corona by serum proteins on nanoparticle post administration have been clinically found to alter the transit properties of the carrier (257). The presence of inner tightly bound corona and outer interchangeable soft corona have been known to affect the stability of the nanocarrier and the biodistribution profiles. The biomolecular corona formation further complicates and reduces the efficiency of the targeted delivery using surface functionalized nanocarriers (258). Protein corona formation has been observed in case of clinically used PEGylated doxorubicin and have been implicated in its altered bio-distribution towards various cancers (259). Such opsonization of the nanocarriers results in immunorecognition by the components of MPS (mononuclear phagocyte system) and increased blood clearance. PEGylation based surface passivation of the nanocarrier have been utilized for the reduction in opsonization and increasing the blood

residence time leading to EPR mediated presentation of the drugs at the tumor. However, it should be noted that pegylation of the surface serves to alter kinetics of opsonization with increased generation of the anti-PEG antibodies upon repeated injections of the nanocarrier (260). Such immune-activation results in the alteration of the pharmacokinetic profile of the nanocarriers and increased clearance referred as ABC (accelerated blood clearance) phenomenon (261). Additionally, the innate immunity activation in response to PEGylated carriers results in clinically observed complement activation-pseudo allergy (CARPA) and treatment failures (262). Clinically tested PEGylated liposomal doxorubicin (Doxil™) presents an excellent example demonstrating the issues associated with such surface engineering approaches. The presentation of doxorubicin in passive targeted nanocarrier results in reduced cardiotoxicity while having improved bio-distribution to intended site and enhanced toxicity due to peripheral deposition in skin (palmar-plantar erythrodysesthesia) (209).

The effective translation of the performance of nanocarriers into clinical realities has been often affected by the EPR effect (98). It is important to note that significant difference has been observed in case of clinical EPR as compared to pre-clinical EPR. The tumor heterogeneity, degree of genetic alterations, surface overexpression of proteins and role of epigenetic activities have been found to modulate clinical EPR associated with these aggressive neoplasms (97). Additionally, the level of angiogenesis, presence of CSC, tumor fibrosis, presence of pro-immunity factors and degree of EMT affects the EPR mediated uptake of the nano-formulations (263). Consequently, modulation of the vasculature, alteration of tumor microenvironment, overcoming the positive tumoral pressure, stimuli-based sensitization and identification of exclusive pharmacological targets need to be evaluated to improve the EPR of nanoparticulate formulations for improved therapeutic outcomes (264).

The efficiency of passive accumulation has been affected by additional barrier presented by tumor microenvironment to nanocarrier uptake in tumors after EPR mediated accumulation in endothelial cells (265). The presence of extracellular matrix (ECM) fibroblasts has been associated with the macrophage mediated uptake of the nanocarriers and prevention of the tumor internalization (266). PEGylated liposomal doxorubicin has been found to present PEG-induced steric hindrance to tumor cell uptake and preferential affinity towards the ECM as compared to neoplastic cells (267). Further, both NSCLC and TNBC exhibit inter-subunit

variability at macro/micro levels of cellular organization which has been implicated for improved survival of the tumor cells (268). Such tumor heterogeneity is associated with modulation of the cellular surface, altered molecular targets and reduced recognition by the therapeutic agents along with increased multidrug resistance (269). The heterogenous tumor remodelling leads to variability in the intended therapeutic outcomes, necessitating the need of identification suitable surface engineering and evaluation of combinatorial targeting of multifunctional components (270, 271). The ineffective clinical treatment using Doxil™ in TNBC and NSCLC have been associated with aforementioned factors and necessitate the need of further modifications for improved nanocarrier mediated delivery of drugs (267, 272). Despite, the shortcomings associated with the liposomal doxorubicin treatment in both these diseases, the nanocarrier has been widely explored in combination with older/newer drugs in clinical studies as indicated in Table 5.

An important aspect in pre-clinical translation failures has been the development of efficient design of the nanocarriers customized for the drug delivery to NSCLC and TNBC tumors. Liposomal Cisplatin formulations (SPI-077-Alza corporation and Lipoplatin- Regulon) had exhibited superior pre-clinical efficacy profiles and improved safety/toxicity profiles in phase I/II clinical trials. However, both formulations failed to elicit the desired therapeutic outcomes when tested as single agent or in combination in Phase III clinical trials. Such therapeutic outcomes have been attributed to failure of the drug release with the desired pharmacokinetics to meet the primary end point (273, 274).

The effective translation of therapeutic efficacy in pre-clinical set up to clinical usage has been observed in case of albumin bound paclitaxel nanoparticles. As detailed earlier, the efficacy of the formulation has been potentiated due to the non-covalent reversible binding of human albumin prior to administration along with the exchange between the nanocarrier and plasma albumin during systemic transit (129). This albumin exchange results in reduction of the hydrodynamic diameter of the formulation from 130 nm to 10-24nm leading to improved transcytosis across tumor cellular endothelium (275). The increased accumulation in TNBC and NSCLC cells have been attributed to the active albumin transport mediated through albondin receptor along enhanced interaction with overexpressed SPARC protein (276). The albumin-based accumulation of paclitaxel has decreased the Pgp mediated MDR of the active

drug along with reduction in the levels of cancer-stem cells leading to improved efficacy in metastatic conditions of these cancers (277, 278).

2.7.2 Challenges and Opportunities

The treatment landscape for TNBC and NSCLC has seen radical change in the last decade with the emergence of immunotherapeutics and target-specific drugs. Although, these newer agents have presented a plethora of options to the clinicians for the treatment of these cancers, the newer drugs have been tested/approved for usage along with the traditionally used Abraxane™ and Doxil™ in the therapeutic regimens. The most recent regulatory approval of immune checkpoint inhibitor atezolizumab indicates the usage in NSCLC and TNBC in combination with Abraxane™ (279). Importantly, the use of nanocarriers to deliver the drug payload to intended sites have been included as a part of multiple clinical trials and have been mechanistically implicated to provide an integrative tumor kill approach (280). Further, the challenges and opportunities offered by the nanotechnology-based therapeutics in better alleviations of the disease conditions of NSCLC and TNBC need to be evaluated.

2.7.2.1 Challenges in design and manufacturing

As illustrated in the previous sections, the delivery of various therapeutic agents has been modulated using promising nanocarrier platforms to present tailored pharmacokinetic and suitable tumor distribution profile. The translation of single and combinatorial nano-drone approach for effective delivery of the agents to the lacunae of action requires establishment between of molecular balance between tumor conditions and nanocarrier properties (281). It is important to note that there can be no single nanocarrier design which can provide same therapeutic effect for different drugs in both NSCLC and TNBC (256). Consequently, it is important to understand the challenges to the development of suitable nanocarrier based drug delivery systems. The design and choice of appropriate nanocarrier for controlled delivery of the single as well as combinatorial (dual/ multiple) drugs at sub-cellular sites would require an understanding of the biophysiological characteristics of both these tumors (282). The intended physicochemical characteristics of such nanocarriers would depend on component factors such as lipophilicity of the drug(s), possibility of interactions between drugs and excipients (lipids/polymers/stabilizers); molecular weight of polymers and degree of saturation/ chain

length of lipids; and intended drug positional arrangements (surface or embedded in matrix/aqueous or lipid layer) (283). For nanocarrier designing against specific molecular targets, the assessment of intended surface properties such as zeta potential, morphology, hydrodynamic size, engineering using targeting moieties and generation of drug-component conjugates need to be done (284). Further, formulation properties for clinically relevant nanocarrier design would include encapsulation efficiency; intended release profiles and temporospatial presence and stability of the formulations (285). The various important considerations for nanocarrier design and different polymeric as well as lipidic nanocarrier platforms are depicted in Figure 13.

Additionally, the in-transit biopharmaceutical properties of the nanocarrier would affect efficient delivery of the therapeutics to tumor site. Careful consideration of the properties such as preferential localization sites, plasma circulation time (presence of PEGylation- long or short circulation), elimination kinetics and dissemination potential to extremities in human system needs to be evaluated (265). Evaluation of intended biological properties for appropriate nanocarrier design would include non-opsonization; reduced uptake by organs of RES; EPR mediated passive targeting potential; feasibility of ligand mediated active targeting of tumor surface proteins and relative specificity/density of such receptors on tumors in comparison with normal cells (286). The potential reasons of the failure in effective pre-clinical to clinical translations has been assigned to lack of sufficient pharmacological evaluation, improper identification of potential biomarkers and clinical design (287). These necessitate the establishment of in-vitro 2D/3D tumor models, in-vivo animal models simulating the NSCLC and TNBC disease conditions including the surface overexpression of the receptors and the components of the tumor microenvironment which may prevent the efficient uptake of the nanocarriers (288). Additionally, identification of the potential ways for overcoming the pathophysiological heterogeneity of TNBC and NSCLC for enhancing the EPR mediated uptake need to be done. Further, the immunological and the toxicity profiles (known/un-intended) of the nanocarrier mediated drug delivery needs to be evaluated considering the pathophysiology associated with these tumors (89). Consequently, a balanced rationalistic approach for incorporating the aforementioned factors needs to be taken while considering the risk-benefit ratio of nanocarrier based drug against these cancers. The passive targeted single

drug nanocarriers (Abraxane™, Doxil™) provide suitable insights in establishing the correlation between the choice of suitable nano-constructs for individual drugs and their therapeutic efficacy. The effective design of siRNA based nanocarrier therapeutic necessitate the choice of components which ensure properties such as long blood circulation for enhanced tumor accumulation; target specific delivery, high encapsulation, endosomal escape and reduced toxicity potential.

The design of combinatorial (drug-drug/drug-gene) nanocarriers require establishment of synergism between the therapeutics along with choice of appropriate components which can ensure sufficient encapsulation and stability of the nanocarrier during storage and the during transit to the tumor site. As detailed in previous sections, NSCLC and TNBC have presented specific overexpression of surface biomarkers/proteins. The development of such active targeting nanocarriers further present additional surface engineering challenges (289). The levels of surface overexpression of such receptors along with identification of specific ligands with validated targeting capabilities are necessary prior to nanoparticulate design. Surface engineering parameters such as conjugation chemistry, choice of spacer subunits and insertion techniques are important when selecting the matrix components (290). Importantly, such nano-design should ensure non-interaction with structural components of long circulation and suitable surface presentation to the receptor while avoiding opsonization by MPS during transit (291). Presentation of the ligand for receptor binding and efficient receptor-mediated cellular uptake are affected by the conjugation chemistry as well as incorporation techniques of the surface ligands. Further, the development of validated physicochemical and pharmacological quantification techniques needs to be done for effective design of such active targeted nanocarriers (292).

Thus, identification of suitable CPP (critical process parameters) and CMA (critical material attributes) present significant challenges for the development of scalable/reproducible manufacturing of nanocarriers. Establishing a suitable correlation between CPP and CMA may help in achieving the QTPP (quality target product profile) of effective clinical usage in NSCLC and TNBC.

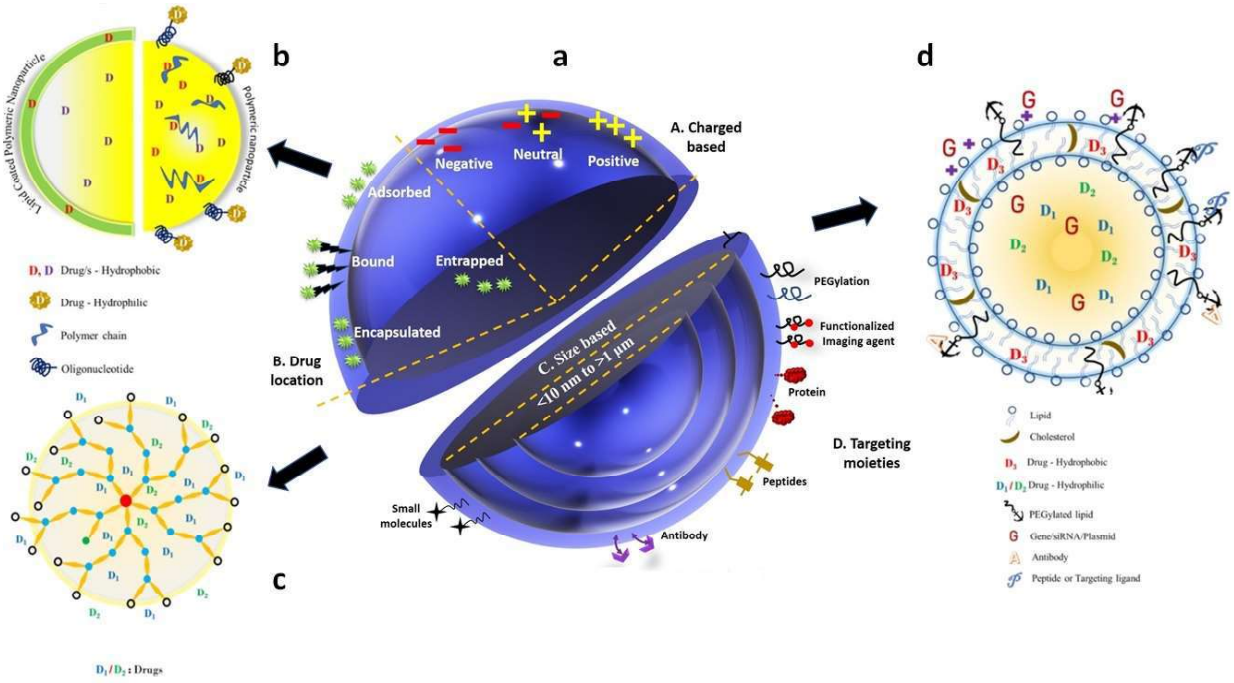

Figure 13: Important characteristics and properties to be considered for design of nanocarriers (a) including charge (A), drug location (B), hydrodynamic size (C) and targeting moieties (D). Different types of passive/ active targeted, single drug/combinatorial polymeric and hybrid lipid/polymer nanocarrier (b); polymeric micelles (c) and liposomes (d). [Adapted with permission from (12, 147)]

2.7.2.2 Opportunities

The landscape of NSCLC and TNBC therapy has been obliterated by the increased chances of mutations and the loss of therapeutic activity leading to heterogenous immune-resistant neoplasms to drug monotherapy. Consequently, a frequent strategy adopted by the clinicians involve the use of synergistic combination of the drugs acting against newer targets with traditional therapeutics to eliminate the cancer survival pathways (293). Nanocarrier based passive and active targeted controlled delivery of therapeutics present suitable opportunities for improving the treatment efficacy of such drug combinations in these cancers (294). QSAR (Quantitative-structural activity relationship) and artificial intelligence (AI) based drug discovery with improved activity against the specific cellular biomarkers of these neoplasms

may help in improving the therapeutic outcomes. Identification of newer drug targets such as immune check point inhibitors, upstream-downstream modulators of cellular kinases as well as epigenetic drugs among others having specificity to the NSCLC and TNBC present suitable opportunities for effective treatment (295, 296). Systemic poly-pharmacology based identification and validation of synergistic drug combinations against newer targets Aurora B and ZAK kinase have presented suitable personalized targets in p53/p38 cross talk network pharmacology of TNBC (297). Similar poly-pharmacology based identification of suitable drug candidates against MET, ALK, EGFR, HER2 mutations have improved the efficacy of drug combinations against NSCLC (298). Bioengineered alteration of gene expression mediated through gene delivery presents opportunities for drug resistance reversal and conditioning of the apoptotic as well as necrotic pathways leading to improved tumor cell reduction (299). The single-guide RNA (sgRNA) based genomic modulation of specific targeted loci mediated through CRISPR-Cas9 (clustered regularly-interspaced short-palindromic repeat associated protein 9) has emerged as a powerful tool for the knockdown of multiple genes affecting the disease outcomes of NSCLC and TNBC. Engineered cationic DOTAP lipid-based nanoparticle delivery of such sgRNA presented superior knockout of mutated genes in transgenic mice resulting improved NSCLC tumor regression and prevention of metastasis (300). Similar efficacy results were obtained in case of TNBC with genome editing of mutations of BRCA1/PARP1 (associated with chemoresistance) and CXC4/CXC7 (known for disease progression and metastasis); growth promoting Lipocalin 2 (Lnc2) as well as cellular progression of MSC when presented using nanocarriers (301-304). Further, lessons learnt from the liposome mediated delivery of RNA interferance (RNAi- Onpattro™) may be implemented in development of successful gene-based drug delivery systems against these two cancers (305).

Additionally, epigenetic de-regulation has been implicated in TNBC as well as NSCLC for tumor growth, invasion and immune-resistance leading to altered disease outcomes (306). DNA methyltransferase and Histone deactylase inhibitors (HDAC) loaded single as well as combinatorial targeted nanocarriers present promising strategies for the improved efficacy in TNBC and NSCLC while circumventing the issue associated with the delivery of naïve agents to the solid tumors (307). Lysophosphatidic acid surface protein (LPAR1) and lymphocyte

expressed G protein (G_2A) have been implicated for the development of the autoimmunity of TNBC cells. Lysophatidic acid and lyophosphatidylcholine surface modified nanoemulsion co-loaded with decitabine and panobinostat on systemic targeting against these receptors showed improved tumoral uptake, enhanced distribution and tumor regression in MDA-MB 231 model in athymic nude mice as compared to free drugs. Further, the nanocarrier targeted the growth of mesenchymal TNBC cells by alleviation of CDH1/E-cadherin expression and suppression of FOXM1 (forkhead box M1) while having no such effects on the epithelial cells (308). Drug resistance of NSCLC cells against gefitinib has been associated with the mutation of $EGFR^{T790M}$. The delivery of dual ligand functionalized liposomal delivery of vorinostat with gefetinib against HER-2 overexpressed NSCLC cells and mannose proteins on TAM was attempted. The nanocarrier presentation resulted in the reprogramming of the tumor cell death cascade, reversal of gefitinib resistance and improved tumor regression (309). Similarly, co-delivery of 4-phenylbutyric acid with curcumin in hyaluronic acid nanocarrier showed improved apoptosis and tumor cell death in CD44 overexpressed A549 model in mice (310)

As illustrated earlier, passive as well as active targeted lipid and polymeric nanocarriers have exhibited good promise on the pre-clinical set up in improving the effectiveness of TNBC and NSCLC treatment. Further exploration of such opportunities while incorporating the learnings of success/failure of EPR mediated approved therapeutics (Abraxane™, Doxil™) may help in improving the clinical outcomes (311). Generation of multifaceted engineered immune-nanocarriers may present enhanced targeting against surface epitope and cellular uptake leading to reduced drug resistance as well as metastasis (312). Combinatorial nanocarrier approach has provided new platform to integrate conventional chemotherapy with futuristic drug/gene therapy generating personalized medicine to ensure tumor specific treatment (313).

Another emerging approach for improving the chemotherapeutic efficacy against these cancers would include localised and stimuli-responsive delivery of drugs. Drug-device combination delivering aerosolized lipid and polymer-based nano-formulations similar to that of liposomal amikacin (Arikayce™) may be explored for more efficient delivery of chemotherapeutics (314). The delivery of doxorubicin, paclitaxel, cisplatin and docetaxel through non-targeted and targeted aerosolized nanocarriers have shown promising results in pre-clinical set up against NSCLC. However, the effective translation of such therapies for clinical usage would

depend on the stage specific pathophysiology of NSCLC, physicochemical properties of the formulation, choice of appropriate device and availability of in-vitro-in-vivo correlation methods (315, 316). Localized stimuli-sensitive nano carrier delivery of chemotherapeutic drugs may be accomplished by careful evaluation of pre-clinical to clinical failures of Thermodox™ (125).

Thus, nanocarrier based delivery of single/multiple chemotherapeutic agents have opened newer avenues for exploration of the physiological conditions and genomic composition of both these cancers. This has led to the promising development of passive/active targeted emerging opportunities for effective treatment of both NSCLC and TNBC.

2.8 Conclusion

TNBC and NSCLC as a group of neoplasms present two of the most aggressive forms of cancer highlighted by treatment failures, low overall survival and progression-free survival with patients having poor-quality-of-life. The late detection of these neoplasms has been coupled with lack of efficient therapeutic response due to genetic mutations, immune-resistance, chemotherapeutic resistance, EMT as well as cancer-stem cells leading to disease relapse and progression. Owing to the pathophysiology of these diseases, various newer agents have been identified to target specific surface overexpressed proteins and CSC. Current treatment regimen involving the use of such new therapeutics in combination with conventional agents have met with limited clinical success in improving the efficacy against these neoplasms. Nanocarrier based conventional drug delivery have formed an integral part of any such newer regimens being tested and used clinically. Active and passive targeted single/ combinatorial nanocarriers have presented suitable controlled platform for effective as well as dynamic delivery of anticancer agents against the issues of treatment failure in NSCLC and TNBC. Although these target-based nano-formulation therapies have borne minimal translation from pre-clinical research to clinical usage, they have provided enumerable opportunities for improved transfection potential with minimal toxicity. Despite, the presence of multiple unexplored areas and challenges to building clinically effective nanocarrier therapies for leveraging the maximum benefits of combination therapy, we believe nano-formulation approaches may

present more efficient treatment options against NSCLC as well as TNBC to clinicians and oncologists.

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