Chapter 2

Problem Statement, and Objective

2. Problem statement and objective of the research

2.1. Problem statement and rationale

TNBC is characterized by the absence of expression of ER, PR, and HER2. As a consequence, hormone–targeted therapies such as tamoxifen and aromatase inhibitors, as well as HER2–targeted drugs like trastuzumab, are ineffective in the treatment of TNBC. Subsequently, chemotherapy stands as the principal therapeutic approach for TNBC [72]. Taxanes, including paclitaxel (PTX) and docetaxel (DOC), are extensively utilized chemotherapeutic agents for the treatment of TNBC [27]. The primary mechanism of action for taxanes is the facilitation of microtubulin assembly and the stabilization of polymers to prevent depolymerization, thereby impeding microtubule dynamics (Figure 2. 1). In various murine and human tumor cell lines, DOC has exhibited cytotoxicity 1.3 to 12 times greater than that of PTX. Furthermore, DOC has demonstrated a 10–fold higher potency than PTX in phosphorylating Bcl–2, potentially accounting for the differential pro–apoptotic activity observed between the two compounds [73]. The pharmaceutical and pharmacokinetic profile of DOC is implicated in tables 2. 1 and 2. 2, respectively.

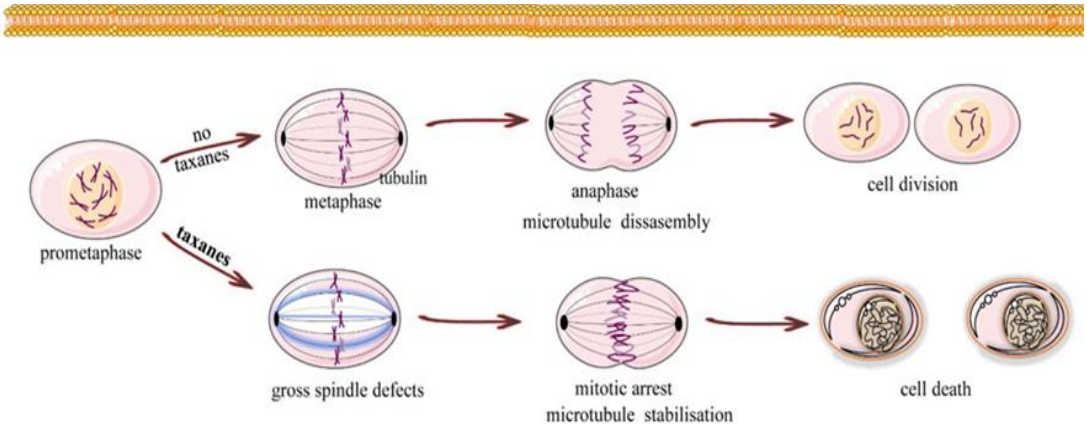

Figure 2. 1: Mechanism of action of DOC. Adapted from Ref. [27]

Table 2. 1: Pharmaceutical profile of DOC

Parameters	Description
Chemical Formula	$C_{43}H_{53}NO_{14}$
Molecular weight	807.9 g/mol
Melting point	232°C
Therapeutic class	Taxane; Antineoplastic

Log P	2.4
Solubility	Insoluble in water (0.0127 mg/ml), soluble in organic solvents, namely ethanol (1.5mg/ml), DMSO (5mg/ml), and DMF (5mg/ml)

Table 2. 2: Pharmacokinetic profile of DOC

Parameters	**Description**
BCS class	IV (low solubility and low permeability)
Bioavailability	~8 %
Half–Life	The terminal elimination half–life was 116 h
Distribution	DOC has a steady-state volume of distribution of 113 L. 94 % of DOC is bound to proteins, mainly α–1–acid glycoprotein, albumin, and lipoproteins
Metabolism	DOC undergoes hepatic metabolism by the CYP3A4 isoenzyme
Excretion	DOC was eliminated in urine and faeces following oxidative metabolism of the tert–butyl ester group, but fecal excretion was the main elimination route
Therapeutic dose	75 mg/m^2 IV for a single dose on day 1 of a 21–day cycle

DOC is administered via intravenous infusion, which may lead to significant side effects such as muscle and joint pain, anemia, immunosuppression, bone marrow suppression, peripheral neuropathy, diarrhea, and alopecia [74]. This compels us to replace the intravenous route with the oral route, which is the most patient–compliance route of administration.

Furthermore, extensive research has revealed that TNBC represents a complex network of signaling pathways orchestrated by a cascade of events. Certain pathways are rigorously regulated and are vital for the proliferation, viability, invasion, and advancement of TNBC. TNBC displays intertumoral heterogeneity arising from discrete tumor subtypes characterized by unique molecular genetic profiles, morphology, and the expression of specific markers. Additionally, TNBC demonstrates intratumoral heterogeneity, as evidenced by tumor variances, encompassing cells with diverse functional properties and distinct biomarker expression patterns [75]. While single–agent therapy has demonstrated

favorable outcomes in cell lines and pre–clinical models, it has not yielded promising results in clinical trials for combating aggressive TNBC, primarily due to its heterogeneity, rapid metastasis, and acquired drug resistance. To address this challenge, combined drug therapy has emerged as a compelling strategy against TNBC. Approximately 80 % of clinical trials are exploring novel therapeutic approaches for TNBC treatment through combination drugs. The benefits of combination therapy encompass synergistic anticancer activity, as it enables the simultaneous targeting of multiple pathways, and a reduction in dose–related side effects, attributed to the use of multiple drugs, thereby diminishing the therapeutic dose of each drug [72].

Clinical trials are underway to assess the efficacy of strategies targeting the EGFR and Src pathways, PARP1 and HDAC inhibitors, and antiangiogenic agents in combination with chemotherapy for treating TNBC. Overexpression of EGFR has been noted in 60–78 % of TNBC cases, indicating its potential as a therapeutic target in this subtype of breast cancer [76]. So, for targeting EGFR, Erlotinib (ERL), a small molecule has been proposed in combination with DOC for TNBC therapy. ERL competes with the binding of ATP to the intracellular tyrosine kinase domain of EGFR, thereby inhibiting receptor autophosphorylation and blocking downstream signal transduction responsible for cell growth, proliferation, angiogenesis, and metastasis (Figure 2. 2) [77]. The pharmaceutical and pharmacokinetic profile of ERL is implicated in tables 2. 3, and 2. 4, respectively.

Table 2. 3: Pharmaceutical profile of ERL

Parameters	Description
Chemical Formula	$C_{22}H_{23}N_3O_4$
Molecular weight	393.4 g/mol
Melting point	167–180°C
Therapeutic class	EGFR inhibitor; small molecular TKIs
Log P	2.7
Solubility	Insoluble in water (0.0891 mg/ml), soluble in organic solvents, namely ethanol (10 mg/ml) and DMSO (100mg/ml)

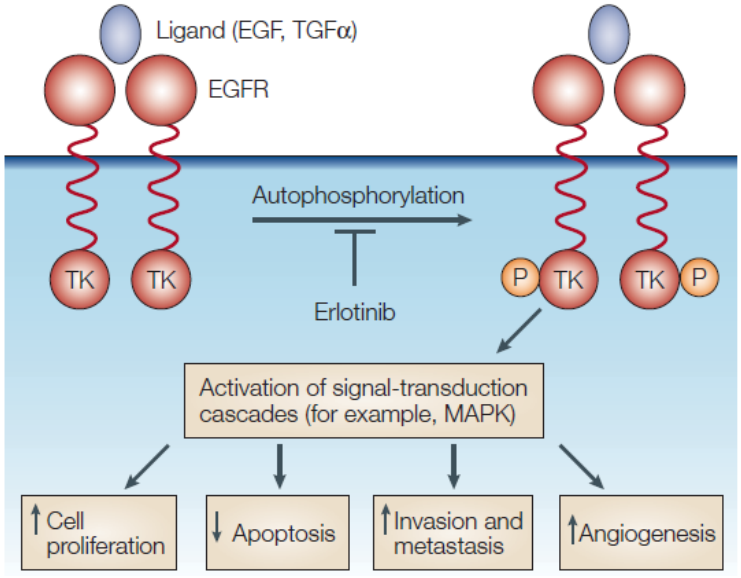

Figure 2. 2: Illustration of signal transduction through the EGFR. Ligand binding leads to receptor dimerization. This results in receptor autophosphorylation, which is inhibited by ERL. Adapted from Ref. [77].

Table 2. 4: Pharmacokinetic profile of ERL

Parameters	Description
BCS class	II (low solubility and high permeability)
Bioavailability	~40 %
Half–Life	The drug elimination half-life was estimated to be around 36 h
Distribution	The apparent volume of distribution of ERL was estimated to be around 232 L. About 93 % of the drug is bound to albumin and α–1 acid glycoprotein
Metabolism	ERL undergoes hepatic metabolism by the CYP3A4 isoenzyme
Excretion	After oral administration, about 83 % of the dose was recovered from feces and 8 % from urine
Therapeutic dose	150 mg per oral daily

Although DOC and ERL demonstrate effective anticancer activity, they cannot be delivered via the oral route because they exhibit poor oral bioavailability due to their poor aqueous solubility, thus restricting their therapeutic efficacy. To overcome these limitations, we proposed developing lipid–based nanoformulations (LNPs) to effectively deliver poorly water–soluble drugs to the tumor site. LNPs help increase oral bioavailability by following lymphatic transport and bypassing first–pass metabolism. Also, the lipid nanocarriers

increase membrane stability and reduce drug–leaching problems associated with conventional formulations. The strategies by which LNPs increase the oral bioavailability of poorly water–soluble drugs are described in table 2. 5 [78]. The different types of LNPs developed for the treatment of TNBC are liposomes, nanoemulsions (NEs), solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), lipid–polymer hybrid nanoparticles (LPH–NPs), and exosomes (Exo), as described in table 2. 6, and figure 2. 3.

Table 2. 5: Strategies employed for increasing the solubility of incorporated poorly water–soluble drugs by using LNPs.

Strategy	Mechanism	References
Enhanced solubilization	The lipids present in the gastrointestinal tract (GIT) increase the excretion of cholesterol and phospholipids (endogenous bile lipids), which further mediates the emulsification of the lipids present within the carrier system and solubilizes the drug	[79]
Alteration in the biochemical barrier	Certain lipids and surfactants can decrease the intestinal secretions of the gastrointestinal wall and prevent the metabolic activity of the enterocytes and lumen by alternating the P–glycoprotein, cytochromes, etc., thereby increasing the absorption of the drugs that are considered substrates of the stated efflux pump, and enzymes	[80]
Alteration in the physical barrier	Some lipids and surfactants can promote intestinal absorption and membrane permeability by fluidizing the intestinal cell membrane and breaching the tight junctions	[81]
Facilitation of lymphatic transport system	Lipids such as LCT (long–chain triglycerides) facilitate lipoprotein formation, further facilitating their lymphatic transport. Hence, it could be stated that LNPs composed of LCT mediate lymphatic transport of poorly aqueous soluble drugs, thus bypassing the first–pass metabolism	[78]

Table 2. 6: Various types of LNPs employed for the treatment of TNBC [82]

LNPs	Composition	Features
Liposomes	Phospholipids and cholesterol	Forms 1–20 phospholipid bilayers (vesicles) with globule size 30–3000 nm. Encapsulate hydrophilic and hydrophobic drugs
Nanoemulsions (NEs)	Oils, surfactants, and cosurfactants	Kinetically stable o/w dispersions. Have a high surface area with small size (50–500 nm). Encapsulate both lipophilic and lipophobic drugs
Solid lipid nanoparticles (SLNs)	Solid lipids (fats) and surfactants	Solid lipids improve the lipidic core and provide stability and mobility to the drug within the lipidic core
Nanostructured lipid carriers (NLCs)	Solid lipids (fats), liquid lipids (fats or oils) and surfactants	NLCs have a distorted structure, which makes the matrix structure imperfect and creates spaces for the accommodation of active compounds
Lipid–polymer hybrid nanoparticles (LPH–NPs)	Polymers, lipids	Hybrid vesicular structures integrate advantageous characteristics of polymers and liposomes in a single moiety
Exosomes (Exo)	Cholesterol, diacylglycerol, surface proteins, heat shock proteins, lysosomal proteins, nucleic acids	Homogenous nanosized vesicles with size ranges from 30–150 nm. Formed by multivesicular bodies (MVB) after fusing with plasma membrane

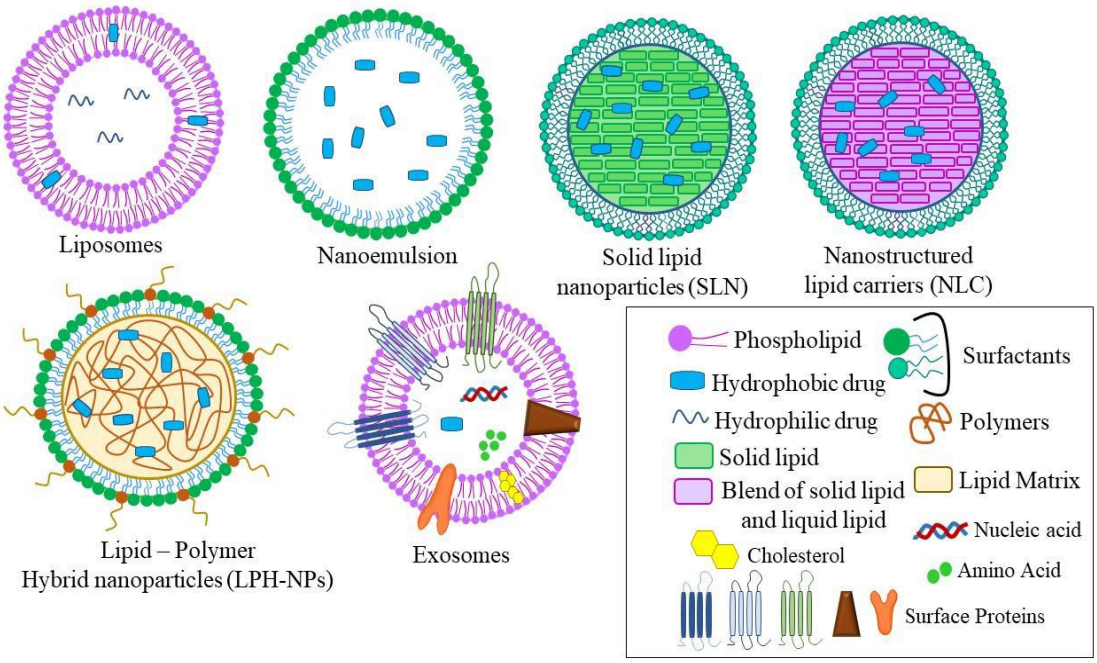

Figure 2. 3: Different types of LNPs used for the treatment of TNBC

Among the various LNPs mentioned above, SLNs and NLCs are selected for the treatment of TNBC, as they are more stable LNPs that offer a controlled drug–release profile. SLNs are o/w type of colloidal nanoparticles consisting of an inner lipid–based phase and an outer aqueous–based phase that are stabilized by surfactants or a mixture of surfactants [83]. It was observed from various studies that SLNs can either solubilize lipophilic chemotherapeutics homogeneously within the lipidic matrix or develop a drug–enriched shell surrounding the lipidic core [84]. The lipid–based phase is solid lipids, including triglycerides, fatty acids, steroids, and waxes [85]. It was further reported that the lipids and surfactants used to prepare SLNs should fall under GRAS (Generally Recognized as Safe) regulations. The most widely used solid lipids are palmitic acid, stearic acid, glyceryl monostearate, compritol 888 ATO, trimyristin, Capmul®MCM C10, soybean lecithin, etc. Regarding structure, SLNs have a solid lipid core providing enhanced stability and controlled release, while emulsions have a liquid oil core that focuses on the solubilization of drugs [86]. It also revealed that the lipids used in the SLNs remain solid at room and body temperatures [87]. Such characteristics help encapsulate lipophilic drugs in the melted lipid phase, which further helps increase the drug loading capacity of SLNs and alter the physicochemical properties of drugs both *in vitro* and *in vivo*. From various studies, it was observed that, typically, SLNs are comprised of 0.1–30 % w/v solid lipid and 0.5–30 % w/v surfactant or surfactant blend. On a similar note, NLCs are composed of a mixture of solid and liquid lipids, further stabilized in the aqueous phase via one or more surfactants [80]. The

incorporation of liquid lipids and solid lipids within the lipid matrix forms a massive crystal imperfection or amorphous structure that facilitates enhanced loading of drugs into the lipid matrix with less pronounced drug expulsion. It was further observed that the NLCs obtained should remain solid at a temperature higher than 40°C. Generally, NLCs encapsulate approximately 5 % w/v of the drug where approximately 3–4 % drug loading is obtained (entrapment efficiency of ~70 %) [88]. The various solid lipids employed in NLCs preparation are glyceryl tripalmitate, softisan 154, glyceryl monostearate, compritol ATO 888, stearic acid, Precirol ATO 5, PEG–DSPE, soybean phosphatidylcholine, etc., and the liquid lipids used for the preparation of NLCs include glyceryl tridecanoate, olive oil, labrafil WL 2609 BS, oleic acid, labrafil M2125 Cs, labrafac PG, polyoxyl castor oil, etc. From various studies, it was observed that the NLCs show high tolerability due to the existence of lipids that are biocompatible and tunable.

All these novel drug delivery systems are passively targeted carriers, and they can sometimes suffer from limitations. An inherent lack of control in uptake within these nanosystems can result in off–target accumulation, potentially leading to the development of multiple drug resistance. Additionally, passive targeting is constrained because certain tumors do not consistently exhibit the EPR effect, and the permeability of their associated blood vessels can display significant heterogeneity. To address these limitations, the concept of active targeting has been introduced. This strategy involves introducing a targeting moiety, such as a ligand, antibody, or peptide, on the nanoparticle's surface to specifically target the overexpressed receptors on tumor cells. Through this mechanism, nanoparticles can recognize and bind to target cells through ligand–receptor interactions, subsequently facilitating their internalization. This approach results in a reduced off–target drug release compared to passively targeted systems [89]. It was revealed that 30 % of the early staged TNBC and 70–80 % of stage IV metastatic TNBC show the overexpression of folate receptor α (FRα), in comparison to little or no expression (0–20 %) in healthy cells; making it an interesting target for cancer therapy. So, surface functionalizing the lipid–based nanoparticles with folic acid will result in their effective binding to the overexpressed FR–α, which then results in increased internalization into the tumor cells via receptor–mediated caveolae–based endocytosis (Figure 2. 3) [90, 91].

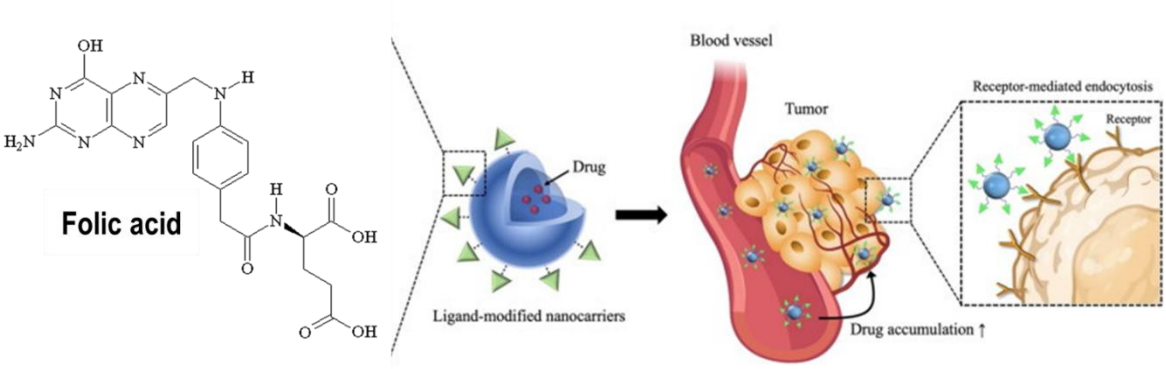

Figure 2. 4: Figure depicting the active targeting of LNPs using folic acid as a targeting ligand

2.2. Objective

The objective is to combine docetaxel and erlotinib to achieve synergistic anticancer effects by developing lipid–based nanoformulations loaded with these drugs. The formulations are surface–functionalized with folic acid to enable targeted and effective therapy for TNBC.