



**University of
Nottingham**

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**Overcoming doxorubicin resistance in
triple-negative breast cancer with
novel mPEG-PCL-DOX nanoparticles**

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**Thesis submitted to the University of Nottingham
for the degree of Doctor of Philosophy**

September 2025

Abstract:

Triple-negative breast cancer (TNBC) is an aggressive subtype characterised by the absence of estrogen, progesterone, and human epidermal growth factor receptors, limiting the use of targeted therapies clocking these proteins. Chemotherapy is still the first line treatment for TNBC and Doxorubicin (DOX) is a commonly used drug, but its effectiveness is limited by dose-dependent cardiotoxicity and multidrug resistance (MDR), often mediated by P-glycoprotein (PgP) efflux. This thesis focused on developing novel pH-responsive methoxy-poly(ethylene glycol)-poly(ϵ -caprolactone) (mPEG-PCL) nanoparticles conjugated with doxorubicin (mPEG-PCL-DOX) via azide–alkyne click chemistry as a new drug delivery system to overcome resistances. The nanoparticles synthesis showed stability at physiological pH with accelerated drug release under acidic conditions. In MDA-MB-231 TNBC cells and resistant derivatives (acquired DOX-resistant and PgP-overexpressing models), mPEG-PCL-DOX showed slower but sustained cytotoxicity compared with free DOX, with improved intracellular accumulation.

Importantly, in 3D spheroid models incorporating basement membrane extract to mimic tumour hypoxia, acidosis, and ECM barriers, mPEG-PCL-DOX showed superior penetration compared with free DOX, particularly in resistant spheroids. These findings demonstrate the ability of pH-responsive polymer–drug conjugates to use the acidic TME, overcome efflux-based resistance, and enhance therapeutic performance in physiologically relevant models.

Overall, this work highlights mPEG-PCL-DOX nanoparticles as a promising strategy to improve the efficacy and safety of DOX in TNBC. While current results are based on *in vitro* studies, they provide a strong ground for *in vivo* evaluation of biodistribution, pharmacokinetics, therapeutic efficacy, and toxicity. This platform may also be adapted to the use of alternative drugs in breast cancer subtypes where DOX is not standard treatment, supporting its broader application in precision nanomedicine.

Acknowledgements:

I would like to express my deepest gratitude to my main supervisor, Professor Cinzia Allegrucci, for her continuous support, invaluable guidance, and unwavering belief in me throughout this project. Her encouragement and inspiring words have been a driving force in helping me overcome the many challenges and difficulties I faced during my PhD journey.

My sincere thanks also go to Professor Cameron Alexander for his expert guidance and advice in the chemistry laboratory, and Prof. Anna Grabowska for training and accessing cell culture laboratory, and to Professor Stuart Paine for his kind support and constructive input.

I am also grateful to my colleagues and fellow PhD group members — Nabeelah Almalki, Mercedes Vázquez, Riba Thomas, and Katlego Modikoane — for their collaboration, insightful discussions, and the supportive environment they helped create.

I am profoundly grateful to my parents, whose love, prayers, and encouragement have accompanied me across the distance from Saudi Arabia to the United Kingdom. Finally, I wish to extend my heartfelt appreciation to my beloved wife, Aljawharah, and my children, Abdulmohsen and Faris, for their patience, love, and sacrifices in sharing this journey with me.

Covid impact statement and extenuating circumstances:

Unfortunately, my PhD project sustained significant delays during the years. First, at the beginning of my PhD I spent six months at home due to the COVID-19 pandemic. At that time, all laboratories were practicing 2-meter social distancing, and priority was given to final-year PhD students to complete their experiments. Additionally, there was limited possibilities to receive lab inductions and training. During these six months I could not do any lab work, which impacted the amount of data generated obtained during the first year. After returning to the labs, I also experienced substantial delays to get my work off the ground as the availability of training continued to be limited and the purchase of laboratory reagents experienced very long delivery times.

To add to these delays, my main PhD supervisor at the time (Prof Stuart Paine) left his position at the University for industry at the end of my second year and only maintained a nominal supervision in the project. This meant that I had to change main supervision, which caused a significant re-adjustment of the project aims and experimental work, as well as adapting to the new situation.

Altogether these circumstances, which were beyond my control, have significantly affected the amount of data I could generate during the project, which I nevertheless tried delivering to the best of my abilities.

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List of Acronyms

Acronym	Definition
ABC	ATP-binding cassette
BME	Basement membrane extract
BSA	Bovine serum albumin
cDNA	Complementary deoxyribonucleic acid
DAPI	4',6-Diamidino-2-phenylindole
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOX	Doxorubicin
DLS	Dynamic light scattering
ECM	Extracellular matrix
ELACRIDAR	Dual P-gp and BCRP inhibitor (GF120918)
FBS	Foetal bovine serum
FTIR	Fourier-transform infrared spectroscopy
HCl	Hydrochloric acid
d.nm	Diameter in nanometer
IC ₅₀	Half-maximal inhibitory concentration
LC-MS	Liquid chromatography–mass spectrometry
MDR	Multidrug resistance
MDR1	Multidrug resistance gene 1
mPEG	Methoxy poly(ethylene glycol)
mPEG-PCL	Methoxy poly(ethylene glycol)-poly(ϵ -caprolactone)
mPEG-PCL-DOX	Methoxy poly(ethylene glycol)-poly(ϵ -caprolactone) conjugated with doxorubicin
MW	Molecular weight
NaCl	Sodium chloride
PBS	Phosphate-buffered saline
PCL	Poly(ϵ -caprolactone)
PEG	Poly(ethylene glycol)
PgP	P-glycoprotein
PDI	Polydispersity index
pH	Potential of hydrogen
PVDF	Polyvinylidene difluoride

qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
RPLP0	Ribosomal protein lateral stalk subunit P0
RPM	Revolutions per minute
RT	Room temperature
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TNBC	Triple-negative breast cancer
WT	Wild type

Chapter 1 Introduction:

1.1 Breast Cancer

Normal living cells reproduce continuously in controlled cycles. But when this controlled cycle is interfered with, it can cause an abnormal cell growth that can transform into the formation of malignant cell growth forming a tumour mass defined as cancer. Malignant tumours can invade nearby and distant tissues by spreading through the circulation to form new tumours and metastatic disease. Even after surgical removal or other form of treatment of the malignant tumour, there is a chance of a minimal residual disease to remain and recurrence to occur. This behaviour creates an important clinical challenge and cancer is still the second cause of total deaths around the world, with 2.3 million new cases were diagnosed in 2020 according to Sung et al. (2021).

Using new and advanced diagnostic tools, scientists managed to identify more than 100 types of cancer. Breast cancer is the most common cancer among women globally and the second most common cancer overall. In 2022, there were approximately 2.3 million new breast cancer cases worldwide, with about 670,000 deaths related to the disease. These numbers are projected to rise significantly, with estimates indicating a 38% increase in new cases and a 68% increase in deaths by 2050. On average, 1 in 20 women globally will be diagnosed with breast cancer during their lifetime, and 1 in 70 will die from it (Kim et al. 2025).

Breast cancer histopathological classification is based on the microscopic appearance of tumour cells, following the World Health Organization (WHO) classification system (Tan et al. 2020). The key histological types include:

- 1) Ductal Carcinoma In Situ (DCIS): Non-invasive, confinement within ducts.
- 2) Lobular Carcinoma In Situ (LCIS): Non-invasive, associated with increased risks.
- 3) Invasive Breast Carcinoma of No Special Type (IBC-NST): known as invasive ductal carcinoma, this is the most common type, characterized by intermediate to high-grade features.
- 4) Lobular Carcinoma: Characterized by loss of E-cadherin expression accounting for 10-15% of breast cancers.
- 5) Special histologic types (~20% of breast cancers) with distinctive features including Tubular carcinoma, Mucinous carcinoma, Medullary carcinoma, Papillary carcinoma, Metaplastic carcinoma, Apocrine carcinoma, Neuroendocrine tumours and carcinomas

This classification is complemented by the assessment of the tumour grade (e.g., Nottingham grading system) assessing key features such as tubule formation, nuclear pleomorphism, and mitotic count to estimate aggressiveness (Bloom and Richardson 1957).

This system is widely used in pathology to inform prognosis and treatment decisions according to tumour type and behaviour (Pareja et al. 2025).

Emerging technologies like artificial intelligence and molecular profiling are complementing traditional histology to refine classification and therapeutic targeting (Cserni 2020).

At the molecular level, breast cancer classification is primarily defined by the presence or absence of three different receptors: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). The expression of these proteins together with the molecular signature associated with tumour gene expression profiling led to the classification of breast cancer into different molecular subtypes, highlighting significant disease heterogeneity. The main subtypes identified are Luminal A and B, HER2 positive, and Triple negative breast cancer (Sørli et al. 2001). Luminal A breast cancer is ER-positive and/or PR-positive, and HER2-negative. It is characterised by low proliferation and therefore these tumours present the best prognosis and respond well to hormone therapy. On the other hand, Luminal B breast cancer is also ER-positive and/or PR-positive but it may be HER2-positive or negative. This subtype presents higher proliferation, and it often requires combined hormone and chemotherapy due its aggressive nature. HER2-enriched breast cancer is: HER2-positive, ER and PR negative or low. These tumours are aggressive, but can be treated with HER2-targeted therapies, including the monoclonal antibody trastuzumab (Herceptin) and pertuzumab (Perjeta). Finally, Triple-negative breast cancer (TNBC) lacks ER, PR, and HER2 expression. These tumours are aggressive and invasive. Due to the lack of expression of the receptors, TNBC is mainly treated with non-targeted therapies including chemotherapy (Carvalho et al. 2025).

1.2 Triple Negative Breast Cancer (TNBC)

Among the different types of breast cancer, triple negative breast cancer (TNBC) represents 15 to 20 % of total new cases (Arroyo-Crespo et al. 2019). Compared to other subtypes, TNBC was characterised by low survival and high relapse rates.

TNBC still represents an unmet clinical need as this form of breast cancer has a poor prognosis being particularly aggressive and lacking effective therapeutic options (Pont, Marqués, and Sorolla 2024).

In addition, TNBC is highly heterogeneous and further classified into 4 different molecular subtypes with different characteristics, as well as clinical and therapeutic profiles (Mahmoud, Ordóñez-Morán, and Allegrucci 2022a).

According to the latest characterisation, TNBC can be classified into the Basal-like 1 (BL1), Basal-like 2 (BL2), Mesenchymal (M) and Luminal androgen receptor (LAR) subtypes (Lehmann et al. 2021).

BL1 tumours are characterized by high expression of genes involved in cell cycle and DNA damage response. They tend to be highly proliferative and show good response to chemotherapy. BL2 tumours are instead enriched for growth factor signalling and metabolic pathways, leading to lower pathologic complete response (pCR) and response to chemotherapy response compared to BL1. M tumours are enriched in genes involved in cell motility, epithelial-to-mesenchymal transition, and growth factor pathways. These are more

aggressive and tend to have poorer prognosis, but they can respond to kinase inhibitors and antiangiogenic therapy. LAR tumours are characterised by androgen receptor signalling and a luminal gene expression profile despite being triple-negative. These tumours are less responsive to chemotherapy but can respond to androgen receptor and agonists and PI3K/mTOR inhibitors (Mahmoud, Ordóñez-Morán, and Allegrucci 2022b). Earlier classifications included two additional subtypes, the immunomodulatory (IM) and mesenchymal stem-like (MSL) (Lehmann et al. 2011a), but these are now considered determined by the contribution of the tumour microenvironment and stromal components to the gene expression profiling (Mahmoud, Ordóñez-Morán, and Allegrucci 2022c).

These different TNBC subtypes demonstrate different response to therapy and differ in sensitivity to chemotherapy, immunotherapy, and targeted agents, as summarised in Figure 1.1.

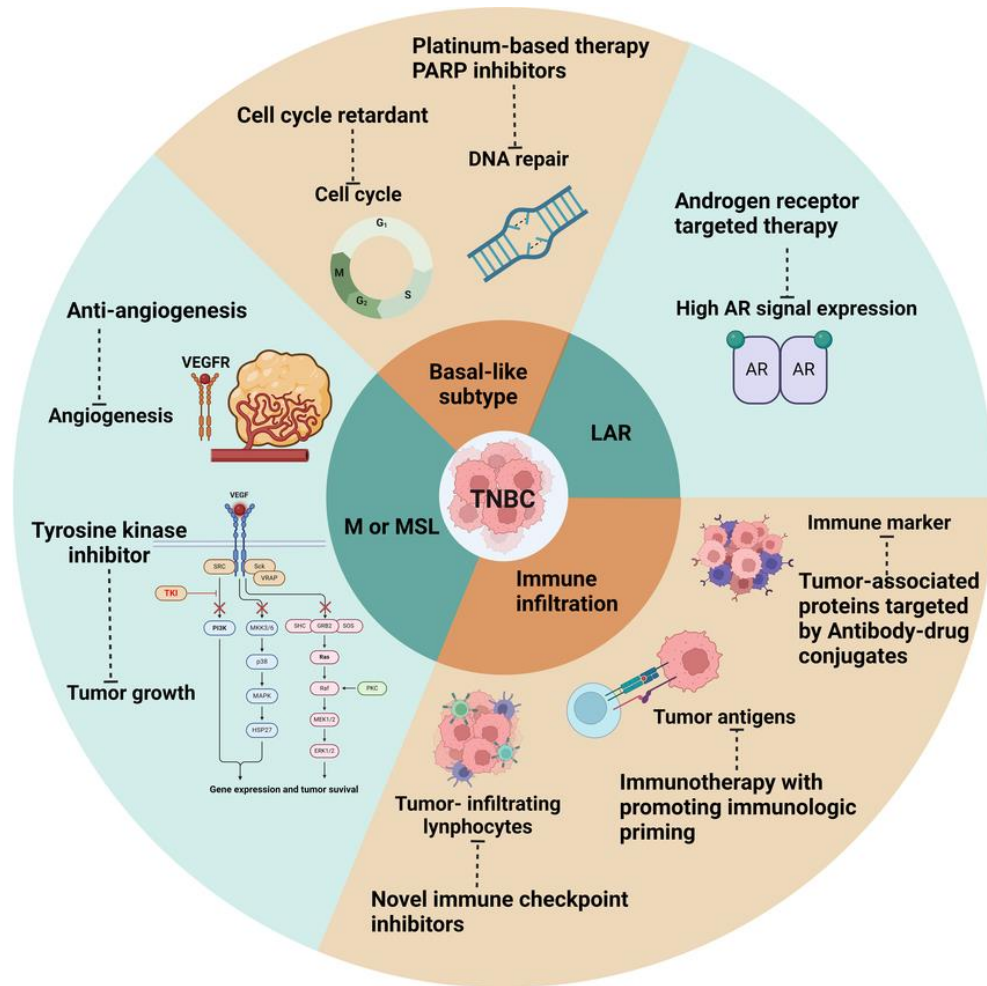


Figure 1.1 TNBC therapies based on molecular subtyping. The figure shows different therapeutic strategies for TNBC subtypes. Figure reproduced from Xiong, Wu, and Yu (2024). Licence: CC BY 4.0.

Therefore, current therapies for TNBC focus on improving effective treatments while reducing toxicity, as well as addressing treatment resistance.

Chemotherapy treatment remains the standard of care treatment of early-stage cancer treatment and is also used as neoadjuvant therapy before surgery to reduce the tumour mass preoperatively and improve surgical outcomes.

Common drugs used include anthracycline and taxanes, often with the addition of carboplatin, which improves pathological complete response and overall survival (Hao et al. 2024).

Recent studies and clinical trials have focused on using shorter, less toxic regimens combining chemotherapy and immunotherapy with radiation, resulting in fewer side effects (Ho et al. 2024).

Immunotherapy with immune checkpoint inhibitors (e.g., pembrolizumab) is integrated into combined treatment for TNBC with immune infiltration and expressing the checkpoint protein PD-L1, improving overall outcomes (Arora et al. 2025). This treatment is also combined with the use of antibody-drug conjugates (ADCs) like Sacituzumab that, when combined with pembrolizumab, show improved progression-free survival in advanced or metastatic TNBC (Abelman et al. 2025).

Another targeted treatment is based on PARP inhibitors (e.g., olaparib, talazoparib), which are the treatment of choice for patients with BRCA1 and BRCA2 mutations. These are frequent mutations in TNBC, with approximately 30 % of TNBC patients carrying either of them (Engel et al. 2018). Additionally, women with BRCA1 mutations are at a higher risk of developing TNBC compared to those with BRCA2 mutations (Chen et al. 2018).

PARP inhibitors target cancer cells' impaired DNA repair mechanisms by blocking poly (ADP-ribose) polymerase, an enzyme critical in repairing single-strand DNA breaks. This leads to accumulation of DNA damage and tumour cell death. This mechanism creates a vulnerability which can be exploited through

synthetic lethality by combining these drugs with chemotherapy to increase the DNA damage beyond repair (Jain, Barge, and Parris 2024).

Therefore, despite the latest advances, chemotherapy still represents the foundation of TNBC treatment, and more research is currently needed to maximise its therapeutic effect while minimising toxicity.

1.3 Chemotherapy in TNBC

Chemotherapy for TNBC typically involves a combination of several classes of drugs, each with distinct mechanisms of action and therapeutic targets. The National Comprehensive Cancer Network (NCCN), a leading US authority in cancer treatment guidelines (<https://www.nccn.org/>), recommends a regimen that includes anthracyclines, taxanes, fluorouracil, cisplatin, and cyclophosphamide. This multi-drug approach is designed to target cancer cells at various stages of their life cycle, thereby maximizing therapeutic efficacy while attempting to limit the development of drug resistance.

1.3.1 Taxanes

Taxanes are a class of drugs including taxol and docetaxel which are widely used in chemotherapy (Shi et al. 2017). Taxol is an antitumor agent extracted from the pacific yew tree *Taxus brevifolia* (Schiff, Fant, and Horwitz 1979). The mechanism of action of taxol is elicited by inhibition of cell division and proliferation through interference in microtubule depolymerization which prevents formation of spindles during mitosis (Yvon, Wadsworth, and Jordan 1999). A gene profiling analysis study of TNBC subtypes conducted by Yin et al.

(2020) suggested that the basal like (BL) subtype has increased expression DNA repair genes and proliferation related genes which can make this specific TNBC subtype sensitive to taxanes. Treating TNBC patients with basal like subtypes (BL1 and BL2) with taxane based chemotherapy showed 4 times higher remission rates than LAR and MSL subtypes (Juul et al. 2010, Bauer et al. 2010).

Taxanes form a key part of neoadjuvant (pre-surgery) and adjuvant (post-surgery) chemotherapy, often combined with platinum agents like carboplatin. Their use in combined regimes result in improved pCR rates (Shi et al. 2024).

1.3.2 Cyclophosphamide

Cyclophosphamide (CP) is a drug used as an immune system suppressor and to treat breast cancer and other types of cancer such as ovarian cancer, lymphoma and leukaemia. CP is a prodrug which is converted into 4-hydroxycyclophosphamide and aldophosphamide by the action of microsomal mixed function oxidases enzymes in the liver (Belfayol-Pisanté et al. 2000). Aldophosphamide is activated by cytochrome P450 enzymes to produce nitrogen mustard which is cytotoxic to tumour cells (Frangou, Bertsias, and Boumpas 2016, Mansilla et al. 2019). The combination of docetaxel and cyclophosphamide (TC) was found to be very effective in the treatment of TNBC, but of limited efficacy on the other subtypes of breast cancer (Nakatsukasa et al. 2017).

1.3.3 Platinum agents

Platinum based chemotherapy such as cisplatin and carboplatin are used as treatment for various cancer types, including breast cancer (Oun, Moussa, and Wheate 2018). A phase II clinical trial performed by J. Zhang et al., 2015 evaluated the efficacy of cisplatin and gemcitabine (GP) on metastatic TNBC (mTNBC) patients. Results of the study showed that the combination of GP significantly improved efficacy and safety profiles, especially in the BL subtypes. Vvon Minckwitz et al., 2014 performed a study on 296 patients to assess the efficacy of adding carboplatin to neoadjuvant therapy for non-metastatic TNBC breast cancer. They found that adding carboplatin to anthracycline chemotherapy and taxane chemotherapy caused a significant increase in pCR rates in TNBC patients compared to the group of patients that did not get carboplatin during their treatment.

Platinum chemotherapy is also a valuable component of TNBC treatment by leveraging the tumour's DNA repair vulnerabilities, especially tumour subtypes carrying mutations of the DNA repair genes to enhance chemotherapy response and improve outcomes (Tian et al. 2021).

1.3.4 Fluorouracil

5-Fluorouracil (5-FU) is an antimetabolites chemotherapy drug for the treatment of solid tumours. Antimetabolite drugs can either incorporate in the DNA or inhibit essential cell biosynthesis processes (Che et al. 2017). 5-FU is converted to the active metabolite flurodeoxyuridine monophosphate by the action of orotate phosphoribosyl transferase (FdUMP), which creates a stable complex with thymidylate synthase (TS) to inhibit the production of deoxythymidine monophosphate (dTMP). The decrease in dTMP production causes cytotoxicity as this molecule is essential for DNA repair and replication (Zhang et al. 2008, Ochiai et al. 2006). Capecitabine is a rationally designed 5-FU prodrug which shows selectivity to tumour cells that have increased expression of thymidine phosphorylase within the tumour cells compared to the surrounding normal tissues (Chintala et al. 2011). In a phase II clinical trial study, results suggested that the combination of cisplatin and capecitabine administration in metastatic TNBC patients pre-treated with taxanes and anthracycline resulted in significant efficacy with acceptable side effects (Li et al. 2015).

1.3.5 Anthracyclines

Anthracyclines represent a critically important class of chemotherapeutic agents, primarily derived from *Streptomyces* bacteria, that are widely recognized for their efficacy in treating a variety of cancers, including breast cancer. This class of drugs holds a prominent position as cancer chemotherapeutics, distinguished by the broad-spectrum activity against more

types of cancers than any other category of chemotherapeutic agents. The World Health Organization (WHO) has acknowledged the significance of anthracyclines by including them in its list of essential medicines, underscoring their vital role in cancer treatment (Mcgowan et al. 2017).

Doxorubicin, a key member of the anthracycline family, is particularly noteworthy for its clinical importance. Marketed under the brand name Adriamycin, doxorubicin operates by disrupting DNA replication during cell division. The chemical structure of doxorubicin, depicted in Figure 1.2, illustrates its intricate molecular composition.

While doxorubicin is highly effective, its use is often accompanied by a range of side effects. Common adverse reactions include mouth inflammation, vomiting, and hair loss. However, more serious complications can arise, with cardiomyopathy being the most severe. This condition, which can lead to congestive heart failure, is known to be dose-dependent, as highlighted in the research by (Chatterjee et al. 2010). Doxorubicin-induced cardiomyopathy is characterized by the enlargement of the cardiac chambers, resulting in both systolic and diastolic dysfunction, as described by Takemura and Fujiwara (2007).

In response to these cardiotoxic effects, an innovative formulation of doxorubicin was developed. This formulation involves encapsulating doxorubicin in a pegylated (polyethylene glycol coated) liposome, marketed under the brand names Doxil and Caelyx. This liposomal form has been demonstrated to significantly reduce the risk of doxorubicin-induced

cardiomyopathy compared to the free form of the drug, as evidenced by the work of (Gabizon et al. 2004).

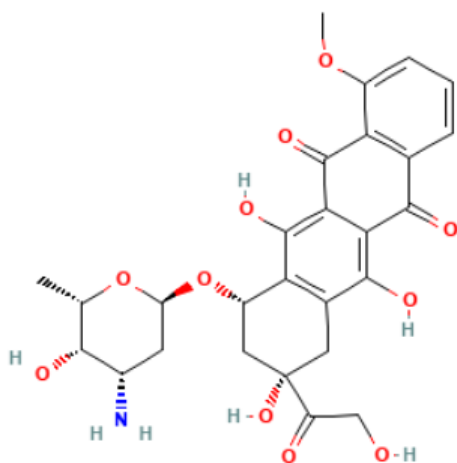


Figure 1.2 Doxorubicin chemical structure. Adapted from (<https://pubchem.ncbi.nlm.nih.gov/compound/Doxorubicin>).

There are two suggested mechanisms of action of doxorubicin within a cancer cell. The first mechanism is by intercalation between two DNA bases, whereas the second mechanism is by formation of free radicals which cause damage to DNA, cell membrane, and proteins (Gewirtz 1999). Intercalation prevents topoisomerase II enzyme function which is responsible for ligating then relaxing supercoils in DNA strands during the transcription process. Doxorubicin's role is to stabilize the topoisomerase II complex regulation of ligated portion in the double helix which will lead to stopping the replication process and cell death (Thorn et al. 2011).

Complementary modes of action also include induction of oxidative stress and chromatin damage. Importantly, anthracyclines can also promote inflammatory responses and antitumor immunity (van der Zanden, Qiao, and Neefjes 2021).

Many researchers have tried to enhance doxorubicin efficacy and reduce its side effects through increasing the selectivity of the drug to target cancer cells. A very promising way is to load the drug into polymeric nanoparticles to form a drug polymer conjugate that can release the drug in cancer cells. In their study, Pearce et al., (2020) synthesized different polymeric materials based on 2-hydroxypropyl methacrylamide (HPMA) with star (Star-SS-L 15 nm) and hyperbranched (HB-SS-HH 10 nm) architectures to investigate the ability of these polymers to deliver doxorubicin to *in vitro* 2D and 3D models using the MDA-MB-231 TNBC cell line, and *in vivo* in murine orthotopic models of human TNBC. Both polymer doxorubicin conjugates showed significant improvement in therapeutic effect compared to the free drug. Also, the polymer doxorubicin conjugates showed decrease in IC₅₀ values compared to the free doxorubicin in TNBC 3D spheroids.

In addition, the analysis of tumour mass and volume in the murine orthotopic model treated with both polymer DOX conjugates showed significant decrease mass and volume and slower rate of tumour regrowth compared to the treatment with free DOX (Pearce et al. 2020). This study showed that it is possible to direct nano materials with different conformations to the desired

tissues and enhance the therapeutic effect by loading the drug to these nano polymers.

1.4 TNBC and drug resistance

Although new therapies are continuously being developed, drug resistance continues to be a significant limitation. TNBC exhibits multiple mechanisms of drug resistance which contribute to its aggressive nature and poor prognosis.

Drug resistance is complex and multifactorial, involving different genetic, epigenetic, and microenvironmental factors. Therefore, understanding these mechanisms is required to develop new treatments and drug formulations aiming to sensitize tumours and improve patient outcomes.

The highly heterogeneous nature of TNBC plays a critical role in drug resistance as TNBC tumours comprise diverse cell populations with variable responses to therapy (Nedeljković and Damjanović 2019a). Some TNBC subtypes, like the M subtype, are particularly enriched in cancer stem cells which are inherently drug resistant, thus leading to cancer cells surviving treatment and causing disease relapse (Mahmoud, Ordóñez-Morán, and Allegrucci 2022d).

Overexpression of drug efflux pumps like ABCG2/BCRP and defects of drug internalisation also leads to both intrinsic and acquired multidrug resistance by removing chemotherapeutic agents from cancer cells (Gautam et al. 2025).

Overcoming apoptosis is another major mechanism involved in TNBC drug resistance.

TNBC cells often activate signalling pathways that promote survival and resist apoptosis, such as the PI3K/AKT/mTOR and other key developmental pathways like the Wnt, Notch, Hedgehog, JAK/STAT and TGF beta signalling pathways (Nedeljković and Damjanović 2019b) (Figure 1.3).

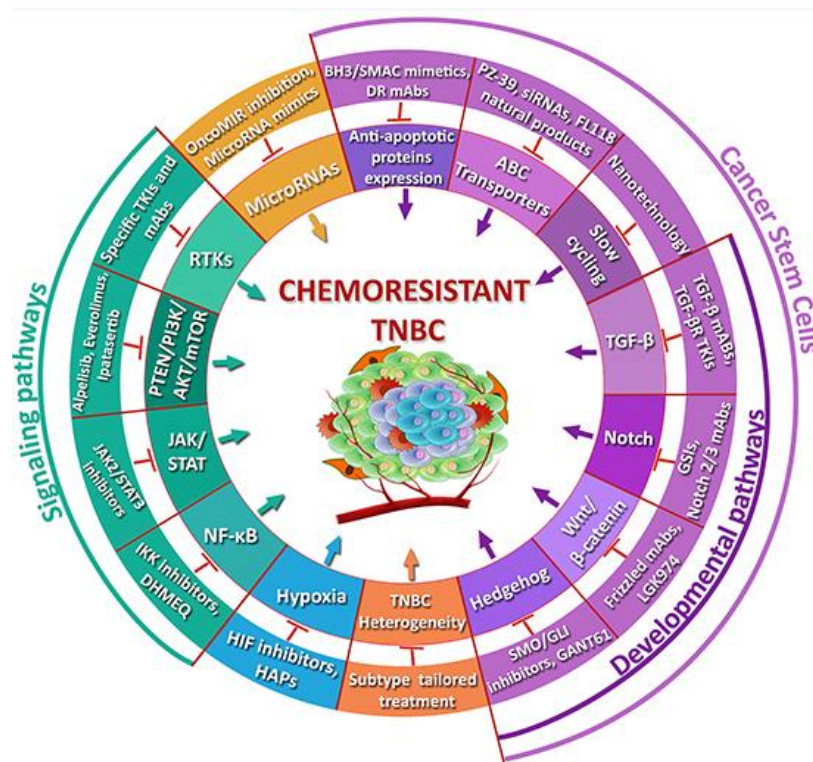


Figure 1.3 Mechanisms regulating chemoresistance in TNBC and therapeutic approached for overcoming the signals involved. Figure reproduced from (Nedeljković and Damjanović 2019c). License CC BY 4.0.

Besides mechanisms inherent to cancer cells, the tumour microenvironment (TME) is one of the most critical factors contributing to drug resistance. The TME of TNBC is a complex ecosystem that surround the tumour, interacting

with it to aid its survival and growth. Key components of TME are stromal cells, immune cells, blood vessels and extracellular matrix (ECM), altogether provides the tumour with the necessary nutrients and oxygen, in addition to facilitating the metastasis and invading surrounding tissues (Anderson and Simon 2020).

Among stromal cells, the cancer-associated fibroblasts (CAF) communicate with TNBC cells, as well as immune cells, influencing metastasis, cancer stemness and immunosuppression. This communication is mediated by both chemokines and growth factor signalling which can influence cell invasion, cancer stem cell enrichment and invasion of immune suppressive cells, such as tumour associated macrophages and regulatory T cells, all contributing to resistance to therapies (Bai et al. 2021).

The rapid growth of TNBC cells also relies on aerobic glycolysis to generate energy, producing large amounts of lactate and protons which are exported out to the ECM using monocarboxylate transporters (MTCs) and leading to the acidification of ECM. In addition, limited oxygen and nutrients deep within the tumour core result from poor vascular supply leading to hypoxia driven cell death and the formation of a necrotic core, which also stimulate glycolysis and lactate production with generation of protons. All together these conditions create a pH gradient within the tumour with a higher pH in the edges and lower pH towards the hypoxic core (Estrella et al. 2013).

Both hypoxia and altered pH enhance TNBC stem cell properties and immune evasion, thus significantly contributing to drug resistance (Bai et al. 2021).

To overcome all the mechanisms, current research aims to target the described resistance pathways with small molecule inhibitors and immunotherapy. Another innovative approach is through the development of novel drug delivery systems (e.g., redox-responsive nanoparticles) to be used in combination therapies that can inhibit survival pathways, and in immunotherapy enhancements to overcome resistance.

Drug delivery systems based on the use of nanoparticles offer new avenues to overcome drug resistance by improving drug bioavailability, enhancing targeted delivery, and modulating the TME.

1.5 Nano drug delivery and polymers

Nano drug delivery systems have been first introduced to overcome drug delivery constraints due to their chemical properties affecting absorption, solubility, biodistribution, and intensive metabolism resulting in loss of therapeutic effect and unwanted drug clearance pharmacokinetics (Duncan and Gaspar 2011, Gaspar and Duncan 2009). Nano medicines and nano based drug delivery systems are also the focus of intense research to assess their effectiveness as curative agents in addition to delivering drugs to the targeted tissue (Patra et al. 2018).

Nanomaterials are defined by any particles that range between 1 and 100 nm in size; this size range gives nanomaterials special chemical, structural and biological properties that can improve therapeutic efficacy and reduce side effects (Agha Zeeshan Mirza and Farhan Ahmed Siddiqui 2014). There are many

advantages of using nano scale materials as they are readily internalised inside cells as there is an inverse relationship between the absorption and the size of the nanoparticle (Pearce et al. 2020). Also, using them as drug carriers for cytotoxic agents can reduce the side effects and toxicity in normal cells (Guo and Jiang 2020). In addition, poorly soluble drugs can be loaded in the hydrophobic inner core of the nanocarrier acting as a reservoir for the drug to increase their solubility through exposing the outer hydrophilic shell to the aqueous environment (Wang, Soltero, and Saihaan 2005, Yan et al. 2011). Nanoparticles have also been used in gene therapy by delivering nucleic acids to tumour cells and prevent their degradation by nuclease enzymes (Yingyuad et al. 2014).

Polymers are one of the most researched nanomaterials; they consist of repeated monomers that contain both hydrophobic and hydrophilic regions, where the drug of interest is loaded into the hydrophobic region and the hydrophilic region gives the polymer its ability to disperse in blood and aqueous media (Zhou et al. 2014). Homopolymers are composed from only one type of monomer (e.g. A-A-A-A-A), whereas copolymers are formed by two different types of monomers, which can form a statistical copolymer if these monomers are randomly repeated in their structure (e.g. A-A-B-A-B-B). Also, if monomers of one type are joined together and monomers of another type are joined together, they will link to form a block copolymer. Block copolymers can be classified according to the number of types of monomers e.g. A-A-A-A-B-B-B-B would be a diblock copolymer and A-A-B-B-A-A or A-A-B-B-C-C would be a triblock copolymer etc. (Nostrum 2011). Polymers can form in different shapes

such as branched, hyperbranched, and linear, and they are bound together through covalent bonds or electrostatic interactions (Nostrum 2011) (Figure 1.4).

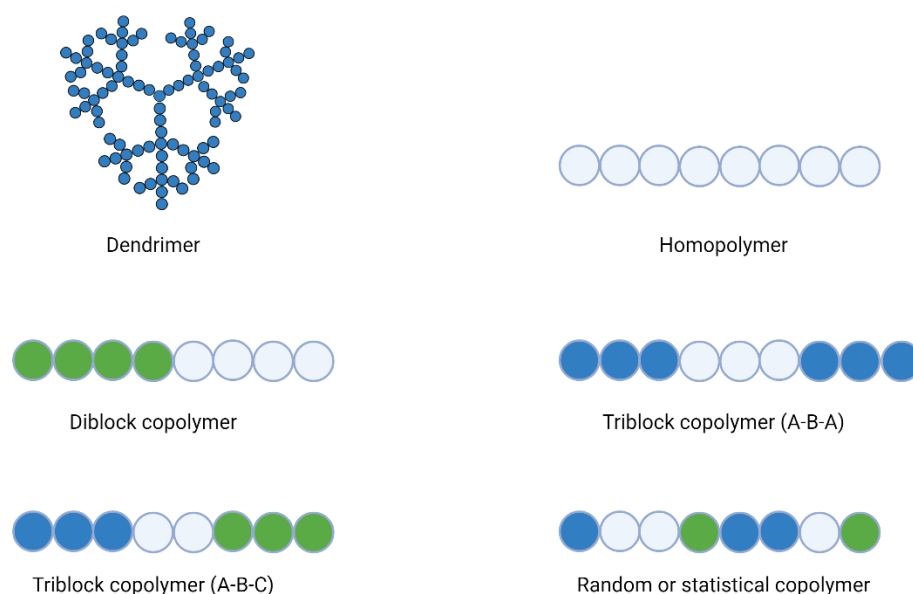


Figure 1.4 Examples of different arrangements of linear homopolymer, diblock copolymer, triblock A+B+A and A+B+C triblock copolymers, random or statistical copolymer, and dendrimer. Adapted and modified from (Bansal et al. 2016).

1.5.1 Different polymer morphologies:

Polymeric materials are preferred for drug delivery as they have great potential for surface modification to give them better pharmacokinetic control and attach different types of drugs to enhance the delivery of these drugs. Venditti, (2019) suggested that different types of morphologies are to be used as they are easier to modify the surface in terms of functionality and charge. An

example of these polymer morphologies includes polymeric nanoparticles, dendrimers, micelles, vesicles and nanocapsules.

1.5.1.1 Dendrimers:

Dendrimers are highly and regularly branched nano polymers that are 1-10 nm in diameter which contain an inner core. Dendrimer structures consist of the following: a) inner core, b) multiple layers of repeating units called generations, c) large number of functional groups present on its surface that plays a significant role in the dendrimer's properties (Noriega-Luna et al. 2014) . The core of the dendrimer is composed of a single or group of atoms and branches of carbon added together through chemical reactions to form the dendritic spherical structure. The dendrimer's core is surrounded by extensive branching which eventually leads to the formation of many interior layers composed of repeating units (Sherje et al. 2018). The generation number is determined by how many branches are present from the central core to the dendrimer.

The number of terminal groups of the dendrimer that are available for drug attachment is increased as the number of generations increase, resulting in increased drug loading. Sherje et al., 2018 suggested that the drug molecules are loaded into the dendrimers structure in three main ways: a) physical encapsulation of the drug molecules within the voids between dendrimer branches, b) conjugation of drug molecule with the terminal groups of the dendrimers, c) electrostatic interactions.

Different types of dendrimers have been investigated in the literature and found to enhance the drug delivery of many drugs in cancer therapy.

Chittasupho et al., 2017 investigated polyamidoamine (PAMAM) doxycycline conjugate (LFC131-DOX-D4) cytotoxicity and cellular binding on T47D and BT-549-Luc breast cancer cell lines. The PAMAM dendrimer conjugate was functionalized with an LFC131 peptide which has the ability of recognizing the increased CXCR4 receptor expression on the breast cancer cell surface. Their results showed significantly improved in vitro cytotoxicity of LFC131-DOX-D4 compared to non-targeted dendrimers.

1.5.1.2 Polymeric micelles and vesicles

Covalently linked block copolymers can self-assemble to produce polymeric micelles or vesicle. Polymeric micelles consist of two layers, an inner hydrophobic core and an outer hydrophilic shell, whereas polymeric vesicles (polymersomes) consist of a hollow compartment surrounded by three layers from the hollow to the shell, which are: 1) hydrophilic region, 2) hydrophobic region, 3) hydrophilic shell (Mu et al. 2019).

Drug delivery using polymeric micelles and vesicles has proven to be an excellent way for delivering drugs to site of action, benefiting from their amphiphilic properties. Water insoluble drugs can be loaded in the micelle hydrophobic core or hydrophobic part of a vesicle, and hydrophilic drugs are usually enclosed into the hydrophilic hollow of the vesicle to increase the drug solubility (Yan et al. 2011, Bansal et al. 2016), while the hydrophilic shell plays an important role in protecting the hydrophobic region of the micelle and vesicle from the aqueous biological environment (Ahmad et al. 2014). In their study Soo Choi et al. (2007) suggested that the nanoparticles renal threshold is

approximately 5.5 nm, and because of the size of polymeric micelles and vesicle are greater than 5.5 nm, these polymeric nanoparticles are able to carry different drugs and prolong systemic circulation time through avoiding kidney filtration. Different drugs can be covalently bound to polymers or can be linked through a spacer such as amino acids and peptides which provides fabricability and flexibility for polymers (Etrych et al. 2002, Khandare and Minko 2006). Polymer drug conjugate is a term used to describe drug molecules which are attached to a polymer, and most of the time for the drug to be active the drug must be released from the polymer, and in this case the polymer drug conjugate is called a macromolecular pro-drug.

1.5.1.3 Stimuli responsive polymers

Many strategies have been investigated to synthesize 'smart' or 'intelligent' nano materials which have the ability to load different drugs and release them to a specific target using internal or external stimuli. Examples of stimuli responsive polymers are thermo-responsive, pH sensitive, enzymes, magnetic, light, and ultrasound sensitive polymers.

Thermo-responsive polymers are one of the most investigated smart systems that have been investigated, and the first thermo-responsive polymer studied was poly(N) isopropyl acrylamide (Heskins and Guillet 2006). They are synthesised using a very fine balanced ratio between the hydrophilic and hydrophobic groups, and because of that, any change in the temperature of the aqueous polymer solution can cause a different arrangement and conformation of the water molecules and the polymer blocks (Bajpai et al.

2008). Thermo-responsive polymers can be divided into two main groups: a) polymers possessing a Lower Critical Solution Temperature (LCST), b) polymers possessing an Upper Critical Solution Temperature (UCST). Thermo-responsive polymers which are below LCST are water soluble and above LCST water insoluble (Jeong, Bae, and Kim 2000). These polymers are switched on/off by changing the temperature. Above a certain temperature, thermos-responsive polymeric micelles undergo a dramatical change in their structure by shrinking of the shell causing the drug to be released by diffusion (Nakayama et al. 2006). While UCST polymers operate in the opposite way of LCST polymers, they are soluble above a certain temperature and insoluble below that temperature (Matanović, Kristl, and Grabnar 2014).

Thermo-responsive polymers as a drug delivery system have been investigated for their ability to treat inflammation sites as there is increased temperature at the site of inflammation compared to other tissues reaching up to 4° to 5° C increase in some cases (Chanmugam et al. 2017). Also, they can be used by applying external heat to create a mild hyperthermia in the targeted site of the body or solid tumours by ultrasound probes, microwave irradiation or near infrared irradiation (Zhang et al. 2014).

Another type of stimuli responsive polymers are pH-responsive systems; they are one of the most investigated polymers and are well characterised. Their characteristics such as solubility, structural conformation or configuration and volume could be reversibly manipulated through the pH of the surrounding environment they are in. One of the most used pH responsive polymers is the

methacrylic acid copolymers, which are used in the gastrointestinal tract (GIT) drug delivery according to the pH gradient present. Khan, Prebeg, and Kurjaković 1999 suggested that colon targeted oral drug delivery could be achieved using methacrylic acid copolymers which are only triggered to release the drug at pH of 7-8, taking advantage of progressively increasing pH along GIT from the stomach (pH 2-3), small intestine (6-7), and the colon (7-8). Also, pH value can vary in cancerous tissue and compared to normal tissues as cancer cells are acidic, with pH ranging between 6 to 7.2 in the cytoplasm and 4.5-6 in subcellular endosomes. This pH difference provides another application of pH responsive polymers for selective targeting of cancer cells (Su et al. 2011).

The last stimuli responsive polymers to be mentioned are redox-polymers which are sensitive to the reducing environment inside the cells. These polymers are usually made with sulphide groups, and they are easily cleaved by the activity of glutathione when they enter the cells (Guo et al. 2018). Compared to normal tissues, tumour tissues have different microcellular environment such as reducing environment, enzyme environment and acidic environment. The reducing environment in tumour tissues is controlled by the oxidation and reduction state of glutathione (GSH). It was reported that the intracellular concentration of GSH is up to 10 mM and the extracellular concentration of GSH ranges from 2 to 20 μ M in normal tissues (Mura, Nicolas, and Couvreur 2013). On the other hand, it was suggested that GSH concentration in tumour tissues was 4 times higher than in normal tissues (Kuppusamy et al. 2002, Meng, Hennink, and Zhong 2009). These differences in GSH concentrations allows redox responsive nanocarriers to selectively

degrade inside tumour cells by cleaving the disulphide bonds to sulfhydryl groups and releasing the drug inside the cell cytoplasm of tumour cells. Because of this property, redox responsive nanocarriers have three main advantages. First, they are stable in normal tissues, which will eventually cause a reduction of the side effects and cytotoxicity of both nanocarrier and drug. Second, they show rapid release of drug because of responding to high GSH concentration which normally range between few minutes to hours. Finally, the release of the drug inside the cytoplasm is expected to give a better therapeutic effect (Meng et al. 2012).

Taken altogether, the stimuli responsive polymers mentioned above undergo sharp modification in their structure following stimulation. This stimulation and conformational change can release the loaded drug rapidly to the desired target, minimizing the side effects of the drug in normal tissues.

1.5.1.4 Polystyrene nanoparticles

Polystyrene is one of the most studied nanoparticles and many protocols used to synthesize polystyrene beads (PB) are available. A commonly used method is the dispersion polymerization (Cho et al., 2016). Compared to other nanomaterials, polystyrene beads are relatively easy to synthesize, less expensive, and the size of particles produced lies within a narrow distribution of size, which can range between few microns to 20 nm. Other advantages of polystyrene beads are that they can be labelled easily with luminescent and fluorescent dyes for detection using spectroscopy methods. In addition, they are visible under an electron microscope without the need of any labelling, and

surface modification can be done readily. As a result of all these advantages, they are extensively used in research to understand the safety of nanomaterials in endocytosis studies (Johnston et al. 2010). Many studies were performed on the cellular uptake of polystyrene beads which suggest that internalization pathways are mainly influenced by the type of cell lines used in the experiment. In addition, the presence of polystyrene beads enhances the uptake of other nanomaterials (Zauner, Farrow, and Haines 2001, dos Santos et al. 2011).

An interesting study performed by (Tchoryk et al. 2019) used colorectal cancer spheroids (HCT116) to investigate the effect of different physiochemical properties of nanoparticles on the penetration and uptake in HCT116 spheroids. In the study, a range of plain polystyrene nanoparticles with different sizes of 30 nm, 50 nm, and 100 nm were used, in addition to 50 nm polystyrene with different surface chemistries (uniform plain, aminated, and carboxylated). The polystyrene size-dependent penetration and localization in HCT116 were qualitatively presented by confocal images and quantified by fluorescence activated cell sorter (FACS) analysis using flow cytometry method. Results showed that the 30 nm polystyrene were taken up in over 70% of cells, and the 50 nm were taken up in over 80% of cells after 2 hours of incubation and increasing to over 90% of cells after 6 hours incubation in the spheroid. On the other hand, a different penetration profile was seen in the 100 nm polystyrene, after 2 hours of incubation, only 10% of cells showed uptake of the 100 nm polystyrene, increasing to 22% after 24 h of incubation. Also, 30 nm and 50 nm polystyrene showed even distribution between the various concentric cell population segments, while there was no significant penetration

to the core of the HCT116 spheroid in the 100 nm polystyrene and only found on the periphery of the spheroid.

Also, the effect of the different surface chemistries in the 50 nm polystyrene (positively charged aminated and negatively charged carboxylated) on the penetration was compared to the unmodified plain polystyrene. The unmodified polystyrene penetration in HCT116 spheroid was 2X higher than aminated and 8X higher than the carboxylated polystyrene.

Therefore, the (Tchoryk et al. 2019) study highlighted the importance of controlling the physiochemical properties such as the size and the surface modification of nanoparticles in order to enhance the delivery of different drugs into tumours.

1.6 Nanotherapeutics and drug resistance

The development of new materials and technologies for nanocarriers that respond to specific stimuli is making significant progress to overcome therapeutic resistance for cancer treatment. The nano-based technology is currently surmounting many hurdles common to conventional drug delivery systems, including poor distribution, indiscriminate toxicity and non-targeted effects. They allow instead increased drug penetration, precise tumour targeting and delivery of drugs that can directly target cancer pathways and drug resistance gene networks, as shown in Figure 1.5.

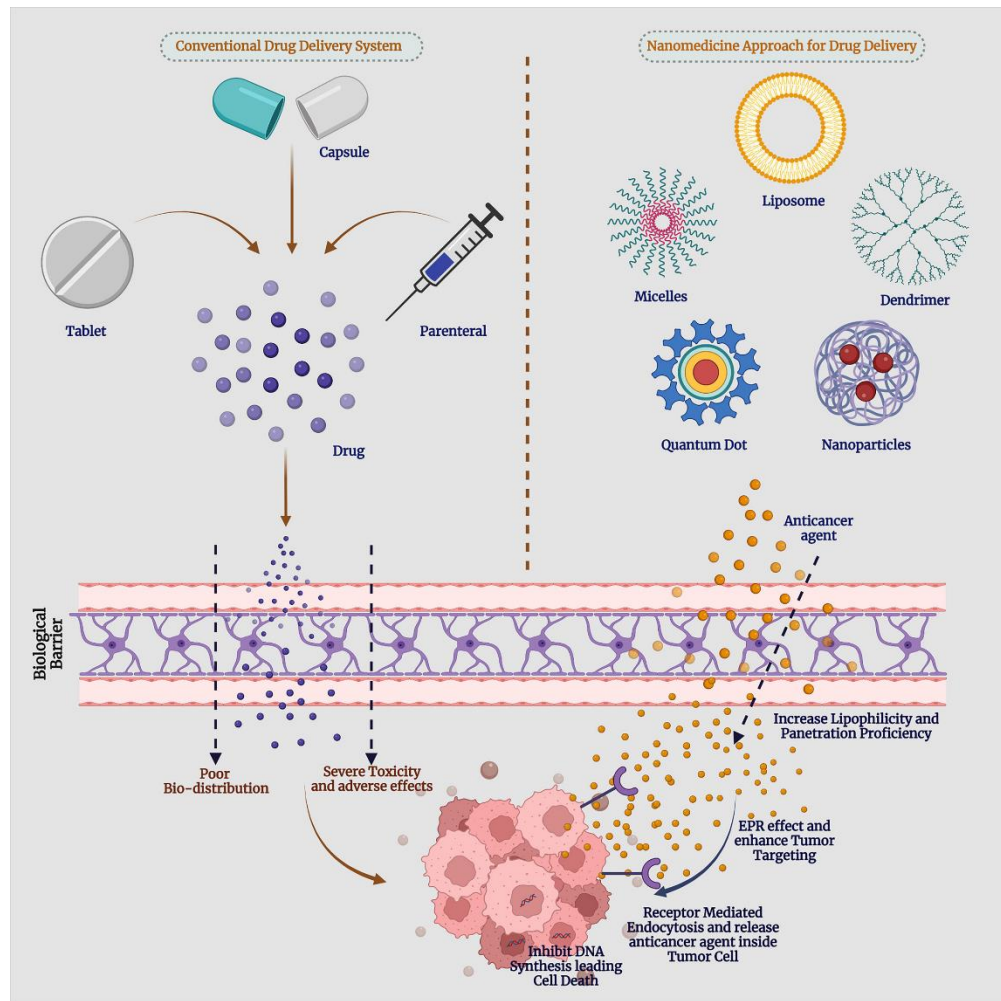


Figure 1.5. Nanotherapeutics approach to cancer treatment. Figure reproduced from (Ashique et al. 2023). Licence CC BY 4.0.

1.7 Hypothesis and project aim

Treatment of breast cancer patients with doxorubicin (DOX) is associated with important toxic effects, including cardiotoxicity. In addition, even after treatment with such a potent drug breast cancer can reoccur due to the development of resistance to its cytotoxic effects, mostly due to acquired mechanisms preventing the drug from entering the cell. One of these mechanisms is by increasing the expression of efflux transporters such as MRP1, BCRP, and P-gp which leads to a decrease of drug intracellular concentrations in the cancer cells. One of many ways of overcoming these issues is by using conjugated drugs to nanoparticles, which are molecules ranging from 1 to 100 nm in size.

Many drug polymer conjugates in the literature have shown enhanced toxicity against cancer cells compared to administration of the drug alone. In this project, novel nanoparticles conjugated to doxorubicin will be investigated and it is hypothesised that they will enhance penetration and delivery of the cytotoxic agent in triple-negative breast cancer (TNBC) cells resistant to therapy. In addition, it is hypothesised that delivery of DOX through nanoparticles would sustain the release of DOX in the tumours without the need to increase or repeat free DOX dosage delivery, which are responsible for undesired toxic effects to vital organs.

Therefore, the aim of this project is to perform *in vitro* studies using monomethoxy poly(ethylene glycol)-poly(ϵ -caprolactone) nanoparticles conjugated to DOX (mPEG-PCL-DOX) conjugates to test and select the best drug

delivery that could be extended to *in vivo* biodistribution studies. The *in vitro* studies will involve testing the polymer conjugate in the TNBC cancer cell line MDA-MB-231 cells sensitive and resistant to DOX to investigating the rate at which the polymer will undergo endocytosis and exert a cytotoxic effect on cells cultured in 2D and 3D spheroids cell culture models.

Chapter 2 Material and methods:

2.1 Materials and reagents

MDA-MB-231 cells were obtained from the NCI60 collection banked at the Biodiscovery Institute at the University of Nottingham under Prof. Grabowska's material transfer agreement. Genetically modified MDA-MB-231 cells expressing luciferase (MDA-MB-231 Luc) were provided by Prof. Grabowska. Cell lines were regularly washed, passaged, and fed with fresh medium. Hanks' Balanced Salt Solution (HBSS) was used for washing cells. Cell culture medium was prepared by adding 50 ml foetal bovine serum (FBS) and 5 ml L-Glutamine to a 500 ml RPMI medium bottle. Trypsin EDTA (0.25%) was used for cell disassociation. Cells were regularly tested for Mycoplasma contamination using the Plasmotest™ from InvivoGen.

The experiments were performed in aseptic conditions using a microbiological safety cabinet class II (MSC) which was disinfected with IMS 70% and Distel medical level surface disinfectant before and after use. Costar 96 black, clear bottom, sterile, and tissue culture treated well plates were used for plating MDA-MB-231 Luc and taking fluorescence reads. Doxorubicin hydrochloride (DOX) with molecular weight of 579.98 g/mol was purchased from Merck (D1515). The PrestoBlue cell viability reagent (A13262) was purchased from ThermoFisher Scientific. The live/dead stain kit was also from ThermoFisher Scientific (Fisher L3224).

2.2 Preparation of mPEG-PCL-DOX pH responsive nanoparticles:

The method of synthesis for the methoxy poly(ethylene glycol)-poly(ϵ -caprolactone) (mPEG-PCL) nanoparticles was originally developed by Dr Christina Parisi, a PhD graduate from School of Pharmacy, University of Nottingham. The mPEG-PCL-DOX nanoparticles synthesis was replicated for this project using the mPEG-PCL polymer stock and used within a week to ensure the nanoparticles' stability across all experiments.

2.3 Preparation of empty mPEG-PCL micelles:

From the mPEG-PCL stock, 10 mg were measured and dissolved in 1 ml of N-methyl-2-pyrrolidone (NMP) and kept for 5 min at 37°C. After that, the solution was added dropwise to 5 ml of deionised water at a rate of 0.2ml/min in a scintillation vial using a syringe pump while the solution was stirring at 1000 rpm. Finally, the solution was dialysed for 24 h in 1 L of deionised water using a 3500 g/mol molecular weight cut-off (MWCO).

2.4 Preparation of mPEG-PCL-DOX conjugated nanoparticles:

mPEG-PCL-DOX nanoparticles were prepared using the copper catalysed azide-alkyne cycloaddition click chemistry reaction. First, 2.5 mg of modified DOX and 10 mg of mPEG-PCL were dissolved in 1 ml NMP for 5 min at 37°C. Once dissolved, the solution was added dropwise to 5 ml of deionised water at a rate of 0.2ml/min in a scintillation vial using a syringe pump while the solution

stirring was at 1000 rpm. After that, 0.44 mg of sodium ascorbate and 0.359 mg of Copper (II) sulphate were added to the solution, which was left stirring overnight. Then 6.4 mg of EDTA were added to the solution to chelate all copper ions found in the mixture, and the formulation was left stirring for another 2 h. Finally, the solution was dialysed for 24 h in 1 L of phosphate buffered saline (PBS) solution, pH 7.4 using a 3500 g/mol molecular weight cut-off (MWCO) membrane.

2.5 Confirmation of mPEG-PCL-DOX nanoparticles synthesis using Fourier Transform Infrared (FTIR) spectroscopy:

The Agilent Cary630 FTIR machine was used to characterise the chemical structure of the empty mPEG-PCL or mPEG-PCL-DOX. For this, 2 ml of the synthesised mPEG-PCL-DOX or empty mPEG-PCL nanoparticles were freeze-dried overnight. A small amount of the solid nanoparticles were then pressed to form a thin pellet. The samples were then analysed using a spectral range of 4000-650 cm^{-1} to record the vibrational fingerprint of the functional groups present. Results were analysed using the SpectraGryph 1.2 software.

2.6 Measurement of nanoparticles average size and polydispersity index (PDI):

The Z-average particles diameter (Z-avg) and the polydispersity index (PDI) of the empty and drug conjugated nanoparticles were determined using dynamic light scattering (DLS) Zetasizer Nano ZS (Malvern Panalytical).

2.7 Quantification and pH dependent drug release of DOX from the nanoparticle's formulation:

To quantify doxorubicin in the prepared formulation, 100 μ l aliquot of mPEG-PCL-DOX NPs was mixed with 100 μ l of Trifluoroacetic acid (TFA) to cleave DOX from the NPs. After that, the mixture was incubated in a water bath at 37°C for 4 hours. A calibration curve for DOX was prepared for quantifying DOX concentration released from the nanoparticles. The initial stock DOX concentration was 12.5 μ g/ml, then a series of 1:2 serial dilutions were generated to establish a range of concentration standards. The analysis of DOX release was performed by adding the samples into a black flat bottom 96-well plate, suitable for fluorescence measurement. The quantification of DOX was performed by a Tecan plate reader, which measured fluorescence intensities at an excitation wavelength of 470 nm and an emission wavelength of 595 nm. The fluorescence data obtained from the mPEG-PCL-DOX samples were then interpolated against the calibration curve of free DOX. Trendline equation derived from the DOX calibration curve was used to quantify DOX in the formulation.

Additionally, DOX release in pH 7.4 and pH 5.5 were investigated over 7 days. For this, the acidic buffer was prepared by adding 1 M HCL to PBS until reaching the desired pH (5.5). Then, in a falcon tube 1 ml of mPEG-PCL-DOX was added to a 3500 g/mol molecular weight cut-off (MWCO) membrane and placed in falcon tubes containing 9 ml of either pH 5.5 or pH 7.4 PBS. After that, 1 ml of

each tube were aspirated and replaced with fresh PBS of the same pH. The aspirated samples were placed in black 96 well plate and DOX fluorescence reads were taken for each day and plotted against the DOX standard curve. After collecting readings for all days, the DOX cumulative release was calculated using the following equation:

$$\text{Cumulative Release (\%)} = \left(\frac{(\text{Amount of drug released})t_1 + t_2 + \dots}{\text{Total drug loaded initially}} \right) \times 100$$

Equation 1: Cumulative release % formula.

2.8 Empty mPEG-PCL toxicity on wild type MDA-MB-231 (WT) cell line:

To assess mPEG-PCL nanoparticles toxicity, 10,000 cells/ well were seeded in a 96 well plate incubated overnight to allow cells attachment. On the following day, empty mPEG-PCL was added to the cells at a concentration of 1 mg/ml. WT cells viability were followed up at day 2, day 4 and day 6 and compared to non-treated cells. At each time point, 20 ul of PrestoBlue reagent were added to each well and incubated for 15 minutes before transferring to an Omega Fluorostar fluorescence plate reader. Viability reads were taken using 540 nm excitation and 590 nm emission. Results were analysed using GraphPad Prism two-way ANOVA test.

2.9 Development of the resistant MDA-MB-231 cell line by DOX exposure:

The protocol used for the development of MDA-MB-231 resistant cells (R) was adapted from Paramanantham et al. 2021. According to this, cells were exposed to increasing concentrations of doxorubicin. The cells were exposed to 10 nM DOX at the start of the experiment, reaching 50 nM at the end. Each concentration was kept the same for a number of passages, considering each passage was 3-4 days in total depending on cell confluency. The concentrations and number of passages are shown in Figure 2.1. The R cells were finally kept in 50 nM doxorubicin RPMI media to preserve the developed resistance.

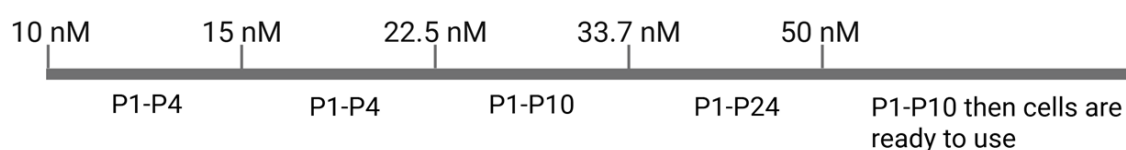


Figure 2.1 Schedule for developing MDA-MB-231 resistant cell line. MDA-MB-231 cells were treated with increasing concentrations of DOX starting from 10 nM to 50 nM. P1,P2... represent the number of passages that DOX was maintained at the same concentration.

2.9 Expression of PgP efflux transporters in MDA-MB-231 cells:

Expression of PgP was obtained by transduction of a lentiviral plasmid coding for the human MDR1 gene. For this, bacterial stabs containing pHaMDRwt (Plasmid #10957), and pMD2.G (Plasmid #12259) were obtained from

Addgene. The bacterial stabs were spread over a plate containing Ampicillin (100 µg/mL) using a sterile loop and incubated overnight at 37°C.

From the LB agar plate, a single colony was taken using a sterile loop and inoculated in LB medium (BD, 244610) supplemented with Ampicillin (100 µg/mL). The liquid culture was incubated overnight at 37°C in a shaking incubator. Glycerol stocks were prepared by taking 600 µL of overnight cultures mixed with 400 µL of sterile 50% glycerol solution (diluted in distilled water). The mixture was vortexed, immersed in liquid nitrogen and stored at -80°C.

The plasmids used for transfections into mammalian cells were recovered from 100 to 200 mL of overnight cultures using the Plasmid Midi Kit (QIAGEN, 12143).

For the lentivirus production, 3×10^6 HEK293T cells were seeded in a T25 flask and incubated overnight until cells reached ~90% confluence. The medium of the cells was changed before transfection. Following the XT360 transfection protocol, the viral envelope vector (1.2 µg of pMD2G (Addgene#12259)) and the viral vector (4.6 µg of pHaMDRwt, Addgene#10957) were added to the transfection reagent diluted in OptiMEM medium in an Eppendorf tube. The transfection mixture was vortexed and incubated at room temperature for 15 min. The transfection mixture was added dropwise onto the flask, and the cells were incubated for 2 days. The virus-containing medium was collected 48 hours after transfection and was filtered using a 0.45 µm low protein-binding filter.

One day before the transduction, 2×10^5 MDA-MB-231 cells were seeded on three wells of a 6-well plate and incubated overnight. Next day, the filtered

medium containing the viral particles was added to the MDA231 cells, and cells were incubated overnight. The following day, the viral medium was removed and replaced with a fresh RPMI medium.

To select resistant cells, MDA-MB-231 cells were treated with 10 or 50 nM DOX for 10 days. After that, the treated cells were seeded into T75 flask until confluent for further experiments.

2.10 Western blotting:

Protein extraction was performed using the radioimmunoprecipitation assay (RIPA) buffer (ThermoFisher #89900). For each cell line (MDA-MB-231-W, MDA-MB-231-R, MDA-MB-231-PgP, MDA-MB-231-PgP-10nM, and MDA-MB-231-PgP-50nM), cells pellets were collected and resuspended in RIPA buffer and incubated on ice for 30 minutes. The cells lysate was then centrifuged at 12,000 RCF for 20 minutes at 4°C to separate the soluble protein fraction. After that, the supernatant was aspirated for each cell line and transferred to a new Eppendorf tube and stored at -80°C. The total protein concentrations were measured using the Bicinchoninic Acid (BCA) assay (ThermoFisher A55860). For each cell line sample, a 1:5 dilution was prepared by mixing 2 ul of the protein sample and 8 ul of dH₂O, producing a total volume of 10 ul. Then 1:50 ration of reagent A to B of BCA working mix was prepared and 10 ul of each diluted sample was combined with 200 ul of the prepared BCA working mix in a 96 well plate, covered and incubated at 37 C° for 30 minutes. After that, the plate placed in in a microplate reader and the absorbance were measured at 580 nm.

Protein concentrations in the samples were calculated using a known standard curve.

To prepare samples for the western blotting, the protein concentration was determined for each cell lysate to load 20 ug of protein per sample, while each sample was prepared to total volume of 40 ul, consisting of protein lysate, dH₂O, and buffers (BOLT LDS Sample buffer (4×) and Bolt reducing Agent (10×)) (ThermoFisher). The sample were then vortexed and subjected to 95-100 C° heat for 5 minutes to denature and reduce the proteins. Following that, the samples were placed on ice for 1 minute and centrifuged and stored on ice until loading.

Electrophoresis was conducted using a mini gel tank system (ThermoFisher). The tank was filled with 1× running buffer, which was prepared by adding 50 ml of SDS running buffer (20X) to 950 ml of dH₂O. A precast polyacrylamide gel (12%) gel was prepared and wells rinsed with running buffer 3 times before placing into the cassette clamps. 5 ul of protein standard ladder mixture (LiCor Cameleon) was added to the first well, followed by 20 ul of each protein lysate samples. The gel was run at 160 V for 50 minutes and after separation of proteins, the gel was transferred onto a PVDF membrane using a BioRAD Trans-Blot Turbo transfer system (30 minutes at 25V). After the transfer, the membrane was blocked using 5% skimmed milk prepared with Tris buffered saline buffer (TBS-50 mM Tris, 150 mM NaCl, pH of 7.4) containing 20% -Tween-20 (TBST) and added to the membrane until it is fully covered and incubated for 1 hour at room temperature. After incubation, the blocking solution was

discarded, and the membrane were transferred to a falcon tube. MDR-1 (D11) primary antibody (1:1000) (Santa Cruz Biotechnology #sc-55510) and B-actin control antibody (1:1000) (Cell signalling #4970) were prepared in 5% skimmed milk in TBST and added to the membrane and the falcon tube were incubated overnight at 4 C° on a rotating rocker. On the next day, the primary antibody solution was discarded, and the membrane was washed 3 times with TBST. Anti-rabbit secondary antibody (1:10,000) (Cytiva #NA934) was prepared in milk/TBST and added to the membrane and incubated at room temperature for 1 hour away from light. The membrane was then washed three times with TBST and transferred to the LiCor Odyssey M imaging system.

2.11 qPCR:

2.11.1 Total RNA extraction

RNA was extracted from the cell pellets using the NucleoSpin RNA Midi kit (Qiagen, #74134) according to the manufacturer's instructions. To extract the RNA, 350 µL of Buffer RA1 containing 10% β-mercaptoethanol were added to each cell pellet and vortexed. The solution was loaded onto a NucleoSpin® filter and centrifuged for 1 minute at $11.000 \times g$. The filter was discarded, and 350 µL of Ethanol (70%) was added to the flow-through and vortexed. The mixture was loaded into a NucleoSpin® RNA column and centrifuged for 30 seconds at $11.000 \times g$. The column was placed in a new collection tube and 350 µL of MDB (Membrane Desalting Buffer) was added and centrifuged again for 1 minute at $11.000 \times g$. After this, contaminant DNA was removed by adding

95 μ L rDNase solution onto the column (10 μ L reconstituted rDNase and 90 μ L dilution buffer). and incubated at room temperature for 15 minutes. Columns were washed by adding 200 μ L of Buffer RAW2 and centrifuging it for 30 seconds at $11.000 \times g$. This was followed by another wash with 200 μ L of Buffer RA3 and finally by another with adding 250 μ L of Buffer RA3, always centrifuging it at $11.000 \times g$. Finally, the RNA was eluted in 60 μ L RNase-free H₂O by centrifugation for 1 minute at $11.000 \times g$.

The RNA concentration was determined using a Nanodrop 2000 spectrophotometer (Thermo Scientific) measuring the absorbance at 260nm and RNA purity was assessed as the A₂₆₀/A₂₈₀ nm absorbance ratio (1.8-2.0 was accepted as good quality). The RNA samples obtained were stored at - 80°C until further analysis.

2.11.2 cDNA synthesis:

The SuperScript™ IV First-Strand Synthesis kit (Invitrogen™) was used for cDNA synthesis according to the manufacturer's instructions. A maximum amount of 5 μ g of the template RNA was used in the reaction. For primer annealing, the following reagents were added to a PCR tube: 1 μ L of 50 μ M random hexamers primer, 1 μ L of 10 μ M dNTP, < 5 μ g of template RNA, and DEPC-treated water, to a final volume of 13 μ L. The reaction was heated at 65°C for five minutes and then incubated on ice. For the reverse transcription reaction, the following components were then added to each tube: 4 μ L of 5x SSIV buffer, 1 μ L of 100 mM DTT, 0.5 μ L of RNaseOUT recombinant RNase inhibitor, 0.5 μ L ddH₂O, and 1 μ L of SuperScript™ IV reverse transcriptase enzyme. The tube was then

incubated at 50°C for 10 minutes, followed by incubation at 80°C for 10 minutes to inactivate the enzyme. cDNA samples were stored at - 20°C.

2.11.3 Quantitative real-time PCR (qPCR):

For qPCR analysis, a SYBR green master mix (Applied Biosystems, #A25742) was prepared for each primer set. The final volume of each reaction was 15 µL and contained 7.5 µL SYBR Green, 0.3 µL of each forward and reverse primer, 0.75 µL cDNA, and 6.2 µL ddH₂O. PCR amplification was performed using the 7500 Real-Time PCR Systems (Thermo Fisher) with an amplification program following the following steps:

- 1) initial denaturation stage: 2 minutes at 95 °C
- 2) 40 cycles of 15 seconds of denaturation at 95 °C, annealing for a minute at 59 °C followed by a melting curve.
- 3) extension of 7 minutes at 72°C.

Threshold cycle (Ct) values and melting curves were obtained upon programme completion. The Ct values were then exported to a Microsoft Excel spreadsheet for further analysis. The graphs were created by GraphPad Prism 9 software. The $\Delta\Delta C_t$ method of relative quantification was used to calculate the Fold changes in gene expression using the 60S acidic ribosomal protein P0 (RPLP0) as the housekeeping gene (primers listed in Table 2.1). All experiments were performed using three biological replicates.

Table 2-1: Primers used for qPCR.

Gene	Primer	Sequences (5'-3')
<i>RPLP0</i>	Forward	ACAACCCAGCTCTGGAGAAA
	Reverse	TGCCCCTGGAGATTTTAGTG
<i>DMR1</i>	Forward	GCTGTCAAGGAAGCCAATGCCT
	Reverse	TGCAATGGCGATCCTCTGCTTC

2.12 Half maximum inhibitory concentration (IC₅₀) studies in 2D models:

A 96-well plate was prepared by seeding each well with a density of 10,000 cells. The cells were then incubated at 37°C with 5% CO₂ overnight to allow cells adhesion. Subsequently, the treatment solutions of free DOX (300 nM) and mPEG-PCL-DOX (1mg/ml) were prepared. The initial top concentration of each treatment was established at 2 µM. From this stock solution, a series of 1:2 serial dilutions were created to obtain a range of concentrations for the treatment assay. These dilutions were then added, and the plate was incubated for a period of 48 hours. Following the incubation period, a volume of 20 µl of Presto Blue reagent was added to each well. The plates were then incubated for an additional 15 minutes, then fluorescence was measured in an Omega Fluorostar fluorescence plate reader using 560/590 nm (excitation/emission). Results were analysed with the Graph Pad Prism software using inhibitor vs response analysis.

2.13 2D cellular uptake images using EVOS system for free DOX and mPEG-PCL-DOX:

The EVOS M7000 imaging system was used to investigate free DOX and mPEG-PCL-DOX cellular uptake. In a 96 well plate, 10,000 cells were seeded and incubated overnight. On the next day, 300 nM of DOX or mPEG-PCL-DOX (1 mg/ml) were added to the wells and incubated for 2 h, 4 h, 8 h, and 24 h. At each time point, the cells were fixed with 2% paraformaldehyde (PFA) and incubated for 15 minutes. Cells were washed with PBS and the cell's nuclei were next stained using 0.1 ug/ml DAPI nuclear stain prepared in PBS (incubated 10 minutes). After that, the plate was transferred to the EVOS and images were captured using the DAPI (blue) and RFP (red) channels.

2.14 DOX and mPEG-PCL-DOX cytotoxic effect on 2D models viability:

In a 96 well plate, 1000 cells/ well were seeded and incubated for 24 h at 37°C with 5% CO₂ to allow cell adhesion. On the following day, 300 nM of free DOX or mPEG-PCL-DOX (1 mg/ml) were added to the plate and returned to the incubator. After each 2 days, 20 µl of Presto Blue reagent was added to each well. The plates were then incubated for an additional 15 minutes, then fluorescence was measured in an Omega Fluorostar fluorescence plate reader using 560/590 nm (excitation/emission). After collecting the viability of all time points, the data were analysed using GraphPad Prism two-way ANOVA test.

2.15 Preparation of pH 6.5 acidic media:

To prepare the acidic media, 15 ml of HEPES buffer and 15 ml of MES buffer were added to 500 ml of RPMI medium. The solution was mixed and incubated in a water bath for minimum 1 hour. Using a pH meter, the pH of the medium was altered using NaOH and HCL to reach pH 6.5. After that, 50 ml of FBS and 5 ml of L-Glutamine were added to the medium. Finally, the medium was filter sterilized using 0.22 um filters. Before each use, the pH was checked again using the pH meter.

2.16 Spheroids culture:

To optimise the cell seeding, cells were prepared in 200 ul of medium and seeded under the following conditions in an Ultra Low Attachment 96 well plate.

- 1) 1000 cells/200 ul + 100 ug/ml BME,
- 2) 2000 cells/200 ul + 100 ug/ml BME,
- 3) 1000 cells/200 ul without addition of BME.

Images for each condition were taken using the EVOS microscope on day 3 and day 7.

Following cell number optimisation, 2000 cells/well in addition to 100 ug/ml or 200 ug/ml BME were used to optimise the BME concentration. On day 7, cells were labelled with the live/dead stain kit . The spheroids were washed twice with PBS, removing as much liquid possible at each time without damaging the

spheroids. After that, enough staining solution was prepared for a volume of 100 μ l/spheroid (the concentration needed was 2 μ l/ml Ethidium homodimer and 0.5 μ l/ml calcein AM prepared in PBS and covered in foil to protect it from light). After this, the staining solution was carefully added to the spheroids, and the plate was incubated in dark at 37 C° for at least 15 minutes. Finally, the plate was transferred to the EVOS fluorescence microscope and the RFP channel was used to visualize dead cells (red) while the GFP channel was used for live cells (green).

2.17 DOX and mPEG-PCL-DOX uptake in spheroids models:

For each MDA-MB-231 model, the spheroids were formed as explained above. On day 4, 300 nM of free DOX or mPEG-PCL-DOX nanoparticles (1 mg/ml) were added to the spheroids. The red fluorescence of DOX was captured using the EVOS fluorescence microscope at day 8 using RFP channel.

2.18 DOX and mPEG-PCL-DOX cytotoxic effect on spheroids viability:

A 96 ultra-low attachment wells plates were prepared, each well containing 2,000 cells/well with addition of 100 μ g/ml BME. The plates were then centrifuged at 300 g for 10 minutes to facilitate uniform spheroid formation. Post-centrifugation, the plates were incubated for 4 days to allow for the spheroids to form before any treatment was applied. On day 4, the first plate

received 0.3 μM of Free DOX and 0.3 μM mPEG-PCL-DOX. The viability of spheroids was assessed 2 days post-treatment using 20 μl of Presto Blue reagent and an Omega Fluorostar fluorescence plate reader using 560/590 nm (excitation/emission). After measuring the viability of all time points, the data were analysed using GraphPad Prism two-way ANOVA test.

2.19 DOX quantification using LC-MS:

To quantify intracellular concentrations of DOX, 8×10^5 cell/well of WT, R and PgP cells were seeded in 6 well plates and incubated overnight. On the following day, free DOX (300 nM) or mPEG-PCL-DOX nanoparticles (1mg/ml) were added to each well and returned to the incubator. After reaching the required time points (2 h, 4 h, 8 h and 24 h), cells are washed twice with PBS and then lysed using ice cold LC-MS grade methanol for 15 minutes. After this, 0.5 ml of the lysate was transferred into an LC-MS vial for analysis.

The Shimadzu SIL-HTc Autosampler coupled with QTRAP 4000 mass spectrometer was used to quantify DOX in the samples using an Acquity UPLC BEH C18 column (1.7 μm , 2.1 $\times$ 100 mm). The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B) delivered in a binary flow at 0.15 ml/min. The gradient program was as follows: 5% B (0–1 min), increased to 90% B at 3 min, ramped to 95% B at 5 min, held until 6 min, then returned to 5% B at 6.1 min and re-equilibrated until 9.5 min. The injection volume was 20 μl . Also, a range of known DOX concentrations from 0 to 500 nM was used to create a standard curve to calculate DOX concentrations in the

samples. Quantification was based on peak area response, and all analyses were conducted in triplicate to ensure reproducibility.

Chapter 3 : Synthesis and characterisation of novel pH-responsive mPEG-PCL-DOX nanoparticles delivery system:

3.1 Introduction:

Nanoparticles-based drug delivery systems are becoming an important area of scientific research for cancer treatment due to their tumour-specific delivery and reduced unwanted toxicities and side effects. Among nanoparticle systems are the methoxy poly(ethylene glycol)-poly(ϵ -caprolactone) (mPEG-PCL) copolymers, which have unique physicochemical properties compared to other nanoparticles. The amphiphilic nature of mPEG-PCL copolymers leads to self-assembly into micelles, with an outer PEG hydrophilic shell and an inner PCL hydrophobic core. This micellar assembly aids in encapsulating hydrophobic drugs like doxorubicin in the hydrophobic core. In addition, outer PEG polymers exhibit enhanced permeability and retention (EPR) properties and help increasing drug bioavailability through stealth behaviour, as well as by minimising reticuloendothelial system (RES) clearance and prolonging bioavailability time *in vivo*.

Among the innovative technologies in mPEG-PCL nanoparticles is the development of pH-sensitive drug systems, which take advantage of the acidic tumour microenvironment (pH 5.0-6.5) compared to the normal tissue environment (pH 7.4). These pH-sensitive systems are designed to selectively

trigger the release of encapsulated or conjugated drugs in the acidic tumour microenvironment, improving drug efficacy and reducing systemic toxicities.

Different methods for chemotherapy drugs conjugation and encapsulation into mPEG-PCL nanoparticles have been described. These include physical encapsulation, in which the drug is trapped within the hydrophobic core of the micelle during self-assembly, through nanoprecipitation, solvent evaporation, or dialysis techniques. The other method is chemical conjugation, in which the drug is covalently linked to the polymer backbone or terminal groups via cleavable linkers, such as ester and Schiff bases, allowing controlled and stimuli responsive release. Each of these methods has advantages and limitations. For example, physical encapsulation is easy, cost effective and does not require any chemical modifications of the drug. However, its drug loading is often low and may exhibit premature release of the drug. On the other hand, chemical conjugation offers stable attachment enables controlled or stimuli-responsive drug release, but its synthesis is complex and requires functionalization and chemical modification of both the drug and polymer, which may compromise the drug's activity.

Several studies have shown the effectiveness of nanoparticle-mediated drug delivery in breast cancer.

An interesting study performed by Carrillo-Castillo et al. 2020 showed successful encapsulation of the Methotrexate (MTX) anticancer drug into mPEG-PCL nanoparticles. A drug release study was performed for 30 days, and enhanced release of MTX was found at pH 5.5 compared to pH 7.4 on day 30.

In the same paper a drug efficacy study was carried out using the MCF-7 breast cancer cell line which demonstrated the MTX/mPEG-PCL IC₅₀ was estimated to be 30 µg mL⁻¹, whereas the IC₅₀ of free MTX could not be reached at the end of the experiment, indicating that nanoparticles were superior in killing cells over the free MTX.

Also, another interesting mPEG-PCL pH-sensitive nanoparticle study was published by Zhai et al. 2018, in which a pH-sensitive mPEG-PCL was conjugated with DOX chemically through an acid-cleavable imine bond. *In vitro* drug release studies were conducted for 48 h in different pH media. Results showed that there was a minimal release at pH 7.4, where only 20% of DOX was released from mPEG-PCL, with a significant increase at pH 6.5 (42.8±1.5 %) and pH 5.0 (60.3±0.8 %), indicating their potential to deliver DOX specifically into the acidic tumour tissues. Also, cellular uptake studies confirmed the internalisation of nanoparticles in the MCF-7 cell line, while *in vitro* cytotoxicity studies indicated a controlled release profile.

Because of mPEG-PCL great potential to deliver anticancer agents to cancer cells, this thesis will focus on investigating novel the efficacy of mPEG-PCL conjugated with doxorubicin in triple negative breast cancer. The particles used in this project will be synthesised by click chemistry, where the formed triazole ring linker between the mPEG-PCL and DOX provides a chemically stable bond at physiological pH, which helps reducing premature drug release in systemic circulation. In addition, the imine cleavable bond allows precise pH responsive release inside endosomes and lysosomes once internalised by cancer cells. This

approach is specifically aimed at overcoming TNBC drug resistance, especially multidrug resistance produced by overexpression of drug transporters, such as the P-glycoprotein (PgP).

3.2 Results

3.2.1 Preparation of empty mPEG-PCL nanoparticles and pH responsive mPEG-PCL-DOX conjugated nanoparticles:

Due to its strong solvating properties, NMP effectively caused dissolution of the mPEG-PCL polymer. Nanoprecipitation of the mPEG-PCL occurred as the solvent quality changed from organic (NMP) to aqueous (deionised water), which lead to rapid precipitation of mPEG-PCL due to its decreased solubility in the aqueous medium as shown in Figure 3.1.

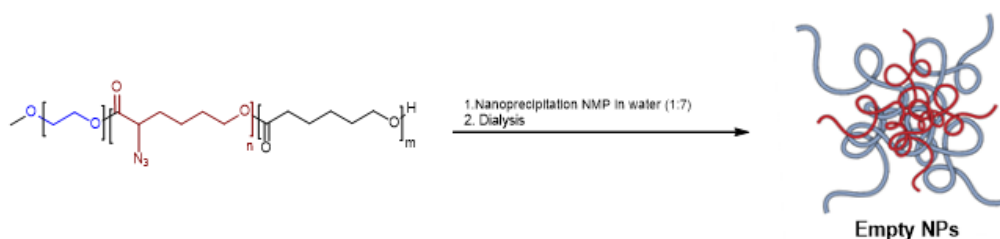


Figure 3.1 Synthesis of empty mPEG-PCL nanoparticles (NPs). Nanoprecipitation of mPEG-PCL molecules, which aggregates to form mPEG-PCL nanoparticles.

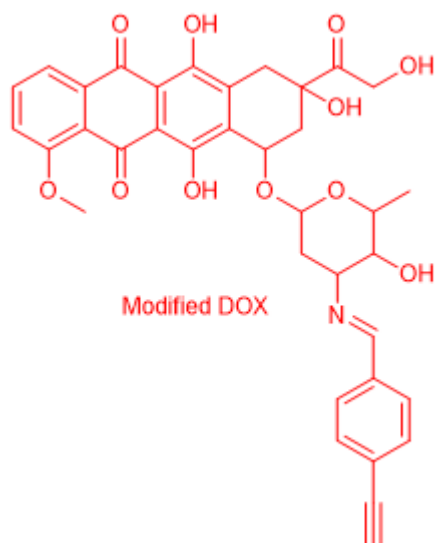


Figure 3.2 Chemical structure of Modified DOX. Alkyne group was added to DOX to facilitate the conjugation to the mPEG-PCL nanoparticles.

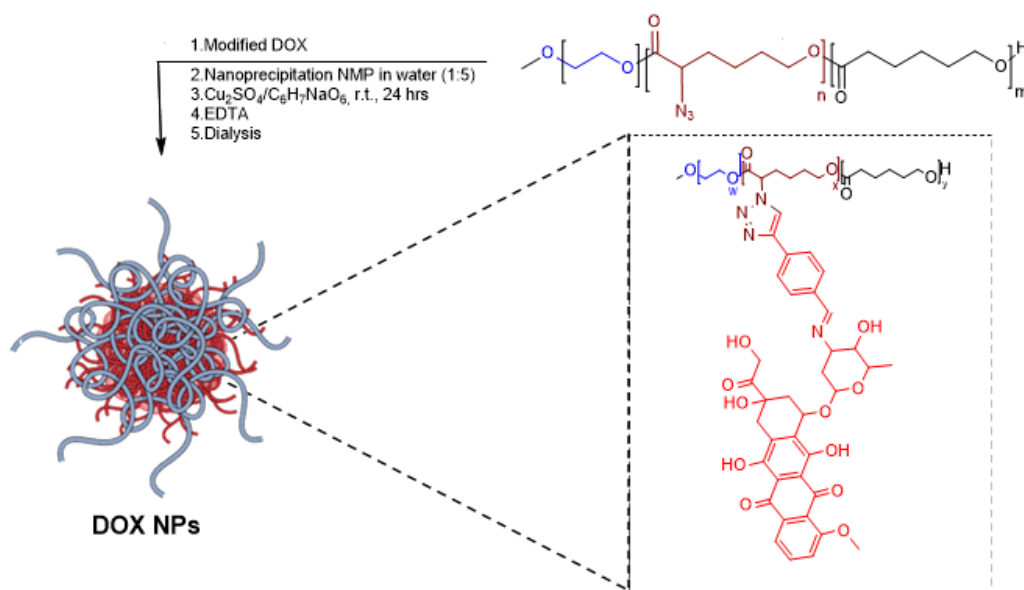


Figure 3.3 Formation of mPEG-PCL-DOX conjugated nanoparticles. Conjugation of DOX to the mPEG-PCL using Azide-Alkyne click chemistry.

The modified DOX shown in Figure 3.2 contains an alkyne group reacted with the azide group in mPEG-PCL by click chemistry, as described in Figure 3.3. This

reaction involved the cycloaddition of an azide to an alkyne to form a 1,2,3-triazole linker. This process is catalyzed by copper (Cu(I)) in the most widely used version of this reaction, known as the Cu-catalyzed azide-alkyne cycloaddition (CuAAC). The imine group (-C=N-) found in Schiff base in the modified doxorubicin would be responsible for the pH sensitive release of the drug in the acidic microenvironment of cellular compartments in cancer cells such as endosomes and lysosomes

3.2.2 Confirmation of Click reaction using FTIR:

To confirm the final chemical structure and the conjugation between azide mPEG-PCL and alkyne modified DOX, Fourier Transformed Infrared (FTIR) spectroscopy was used. As shown in

Figure 3.4, the FTIR spectrum shows a peak representing azide in mPEG-PCL (blue) that disappeared completely in mPEG-PCL-DOX (red) at $\sim 2100\text{ cm}^{-1}$. Also, a prominent peak at $\sim 1730\text{ cm}^{-1}$ representing ester carbonyl (C=O) in mPEG-PCL was reduced significantly in mPEG-PCL-DOX, accompanied by the appearance of a peak at $\sim 1650\text{ cm}^{-1}$ in mPEG-PCL-DOX. Another interesting observation was the broadening peak in the region $\sim 3200\text{-}3600\text{ cm}^{-1}$ in mPEG-PCL-DOX representing OH stretching, while it was absent in mPEG-PCL spectrum.

The disappearance of the azide peak at $\sim 2100\text{ cm}^{-1}$ in mPEG-PCL-DOX suggests that the azide groups are completely reacted. Also, the peak appeared at $\sim 1650\text{ cm}^{-1}$, suggesting triazole ring formation and confirming reaction completion. The disappearance of the ester carbonyl group at $\sim 1730\text{ cm}^{-1}$ could result from shielding from the formed triazole ring. Lastly, the appearance of a broad band between $\sim 3200\text{--}3600\text{ cm}^{-1}$ in mPEG-PCL-DOX and its absence in the mPEG-PCL spectrum supports the conjugation of DOX, as the broadened band is attributed to the DOX O-H vibrations. All previous observations (Figure 3.4) provide strong evidence for the successful conjugation of DOX onto mPEG-PCL via CuAAC click chemistry.

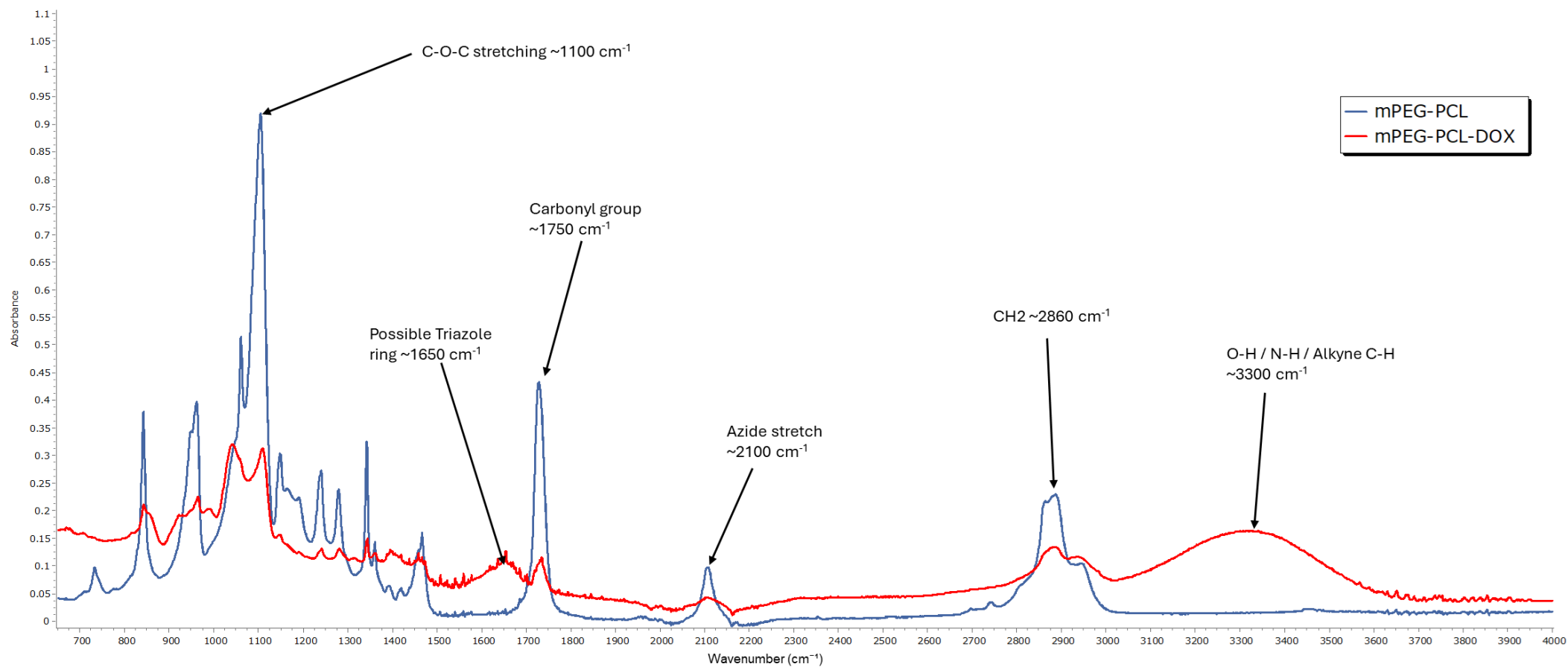


Figure 3.4: FTIR spectra of azide functionalized mPEG-PCL (blue) before conjugation, and after DOX conjugated to mPEG-PCL (red).

3.2.3 Characterisation of mPEG-PCL, mPEG-PCL-DOX, and mPEG-PCL-DOX-Cy5.5 nanoparticles size and PDI (dialysed in PBS):

Although the initial characterisation of the nanoparticles was performed by a formed postgraduate researcher in the lab (Dr Christina Parisi), the nanoparticles size and polydispersity index (PDI) were replicated to ensure accurate nanoparticles synthesis each time. mPEG-PCL-DOX nanoparticles average size was measured to be 63.4 ± 0.7 d.nm (Figure 3.5). Encapsulation efficiency and drug loading for mPEG-PCL-DOX were 46 ± 0.93 % and 11.73 ± 0.73 %, respectively (Table 3-1).

The synthesis of mPEG-PCL nanoparticles was improved from the original protocol by changing the dialysis medium from de-ionised water to PBS buffer to stabilize the pH of the formulation of the nanoparticles.

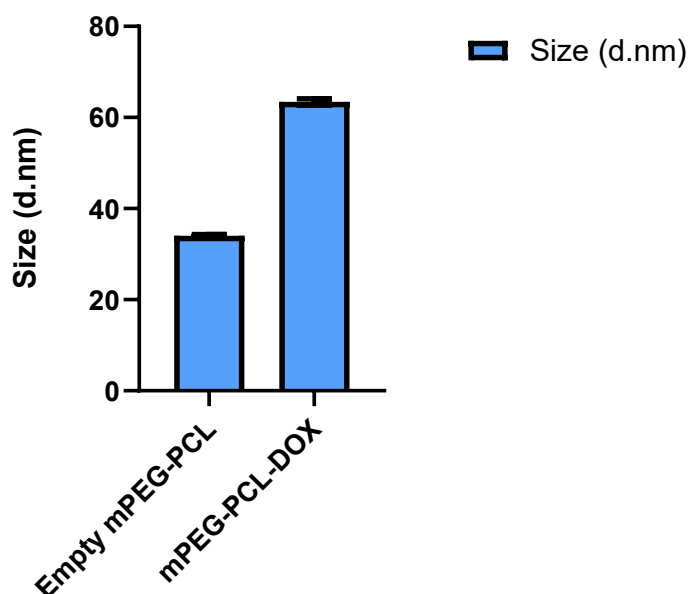


Figure 3.5 : Nanoparticles characterisation showing particles size (d.nm)) for Empty mPEG-PCL and mPEG-PCL-DOX using DLS. (n=3) $\pm$ SD.

Dynamic light scattering (DLS) results shown in Figure 3.5 confirmed the synthesis of the empty and conjugated nanoparticles. The correlogram shown in Figure 3.7 represents the intensity autocorrelation function obtained from Dynamic Light Scattering (DLS) analysis. The DLS technique measures fluctuations in scattered light intensity resulting from the Brownian motion of nanoparticles in suspension. These fluctuations are correlated over time to generate an autocorrelation curve, from which the hydrodynamic diameter and polydispersity index (PDI) are calculated using the Stokes–Einstein equation. The single exponential decay curve of the correlogram shown in Figure 3.7 indicates a monodisperse sample with nanoparticles of similar size.

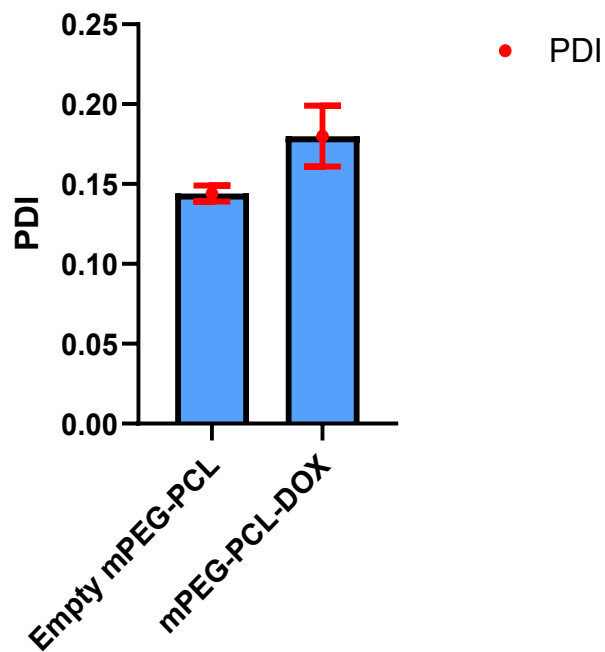


Figure 3.6 Nanoparticles characterisation showing polydispersity index (PDI) for Empty mPEG-PCL and mPEG-PCL-DOX using DLS. (n=3) $\pm$ SD.

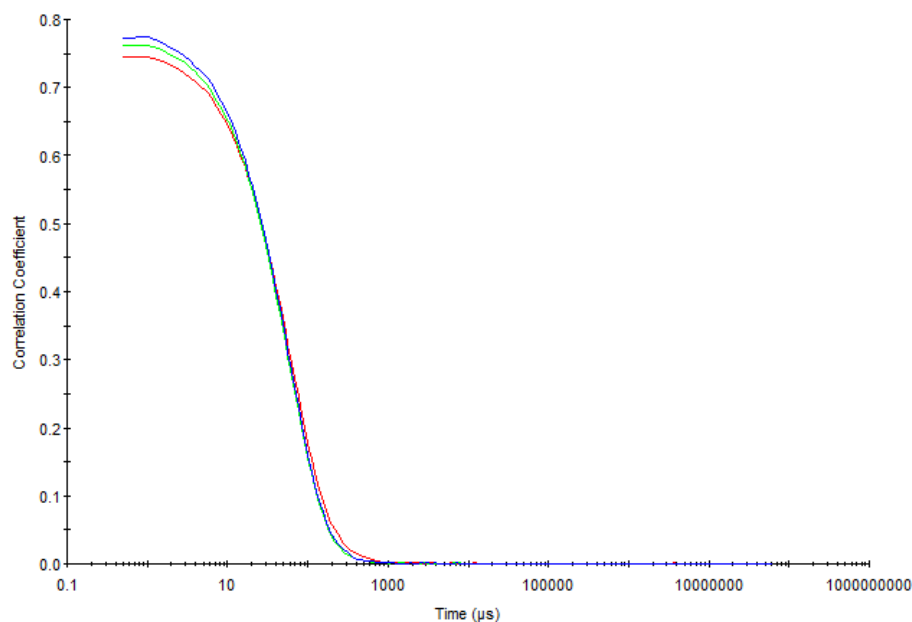


Figure 3.7: mPEG-PCL-DOX nanoparticles correlogram (n=3). Each colour represents a replicate.

3.2.4 Quantification of DOX concentration in the synthesised mPEG-PCL-DOX nanoparticles:

To quantify the amount of DOX encapsulated DOX, mPEG-PCL-DOX were incubated 1:2 with trifluoroacetic acid (TFA), a strong acid with a pH of 0.5, for 4 hours. The highly acidic TFA was used to trigger the release of all DOX from mPEG-PCL nanoparticles.

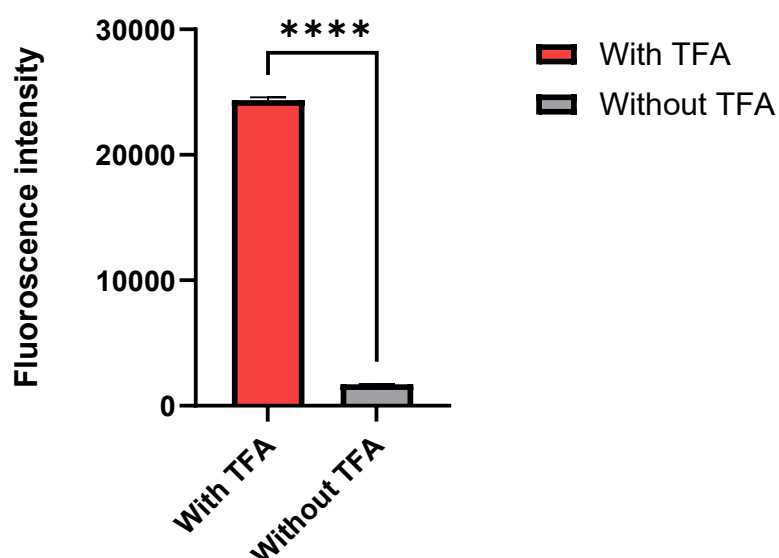


Figure 3.8: Fluorescence intensity readings of mPEG-PCL-DOX nanoparticles with or without incubation with TFA. mean $\pm$ SD (n=3).

As shown in Figure 3.8, the fluorescence intensity of mPEG-PCL-DOX nanoparticles incubated with TFA was very significant ($P < 0.001$) compared to those without TFA.

The DOX standard curve was then used to quantify the DOX amount in the nanoparticle formulation and calculate drug loading and encapsulation efficiency.

As shown in Table 3-1, the concentration of DOX in the formulation was calculated to be 195.6 ± 4.04 ug/ml, and the encapsulation efficiency was 46.92 ± 0.93 %, while the drug loading was 11.73 ± 0.73 %.

Table 3-1: Size and PDI for empty mPEG-PCL, mPEG-PCL-DOX, mean $\pm$ SD (n=3). Encapsulation efficiency and drug loading mean $\pm$ SD of mPEG-PCL-DOX.

NPs	DOX concentration (ug/ml)	Size (nm)	PdI	Encapsulation Efficiency (%)	Drug Loading (%)
Empty mPEG-PCL	-	34.06 ± 0.25	0.144 ± 0.005	-	-
mPEG-PCL-DOX	195.6 ± 4.04	63.4 ± 0.7	0.180 ± 0.019	46.92 ± 0.93	11.73 ± 0.73

3.2.5 mPEG-PCL-DOX release study:

The *in vitro* cumulative release of DOX from the nanoparticles was measured for seven days, as shown in Figure 3.9, using buffers at different pH. In this experiment, pH 7.4 was used to mimic normal physiological conditions, and pH 5.5 was used to mimic the tumour microenvironment.

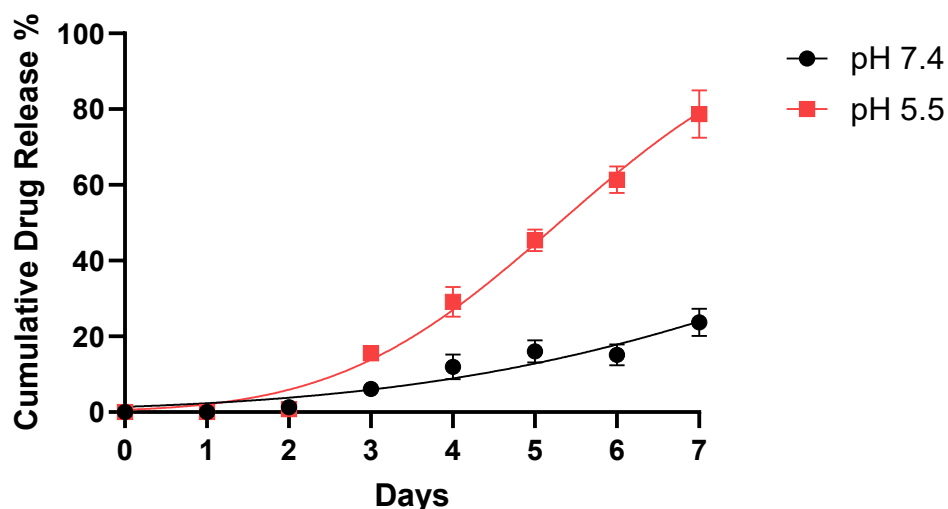


Figure 3.9: mPEG-PCL-DOX nanoparticles drug release study in pH 7.4 (black) and pH 5.5 (red) mean $\pm$ SD over 7 days.

As shown in Figure 3.9, the cumulative DOX release from mPEG-PCL in both pH conditions started on day 2 and exhibited a gradual increase in the release of DOX, but with much higher rates at pH 5.5. For pH 7.4, the maximum release reached was 23.6 % $\pm$ 2.9 %. On the other hand, DOX release from mPEG-PCL was significantly enhanced at pH 5.5 to reach 78.6 % $\pm$ 5.1 %.

Altogether the results of this chapter demonstrate the successful synthesis of the pH sensitive nanoparticles encapsulating DOX. The synthesis of the nanoparticles proved stable after buffering at pH7.4, while the release of the DOX was enhanced by lowering the pH at 5.5, thus mimicking the effective release in the acidic conditions of the tumour microenvironment.

3.3 Discussion

Novel pH-responsive mPEG-PCL-DOX nanoparticles were synthesised and characterised as a potential of a targeted delivery system for TNBC. To this end, modified DOX was conjugated chemically via copper-catalyzed azide-alkyne cycloaddition (CuAAC) click chemistry, which led to the formation of a 1,2,3 triazole ring with a pH-sensitive imine group as a release trigger site designed to hydrolyse an acidic environment.

The size and PDI of empty mPEG-PCL and mPEG-PCL-DOX were characterised using DLS as shown in Table 3-1 and Figure 3.5, demonstrating an average size of 34.06 ± 0.25 d.nm for empty mPEG-PCL and 63.4 ± 0.7 d.nm for mPEG-PCL-DOX. These sizes have been previously demonstrated to be suitable for endocytosis (Sousa De Almeida et al. 2021). The size difference between the empty mPEG-PCL and mPEG-PCL-DOX could be due to the DOX molecules' weight within the nanoparticles, which indicates a successful synthesis. Also, the single exponential decay observed in the DLS correlogram in Figure 3.7 confirmed the monodispersed nanoparticles with a low PDI (0.180 ± 0.019), indicating a uniform size distribution and reproducible nanoparticles synthesis protocol for mPEG-PCL-DOX.

The quantification of conjugated DOX in mPEG-PCL showed significant increase in fluorescence intensity signal ($P < 0.0001$) in nanoparticles treated with TFA compared to non-treated, confirming the release of DOX from mPEG-PCL. The drug loading DOX within the mPEG-PCL nanoparticles was quantified to be 11.73 ± 0.73 % and 46.92 ± 0.93 % for encapsulation efficiency, highlighting

mPEG-PCL-DOX as a candidate to retain and deliver DOX to targeted cells. The *in vitro* drug release profiles were found to be pH-dependent, reflecting the nanoparticles' pH-sensitive behaviour. At physiological pH (7.4), mimicking normal systemic conditions, the release of DOX was minimal, reaching only about $23.6 \pm 2.9\%$ over seven days. On the other hand, at acidic pH (5.5), representing the tumour microenvironment, a significantly higher cumulative release of approximately $78.6 \pm 5.1\%$ was observed. This accelerated release results from the hydrolysis of the Schiff base linker within the nanoparticles at lower pH values, enabling enhanced DOX release specifically within acidic cell compartments such as endosomes or lysosomes and tumour microenvironment.

These finding suggests that mPEG-PCLDOX nanoparticles may be used as a targeted therapy in cancer treatment, benefiting from the acidic microenvironment of cancer to trigger the targeted release of DOX intratumorally, and possibly minimising side effects. The reduction of systemic toxicity not only increases the safety and tolerability of treatment potentially permitting higher, more effective drug dosing but also improves patient quality of life. This approach is increasingly validated in review literature as a critical advantage of nanoparticle-mediated drug delivery in oncology (Cheng et al. 2025).

Chapter 4 : 2D TNBC cell lines studies

4.1 Introduction:

Triple negative breast cancer (TNBC) is one of the most aggressive and challenging subtypes of breast cancer, characterised by high heterogeneity and rapid disease progression. In addition, TNBC is characterised by the lack of expression of molecular targets such as estrogen, progesterone and HER2 receptors, thus limiting therapeutic options for treatment. Chemotherapy is still the first line of treatment for TNBC, however not all patients respond due to inherent and acquired resistance. Indeed, chemotherapy drugs such as doxorubicin, which are part of the main treatment in TNBC, become less effective due to the rapid emergence of multidrug resistance. This resistance is driven by various mechanisms, including the overexpression of ATP-binding cassette (ABC) transporters, alterations in apoptotic pathways, enrichment of cancer stem cells, and the unique features of the tumour microenvironment, such as hypoxia and acidosis (Raman et al., 2025a; Nedeljković and Damjanović, 2019). For these reasons, patients' survival rates decrease within 3-5 years of diagnosis, as the survival rates of TNBC patients are only 77% compared to 90% for other types of breast cancer (Ossovskaya et al. 2011, Wei et al. 2024).

Expression of drug efflux mediated by ATP-binding cassette (ABC) transporters is one of the most studied mechanisms of resistance in TNBC. Three main ABC transporters are significantly overexpressed in TNBC compared to other breast cancer subtypes, the ABCC1/MRP1 transporter (multidrug-resistant protein

1), the ABCG2/BCRP transporter (breast cancer resistance protein), and the ABCC11/MRP8 transporter (multidrug-resistant protein-8). Expression of ABCC1 confers resistance to various drugs, including taxanes and anthracyclines. On the other hand, ABCG2 specifically induces chemoresistance in cancer stem cells by effluxing various drugs, such as 5-fluorouracil and doxorubicin (Raman et al., 2025b). In addition, the P-glycoprotein (PgP), encoded by the ABCB1 gene, is another type of ABC transporter that plays a major role in TNBC resistance. Its structure comprises two nucleotide-binding domains and two transmembrane domains, with multiple drug-binding sites that allow it to recognise, bind, and efflux a broad range of substrates, including drugs like anthracyclines and taxanes, thereby reducing their intracellular accumulation and diminishing their cytotoxic effect. Overexpression of PgP is triggered by the cell's exposure to chemotherapy itself and epigenetic changes induced by effectors within the tumour microenvironment (Ambudkar et al. 2003, Wu et al. 2016). To counteract PgP-mediated drug resistance in TNBC, research has focused on several strategies, such as nanoparticles-based delivery systems which facilitate drug entry through endocytosis rather than passive diffusion, thereby bypassing membrane efflux pumps (Mirzaei et al. 2022). Other strategies include direct inhibition of the PgP ATP-binding domains. For example, ELACRIDAR (GF120918) is a potent PgP inhibitor which acts as a non-competitive inhibitor, which binds to an allosteric site on PgP and modifies its ATPase activity, leading to the prevention of PgP from pumping drugs out of the cells and allowing higher intracellular accumulation and cytotoxicity of drugs. In their study, Chen et al. 2017 found that ELACRIDAR was

able to restore sensitivity in docetaxel-resistant non-small cell lung cancer (NSCLC) cell lines overexpressing PgP, as results showed significant reduction in IC50 values when cells were treated with the inhibitor. Although ELACRIDAR showed promising results in preclinical trials, its clinical translation has been limited, as its ability to overcome drug resistance in patients is moderate, due to the complexity of tumour biology and pharmacokinetics challenges (Lai et al. 2020).

In this project, we aimed to test the effect of nanoparticles to deliver doxorubicin to bypass drug resistance in TNBC. Therefore, the aim of this chapter was to test the effect of pH-sensitive nanoparticle to mediate intracellular delivery of doxorubicin in control (wild type) and drug-resistant TNBC cell lines cultured in conventional two dimension culture conditions.

4.2 Results:

4.2.1 Empty mPEG-PCL toxicity in wild type MDA-MB-231

(WT) cells:

As it is well-documented that certain nanoparticles may induce cytotoxic effects independently of any therapeutic payload (Xuan et al. 2023), it was first necessary to determine whether any potential cytotoxicity observed in treated samples could be attributable to the conjugated doxorubicin (DOX) rather than to the mPEG-PCL carrier system itself. Therefore, empty mPEG-PCL nanoparticles were first tested by treating the WT cell line with the empty particles for up to 6 days.

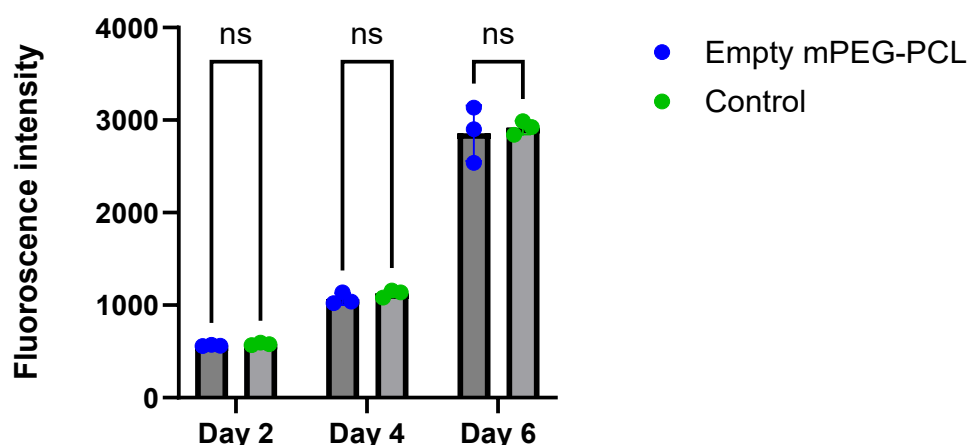


Figure 4.1: Viability assay of empty mPEG-PCL in WT cells compared to untreated (Control) cells. Figure shows fluorescence intensity on days 2, 4, and 6. Data are expressed as mean $\pm$ SD (n=3). Data were analysed by two-way ANOVA test relative to control cells at each time point.

As shown in Figure 4.1, there was no statistically significant viability difference between cells treated with empty mPEG-PCL and non-treated (control) across days 2, 4, and 6, confirming no toxic effect mediated by the carrier particles.

4.2.2 DOX vs mPEG-PCL-DOX nanoparticles IC_{50} in WT:

The next step was to examine the cytotoxic efficacy of free doxorubicin (DOX) in WT cells. To this end, dose-response experiments were performed with cells treated with concentrations ranging from 1 nM to 2 μ M of free DOX for 48 hours. Cells viability was calculated, and dose vs response curves were plotted using GraphPad prism to calculate the half-maximal inhibitory concentration (IC_{50}) values (Figure 4.2).

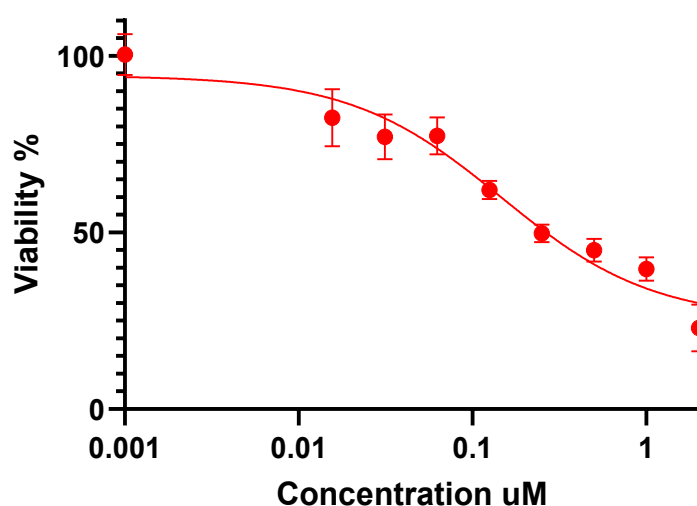


Figure 4.2: Dose vs response curves of free DOX. The figure shows WT cells viability measured by PrestoBlue viability assay after the exposure to free DOX (red). The figure shows mean IC_{50} and 95% CI.

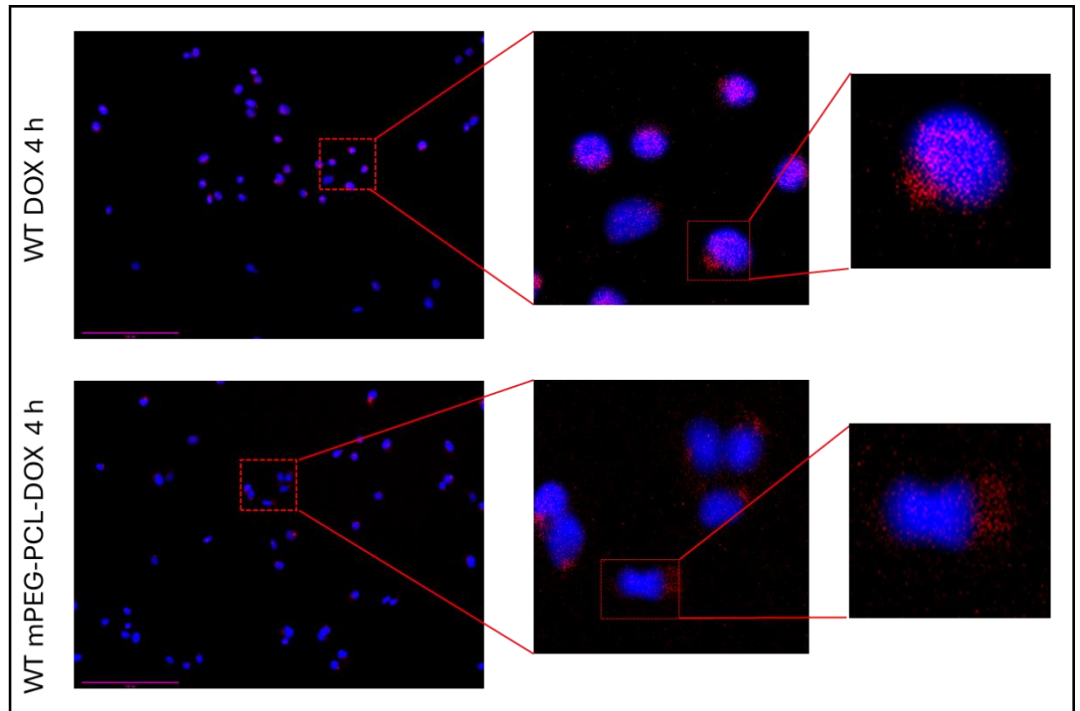
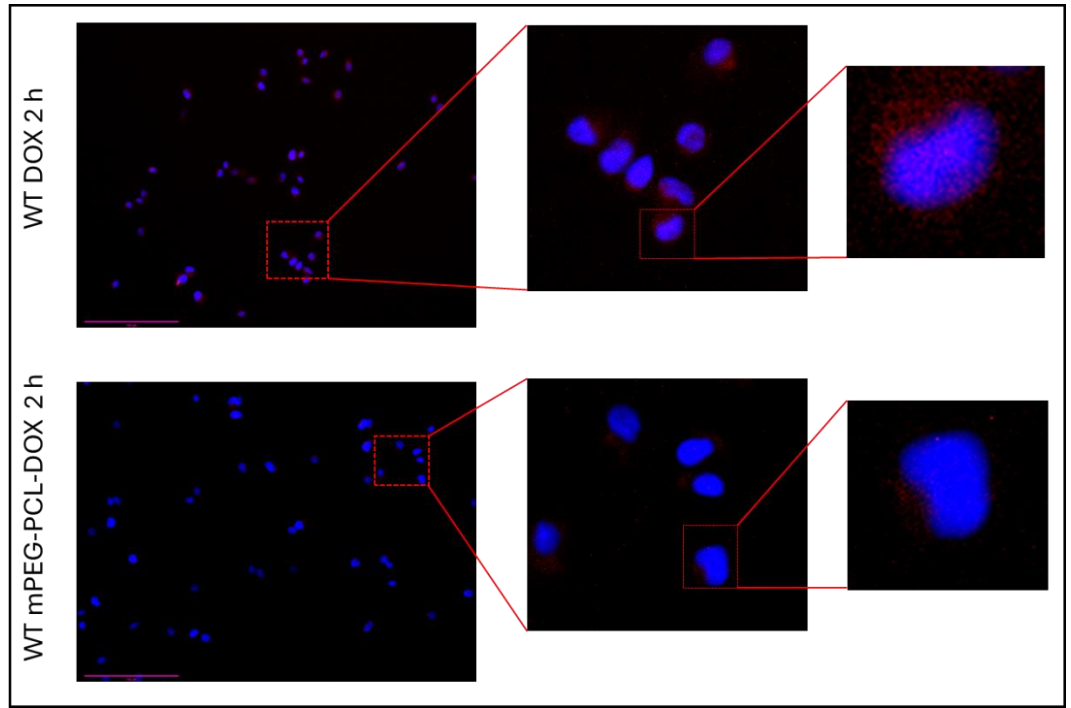
As shown in Table 4-1, the calculated IC₅₀ for free DOX was 0.305 μ M with a 95% confidence interval (CI) ranging from 0.245 to 0.379 μ M.

Table 4-1: Calculated IC₅₀ values for DOX.

	Free DOX
IC ₅₀ (uM)	0.305
95% CI (uM)	0.245 to 0.379

4.2.3 Uptake of free DOX and mPEG-PCL-DOX nanoparticles in WT cells:

The cellular uptake of DOX and mPEG-PCL-DOX nanoparticles was next examined using fluorescence microscopy. For this, WT cells were treated with 300 nM (IC₅₀ concentration) with either free DOX or mPEG-PCL-DOX, and fluorescence images were captured at 2, 4, 8, and 24 hours post-exposure. The 300 nM. To ensure like-with-like comparisons between free DOX and DOX–NPs, the free DOX concentration was matched to the DOX-equivalent present in the nanoparticle dose. The DOX-equivalent was experimentally determined by complete release of DOX from nanoparticles using trifluoroacetic acid (TFA), followed by fluorescence quantification against a DOX calibration curve.



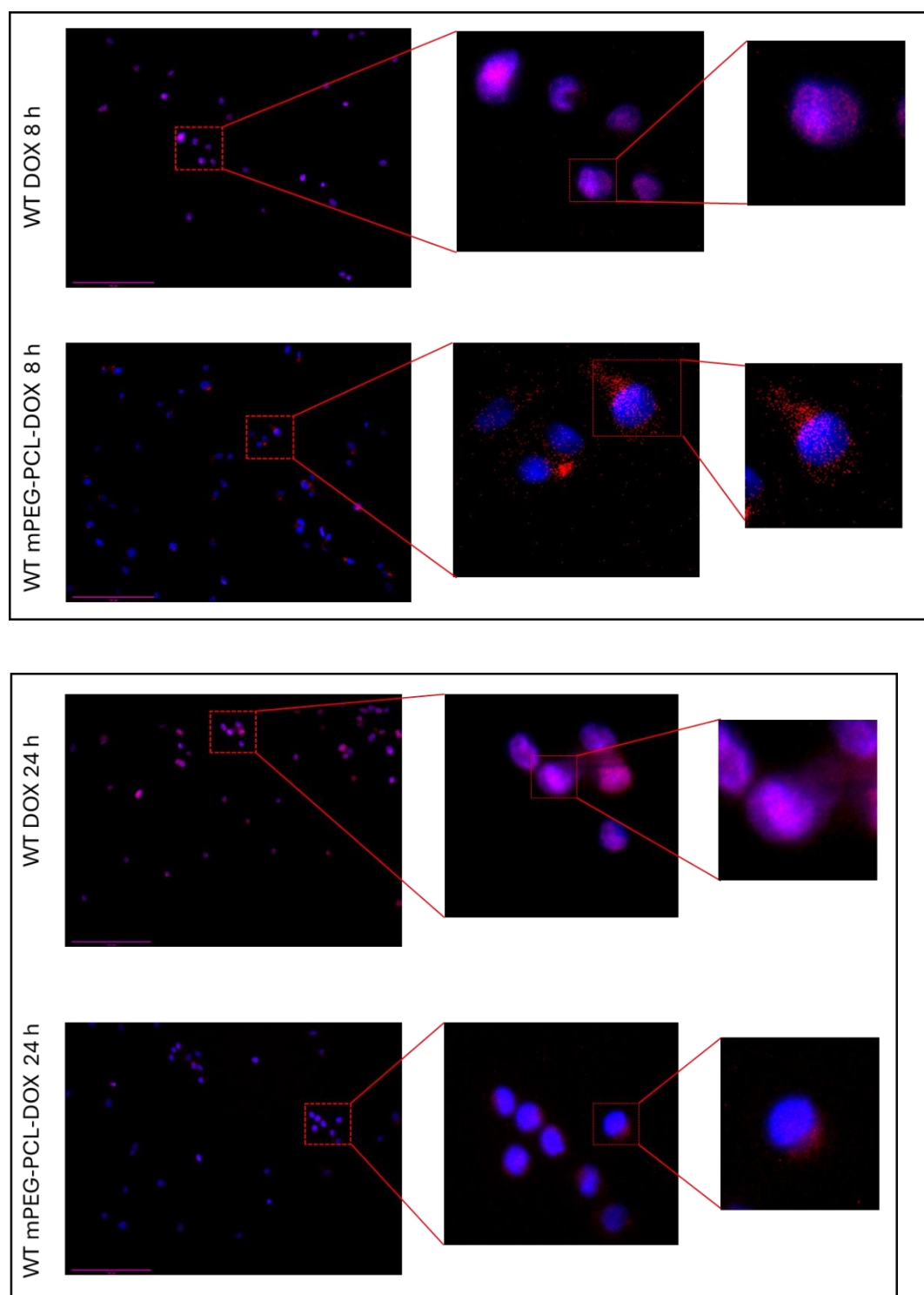


Figure 4.3: Fluorescence microscopy images of WT cells treated with free DOX or mPEG-PCL-DOX nanoparticles. Images were captured using an EVOS microscope at 2, 4, 8, and 24 hours post-treatment. DOX was visualized via the RFP channel (red), and cell nuclei were stained with DAPI (blue). Scale bar = 150 μm (20 $\times$ magnification).

As shown in Figure 4.3, both free DOX and mPEG-PCL-DOX exhibited time-dependent uptake into WT MDA-MB-231 cells. However, the fluorescence signal intensity associated with free DOX was consistently higher than that of mPEG-PCL-DOX, particularly at later time points (8 and 24 hours). Additionally, cells treated with free DOX displayed more pronounced nuclear-associated fluorescence, as shown by the overlap between the red (RFP) and blue (DAPI) channels. In contrast, cells exposed to mPEG-PCL-DOX showed comparatively lower nuclear DOX fluorescence intensity across all time points, with DOX fluorescence localised both around the nucleus and cytoplasm.

The intracellular concentration of DOX as free drug or drug released from the nanoparticles was further quantified by liquid chromatography-mass spectrometry (LCMS) at 2, 4, 8 and 24 hours. The measured concentration represents total cell-associated DOX extracted from lysed cells, including both released and residual nanoparticle-associated drug.

The concentration of DOX measured was extrapolated from a standard curve, which was first created by detection of known drug concentrations (Figure 4.4).

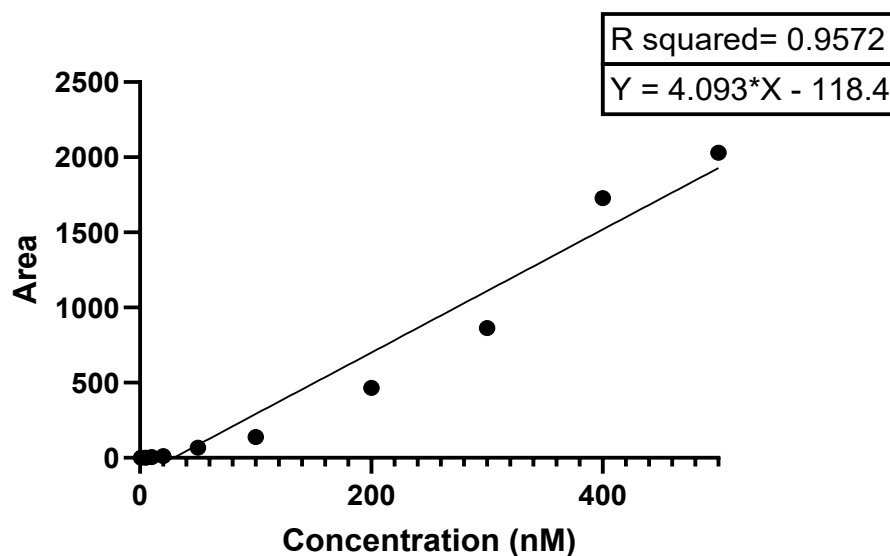


Figure 4.4: LCMS standard curve for DOX concentration determination.

The quantified intracellular DOX concentration was time dependent for both the free form and the nanoparticles conjugated form. WT cells treated with mPEG-PCL-DOX showed a higher intracellular accumulation of DOX when the cells were treated with free DOX compared to mPEG-PCL-DOX, with statistically significant difference at 4 h ($P < 0.03$), 8 ($P < 0.003$) and 24 h ($P < 0.02$) as shown in Figure 4.5.

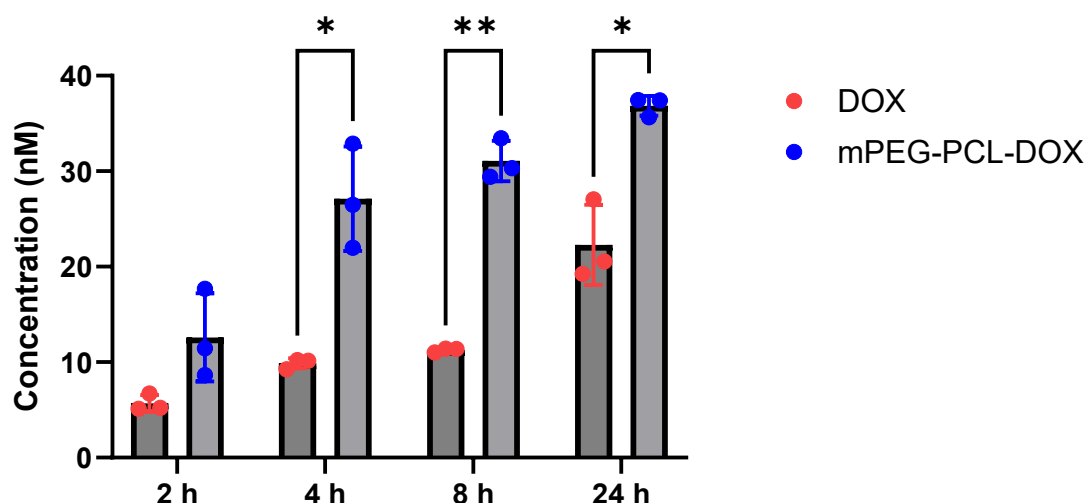


Figure 4.5: Intracellular concentration of free DOX and nanoparticles conjugated DOX in WT cells. Quantification was performed by LCMS at 2, 4, 8 and 24 h, and DOX was quantified using a DOX calibration curve. Data were analysed using two-way ANOVA and are presented as mean $\pm$ SD (n = 3).

These data suggest that the nanoparticles are efficiently internalised, and they produce intracellular concentrations of DOX over time to levels which are comparable and superior to the free drug.

4.2.4 WT viability after exposure to free DOX and mPEG-PCL-DOX nanoparticles:

To evaluate the effect of long exposure to treatment, WT cells were exposed to 300 nM of free DOX or mPEG-PCL-DOX nanoparticles. Viability readings were calculated on days 2, 4, 6, 8, 10 and 12, and compared to untreated WT control cells.

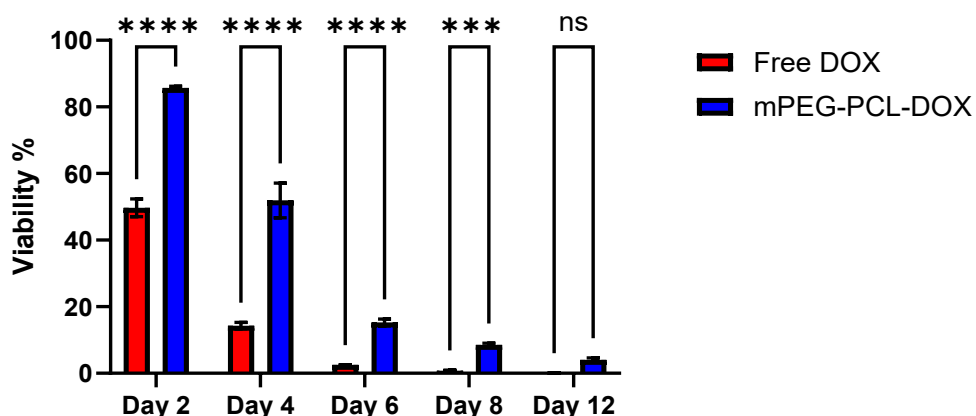


Figure 4.6: Viability of WT MDA-MB-231 cells treated with free DOX or mPEG-PCL-DOX nanoparticles over a 12-day period. Cell viability was measured on days 2, 4, 6, 8, and 12 post-treatment. Data were analysed using two-way ANOVA and are presented as mean $\pm$ SD (n = 3).

A time dependent decrease in WT cells viability was observed for both DOX and mPEG-PCL-DOX, as presented in Figure 4.6. The cytotoxic effect of free DOX was significantly higher at days 2, 4, and 6 ($P < 0.0001$) compared to mPEG-PCL-DOX nanoparticles. The mPEG-PCL-DOX showed a slower toxic effect that was sustained over time.

Considering the results of the drug uptake, there was no direct correlation between uptake and rate of cytotoxicity could be observed.

4.2.5 Establishment of resistant MDA-MB-231 (R) via chronic exposure to DOX:

To assess whether the delivery of doxycycline with nanoparticles could be beneficial in the treatment of cancer developing drug resistance, the next step was to develop MDA-MB-231 cells resistant to doxycycline. This was achieved by treating the WT MDA-MB-231 cells for a period of six months with increasing concentrations of the drug up to maximum concentration of 0.5 μM and by maintaining the resistant cell line in the presence of the drug.

As shown in Figure 4.7, during this time the resistant cells maintained a normal morphology, although cell growth was dependent on the presence of DOX.

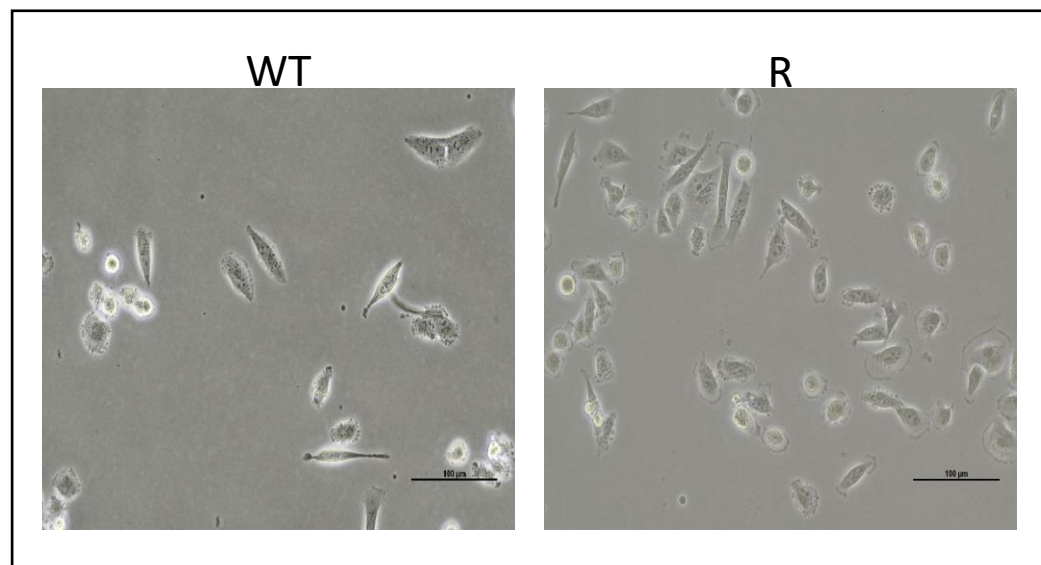


Figure 4.7 Morphology of R cells cultured for 6 months with increasing concentrations of DOX. Bar=100 μm

4.2.6 Resistant MDA-MB-231 (R) IC₅₀:

To verify the development of doxorubicin resistance, an IC₅₀ study was performed on the MDA-MB-231-Resistant (R) cell line following exposure to increasing concentrations of DOX ranging from 1 nM to 8 μ M. Cell viability was evaluated using the PrestoBlue assay after 48 hours of treatment.

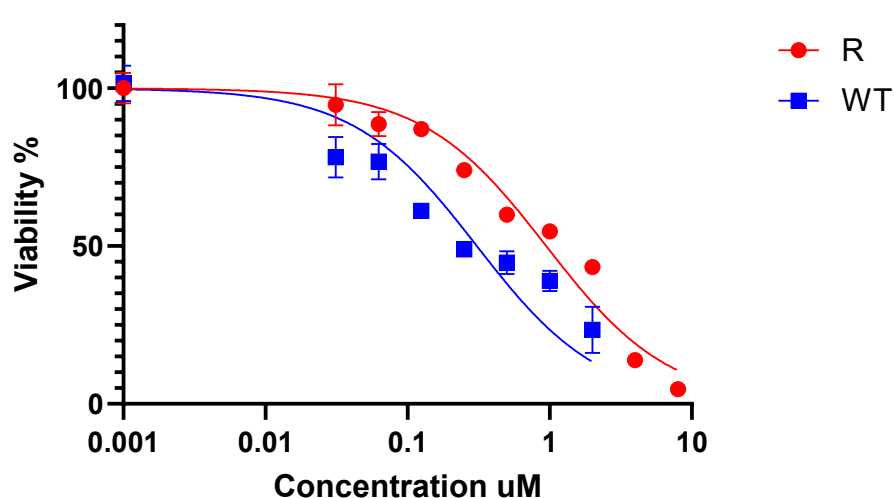


Figure 4.8 Dose vs response curve for DOX in R and WT cells. Dose–response curve for free DOX in MDA-MB-231-R (R) cells. Cell viability was measured using the PrestoBlue assay following treatment with increasing concentrations of DOX, starting from 8 μ M with 1:2 serial dilutions. Data are presented as mean $\pm$ SD (n = 6).

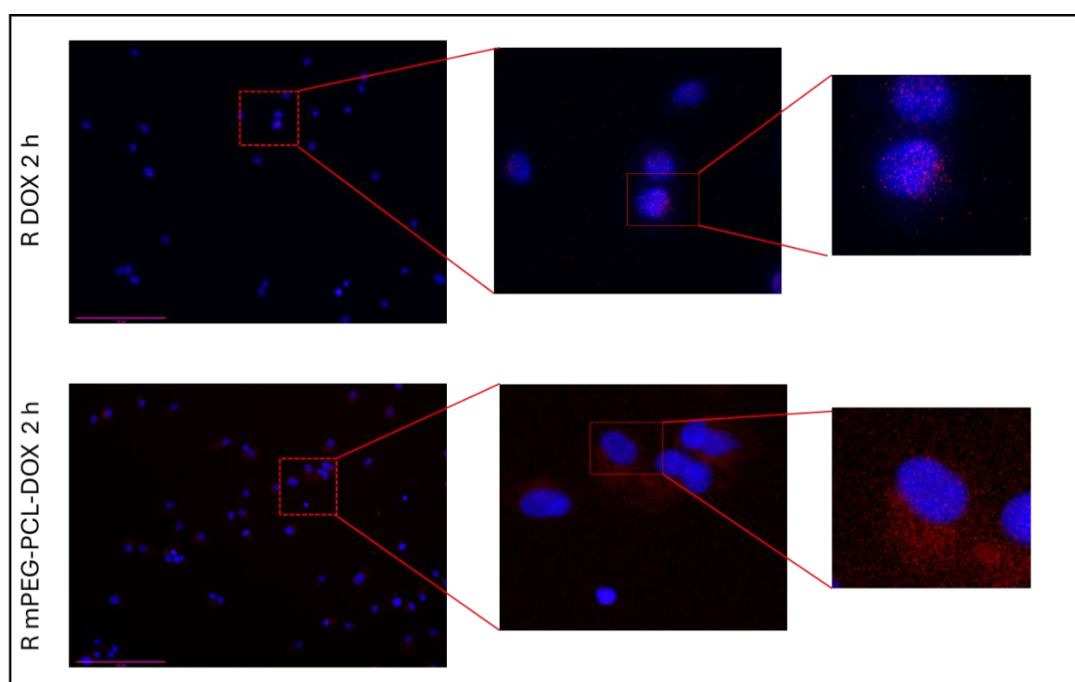
Table 4-2: IC₅₀ mean and 95% CI values in μ M for free DOX in R cells (n = 6).

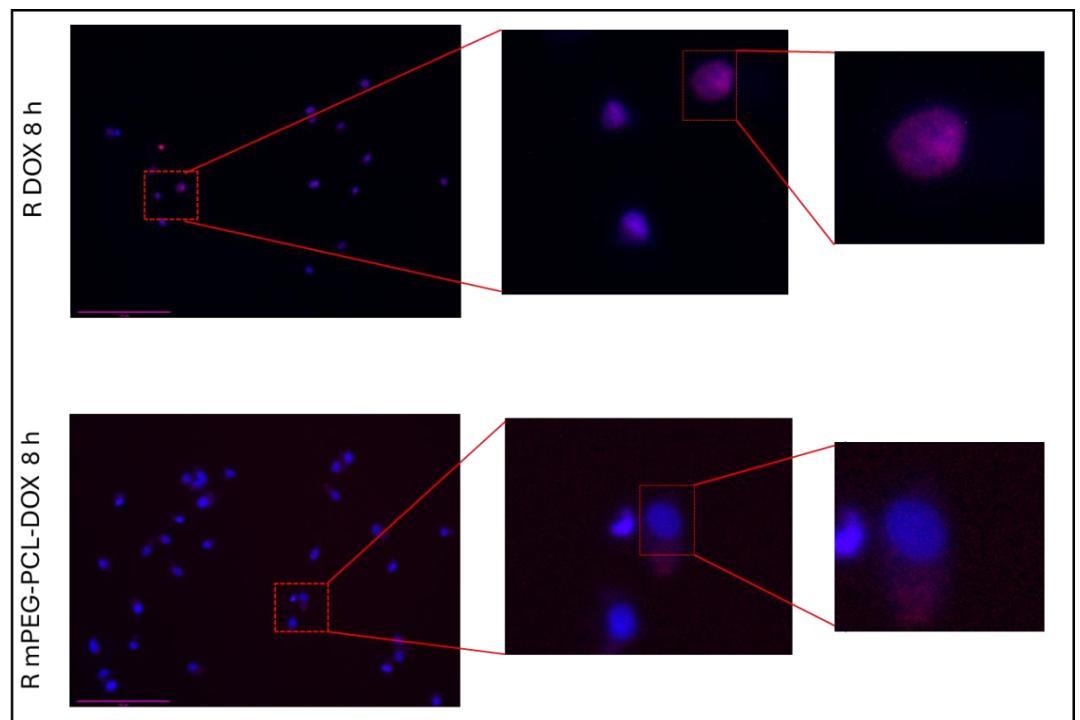
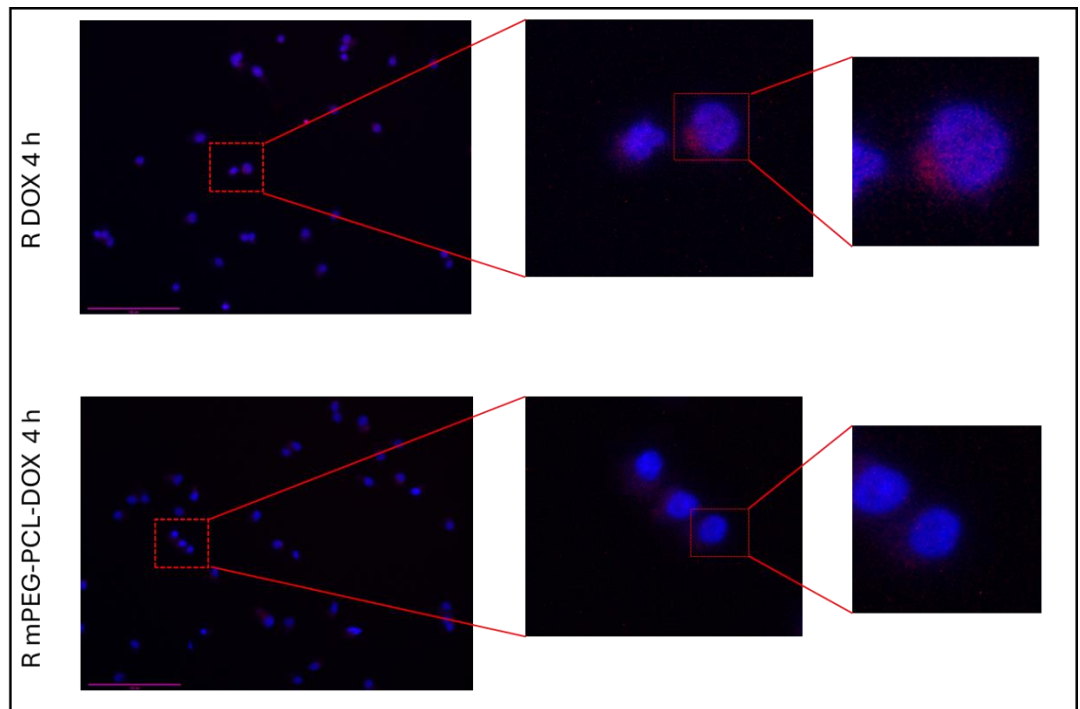
	R	WT
IC ₅₀ (uM)	1.405	0.305
95% CI (uM)	1.054 to 1.882	0.245 to 0.379

As shown in Figure 4.8 and Table 4-2, the mean IC_{50} for free DOX in R cells was determined to be 1.405 μ M, with a 95% confidence interval (CI) ranging from 1.054 to 1.882 μ M ($n = 6$). This value was approximately 4.6 fold higher compared to that measure in WT cells, confirming the acquisition of resistance when compared to the parental wild-type cell line.

4.2.7 Uptake of free DOX and mPEG-PCL-DOX nanoparticles in R cells:

To investigate the intracellular uptake behaviour of free DOX and mPEG-PCL-DOX nanoparticles in DOX-resistant MDA-MB-231-R (R) cells, fluorescence imaging was performed using the EVOS system. Cells were treated with 300 nM of either free DOX or mPEG-PCL-DOX for varying durations. The red fluorescence signal (RFP channel) corresponded to DOX fluorescence, while nuclei were counterstained with DAPI (blue).





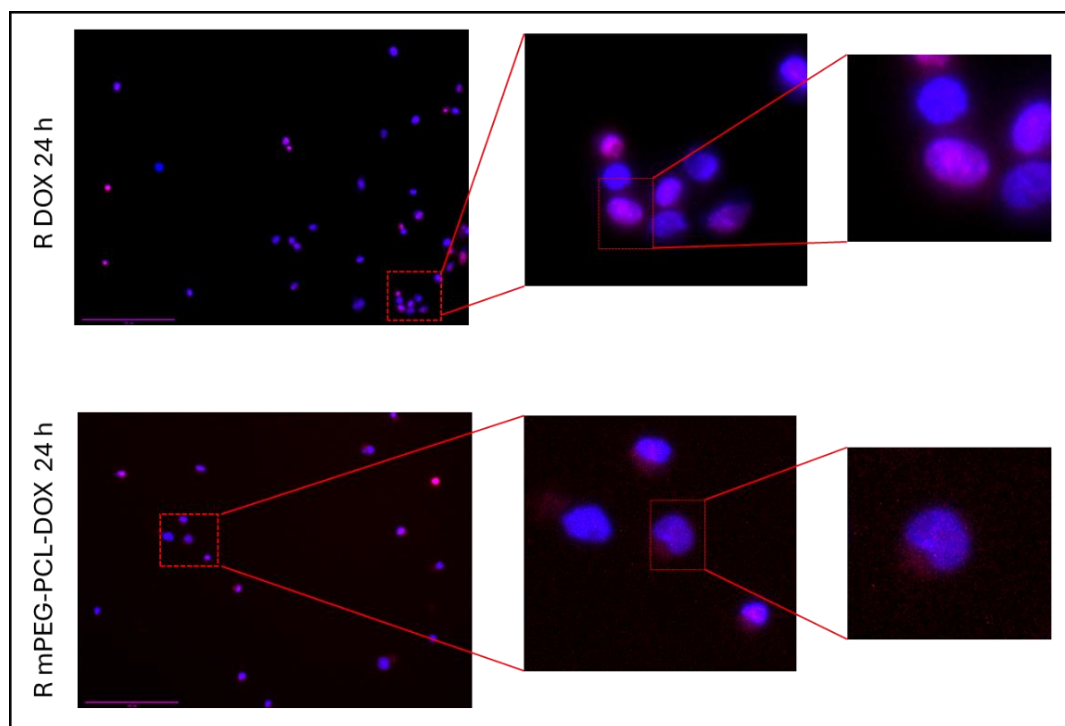


Figure 4.9 : Fluorescence microscopy images of R cells treated with free DOX or mPEG-PCL-DOX nanoparticles. Images were captured using the EVOS system at 2, 4, 8, and 24 hours post-treatment. DOX was visualized via the RFP channel (red), and cell nuclei were stained with DAPI (blue). Scale bar = 150 μ m (20 $\times$ magnification).

As shown in Figure 4.9, both free DOX and mPEG-PCL-DOX showed time-dependent uptake in MDA-MB-231-R cells. However, noticeable differences in fluorescence distribution were observed between the two treatments. Free DOX produced a strong and diffuse red fluorescence signal that prominently overlapped with the DAPI-stained nuclei, indicating efficient nuclear accumulation. On the other hand, cells treated with mPEG-PCL-DOX exhibited weaker red fluorescence with limited nuclear overlap.

Importantly, the rate of uptake in R cells was lower in R cells compared to WT for both the free DOX and mPEG-PCL-DOX, as a weak signal started to be observed only after 8 hours and 24 hours for DOX and mPEG-PCL-DOX, respectively.

Consistent with this, the intracellular concentrations of DOX measured by LCMS over time were also lower, both with the free drug and nanoparticle delivery. However, the concentration of DOX with the nanoparticles was still higher than that with the free drug (Figure 4.10).

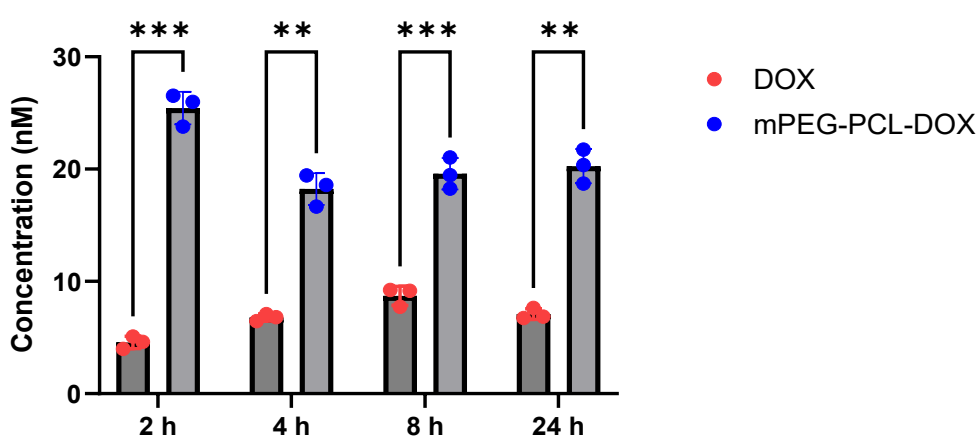


Figure 4.10: R cells intracellular concentration of free DOX and nanoparticles conjugated DOX. Quantification was performed LCMS at 2, 4, 8 and 24 h, and DOX was quantified using a DOX calibration curve. Data were analysed using two-way ANOVA and are presented as mean $\pm$ SD (n = 3).

4.2.8 Viability of R cells after exposure to free DOX and mPEG-PCL-DOX nanoparticles

To evaluate the ability of mPEG-PCL-DOX nanoparticles to deliver DOX and overcome acquired resistance, a long-term viability study was conducted with MDA-MB-231-R (R) cells. Cells were treated with either free DOX or mPEG-PCL-DOX at a concentration of 300 nM. Viability was assessed over a 12-day period on days 2, 4, 6, 8, and 12 using the PrestoBlue viability assay.

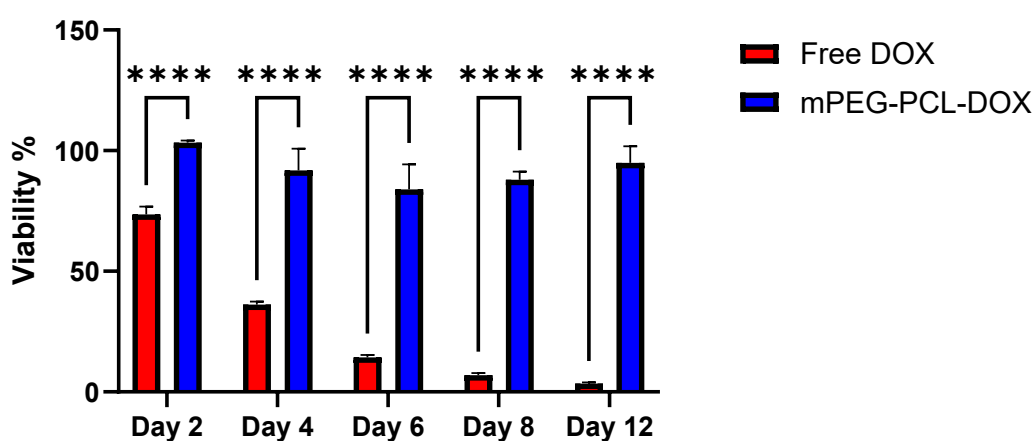


Figure 4.11: Viability of R cells treated with free DOX or mPEG-PCL-DOX nanoparticles over a 12-day period. Cell viability was measured on days 2, 4, 6, 8, and 12 using a fluorescence-based assay. Data were analysed using two-way ANOVA and are presented as mean $\pm$ SD (n = 3).

As shown in Figure 4.11, a marked difference in cell viability was observed between treatment groups over time. Free DOX-treated R cells showed consistently low viability, whereas cells treated with mPEG-PCL-DOX unexpectedly maintained significantly higher viability across all time points.

For this resistant cell line, there was no direct correlation between uptake and rate of cytotoxicity in both wild type and DOX resistant cells could also be observed.

4.2.9 Establishment of resistant MDA-MB-231 by expression of PgP

To further investigate the effect of different mechanisms of drug resistance on the drug uptake, a second doxorubicin-resistant cell model was developed by introducing the MDR1 gene into wild-type MDA-MB-231 cells through lentiviral transduction to cause overexpression of the P-glycoprotein (PgP) efflux transporter. To select resistant cells, the transduced cell population was treated with two different concentrations of DOX (either 10 nM or 50 nM) to eliminate non-resistant cells.

Resistant cells presented a normal morphology over a number of passages after selection, similar to the WT cell line (Figure 4.12).

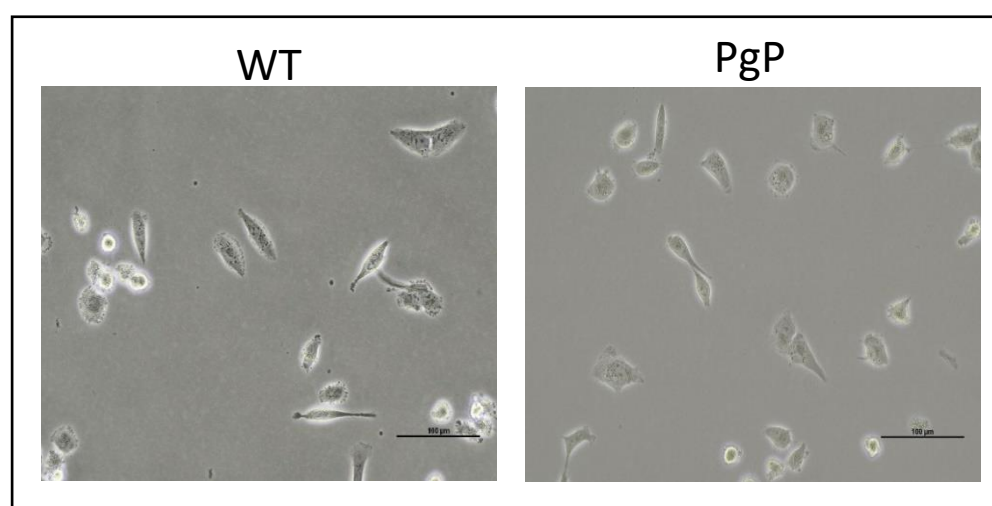


Figure 4.12 Morphology of PgP expressing cells selected with 50nM DOX.

Bar=100 μ m.

PgP protein expression was subsequently confirmed by western blot analysis of untreated, 10 nM-treated, and 50 nM-treated cells. For comparison, WT and MDA-MB-231-R cells were included to assess relative MDR1/PgP expression levels, whereas MCF-7 cells, a breast cancer cell line sensitive to DOX, were used as a negative control.

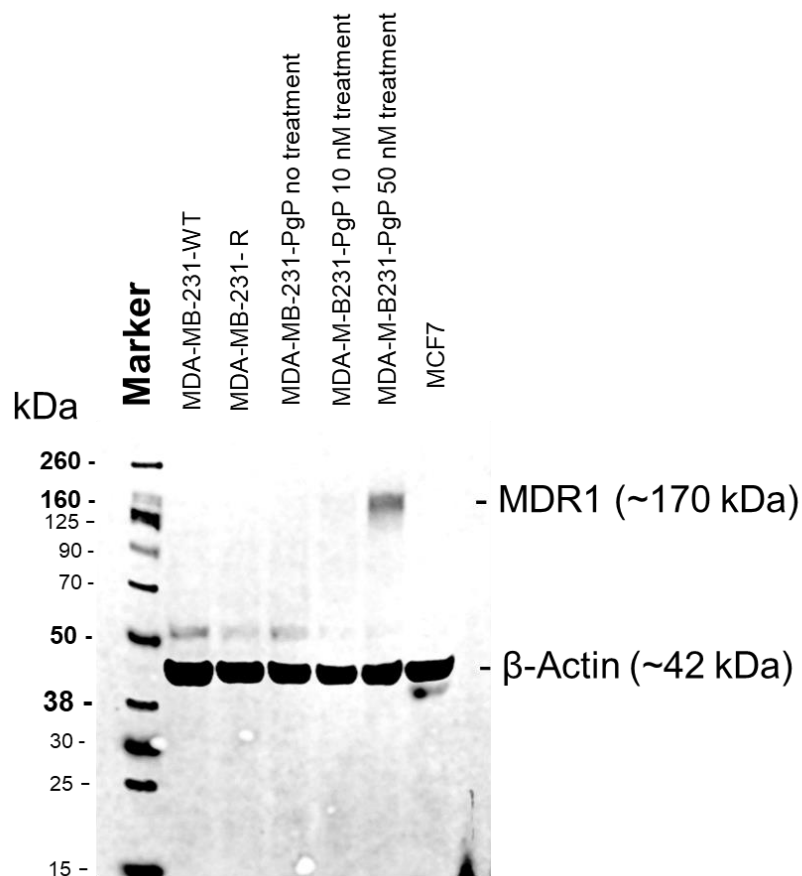


Figure 4.13: Western blotting of PgP (MDR1) expressing cells . Western blotting image showing MDR1 (~170 kDa) expression of MDA-MB-231-WT, MDA-MB-231-R, non-treated MDA-MB-231-PgP, MDA-MB-231-PgP treated with 10 nM, MDA-MB-231-PgP treated with 50 nM. MCF7 served as negative control. β-actin expression was used as sample loading control.

Figure 4.13 shows a strong MDR1 band at ~170 kDa in PgP cells treated with 50 nM DOX, while fainter bands were observed in PgP cells treated with 10 nM DOX. Also, no bands were detected in MCF7 negative control, WT, PgP unselected cells or R cells. β -actin was used as a loading control to confirm equal protein loading across all samples.

To further confirm the overexpression of P-glycoprotein, quantitative PCR (qPCR) was also performed to assess MDR1 mRNA expression in the newly developed MDA-MB-231-PgP cell line. Expression levels were evaluated in cells without DOX selection, as well as in those selected with 10 nM and 50 nM DOX. Comparative analysis was performed against WT MDA-MB-231, MDA-MB-231-R.

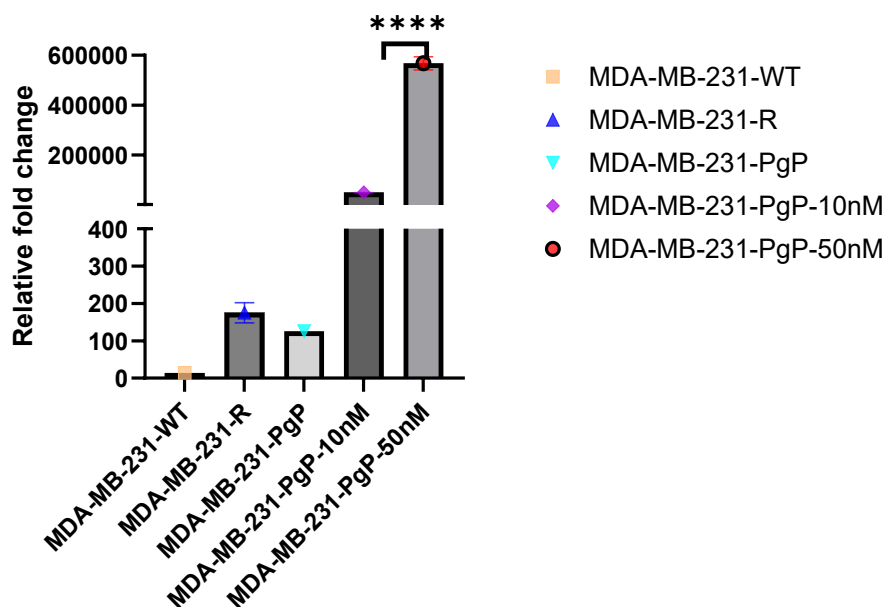


Figure 4.14: Relative MDR1 gene expression levels in various cell lines as determined by qPCR. Figure shows relative fold change of MDR1 expression in different cell lines. Data are presented as mean $\pm$ SD (n=3).

A highly significant difference in the expression was observed in PgP cells treated with 50 nM DOX compared to cells treated with 10 nM DOX ($P < 0.0001$), while there was minimal expression of MDR1 gene in WT, R, DOX non-treated PgP (Figure 4.14).

Based on this result, the MDA-MB-231-PgP cell line treated with 50 nM DOX was used in subsequent experiments to evaluate the uptake of DOX and mPEG-PCL-DOX.

4.2.10 Resistant MDA-MB-231 PgP IC₅₀:

To evaluate the DOX cytotoxic response in PgP cells, a dose response study was performed to determine IC₅₀ values. Cells were treated with increasing concentration of DOX for a period of 48 h. The results are presented in Figure 4.15 and Table 4-3.

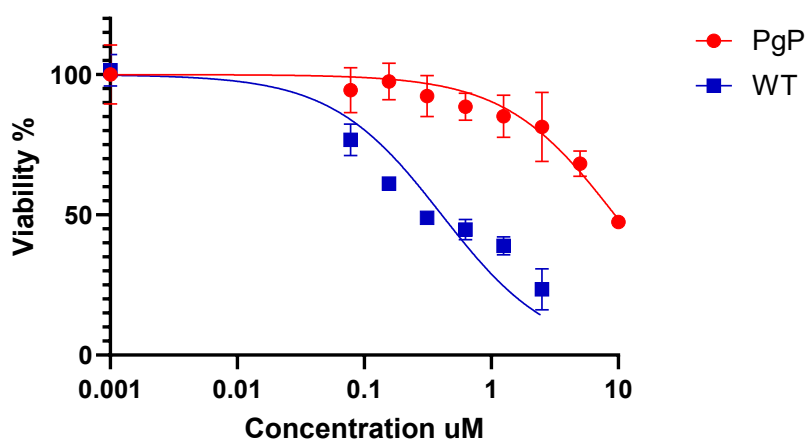


Figure 4.15: Dose vs response curve for DOX in PgP and WT cells. Dose–response curve for free DOX in MDA-MB-231-PgP (PgP) cells. Cell viability was measured using the PrestoBlue assay following treatment with increasing concentrations of DOX. Data are presented as mean $\pm$ SD (n = 3).

Table 4-3: IC₅₀ mean and 95% CI values in uM for free DOX in PgP cells (n = 6).

	PgP	WT
IC ₅₀ (uM)	9.3	0.305
95% CI (uM)	7.6 to 11.7	0.245 to 0.379

As presented in Figure 4.15 and Table 4-3, the IC₅₀ free DOX was calculated to be 9.3 uM with 7.6 to 11,7 uM 95% CI.

To investigate whether inhibition of P-glycoprotein (PgP) could restore drug sensitivity in PgP-overexpressing cells, MDA-MB-231-PgP cells were treated with free DOX in the presence of the drug Elacridar (0.2 μ M), which is a specific PgP inhibitor. Cells were treated with Elacridar at the time of seeding, and viability was assessed after 48 hours.

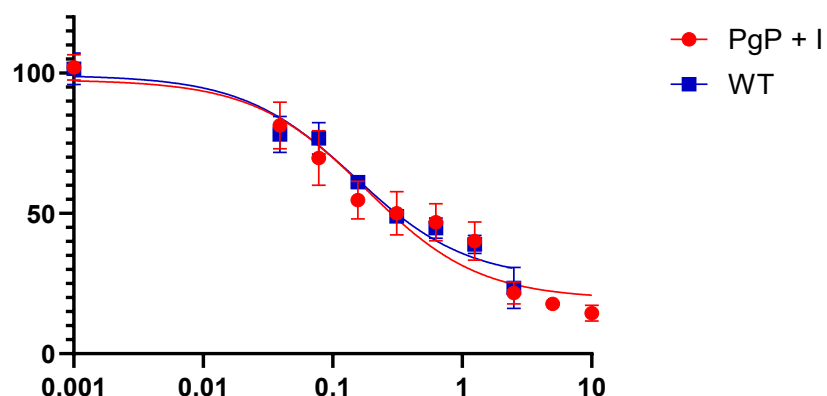


Figure 4.16: Dose–response curves of free DOX and mPEG-PCL-DOX in PgP cells in the presence of Elacridar (0.2 μ M). Cell viability was measured using the PrestoBlue assay following treatment with increasing concentrations of DOX. Data are presented as mean $\pm$ SD (n = 3).

Table 4-4: IC₅₀ values and 95% confidence intervals for free DOX in MDA-MB-231-PgP cells with Elacridar and WT cells.

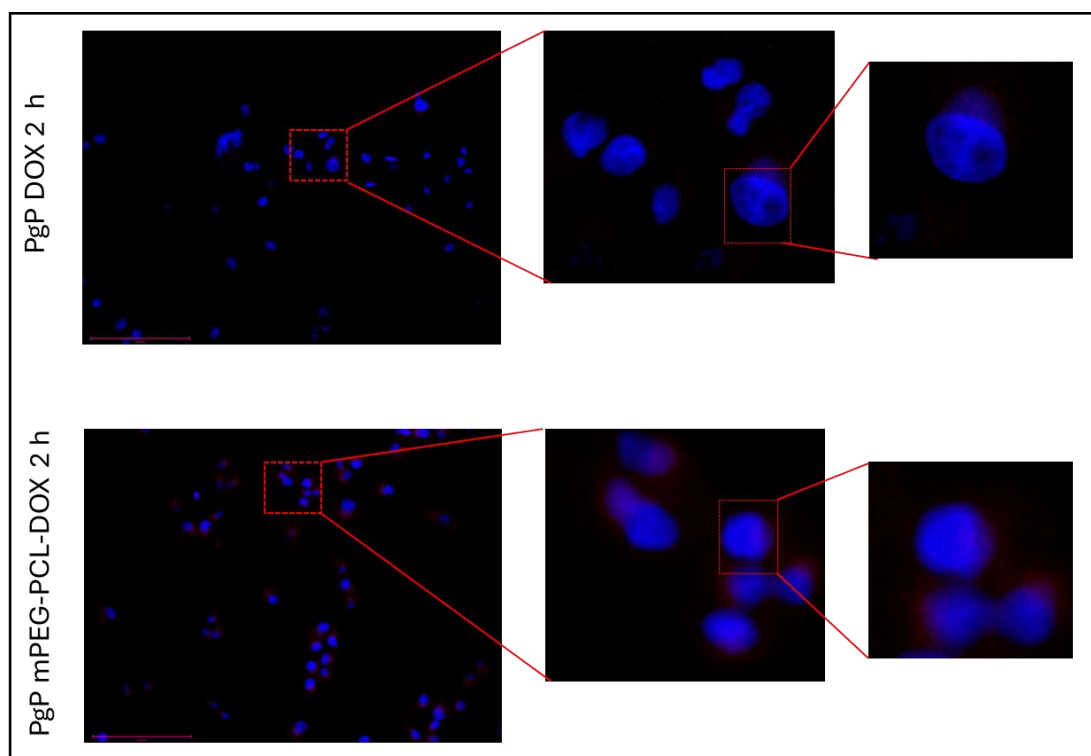
	PgP + I	WT
IC ₅₀ (uM)	0.380	0.305
95% CI (uM)	0.265 to 0.545	0.245 to 0.379

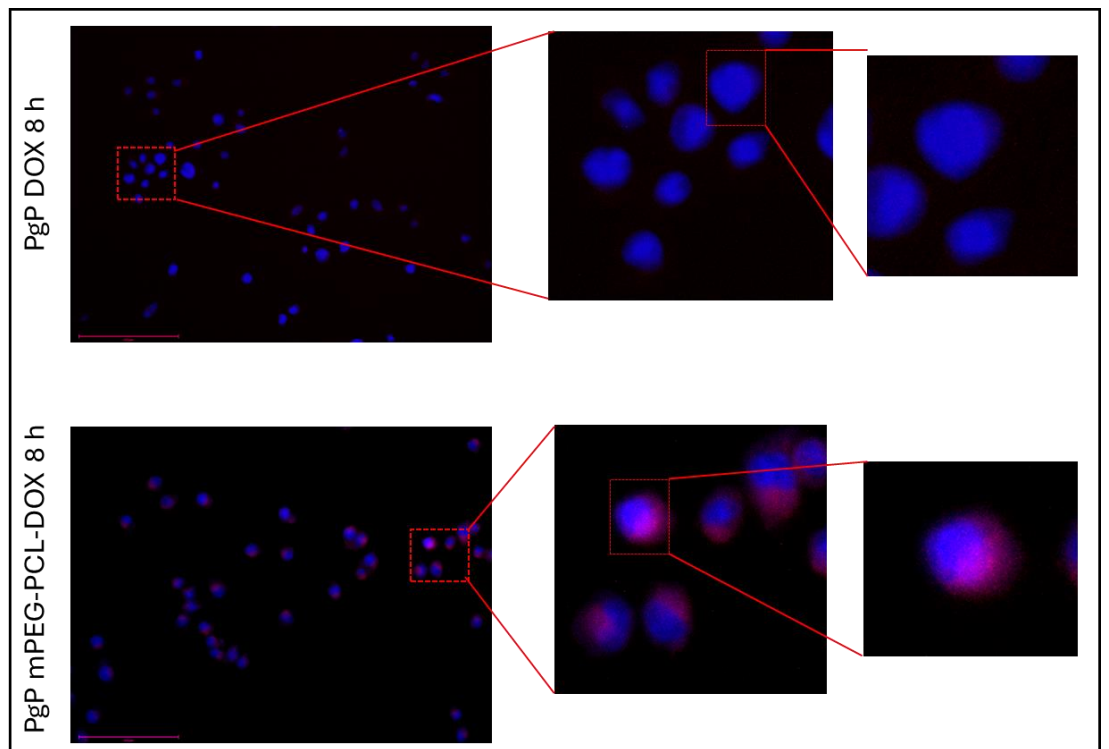
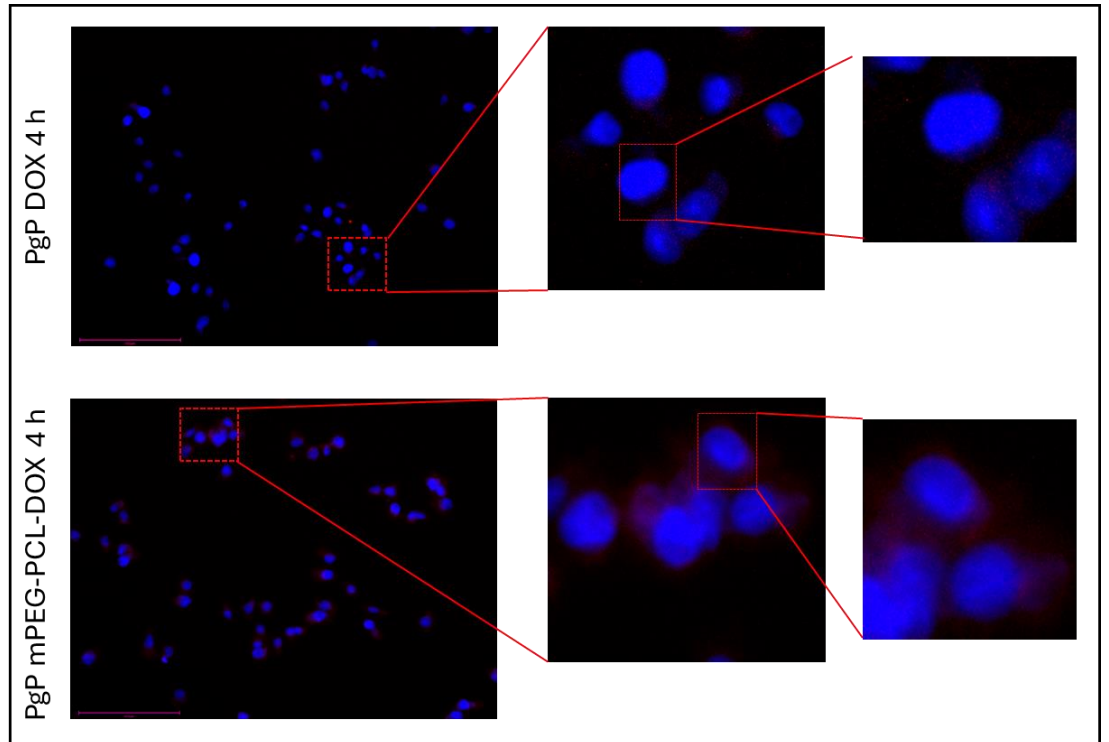
As shown in Figure 4.16 and Table 4-4, co-treatment with Elacridar enhanced the cytotoxicity of DOX in PgP cells. The IC₅₀ of free DOX decreased to 0.380 μ M (95% CI: 0.265–0.545 μ M), which is relatively similar to the WT IC₅₀.

4.2.11 Uptake of free DOX and mPEG-PCL-DOX

nanoparticles in PgP cells:

To examine the influence of PgP overexpression on DOX cellular uptake, MDA-MB-231-PgP cells were treated with 300 nM of either free DOX or mPEG-PCL-DOX. Fluorescence microscopy using the EVOS imaging system was used to visualize DOX fluorescence (red, RFP channel) and nuclear staining (blue, DAPI channel) at 2, 4, 8 and 24 h after exposure.





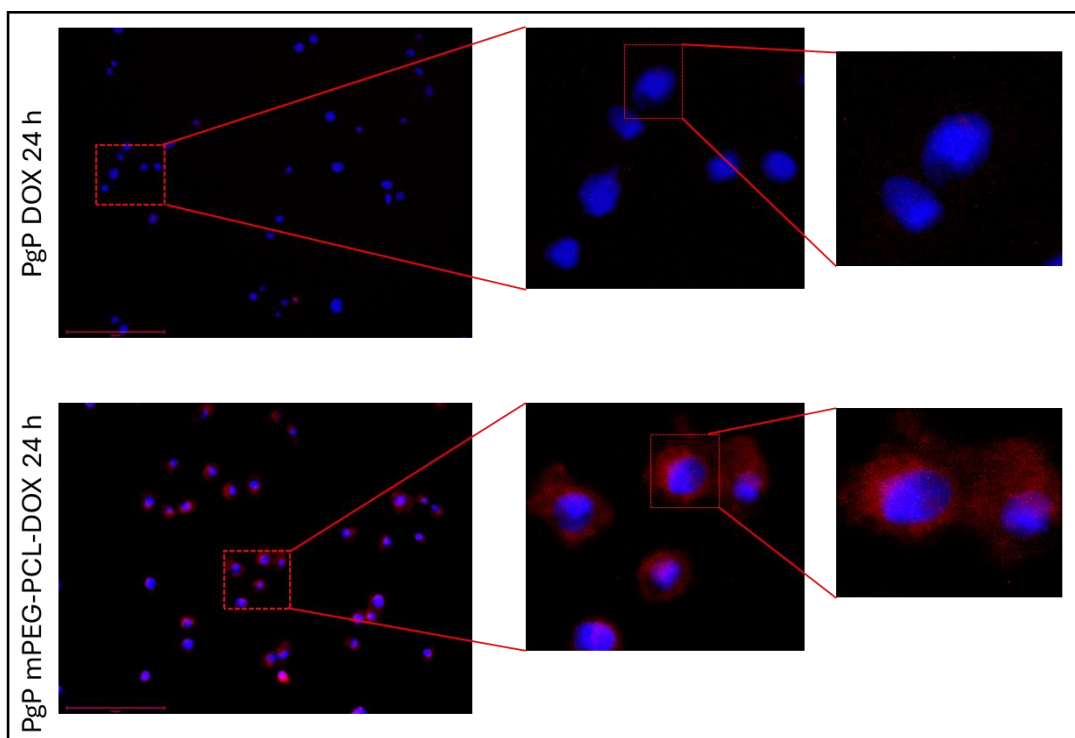


Figure 4.17: Fluorescence microscopy images of MDA-MB-231-PgP cells treated with free DOX or mPEG-PCL-DOX (300 nM). DOX was detected using the RFP channel (red), and cell nuclei were stained with DAPI (blue). Images show intracellular uptake differences between the free and nanoparticle-conjugated drug forms at 2, 4, 8 and 24 h. Scale bar = 150 μm (20 $\times$ magnification).

As shown in Figure 4.17, the uptake of free DOX in MDA-MB-231-PgP cells appeared limited, with minimal red fluorescence and little nuclear accumulation. In contrast, cells treated with mPEG-PCL-DOX exhibited stronger intracellular red fluorescence and nuclear-associated signal.

The effect of efflux transporters in on DOX intracellular concentration was also investigated, as shown in Figure 4.18.

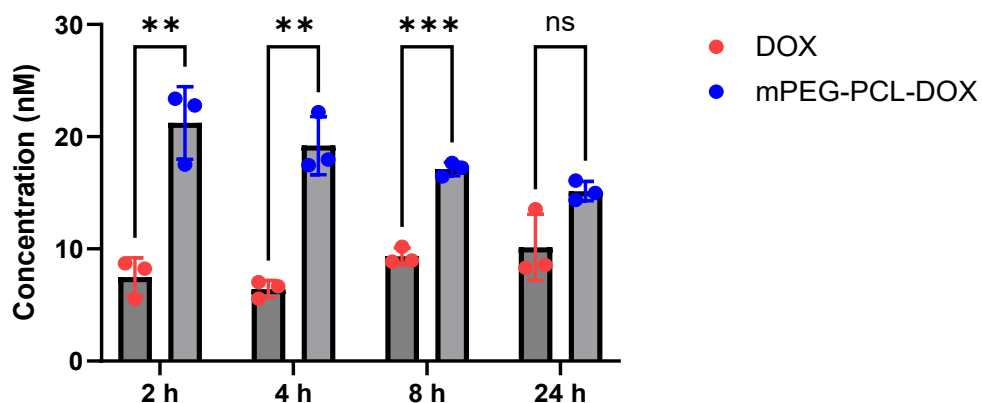


Figure 4.18: PgP cells intracellular concentration of free DOX and nanoparticles conjugated DOX. Quantification was performed LCMS at 2, 4, 8 and 24 h, and DOX was quantified using a DOX calibration curve. Data were analysed using two-way ANOVA and are presented as mean $\pm$ SD (n = 3).

In PgP cells, the intracellular accumulation of DOX was lower than that measured in wild type cells and similar to the levels measured in the DOX resistant cells R). Consistently, the concentration measured after delivery with the nanoparticles was mostly higher than that of the free drug delivery.

4.2.12 Viability of PgP cells after exposure to free DOX and mPEG-PCL-DOX nanoparticles

PgP cells were next treated with 300 nM of either free DOX or mPEG-PCL-DOX nanoparticles to evaluate their long-term cytotoxic effect and sustained release characteristics. Using a fluorescence-based viability assay, cell viability was assessed across eight days at specified time points—days 2, 4, 6, and 8.

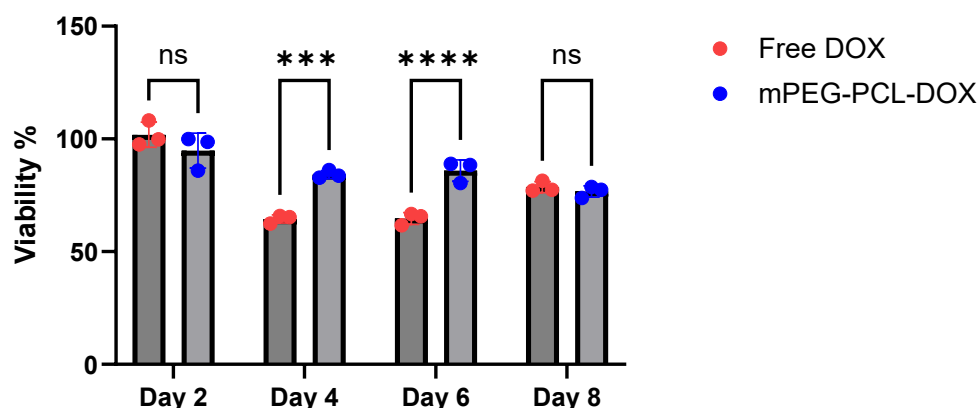


Figure 4.19: Viability of PgP cells treated with free DOX or mPEG-PCL-DOX nanoparticles over a 8-day period. Cell viability was measured on days 2, 4, 6 and 8, using PrestoBlue viability assay. Data were analysed using two-way ANOVA and are presented as mean $\pm$ SD (n = 3).

Figure 4.19 shows the cytotoxicity of free DOX and mPEG-PCL-DOX nanoparticles on PgP cells. At day 2, there was no cytotoxic effect of free DOX or conjugated DOX. The toxicity of free DOX started at day 4 and this was modestly lower than the mPEG-PCL-DOX. This difference was maintained up to day 8, when there was no statistical difference between the two treatments.

To further study the effect of PgP resistance, cells were treated with 300 nM of free DOX or mPEG-PCL-DOX in the presence of Elacridar (0.2 μ M), and viability was evaluated on days 2, 4, and 6.

These results are consistent with the data obtained from the uptake experiment and a direct correlation between uptake and rate of cytotoxicity in both wild type and PgP DOX resistant cells could be observed.

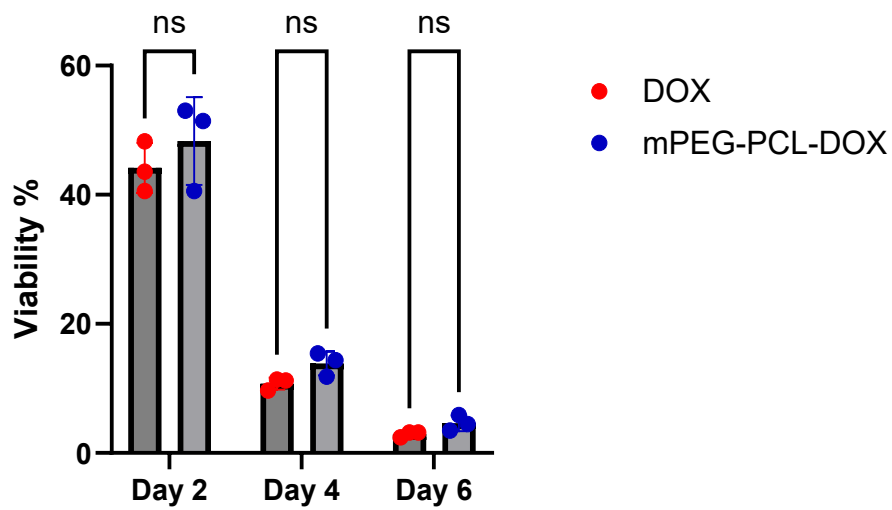


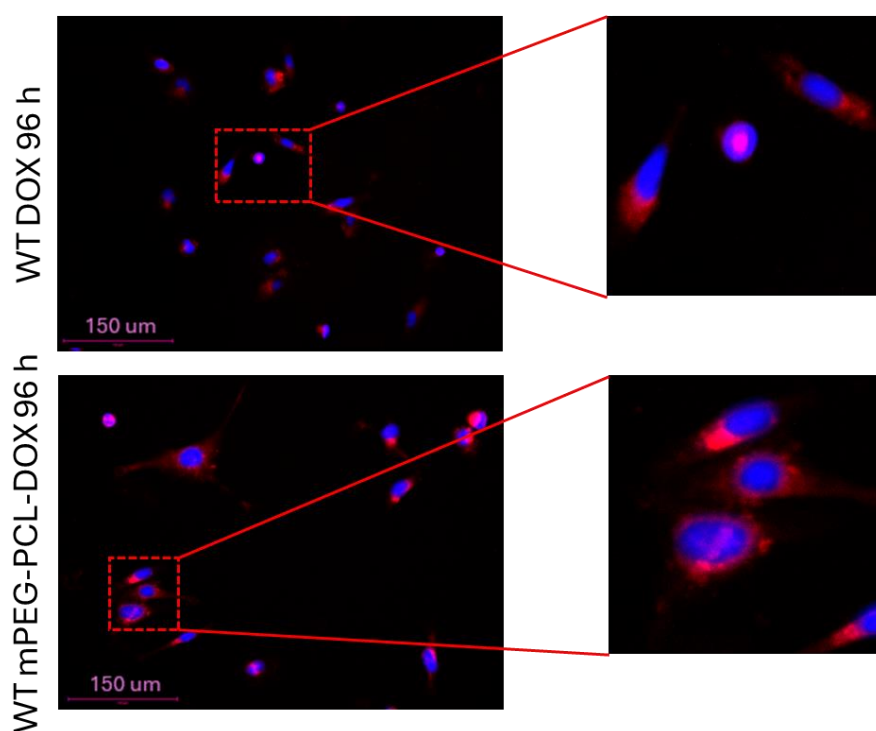
Figure 4.20 Viability of PgP cells plus ELACRIDAR treated with free DOX or mPEG-PCL-DOX nanoparticles over a 6-day period. Cell viability was measured on days 2, 4 and 6, using PrestoBlue viability assay. Data were analysed using two-way ANOVA and are presented as mean $\pm$ SD (n = 3).

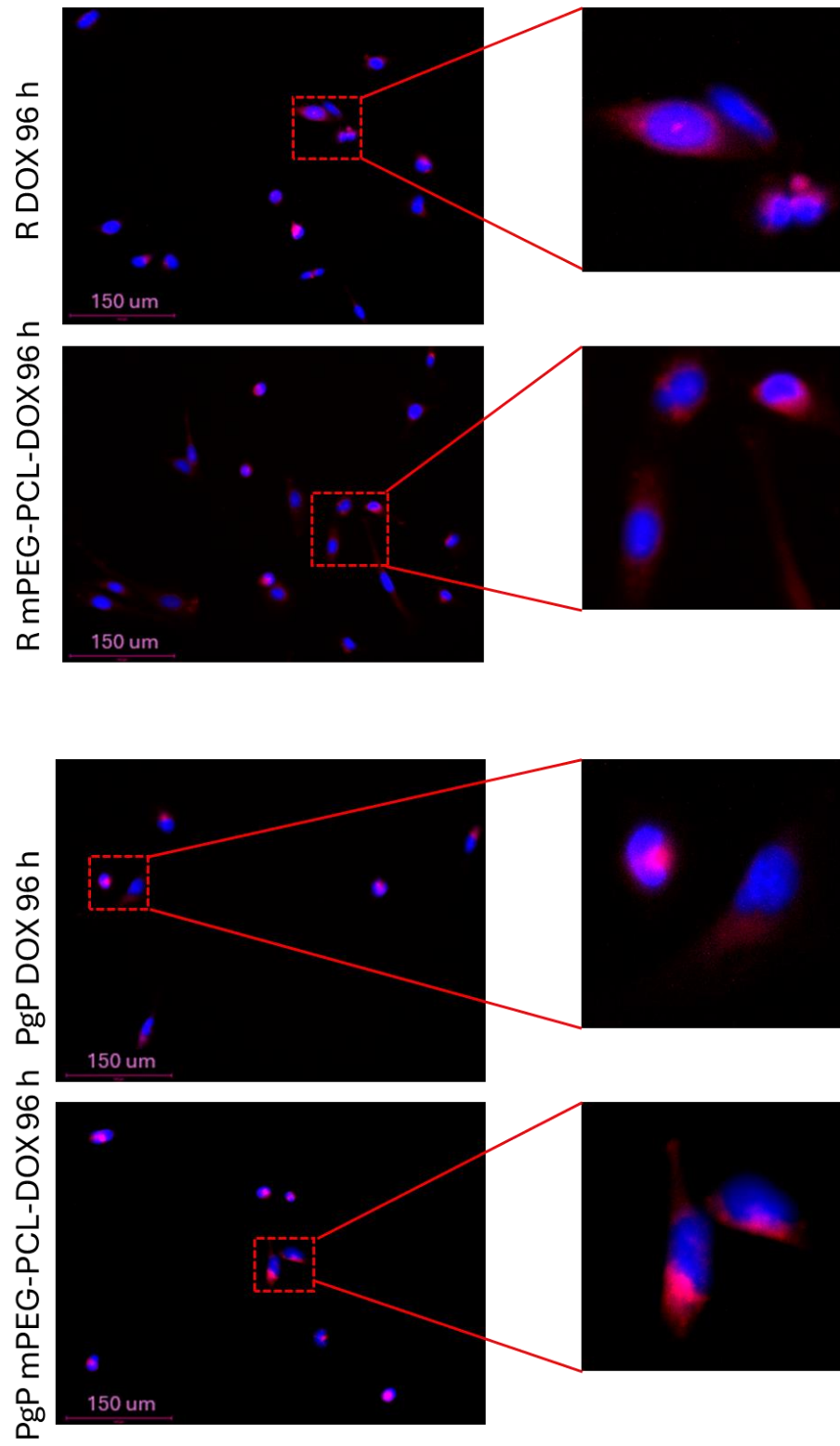
As shown in Figure 4.16, both treatment groups showed reduced viability across the measured time points after co-treatment with the PgP inhibitor. Importantly, no statistically significant differences in viability were observed between the two formulations at any time point, with viability values like those measured in the wild type cells.

4.2.13 Effect of drug treatment in acidosis:

As the release of DOX from the nanoparticles has been designed to be pH dependant, the next step in the project was to evaluate the influence of acidic pH on drug efficacy. To this end, we compared the effect of free DOX and mPEG-PCL-DOX on the different cell lines, either drug sensitive or resistant. Therefore, cells were treated with 300 nM of either drug formulations under mildly acidic conditions (pH 6.5), mimicking the pH conditions found in the tumour microenvironment, which may facilitate the release of DOX from the nanoparticles.

To visualize DOX or mPEG-PCL-DOX nanoparticles uptake in the acidic medium, fluorescence microscopy was used to confirm the different cell lines uptake after 4 days of exposure.





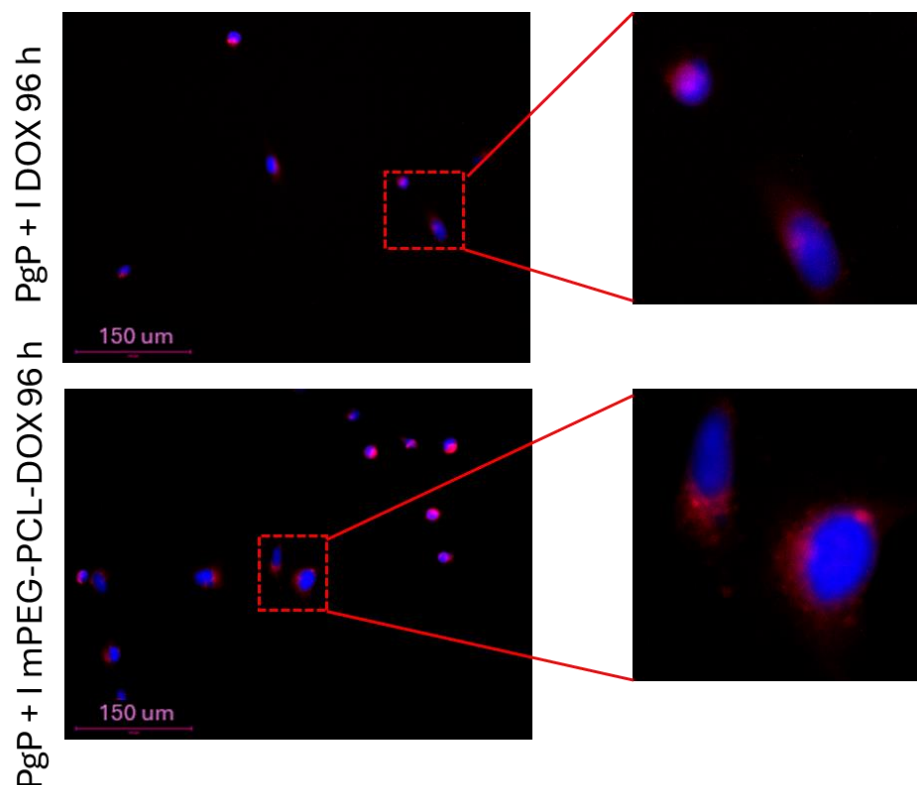


Figure 4.21: DOX and mPEG-PCL-DOX (300 nM) uptake in WT, R, PgP and PgP + inhibitor cell lines cultured in slightly acidic medium. DOX was detected using the RFP channel (red), and cell nuclei were stained with DAPI (blue). Images show intracellular uptake differences between the free and nanoparticle-conjugated drug forms at 96 h. Scale bar = 150 μ m (20 $\times$ magnification).

As shown in Figure 4.21, all cell lines showed uptake of DOX and mPEG-PCL-DOX in the acidic medium, including cells PgP cells treated with the inhibitor.

4.2.14 Cell viability in acidosis

The viability for each cell line was followed for up to 10 days or up to when most of cells were non-viable.

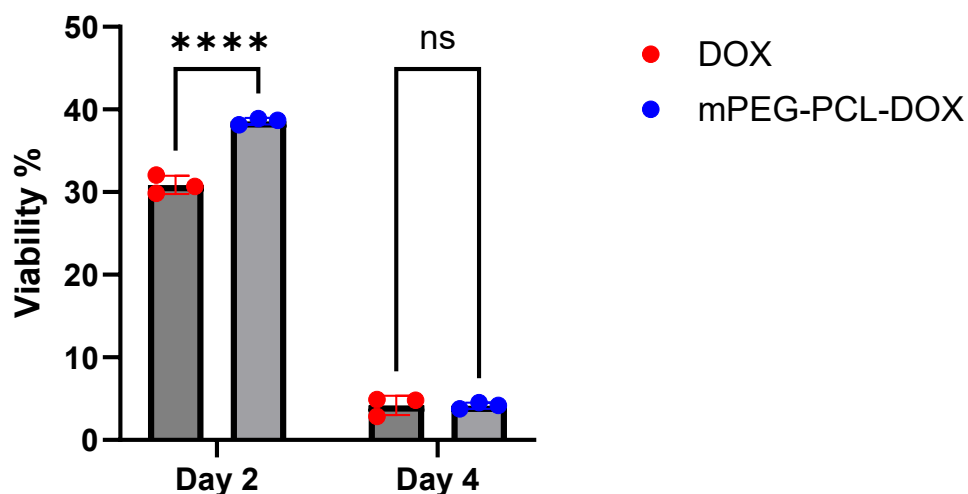


Figure 4.22: WT cells viability on day 2 and 4, after treating cells with either DOX or mPEG-PCL-DOX. Cell viability was measured on days 2, and 4 using PrestoBlue viability assay. Data were analysed using two-way ANOVA and are presented as mean $\pm$ SD (n = 3).

Treating wild type (W) cells with DOX caused a significant cytotoxic effect ($P < 0.0001$) as the mean viability of cells was 30.86 ± 0.89 % compared to 38.57 ± 0.31 % for cells treated with mPEG-PCL-DOX nanoparticles on day 2. On day 4, there was no significant difference in W cells viability and the cells showed very low survival (<10%).

The viability of the DOX resistant cell line (R) was also followed for 10 days as shown in Figure 4.23.

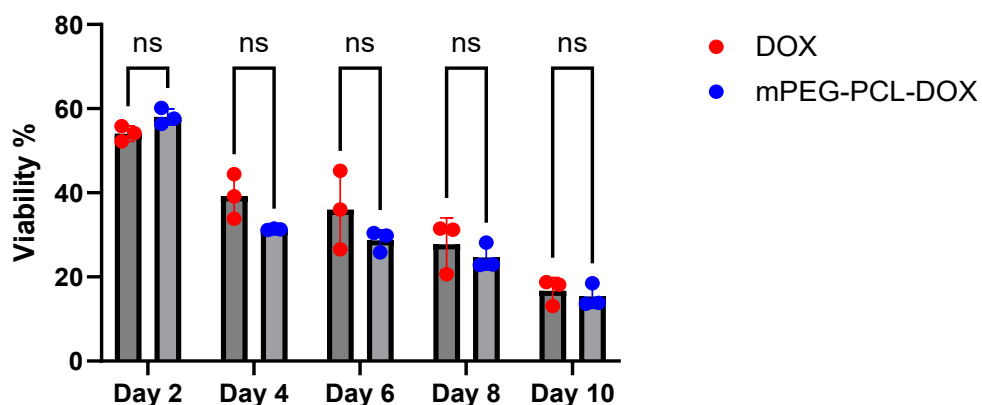


Figure 4.23: R cells viability on day 2, 4, 6 8 and 10, after treating cells with either DOX or mPEG-PCL-DOX. Cell viability was measured on days 2, 4, 6, 8 and 10 using PrestoBlue viability assay. Data were analysed using two-way ANOVA and are presented as mean $\pm$ SD (n = 3).

There was no significant difference observed between R cells treated with DOX or mPEG-PCL-DOX across all days, with viability reducing progressively throughout the treatment.

The viability of PgP-resistant cells also decreased over time, although to a less extent compared to the R cells. However, there were no significant difference in PgP cells viability between DOX or mPEG-PCL-DOX across all days of experiment, as shown in Figure 4.24.

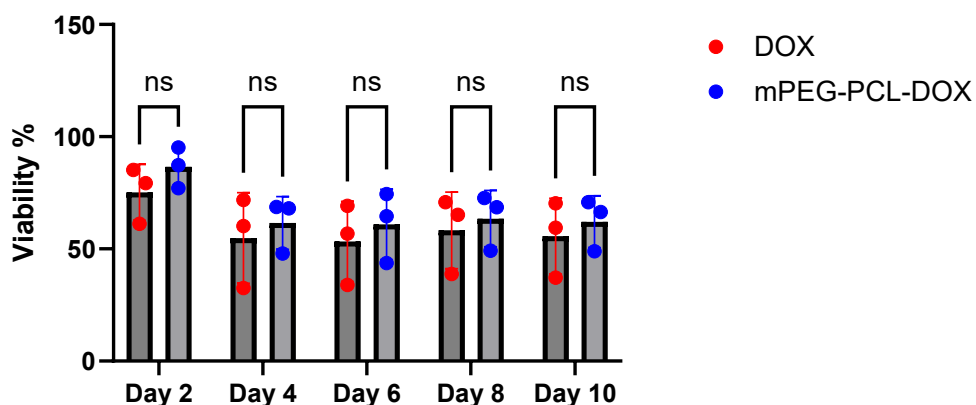


Figure 4.24: PgP cells viability on day 2, 4, 6 8 and 10, after treating cells with either DOX or mPEG-PCL-DOX. Cell viability was measured on days 2, 4, 6, 8 and 10 using PrestoBlue viability assay. Data were analysed using two-way ANOVA and are presented as mean $\pm$ SD (n = 3).

Treatment with the PgP inhibitor ELACRIDAR reversed the sensitivity of the cells to treatment, achieving very low viability on day 4 to an extent that was similar to that measured with WT cells. On day 4, no statistically significant difference in the PgP cells viability was recorded between DOX or nanoparticles treatment (Figure 4.25).

Therefore, these data suggest that differently from the results obtained from experiments performed at physiological pH, no direct correlation between uptake and rate of cytotoxic in both wild type and DOX resistant cells.

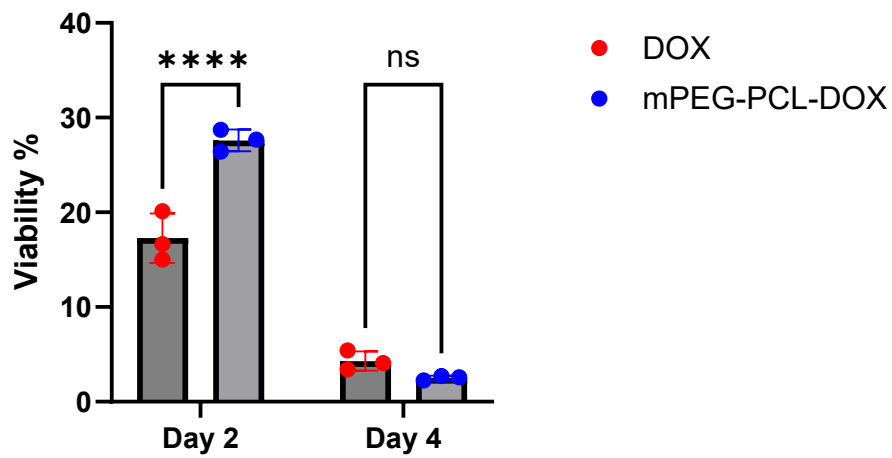


Figure 4.25: PgP + inhibitor cells viability on day 2 and 4, after treating cells with either DOX or mPEG-PCL-DOX. Cell viability was measured on days 2, and 4 using PrestoBlue viability assay. Data were analysed using two-way ANOVA and are presented as mean $\pm$ SD ($n = 3$).

4.3 Discussion:

This study aimed to investigate the cytotoxic effect of novel polymeric mPEG-PCL nanoparticles conjugated with doxorubicin through a pH responsive linker to overcome drug resistance in TNBC. To this end, different of isotypes of MDA-MB-231 cells were created to assess resistance to DOX treatment. The first model created was cells chronically treated with DOX (R), and the second was cells engineered to over express P-glycoproteins through MDR1 gene transduction. These resistant cells were compared to wild type cells (WT). R cells acquired resistant was confirmed by DOX IC₅₀ study, with the IC₅₀ calculated to be 1.4 μ M, compared to the parent WT cells which was 0.3 μ M as shown in Table 4-1 and Table 4-2. For PgP cells, the overexpression of P-glycoproteins was assessed using western blotting which showed a strong band at ~170 kDa in PgP cells selected using 50 nM DOX. For further confirmation, a qPCR study showed significantly higher MDR1 gene expression in PgP cells selected with 50 nM DOX than any other cell lines, confirming successful transduction MDR1 gene into the cells (Figure 4.14). Also, in PgP cells the DOX IC₅₀ value shifted from 0.3 μ M to 9.3 μ M after overexpressing the PgP efflux pump, as shown in Table 4-3.

These two different cell models were created given the different mechanisms of resistance to DOX. Indeed, chronic exposure to DOX has been associated with the upregulation of Epidermal Growth Factor Receptor (EGFR), and activation of its downstream signalling (Paramanantham et al. 2021b, Tian et al. 2023).

On the other hand, resistance to DOX mediated by expression of PgG involves overexpressing transmembrane glycoproteins which actively efflux drugs, keeping low intracellular concentrations, thus lowering its cytotoxicity (Bao et al. 2012). Importantly, similar links to elevated PgP and chemoresistance to DOX have been reported in MDA-MB-231 and other breast cancer cell lines (Mirzaei et al. 2022, Bao et al. 2012).

Before assessing the therapeutic performance of the mPEG-PCL-DOX, it was crucial to exclude the polymeric nanoparticle induced toxicity without the DOX conjugation on the cells, as some nanoparticles may induce toxicity by itself (Xuan et al. 2023). As shown in Figure 4.1, there was no statistically significant difference in the WT cells control and WT cells treated with empty mPEG-PCL after 2, 4 and 6 days of exposure, which subsequently confirms that any cytotoxic effect on cells were driven by the conjugated DOX and not from the nanoparticles itself. This finding aligns with reports that PEG-PCL nanoparticles systems are generally safe biodegradable/biocompatible with minimal intrinsic cytotoxicity at comparable doses, which makes them promising tools in drug delivery (Grossen et al. 2017, Cuong et al. 2010).

Although the internalization of the mPEG-PCL-DOX nanoparticles and the free DOX showed different patterns of accumulation across the different models, all of them showed a time dependent accumulation. The fluorescence images revealed a higher accumulation of free DOX compared to mPEG-PCL-DOX in WT and R models, with more fluorescence signal in WT cell nuclei than R cells (Figure 4.3 and Figure 4.9). In the contrary, the intracellular accumulation of mPEG-

PCL-DOX was much higher than free DOX in PgP cells model (

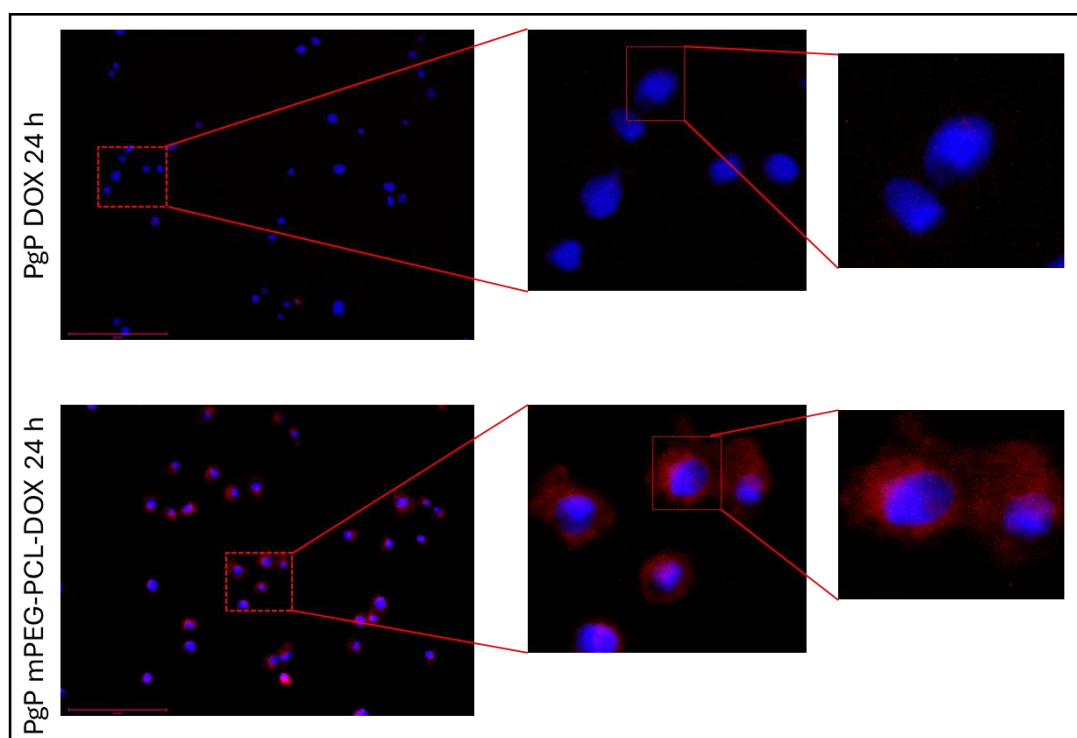


Figure 4.17), as the images showed minimum or no DOX signal in cells treated with free DOX, which suggest that efflux membrane transporters were successful in pumping out the drug and preventing the accumulation inside the cells, while the accumulation of mPEG-PCL-DOX indicate the potential of the nanoparticles to escape the cell membrane efflux transporters. Multiple studies are in line with these finding and showing that the use of nanoparticles can enhance the uptake and accumulation of DOX in cancer cells overexpressing PgP efflux pumps (Dong et al. 2009).

Viability studies revealed that free DOX was more potent than the mPEG-PCL-DOX nanoparticles in WT and R models. It is also worth mentioning that mPEG-PCL-DOX caused almost no cytotoxic effect on R model at all time points of the

study. These observations are in line with EVOS images which showed a better accumulation of free DOX compared to the mPEG-PCL-DOX nanoparticles.

On the other hand, DOX was significantly more potent than mPEG-PCL-DOX in PgP model at days 4 and 6, but on day 8, there was no significant difference observed between the potency of DOX and mPEG-PCL-DOX on PgP cells. Such delayed potency is typical for a pH responsive DOX carriers which are relatively stable at pH 7.4 and gradual accelerated release is observed at endosomal and lysosomal pH (~6.0-5.0) (Nayanathara et al. 2023). Additionally, combination therapy with ELACRIDAR PgP inhibitor enhanced the cytotoxicity of the free DOX and mPEG-PCL-DOX nanoparticles (Figure 4.20), as the inhibitor bind to the transporter's substrate-binding sites and preventing DOX recognition and expulsion. Also, there was no significant difference in the PgP cells viability observed for both treatments at all days of study. These findings confirm evidence that elacridar is a potent third-generation P-gG inhibitor that can resensitise resistant cells (Stasiak et al. 2025).

A promising finding was observed when the viability experiments were replicated in slightly acidic media, mimicking the acidic tumour microenvironment. EVOS fluorescence images captured on day 4 showed a successful accumulation of both free DOX and mPEG-PCL-DOX on and around cell's nucleus (Figure 4.21). As shown in Figure 4.22 and Figure 4.25 the cytotoxic effect of free DOX was highly significant on day 2 in WT and PgP + I cells than the mPEG-PCL-DOX nanoparticles, but this significant difference disappeared on day 4, as the free DOX and the mPEG-PCL-DOX exhibited nearly

the same cytotoxic effect, which was expected because of the nature of the pH responsive nanoparticles, as discussed above. It was also notable that changing the acidity of WT medium enhanced the killing effect (<5 % viable cells) of mPEG-PCL-DOX compared to neutral medium (~50 % viable cells) at day 4 (Figure 4.6 and Figure 4.22). For the R model, viability studies showed enhanced cytotoxic effect in acidic medium compared to neutral medium, especially in cells treated with mPEG-PCL-DOX nanoparticles (Figure 4.11 and Figure 4.23). Similar findings were published by Chen, W. L. et al. 2017 , demonstrating that pH responsive nanoparticles (DOX/DA) showed better accumulation and cytotoxicity in pH 6.8 compared to pH 7.4. Contrarily, the slightly acidic medium caused no significant difference in the viability of the PgP model between the free DOX and mPEG-PCL-nanoparticles treatments (Figure 4.24). This could be due to the overexpression of PgP opposing the enhanced release with a very efficient efflux as the nanoparticle delivery may not affect the ability to pump the realised drug outside of the cells, as previously reported (Dong et al. 2009).

The quantification of DOX intracellular concentration using LC-MS showed that mPEG-PCL-DOX concentrations in general was significantly higher than free DOX in all models. There was better accumulation of free DOX in WT compared to R and PgP models (Figure 4.5, Figure 4.10 and Figure 4.18), which could be a result of the resistant mechanism in actively pumping DOX out of the cells, keeping low concentrations of intracellular DOX. Although mPEG-PCL-DOX accumulation was higher, the viability studies showed a delay in the onset of the cytotoxic effect compared to free DOX. One explanation of that could be

endosomal and lysosomal sequestration, which has been reported extensively in literature (Zhitomirsky et al. 2014, Lee and Tannock 2006), as DOX is a weakly basic drug that becomes protonated and trapped within acidic endosomal and lysosomal compartments. This sequestration reduces its availability in the cytoplasm and nucleus, delaying and limiting its cytotoxic effects on DNA. This can explain the fluorescence images of WT and R models in which the nanoparticles were localized within the cytoplasm and around the nucleus, with gradual release in acidic compartments, while free DOX accumulated on the nucleus directly. Similar findings were reported by Wang et al. (2020), where pH responsive micelle based on mPEG-PCL were used and loaded with DOX. The study found high perinuclear accumulation of the nanoparticles compared to nuclear accumulation of free DOX, consistent with the data presented here.

Altogether, the data presented in this chapter point to a promising effect of the nanoparticles as a delivery system that could be used to overcome drug resistance in TNBC. However, the complex nature of the tumour microenvironment and the different mechanisms of drug resistance need to carefully be considered when designing their chemical properties.

These data presented in this chapter highlight the potential for using novel mPEG-PCL-DOX to selectively release DOX in the acidic tumour microenvironment which triggers the release of DOX to the required site, minimizing side effects on other organs.

Therefore, the next step in the project will be examining the penetration and cytotoxic effect of mPEG-PCL-DOX nanoparticles in TNBC spheroids, representing a closer to patient model compared to cells cultured in 2D.

Chapter 5 : 3D TNBC cell lines studies:

5.1 Introduction:

One of the major challenges that both clinicians and scientists face in cancer research is that the drug efficacy is very different in the patient's body compared to 2D monolayer cells cultured in the laboratory setting and used in preclinical research. This is one of the main reasons why also many clinical trials fail, and cancer drugs present the highest attrition rate in drug discovery (Kuo and Barrett 2024). These simplified models do not reflect or mimic the conditions of the tumour environment in a living organism by lacking the complexity of 3D interactions, oxygen and nutrient gradients as well as the presence of other cell types, which all together make up tumour microenvironment (TME) (Edmondson et al. 2014). Three dimensional (3D)

spheroids represent a more clinically relevant model to study cancer. These spheroids are formed by aggregation of cancer cells into spherical clusters which resemble how tumour form and grow. There are many advantages in using spheroids in cancer research, as they recreate the real tumour features by forming an outer layer consisting of actively growing cells and an inner hypoxic core, due to the limited oxygen and nutrient reaching this part of the tumour growth (Nath and Devi 2016). Also, spheroids models benefit from preserving the natural cell to cell and cell to extracellular matrix (ECM) interactions, which play an important role in tumour growth and response to drugs. These interactions are largely lost in 2D cultures compared to spheroids, which produce their own ECM, activating signalling pathways which are important for TNBC survival and metastasis (Imamura et al. 2015).

In addition, the complexity of 3D spheroid models can be increased by co-culturing cancer cells with stromal, vascular and immune cells to study the TME contribution to resistance to therapy, tumour growth and immune evasion.

Importantly, spheroids can predict treatments outcomes more accurately than 2D monolayer models, therefore they are instrumental in reducing the high failure rates of new cancer therapy as they can better predict and inform drug efficacy in animal models and clinical trials (Lv et al. 2017). Overall, the use of 3D spheroids bridges the gap between the simple monolayer cell culture and the complex animal model, providing an affordable, reliable and realistic way to mimic the complex TME.

Leveraging on this evidence, the aim of this chapter was to use wild type and resistant MDA-MB-231 cells grown in 3D spheroids to test the toxic effect of DOX delivered as free drug or mPEG-PCL-DOX nanoparticles, and to evaluate drug toxicity in the 3D models compared to cell cultured in 2D.

5.2 Results:

5.2.1 Optimization of MDA-MB-231 spheroids growth:

To study the toxic effect of free DOX and mPEG-PCL-DOX nanoparticles on MDA-MB-231 spheroids, an optimization study was carried out to determine the optimal conditions for spheroid formation and growth. Cell plated in low adherent plates failed to form spheroids without the addition of ECM proteins. Therefore, the Cultrex Basement Membrane Extract was added to support the formation of WT MDA-MB-231 spheroids.

As shown in Figure 5.1, the addition BME was essential to the formation of WT spheroids. Without BME, cells aggregated into loose, small and irregular clumps, which lacked cohesion and formed poorly defined spheroids. On the other hand, the presence of BME supported the formation of large, dense, and well-rounded spheroids with well-defined boundaries and regular edges.

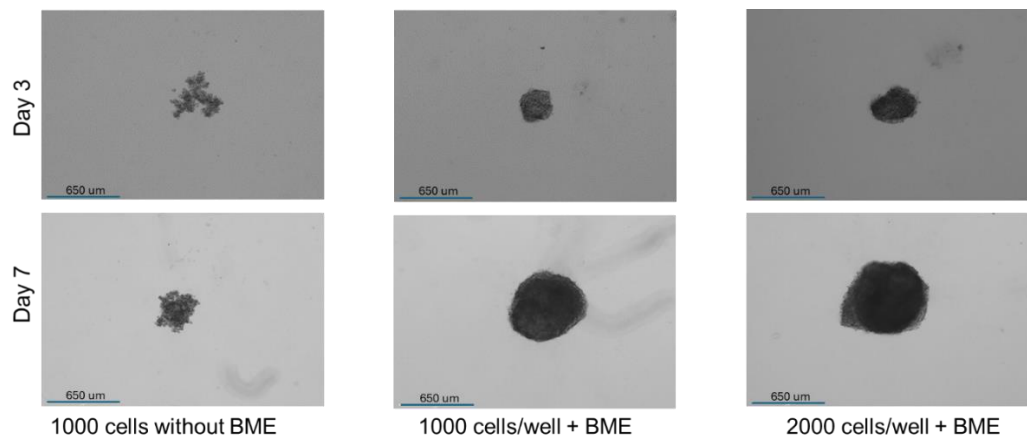


Figure 5.1 WT MDA-MB-231 cells spheroids formation brightfield images.

Images show spheroids formation using different cell seeding number with or without the addition of 100 ug/ml BME. Scale bar = 650 μ m. The figure shows representative images of 2 replicated experiments with 3 spheroids.

Two different concentrations of BME (100 ug/ml or 200 ug/ml) were tested to determine the best conditions to sustain spheroid viability across 7 days of culture.

The fluorescence live/dead stain images of WT spheroids shown in Figure 5.2 indicate that BME could sustain the formation of compact spheroids at both concentrations. Both concentrations also sustained viability, however cell were more viable in 100 ug/ml BME (green fluorescence) and had fewer dead cells (red fluorescence) compared to spheroids formed using 200 ug/ml BME. Therefore, the concentration of 100 ug/ml BME was chosen to support the growth of spheroids as it was shown to be the best concentration with less cell death.

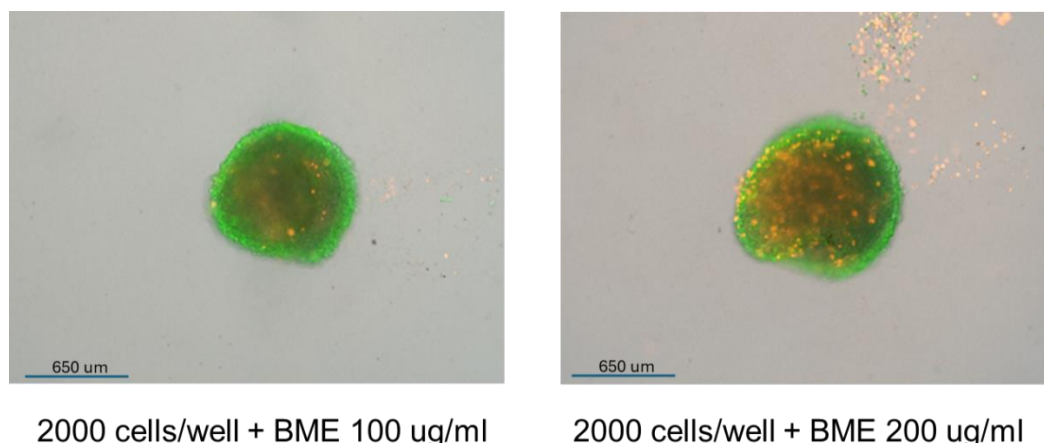


Figure 5.2 WT MDA-MB-231 cell line live/dead staining images, using 100 ug/ml BME or 200 ug/ml BME. Images were captured at day 7. Dead cells are shown in red and live cells are shown in green. The figure shows representative images of 2 replicated experiments with 3 spheroids. Scale bar = 650 μ m.

5.2.2 Determination of DOX IC₅₀ values in spheroids of wild type and resistant cells:

An IC₅₀ study was conducted on WT (wilde type), R (DOX resistant), PgP (PgP resistant) and PgP + inhibitor to determine the impact of 3D cell growth on DOX resistance.

As shown in the dose-response curves in Figure 5.3 and IC₅₀ values in Table 5-1, there were clear differences in the sensitivity to doxorubicin among the four types of spheroids. WT spheroids had the lowest IC₅₀ (0.182 μ M), indicating the highest sensitivity to DOX compared to the resistant spheroids, which showed increased IC₅₀s of about 4-fold. Importantly, the addition of the PgP inhibitor was able to reduce the IC₅₀ to 0.235 μ M, restoring the sensitivity of PgP spheroids to DOX.

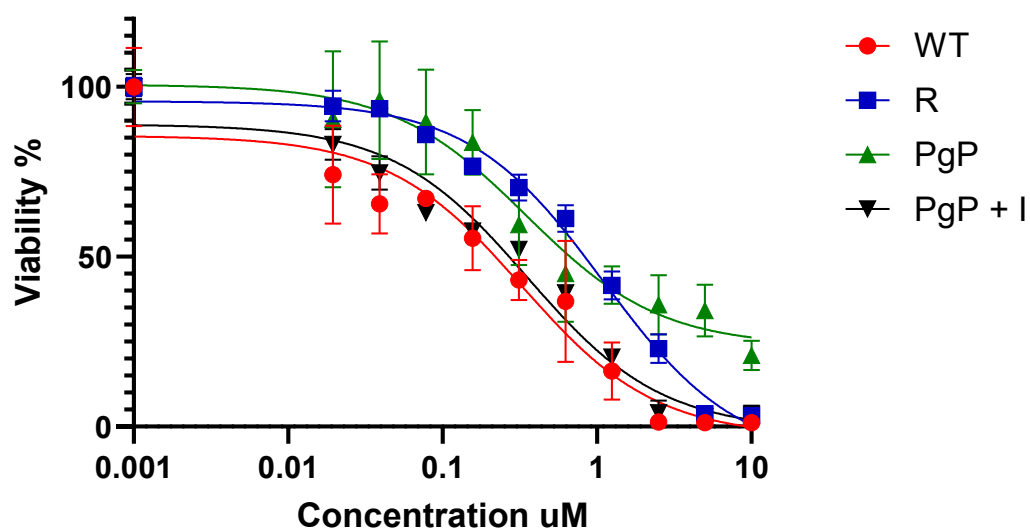


Figure 5.3 Dose vs response curve for DOX in WT, R, PgP and PgP + I spheroids. Cell viability was measured after 3 days of incubation using the PrestoBlue assay following treatment with various concentrations of DOX starting from 10 μ M then 1:2 serial dilutions. Data are presented as mean $\pm$ SD (n = 3).

Table 5-1 IC₅₀ mean and 95% CI values in uM for free DOX in WT, R, PgP and PgP + I spheroids (n = 6).

	WT	R	PgP	PgP + I
IC ₅₀ (uM)	0.182	0.744	0.827	0.235
95% CI (uM)	0.131 to 0.248	0.655 to 0.846	0.563 to 1.226	0.189 to 0.291

5.2.3 Uptake of free DOX and mPEG-PCL-DOX in spheroids:

The uptake of free DOX and mPEG-PCL-DOX nanoparticles uptake into the spheroids was next investigated using fluorescence microscopy. The spheroids were left to form for 4 days, then 300 nM of free DOX or mPEG-PCL-DOX nanoparticles were added and incubated until day 8. The spheroids images were captured at day 4 before adding the treatment, and at day 8 using brightfield, and the RFP channel for DOX detection.

In WT spheroids the uptake of DOX was limited by the dense formation of the spheroids, which restricted the diffusion of DOX and resulted in lower penetration and accumulation. On the other hand, DOX accumulation was slightly enhanced when delivered through mPEG-PCL-nanoparticles compared to free DOX (Figure 5.4).

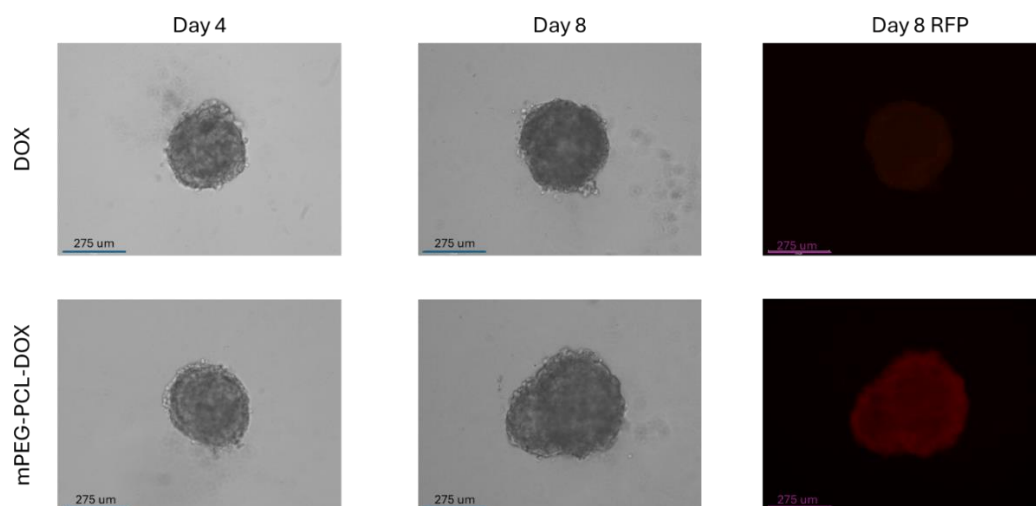


Figure 5.4 Fluorescence microscopy images of WT spheroids treated with free DOX or mPEG-PCL-DOX nanoparticles. Images were captured using the EVOS system at day 8. DOX was visualized via brightfield and the RFP channel (red) Scale bar = 275 µm. The figure shows representative images of 2 replicated experiments with 3 spheroids.

The R spheroids uptake of free DOX exhibited less accumulation than mPEG-PCL-DOX nanoparticles. Notably, post treatment spheroids with DOX or mPEG-PCL-DOX caused spheroids boundaries to become ragged, diffused and disintegrated, accompanied with loss of the compact spherical and more irregular shape, with the presence of cells debris around the spheroids (Figure 5.5).

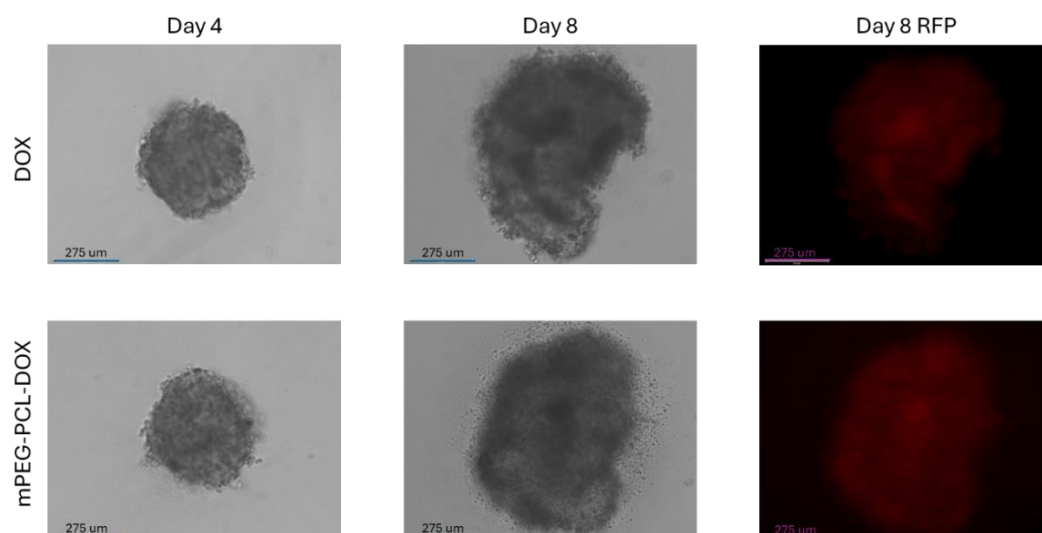


Figure 5.5 Fluorescence microscopy images of R spheroids treated with free DOX or mPEG-PCL-DOX nanoparticles. Images were captured using the EVOS system at day 8. DOX was visualized via brightfield and the RFP channel (red) Scale bar = 275 μm . The figure shows representative images of 2 replicated experiments with 3 of spheroids.

For PgP spheroids uptake, the efflux transporter played a crucial role in preventing free DOX from penetrating the spheroids, as there was no fluorescence detected in spheroids treated with free DOX, while the spheroid structure remained intact and preserved. On the other hand, mPEG-PCL-DOX showed successful penetration and accumulation across all parts of the of the spheroids structure, with spheroids becoming less compact and the edges becoming less defined (Figure 5.6)

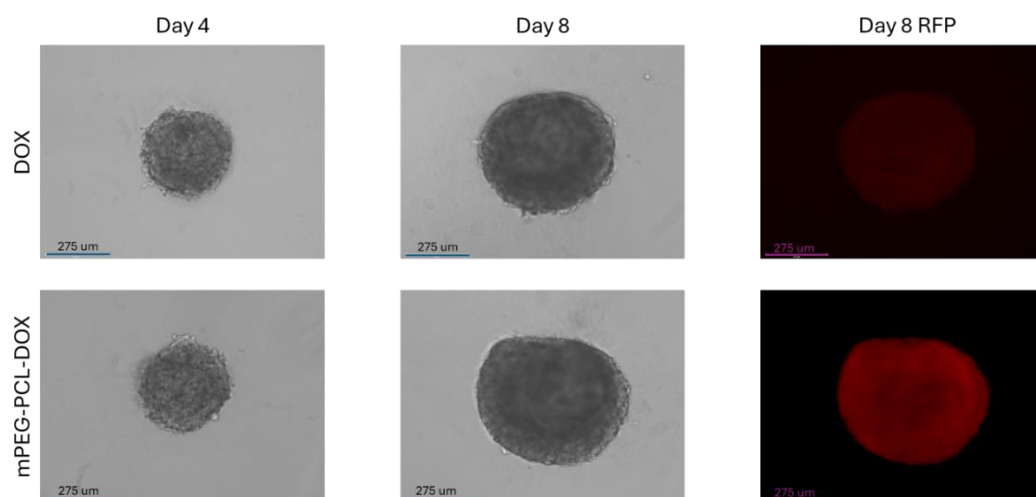


Figure 5.6 Fluorescence microscopy images of PgP spheroids treated with free DOX or mPEG-PCL-DOX nanoparticles. Images were captured using the EVOS system at day 8. DOX was visualized via brightfield and the RFP channel (red) Scale bar = 275 μ m. The figure shows representative images of 2 replicated experiments with 3 spheroids.

The addition of Elacridar (PgP inhibitor) in PgP spheroids caused a great enhancement of the uptake of both DOX and mPEG-PCL -DOX nanoparticles. In spheroids treated with nanoparticles, the DOX red fluorescence was stronger, with deeper penetration, and the spheroids had less defined boundaries and density compared to spheroids treated with free DOX, as shown in Figure 5.7.

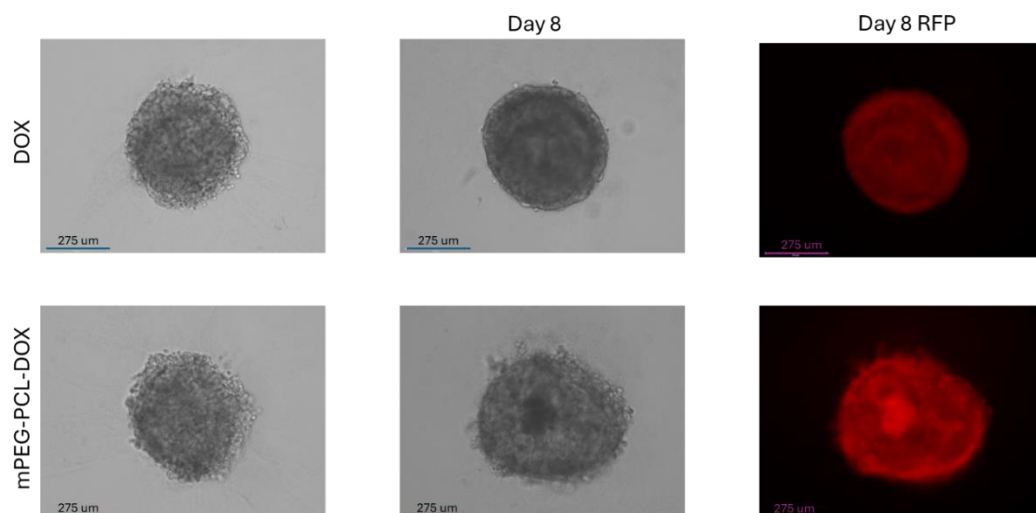


Figure 5.7 Fluorescence microscopy images of PgP + ELACRIDAR spheroids treated with free DOX or mPEG-PCL-DOX nanoparticles. Images were captured using the EVOS system at day 8. DOX was visualized via brightfield and the RFP channel (red) Scale bar = 275 μm . The figure shows representative images of 2 replicated experiments with 3 spheroids.

5.2.4 Spheroids viability in physiological medium:

The effect of prolonged treatment with free DOX and mPEG-PCL-DOX nanoparticles on the viability of the different spheroids was next investigated. The treatment was tested for up to 12 days to allow more time for penetration and DOX release within the spheroids.

As shown in Figure 5.8, WT spheroids treated with either free DOX or mPEG-PCL-DOX showed a progressive reduction in the spheroid's viability over time. Both treatments showed significant cytotoxicity as the viability decreased from day 2 reaching to day 12, indicating a cumulative cytotoxic effect. Notably,

statistical analysis showed non-significant differences between the two formulations at most time points, except for day 12, when mPEG-PCL-DOX achieved a statistically greater reduction in viability.

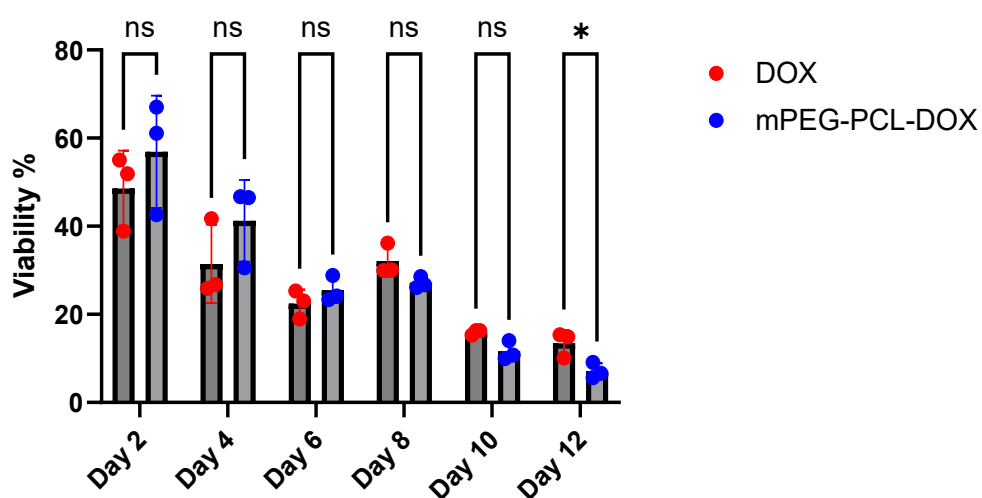


Figure 5.8 *WT* spheroids viability on days 2, 4, 6, 8, 10 and 12 after treating spheroids with either DOX or mPEG-PCL-DOX. Cell viability was measured using PrestoBlue viability assay. Data analysed using two-way ANOVA and presented as mean $\pm$ SD ($n = 3$).

In R spheroids, both treatments were able to reduce viability to similar values at all time points, with no treatment able to reduce the viability below 50%. There was no statistically significant difference in the viability of spheroids treated with free DOX or mPEG-PCL-DOX nanoparticles (Figure 5.9).

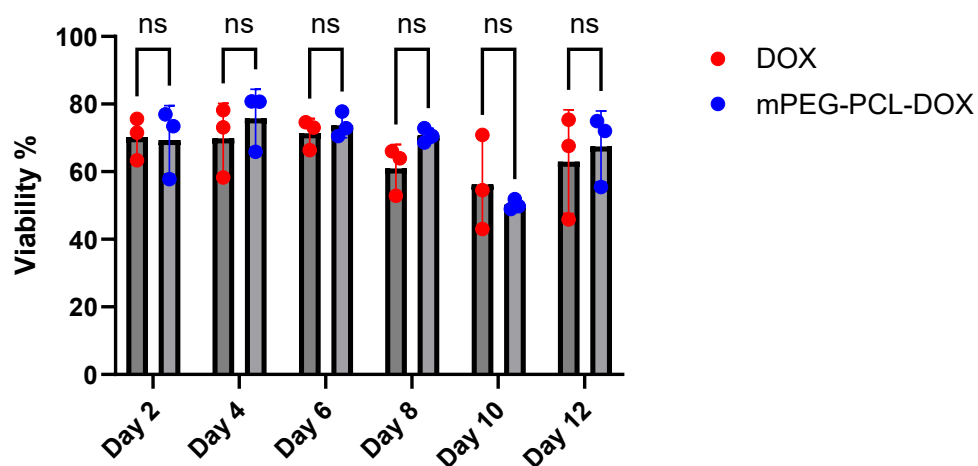


Figure 5.9 *R* spheroids viability on days 2, 4, 6, 8, 10 and 12 after treating spheroids with either DOX or mPEG-PCL-DOX. Cell viability was measured using PrestoBlue viability assay. Data analysed using two-way ANOVA and presented as mean $\pm$ SD ($n = 3$).

For PgP spheroids, there was no significant difference in the viability of spheroids treated with DOX or mPEG-PCL-DOX across all days of experiment. Interestingly, the viability of spheroids dropped at day 8 to below 50 % and remained lower than that measured with *R* spheroids (Figure 5.10).

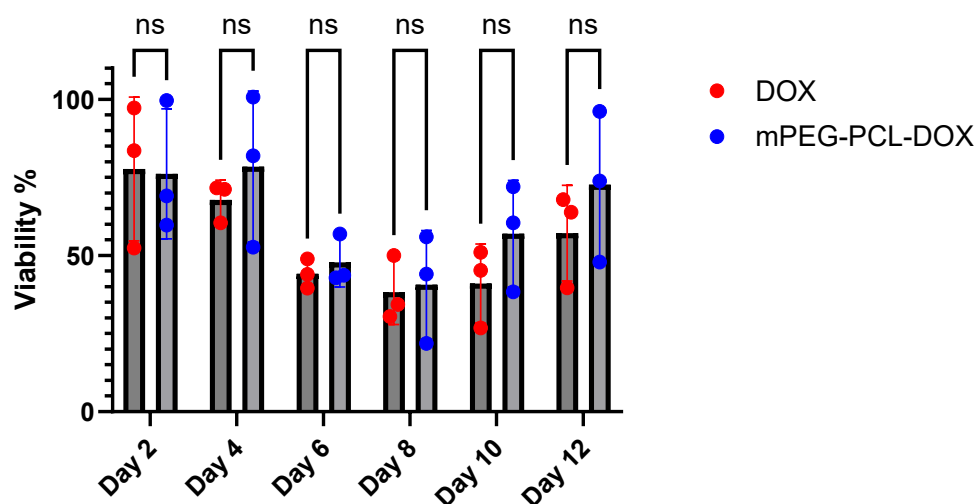


Figure 5.10 PgP spheroids viability on days 2, 4, 6, 8, 10 and 12 after treating spheroids with either DOX or mPEG-PCL-DOX. Cell viability was measured using PrestoBlue viability assay. Data analysed using two-way ANOVA and presented as mean $\pm$ SD (n = 3).

Finally, adding the Elacridar PgP inhibitor enhanced the cytotoxic effect of both free DOX and mPEG-PCL-DOX, as shown in Figure 5.11. Although there was no statistically significant difference between the two treatments on the viability of spheroids, adding PgP inhibitor caused a progressive cytotoxic effect on the spheroid viability to be lower than 10 % at day 8.

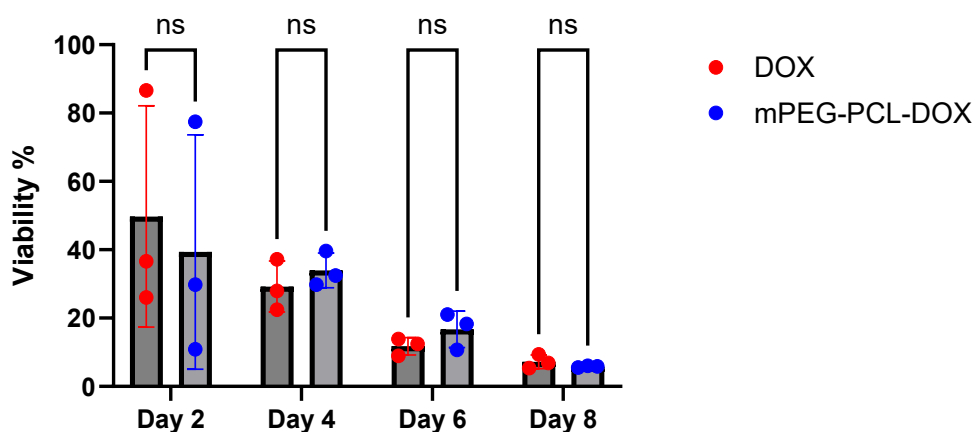


Figure 5.11 PgP + ELACRIDAR spheroids viability on days 2, 4, 6, and 8, after treating spheroids with either DOX or mPEG-PCL-DOX. Cell viability was measured using PrestoBlue viability assay. Data analysed using two-way ANOVA and presented as mean $\pm$ SD (n = 3).

5.2.5 Spheroids viability in acidic medium:

The viability of spheroids was also measured in the acidic medium, mimicking the pH of the tumour microenvironment.

At day 2, free DOX exhibited statistically significant toxicity compared to mPEG-PCL-DOX. This significant difference diminished at day 4, as there was no significant difference between spheroid treated with free DOX or mPEG-PCL-DOX nanoparticles, with viability reduced to very low levels in both conditions (Figure 5.12).

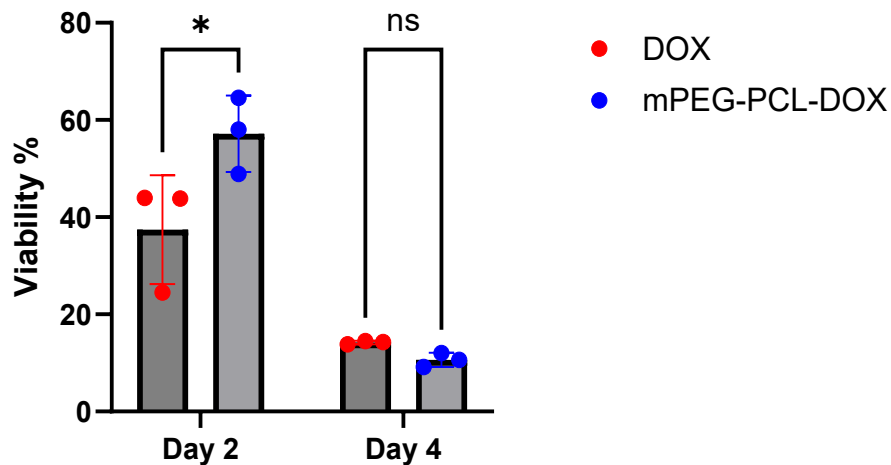


Figure 5.12 WT spheroids viability on days 2 and 4, after treating cells with either DOX or mPEG-PCL-DOX. Cell viability was measured using PrestoBlue viability assay. Data analysed using two-way ANOVA and presented as mean $\pm$ SD (n = 3).

R spheroids showed a cumulative cytotoxic effect in both treatments across all days (Figure 5.13). There was no significant difference between spheroids viabilities treated with free DOX or mPEG-PCL-DOX across all days. Notably, the slightly acidic medium improved the cytotoxic effect of both the free DOX and the nanoparticles.

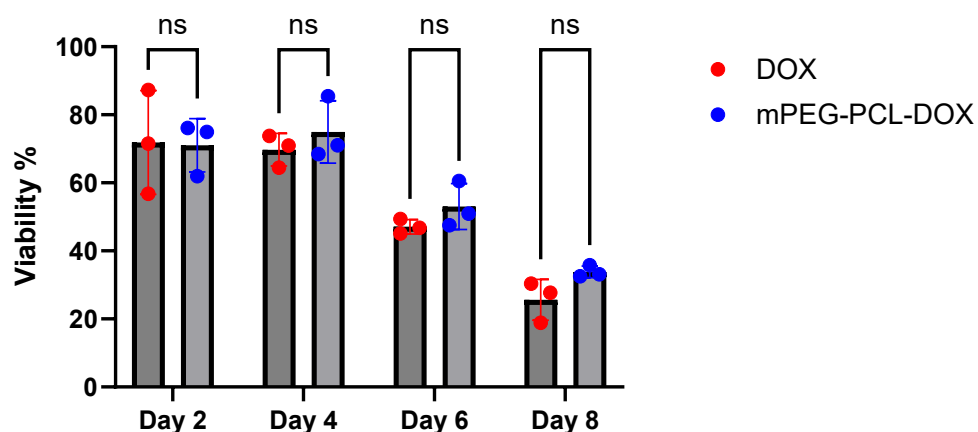


Figure 5.13 R spheroids viability on days 2, 4, 6 and 8, after treating cells with either DOX or mPEG-PCL-DOX. Cell viability was measured using PrestoBlue viability assay. Data analysed using two-way ANOVA and presented as mean $\pm$ SD (n = 3).

PgP spheroids were found to be resistant to both treatments, with no statistical difference in the viability of spheroids treated with DOX or mPEG-PCL-DOX, as shown in Figure 5.14. The experiment was stopped at day 4, as in day 6 the spheroids lost their shape and there were almost no viable cells.

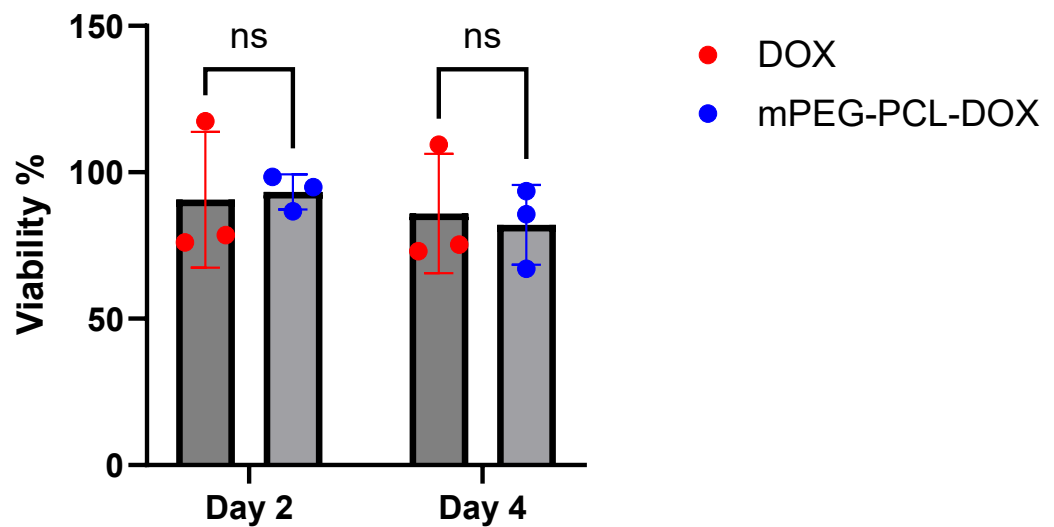


Figure 5.14 PgP spheroids viability on days 2 and 4, after treating cells with either DOX or mPEG-PCL-DOX. Cell viability was measured using PrestoBlue viability assay. Data analysed using two-way ANOVA and presented as mean $\pm$ SD (n = 3).

However, treatment with the Elacridar PgP inhibitor enhanced both free DOX and mPEG-PCL-DOX cytotoxic effect, with significant difference for mPEG-PCL-DOX nanoparticles at day2. The addition of Elacridar instantly reduced the viability of spheroids to less than 20 % at day 2, and less than 10 % at day 4 (Figure 5.15).

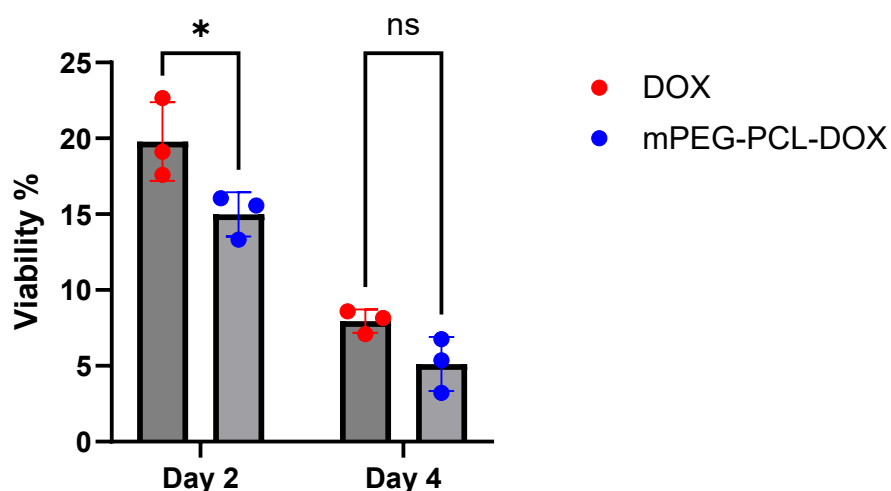


Figure 5.15 PgP + I spheroids viability on days 2 and 4, after treating cells with either DOX or mPEG-PCL-DOX. Cell viability was measured using PrestoBlue viability assay. Data analysed using two-way ANOVA and presented as mean $\pm$ SD (n = 3).

5.2.6 Comparing cytotoxic effect between 2D and 3D models:

To gain better understanding into the therapeutic potential of the mPEG-PCL-DOX nanoparticles, the cytotoxic effects in both 2D and 3D models were compared to free DOX.

In the WT model, both free DOX and mPEG-PCL-DOX were more potent in 2D monolayer compared to 3D spheroids. These differences were observed at days 6 and 8 and were statistically significant ($P < 0.05$) as shown in Figure 5.16.

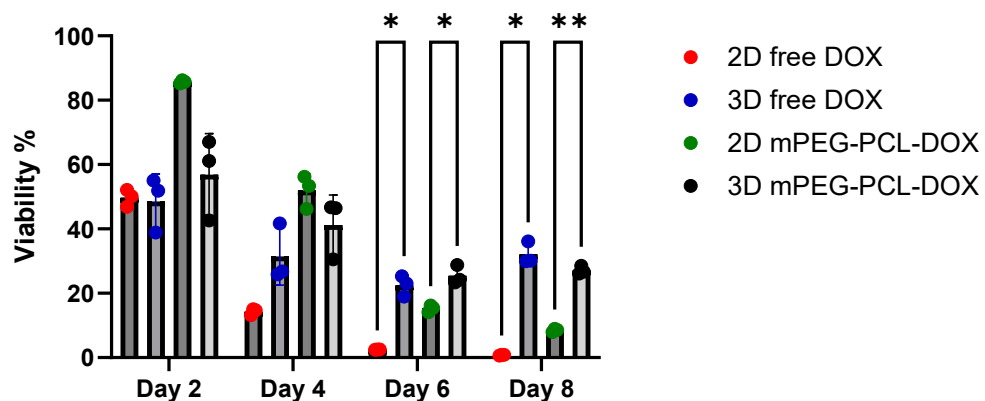


Figure 5.16: WT viability in 2D and 3D models treated with DOX or mPEG-PCL-DOX nanoparticles. Cell viability was measured using PrestoBlue viability assay. Data analysed using two-way ANOVA and presented as mean $\pm$ SD (n = 3).

Free DOX caused significant cytotoxic effect in days 6 and 8 in 2D compared to 3D also in the R model. Interestingly, mPEG-PCL-DOX nanoparticles showed a highly significant potency in R spheroids than 2D cells ($P < 0.001$) at day 8 as shown in Figure 5.17.

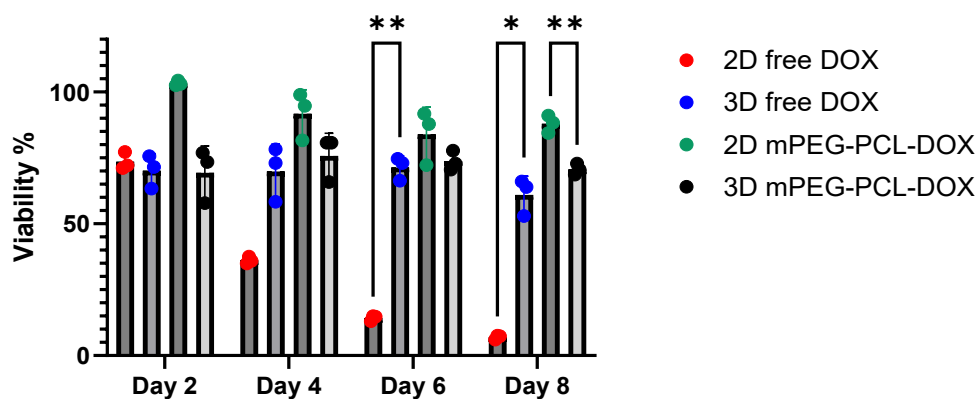


Figure 5.17 R viability in 2D and 3D models treated with DOX or mPEG-PCL-DOX nanoparticles. Cell viability was measured using PrestoBlue viability assay. Data analysed using two-way ANOVA and presented as mean $\pm$ SD (n = 3).

At days 2 and 4, there was no significant difference in the cytotoxicity of DOX and mPEG-PCL between 3D spheroids and 2D PgP cells. At day 6, both free DOX and mPEG-PCL-DOX showed significantly higher toxicity in 3D spheroids ($P < 0.001$). At day 8, free DOX produced significantly higher toxicity in 3D PgP spheroids compared to 2D cells ($P < 0.001$). Also, mPEG-PCL-DOX caused higher cytotoxic effect at day 8, but it was not statistically significant as shown in Figure 5.18

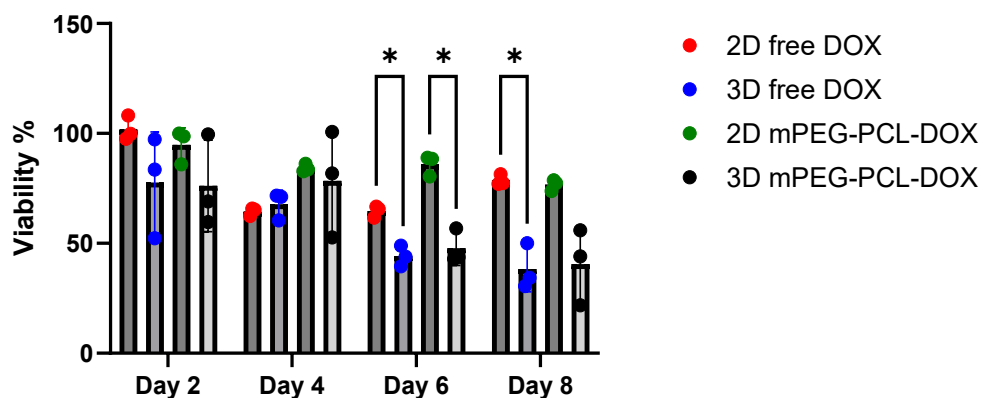


Figure 5.18 PgP viability in 2D and 3D models treated with DOX or mPEG-PCL-DOX nanoparticles. Cell viability was measured using PrestoBlue viability assay. Data analysed using two-way ANOVA and presented as mean $\pm$ SD (n = 3).

The addition of ELACRIDAR resensitized the cytotoxic effect of both free DOX and mPEG-PCL-DOX in 2D and 3D PgP spheroids with no significant differences at day 6 of the study, as shown in Figure 5.19.

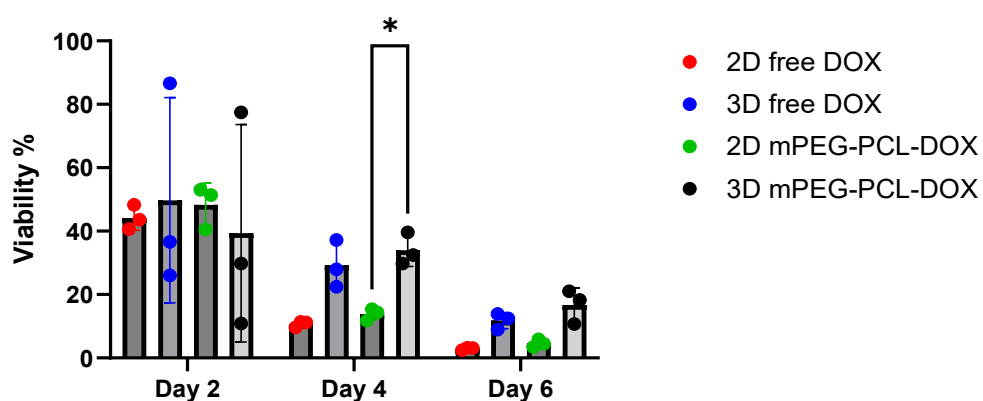


Figure 5.19 PgP plus ELACRIDAR viability in 2D and 3D models treated with DOX or mPEG-PCL-DOX nanoparticles. Cell viability was measured using PrestoBlue viability assay. Data analysed using two-way ANOVA and presented as mean $\pm$ SD (n = 3).

Therefore, these data show that the 3D spheroid model performed well in predicting drug toxicity responses and that sustained drug delivery through nanoparticles could enhance drug sensitivity in drug resistant cells.

5.3 Discussion:

Recapitulating the complex nature of the TME is critical for mimicking the effect of drugs on tumours growing in a patient.

The establishment of viable and compact MDA-MB-231 spheroids in the presence of BME emphasized the critical role of extracellular matrix (ECM) components in maintaining the spheroids' structure and viability. Without BME, the cells aggregated into irregular clumps that lack structural integrity, while adding BME promoted the formation of dense, and round spheroids with well-defined edges as shown in Figure 5.1. This observation agrees with previous reports that ECM proteins such as laminin and collagen are essential components for cohesion and survival, as they help in cell-cell and cell-matrix interactions (Nath and Devi 2016, Keller, Rudolf, and Hafner 2019). Similarly, (Vinci et al. 2012) showed that basement membrane components facilitate the formation of physiologically relevant spheroids that mimic TME.

It was also found that 100 ug/ml BME supported the spheroids viability more effectively than 200 ug/ml, which suggest that higher BME concentrations may obstruct nutrient and oxygen diffusion, which ultimately leads to higher cell death, as shown in Figure 5.2.

The spheroids IC₅₀ results confirmed the resistance of R and PgP models compared to WT model, in which the IC₅₀ was the lowest (0.182 uM) whereas R and PgP models were almost 4 fold higher, reflecting their resistance to free DOX, as shown in Table 5-1 and Figure 5.3. However, inhibition of PgP spheroids with ELACRIDAR restored DOX sensitivity, mirroring the 2D culture results.

Similar findings were reported by a study by (Salata and Lopes 2022a), which found that adding ELACRIDAR to the treatment can effectively resensitize TNBC cells to chemotherapeutics.

Drug uptake experiments using fluorescence microscopy showed that free DOX exhibited limited penetration into WT and R spheroids. In contrast, mPEG-PCL-DOX nanoparticles improved drug penetration and accumulation in WT, and no difference was observed compared to free DOX in R spheroids. Most notably, PgP spheroids treated with free DOX showed minimum penetration, which indicates that the majority of free dux was being pumped out by the PgP transporters, while the mPEG-PCL-DOX showed strong penetration and distribution throughout the spheroid structure. These results strongly suggest that the nanoparticles can overcome efflux-mediated barrier, which is supported by previous studies (Dong et al. 2009, Ho-Lun et al. 2006, Famta et al. 2021).

The prolonged viability studies showed that both free DOX and mPEG-PCL-DOX produced cumulative cytotoxic effect in WT across 12 days, with significantly higher toxicity produced by the nanoparticles at day 12, suggesting that the nanoparticles provide sustained drug release and retention properties. However, in both R and PgP spheroids, there was no significant difference between the two treatments on the viability of the spheroids. This could be due to drug efflux due to enhanced expression of transporters. When viability was assessed in acidic medium (pH 6.5), intended to mimic the tumour microenvironment, cytotoxicity was enhanced for both formulations in all

models. This observation highlights the benefit of pH-responsive release mechanisms, as acidic environments promote hydrolysis and destabilization of nanoparticle–drug linkages, thereby enhancing intratumorally drug release (Nogueira et al. 2020).

In agreement with the PgP model spheroid uptake, the addition to ELACRIDAR enhanced the cytotoxic effect of both free DOX and mPEG-PCL-DOX, reducing the viability of the spheroids to less than 10% at day 8. This finding highlights that efflux transporters represent one of the main barriers to chemotherapy efficacy in TNBC but can be effectively targeted by combination strategies. Similar finding was reported by (Salata and Lopes 2022b), which found that combining ELACRIDAR with paclitaxel nanoemulsion (PE-NETri) increased the cytotoxicity in MCF7 and MDA-MB-231 cells. These findings emphasized the therapeutic potential of combining nanocarriers with efflux inhibitors in resistant breast cancer.

The comparison between 2D and 3D cultures cytotoxic responses were found to be spheroids models specific. In WT, the viability was lower in 2D models compared to 3D spheroids in both free DOX and mPEG-PCL-DOX. This might be as a result of the better penetration in 2D monolayer compared to the more complex structure of the spheroids. The R model was comparable to WT, except that the mPEG-PCL-DOX performed better in 3D nanoparticles at the latest time point, suggesting that more time is required for drug release in 3D. In the PgP model, both treatments worked better in 3D spheroids, which was shown by the enhanced drug accumulation, especially for the nanoparticles as

previously mentioned. Similar work was performed by Oliveira et al. (2016), in which the developed solid lipid nanoparticles loaded with DOX showed a prominent cytotoxicity and penetration of spheroids.

Therefore, based on the results obtained, mPEG-PCL-DOX nanoparticles exhibited superiority over free DOX in penetration and accumulation of 3D spheroids. The enhanced performance of mPEG-PCL-DOX in combination with ELACRIDAR confirm their potential in overcoming efflux mediated multidrug resistance, which might be a promising approach for the treatment of TNBC.

Chapter 6 : General Discussion

Overcoming drug resistance and reducing off-target toxicity of conventional chemotherapeutics is a critical challenge in treating breast cancer. Due to its intrinsic heterogeneity, lack of molecular targets, and tendency to develop MDR, TNBC is the most therapeutically challenging subtype of breast cancer. Chemotherapy, including anthracyclines, taxanes, platinum agents, and cyclophosphamide, have demonstrated limited long-term efficacy, with high relapse rates and poor overall survival compared to receptor-positive breast cancers (Lehmann et al. 2016). Among these agents, doxorubicin (DOX) remains a cornerstone drug due to its broad-spectrum cytotoxicity; however, its clinical utility is severely compromised by dose-dependent cardiotoxicity and the emergence of MDR mechanisms mediated by ATP-binding cassette (ABC) transporters such as P-glycoprotein (PgP/ABCB1) and multidrug resistance-associated proteins (MRPs) (Belger et al. 2023, Mirzaei et al. 2022). The use of nanotechnology for targeted drug delivery has been extensively investigated as a promising strategy to overcome MDR and improve cancer therapy. Nanoparticles such as mPEG-PCL copolymers are especially advantageous due to their amphiphilic nature, controlled size, stealth properties, and modifiability for stimuli responsive release. Therefore, this study aimed to investigate the development and evaluation of novel pH responsive mPEG-PCL nanoparticles conjugated with doxorubicin (mPEG-PCL-DOX) through a cleavable pH sensitive bond for the treatment of resistant models of TNBC.

The nanoparticles were successfully synthesised using azide alkyne click chemistry, confirmed by FTIR through disappearance of the azide peak at $\sim 2100\text{ cm}^{-1}$ and formation of a triazole band at $\sim 1650\text{ cm}^{-1}$, with DLS showing particles size of $48.56 \pm 0.184\text{ d.nm}$ and acceptable PDI (0.180), a size range suitable for tumour accumulation through the enhanced permeation and retention (EPR) effect (Danhier et al. 2012). The nanoparticles release studies confirmed the design principle of pH responsiveness, as the mPEG-PCL-DOX nanoparticles showed stability at physiological pH and significantly enhanced doxorubicin release under acidic conditions, which are representative of the tumour microenvironment and closely aligning with previous reports on acid-sensitive delivery systems (Zhai et al. 2018). The conjugate design in this study therefore fits within the broader category of “smart” nanocarriers that use tumour-specific stimuli to improve drug selectivity.

In vitro studies were conducted on MDA-MB-231 cell line, and two drug resistant models were established, which acquired resistance to DOX through continuous DOX exposure or overexpression of PgP efflux transporters. Both resistance models showed higher IC₅₀ values compared to the parent WT model, and reduced intracellular accumulation of DOX, confirming the development of DOX resistance. In the drug sensitive WT model, results demonstrated that free DOX is more rapidly cytotoxic and accumulates in higher concentrations in the nucleus than mPEG-PCL-DOX at equivalent doses. However, the mPEG-PCL-DOX nanoparticles exhibited a slower, sustained release profile and presented a delayed but significant reduction in viability, especially upon prolonged exposure, a potential for minimizing off-target

toxicity and enabling lower dosing frequencies. In PgP resistant cells, fluorescence microscopy and LC-MS quantification of novel DOX conjugated nanoparticles demonstrated a significant bypass of the PgP-mediated efflux and intracellular accumulation compared to free DOX. These observations align with previous studies where polymer-drug conjugates, such as HPMA–DOX micelles achieved enhanced cytotoxicity in resistant breast cancer cells by avoiding transporter recognition (Pearce et al. 2020). Moreover, the use of Elacridar, a known PgP inhibitor, resensitised PgP-overexpressing cells to both formulations, further confirming the centrality of ABC transporter-mediated resistance and the therapeutic potential of combined therapeutic strategies (nanoparticles delivery + efflux inhibitors).

Under acidic extracellular conditions (pH 6.5), which mimic the tumour microenvironment, both uptake and cytotoxicity of mPEG-PCL-DOX improved further, consistent with the design intent and prior literature reporting the enhanced release and activity of acid-sensitive prodrugs and nanocarriers. Comparable strategies, including pH-sensitive liposomes and micelles, have shown improved therapeutic indices in preclinical TNBC models (Chen, W. L. et al. 2017b). This confirms the necessity of accounting for TME acidity when designing next-generation formulations for solid tumours.

A major advance of this work is the inclusion of 3D spheroid models to better simulate the *in vivo* tumour microenvironment, including hypoxic and acidic gradients, ECM influence, and complex multicellular structures. Consistent with the literature, it was confirmed that ECM components (BME) are essential

for the formation of compact, viable spheroids. The superiority of mPEG-PCL-DOX over free DOX, especially in terms of spheroid penetration and accumulation was pronounced in PgP spheroids, especially when accompanied with ELACRIDAR PgP inhibitor, supporting the hypothesis that polymeric nanoparticles can overcome not only transporter mediated resistance, but also the barriers imposed by the dense 3D tumour. This observation is consistent with reports by Tchoryk et al., (2019), who demonstrated that nanoparticle size and surface chemistry critically determine penetration in 3D models, and by Pearce et al. (2020), who showed that polymer-DOX conjugates reduced tumour mass and delayed regrowth *in vivo* compared with free DOX. By directly demonstrating improved penetration in multicellular spheroids, the project of this thesis provides important evidence that the developed nanoparticles may overcome one of the major barriers limiting chemotherapeutic efficacy *in vivo*.

When compared to the broader landscape of nanoparticle drug delivery systems for TNBC, the present work offers several advantages. Liposomal formulations such as pegylated liposomal DOX (Doxil®) have successfully reduced cardiotoxicity, but shown limited efficacy against resistant TNBC due to poor penetration and lack of responsiveness to tumour specific stimuli (Mi 2020, Zeng et al. 2023). Dendrimer-based conjugates offer high drug loading and surface functionality, but often raise concerns regarding biocompatibility and clearance (Madaan et al. 2014, Kaurav et al. 2023). The relative simplicity,

biocompatibility, and dual targeting features (EPR and pH-responsiveness) of the present system present it as a promising technology that warrant further development. The consistent correlation between improved nuclear accumulation, cellular uptake, and pH-responsive release lays a strong foundation for *in vivo* preclinical studies. These results are extension to the field of nano delivery that pH responsive, biodegradable polymeric nanoparticles can provide an effective strategy to address the fundamental challenge of drug resistance in TNBC and potentially other multidrug-resistant solid cancers. By aligning drug release kinetics to the TME properties, such systems may maximize tumour exposure while minimizing systemic toxicity.

6.1 Thesis limitations:

Although the present study demonstrates considerable potential, several limitations must be addressed. This work was performed using the MDA-MB-231 cell line, but its validity using another cell lines must be investigated. As TNBC is an heterogeneous disease composed by several tumour molecular subtypes, the project would have benefitted by validation using different subtype-like cell lines to establish the general applicability of the nanoparticles.

In addition, *in vitro* models, including 3D spheroids, do not fully capture the complexity of human tumours, including immune interactions, vascular architecture, and pharmacokinetic variability. Therefore, a limitation of this study is the sole focus on *in vitro* models and future work should address this shortcoming by extending the use of the nanoparticles in mouse models of

TNBC. While the present system enhanced DOX delivery, single-agent chemotherapy may be insufficient to achieve durable responses in TNBC, where combination approaches are increasingly favoured (Chalakur-Ramireddy and Pakala 2018). Therefore, additional strategies should have focussed on the use of combination therapies to explore the efficacy of DOX release from nanoparticles and other synergistic drugs to target TNBC vulnerabilities.

6.2 Future work:

As discussed above, this work was performed using the MDA-MB-231 cell line and the novel nanoparticles should be investigated using other TNBC cell lines for their penetration, accumulation and effectiveness. Moreover, the work was limited to *in vitro* systems, and future work should focus on extending these findings into *in vivo* studies to evaluate biodistribution, pharmacokinetics, tumour accumulation, and systemic toxicity in orthotopic TNBC models. *In vivo* biodistribution studies using fluorescent or radiolabelled nanoparticles should assess tumour accumulation versus off-target organ deposition, while pharmacokinetic analyses should determine circulation half-life and clearance. Therapeutic efficacy should be evaluated through tumour regression, recurrence rates, and survival outcomes, with comparisons made to free DOX and liposomal formulations. Cardiotoxicity, a key clinical limitation of DOX, must be carefully assessed through echocardiography, serum biomarkers (e.g., troponins), and histopathology. Additionally, the potential of conjugating alternative drugs should be explored for the treatment of TNBC subtypes

where doxorubicin is not part of the standard therapeutic regimen, such as taxane-based therapies (e.g., paclitaxel, docetaxel) or platinum-based therapies (e.g., cisplatin, carboplatin) that are increasingly used in BRCA-mutated TNBC. Such studies would allow assessment of whether the improved cytotoxicity and penetration observed *in vitro* translate to enhanced tumour regression, delayed recurrence, and reduced cardiotoxicity *in vivo*.

In addition, drug-conjugated nanoparticles could be used in combination with other emerging therapies for TNBC, including PARP inhibitors, checkpoint inhibitors and small molecule inhibitors targeting upregulated signalling pathways in TNBC molecular subtypes.

Altogether, these future studies will validate the effectiveness of the novel nanoparticle drug delivery system and pave the way to better treatment of TNBC for the benefit of breast cancer patients.

Bibliography

- Abelman, R.O., McLaughlin, S., Fell, G.G., Garrido-Castro, A.C., Lynce, F., Trippa, L., Poorvu, P.D., Rosenstock, A.S., Medford, A.J., Wander, S.A., Comander, A.H., Isakoff, S.J., Moy, B., Tung, N.M., Mittendorf, E.A., Ellisen, L., Tolaney, S.M., Bardia, A., and Spring, L. (2025) 'A Phase 2 Study of Response-Guided Neoadjuvant Sacituzumab Govitecan and Pembrolizumab (SG/P) in Patients with Early-Stage Triple-Negative Breast Cancer: Results from the NeoSTAR Trial.' *Journal of Clinical Oncology* [online] 43 (16_suppl), 511–511. available from <https://ascopubs.org/doi/pdf/10.1200/JCO.2025.43.16_suppl.511> [29 August 2025]
- Agha Zeeshan Mirza and Farhan Ahmed Siddiqui (2014) 'Nanomedicine and Drug Delivery: A Mini Review'. *International Nano Letter*
- Ahmad, Z., Shah, A., Siddiq, M., and Kraatz, H.B. (2014) 'Polymeric Micelles as Drug Delivery Vehicles'. *RSC Advances* [online] 4 (33), 17028–17038. available from <[https://pubs-rsc.org.nottingham.idm.oclc.org/en/content/articlehtml/2014/ra/c3ra47370h](https://pubs-rsc.org/nottingham.idm.oclc.org/en/content/articlehtml/2014/ra/c3ra47370h)> [9 November 2021]
- Ambudkar, S. V., Kimchi-Sarfaty, C., Sauna, Z.E., and Gottesman, M.M. (2003) 'P-Glycoprotein: From Genomics to Mechanism'. *Oncogene* [online] 22 (47 REV. ISS. 6), 7468–7485. available from <<https://pubmed.ncbi.nlm.nih.gov/14576852/>> [17 August 2025]

Anderson, N.M. and Simon, M.C. (2020) 'The Tumor Microenvironment'.

Current Biology [online] 30 (16), R921–R925. available from

<<https://pubmed.ncbi.nlm.nih.gov/32810447/>> [25 August 2025]

Arora, A., Seenu, V., Parshad, R., Bansal, V.K., Dhar, A., Mathur, S., Tanwar, P.,

Mishra, P., Kataria, K., Suhani, Gogia, A., Kumar, B., Haresh, K.P., Mallick,

S., Saini, S.K., Mishra, A., Bansal, B., Sharma, J., Saikia, J., Rangarajan, K.,

Dhamija, E., Prasad, C.P., Shamin, S.A., Agastm, S., Kalra, K., Vishvam, D.,

Mani, K., Kumar, A., Bakhshi, S., and Batra, A. (2025) 'A Phase II,

Randomized, Open-Labeled Study to Evaluate Low-Dose Pembrolizumab

in Addition to Neoadjuvant Chemotherapy for Triple-Negative Breast

Cancer (TNBC)'. *Trials* [online] 26 (1). available from

<<https://pubmed.ncbi.nlm.nih.gov/40011935/>> [29 August 2025]

Arroyo-Crespo, J.J., Armiñán, A., Charbonnier, D., Deladriere, C., Palomino-

Schätzlein, M., Lamas-Domingo, R., Forteza, J., Pineda-Lucena, A., and

Vicent, M.J. (2019) 'Characterization of Triple-negative Breast Cancer

Preclinical Models Provides Functional Evidence of Metastatic

Progression'. *International Journal of Cancer* [online] 145 (8), 2267–2281.

available from

<<https://onlinelibrary.wiley.com/doi/abs/10.1002/ijc.32270>> [24 March 2021]

Arroyo-Crespo, J.J., Armiñán, A., Charbonnier, D., Deladriere, C., Palomino-

Schätzlein, M., Lamas-Domingo, R., Forteza, J., Pineda-Lucena, A., and

Vicent, M.J. (2019) 'Characterization of Triple-Negative Breast Cancer

Preclinical Models Provides Functional Evidence of Metastatic Progression'. *International Journal of Cancer* 145 (8), 2267–2281

Ashique, S., Garg, A., Hussain, A., Farid, A., Kumar, P., and Taghizadeh-Hesary, F. (2023) 'Nanodelivery Systems: An Efficient and Target-Specific Approach for Drug-Resistant Cancers'. *Cancer Medicine* [online] 12 (18), 18797–18825. available from </doi/pdf/10.1002/cam4.6502> [29 August 2025]

Bai, X., Ni, J., Beretov, J., Graham, P., and Li, Y. (2021) 'Triple-Negative Breast Cancer Therapeutic Resistance: Where Is the Achilles' Heel?' *Cancer Letters* [online] 497, 100–111. available from <<https://www.sciencedirect.com/science/article/abs/pii/S0304383520305425>> [29 August 2025]

Bajpai, A.K., Shukla, S.K., Bhanu, S., and Kankane, S. (2008) 'Responsive Polymers in Controlled Drug Delivery'. *Progress in Polymer Science* 33 (11), 1088–1118

Bansal, K., Sasso, L., Makwana, H., Awwad, S., Brocchini, S., and Alexander, C. (2016) 'Nanopharmacy: Exploratory Methods for Polymeric Materials'. *Pharmaceutical Nanotechnology: Innovation and Production* [online] 231–270. available from <https://www.researchgate.net/publication/311661945_Nanopharmacy_Exploratory_Methods_for_Polymeric_Materials_Innovation_and_Production> [9 November 2021]

- Bao, L., Hazari, S., Mehra, S., Kaushal, D., Moroz, K., and Dash, S. (2012) 'Increased Expression of P-Glycoprotein and Doxorubicin Chemoresistance of Metastatic Breast Cancer Is Regulated by MiR-298'. *The American Journal of Pathology* [online] 180 (6), 2490. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC3378910/>> [16 August 2025]
- Bauer, J.A., Chakravarthy, A.B., Rosenbluth, J.M., Mi, D., Seeley, E.H., Granja-Ingram, N.D.M., Olivares, M.G., Kelley, M.C., Mayer, I.A., Meszoely, I.M., Means-Powell, J.A., Johnson, K.N., Tsai, C.J., Ayers, G.D., Sanders, M.E., Schneider, R.J., Formenti, S.C., Caprioli, R.M., and Pietersen, J.A. (2010) 'Identification of Markers of Taxane Sensitivity Using Proteomic and Genomic Analyses of Breast Tumors from Patients Receiving Neoadjuvant Paclitaxel and Radiation'. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research* [online] 16 (2), 681–690. available from <<https://pubmed-ncbi-nlm-nih-gov.nottingham.idm.oclc.org/20068102/>> [25 November 2021]
- Belfayol-Pisanté, L., Guillevin, L., Tod, M., and Fauvelle, F. (2000) 'Pharmacokinetics of Cyclophosphamide (CP) and 4-OH-CP/Aldophosphamide in Systemic Vasculitis'. *Fundamental & Clinical Pharmacology* [online] 14 (4), 415–421. available from <<https://pubmed-ncbi-nlm-nih-gov.nottingham.idm.oclc.org/11030450/>> [25 November 2021]
- Belger, C., Abrahams, C., Imamdin, A., and Lecour, S. (2023) 'Doxorubicin-Induced Cardiotoxicity and Risk Factors'. *International Journal of*

Cardiology. Heart & Vasculature [online] 50, 101332. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC10784684/>> [28 August 2025]

Bloom, H.J. and Richardson, W.W. (1957) 'Histological Grading and Prognosis in Breast Cancer a Study of 1409 Cases of Which 359 Have Been Followed for 15 Years'. *British Journal of Cancer* [online] 11 (3), 359–377. available from <<https://www.nature.com/articles/bjc195743>> [29 August 2025]

Carrillo-Castillo, T.D., Castro-Carmona, J.S., Luna-Velasco, A., and Zaragoza-Contreras, E.A. (2020) 'PH-Responsive Polymer Micelles for Methotrexate Delivery at Tumor Microenvironments'. *E-Polymers* 20 (1), 624–635

Carvalho, E., Canberk, S., Schmitt, F., and Vale, N. (2025) 'Molecular Subtypes and Mechanisms of Breast Cancer: Precision Medicine Approaches for Targeted Therapies'. *Cancers* 2025, Vol. 17, Page 1102 [online] 17 (7), 1102. available from <<https://www.mdpi.com/2072-6694/17/7/1102/htm>> [29 August 2025]

Chalakur-Ramireddy, N.K.R. and Pakala, S.B. (2018) 'Combined Drug Therapeutic Strategies for the Effective Treatment of Triple Negative Breast Cancer'. *Bioscience Reports* [online] 38 (1), BSR20171357. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC5789156/>> [28 August 2025]

- Chanmugam, A., Langemo, D., Thomason, K., Haan, J., Altenburger, E.A., Tippet, A., Henderson, L., and Zortman, T.A. (2017) 'Relative Temperature Maximum in Wound Infection and Inflammation as Compared with a Control Subject Using Long-Wave Infrared Thermography'. *Advances in Skin and Wound Care* [online] 30 (9), 406–414. available from <https://journals-lww-com.nottingham.idm.oclc.org/aswcjournal/Fulltext/2017/09000/Relative_Temperature_Maximum_in_Wound_Infection.4.aspx> [16 November 2021]
- Chatterjee, K., Zhang, J., Honbo, N., and Karliner, J.S. (2010) 'Doxorubicin Cardiomyopathy'. *Cardiology* [online] 115 (2), 155. available from <[pmc/articles/PMC2848530/](https://pubmed.ncbi.nlm.nih.gov/22848530/)> [26 November 2021]
- Che, J., Pan, L., Yang, X., Liu, Z., Huang, L., Wen, C., Lin, A., and Liu, H. (2017) 'Thymidine Phosphorylase Expression and Prognosis in Colorectal Cancer Treated with 5-Fluorouracil-Based Chemotherapy: A Meta-Analysis'. *Molecular and Clinical Oncology* [online] 7 (6), 943. available from <[pmc/articles/PMC5740914/](https://pubmed.ncbi.nlm.nih.gov/2740914/)> [25 November 2021]
- Chen, H., Shien, K., Suzawa, K., Tsukuda, K., Tomida, S., Sato, H., Torigoe, H., Watanabe, M., Namba, K., Yamamoto, H., Soh, J., Asano, H., Miyoshi, S., and Toyooka, S. (2017) 'Elacridar, a Third-Generation ABCB1 Inhibitor, Overcomes Resistance to Docetaxel in Non-Small Cell Lung Cancer'. *Oncology Letters* [online] 14 (4), 4349. available from <[https://pubmed.ncbi.nlm.nih.gov/articles/PMC5607652/](https://pubmed.ncbi.nlm.nih.gov/2607652/)> [5 July 2025]

- Chen, H., Wu, J., Zhang, Z., Tang, Y., Li, Xiaoxuan, Liu, S., Cao, S., and Li, Xianzhu (2018) 'Association between BRCA Status and Triple-Negative Breast Cancer: A Meta-Analysis'. *Frontiers in Pharmacology* [online] 9 (AUG). available from <<https://pubmed.ncbi.nlm.nih.gov/30186165/>> [25 August 2025]
- Chen, W.L., Li, F., Tang, Y., Yang, S. Di, Li, J.Z., Yuan, Z.Q., Liu, Y., Zhou, X.F., Liu, C., and Zhang, X.N. (2017a) 'Stepwise PH-Responsive Nanoparticles for Enhanced Cellular Uptake and on-Demand Intracellular Release of Doxorubicin'. *International Journal of Nanomedicine* [online] 12, 4241. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC5473598/>> [17 August 2025]
- Chen, W.L., Li, F., Tang, Y., Yang, S. Di, Li, J.Z., Yuan, Z.Q., Liu, Y., Zhou, X.F., Liu, C., and Zhang, X.N. (2017b) 'Stepwise PH-Responsive Nanoparticles for Enhanced Cellular Uptake and on-Demand Intracellular Release of Doxorubicin'. *International Journal of Nanomedicine* [online] 12, 4241. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC5473598/>> [28 August 2025]
- Cheng, H., Liao, J., Ma, Y., Sarwar, M.T., and Yang, H. (2025) 'Advances in Targeted Therapy for Tumor with Nanocarriers: A Review'. *Materials Today Bio* [online] 31, 101583. available from <<https://www.sciencedirect.com/science/article/pii/S2590006425001413>> [13 August 2025]

- Chintala, L., Vaka, S., Baranda, J., and Williamson, S.K. (2011) 'Capecitabine versus 5-Fluorouracil in Colorectal Cancer: Where Are We Now?' *Oncology Reviews* [online] 5 (2), 129–140. available from <<https://link-springer-com.nottingham.idm.oclc.org/article/10.1007/s12156-011-0074-3>> [26 November 2021]
- Chittasupho, C., Anuchapreeda, S., and Sarisuta, N. (2017) 'CXCR4 Targeted Dendrimer for Anti-Cancer Drug Delivery and Breast Cancer Cell Migration Inhibition'. *European Journal of Pharmaceutics and Biopharmaceutics* 119, 310–321
- Cho, Y.S., Shin, C.H., and Han, S. (2016) 'Dispersion Polymerization of Polystyrene Particles Using Alcohol as Reaction Medium'. *Nanoscale Research Letters* [online] 11 (1), 1–9. available from <<https://nanoscalereslett.springeropen.com/articles/10.1186/s11671-016-1261-8>> [22 November 2021]
- Cserni, G. (2020) 'Histological Type and Typing of Breast Carcinomas and the WHO Classification Changes over Time'. *Pathologica* [online] 112 (1), 25. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC8138497/>> [29 August 2025]
- Cuong, N. Van, Jiang, J.L., Li, Y.L., Chen, J.R., Jwo, S.C., and Hsieh, M.F. (2010) 'Doxorubicin-Loaded PEG-PCL-PEG Micelle Using Xenograft Model of Nude Mice: Effect of Multiple Administration of Micelle on the Suppression of Human Breast Cancer'. *Cancers* [online] 3 (1), 61.

available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC3756349/>>

[16 August 2025]

Danhier, F., Ansorena, E., Silva, J.M., Coco, R., Le Breton, A., and Préat, V.

(2012) 'PLGA-Based Nanoparticles: An Overview of Biomedical Applications'. *Journal of Controlled Release* [online] 161 (2), 505–522.

available from

<<https://www.sciencedirect.com/science/article/pii/S016836591200075>

2> [26 August 2025]

Dong, X., Mattingly, C.A., Tseng, M.T., Cho, M.J., Liu, Y., Adams, V.R., and

Mumper, R.J. (2009) 'Doxorubicin and Paclitaxel-Loaded Lipid-Based Nanoparticles Overcome Multi-Drug Resistance by Inhibiting P-Gp and Depleting ATP'. *Cancer Research* [online] 69 (9), 3918. available from

<<https://pmc.ncbi.nlm.nih.gov/articles/PMC2758164/>> [16 August 2025]

Duncan, R. and Gaspar, R. (2011) 'Nanomedicine(s) under the Microscope'.

Molecular Pharmaceutics [online] 8 (6), 2101–2141. available from

<[https://pubs-acsc-](https://pubs-acsc)

[org.nottingham.idm.oclc.org/doi/abs/10.1021/mp200394t](https://pubs-acsc.org.nottingham.idm.oclc.org/doi/abs/10.1021/mp200394t)> [27

September 2021]

Edmondson, R., Broglie, J.J., Adcock, A.F., and Yang, L. (2014) 'Three-

Dimensional Cell Culture Systems and Their Applications in Drug

Discovery and Cell-Based Biosensors'. *Https://Home.Liebertpub.Com/Adt*

[online] 12 (4), 207–218. available from

</doi/pdf/10.1089/adt.2014.573?download=true> [23 July 2025]

- Engel, C., Rhiem, K., Hahnen, E., Loibl, S., Weber, K.E., Seiler, S., Zachariae, S., Hauke, J., Wappenschmidt, B., Waha, A., Blümcke, B., Kiechle, M., Meindl, A., Niederacher, D., Bartram, C.R., Speiser, D., Schlegelberger, B., Arnold, N., Wieacker, P., Leinert, E., Gehrig, A., Briest, S., Kast, K., Riess, O., Emons, G., Weber, B.H.F., Engel, J., and Schmutzler, R.K. (2018) 'Prevalence of Pathogenic BRCA1/2 Germline Mutations among 802 Women with Unilateral Triple-Negative Breast Cancer without Family Cancer History'. *BMC Cancer* [online] 18 (1). available from <<https://pubmed.ncbi.nlm.nih.gov/29514593/>> [25 August 2025]
- Estrella, V., Chen, T., Lloyd, M., Wojtkowiak, J., Cornnell, H.H., Ibrahim-Hashim, A., Bailey, K., Balagurunathan, Y., Rothberg, J.M., Sloane, B.F., Johnson, J., Gatenby, R.A., and Gillies, R.J. (2013) 'Acidity Generated by the Tumor Microenvironment Drives Local Invasion'. *Cancer Research* [online] 73 (5), 1524–1535. available from <[/cancerres/article/73/5/1524/586608/Acidity-Generated-by-the-Tumor-Microenvironment](https://cancerres.aacrjournals.org/article/73/5/1524/586608/Acidity-Generated-by-the-Tumor-Microenvironment)> [25 August 2025]
- Etrych, T., Chytil, P., Jelínková, M., Íhová, B.R., and Ulbrich, K. (2002) 'Synthesis of HPMA Copolymers Containing Doxorubicin Bound via a Hydrazone Linkage. Effect of Spacer on Drug Release and in Vitro Cytotoxicity'. *Macromol. Biosci* 2, 43–52
- Famta, P., Shah, S., Chatterjee, E., Singh, H., Dey, B., Guru, S.K., Singh, S.B., and Srivastava, S. (2021) 'Exploring New Horizons in Overcoming P-Glycoprotein-Mediated Multidrug-Resistant Breast Cancer via Nanoscale

Drug Delivery Platforms'. *Current Research in Pharmacology and Drug Discovery* [online] 2, 100054. available from
<<https://www.sciencedirect.com/science/article/pii/S2590257121000419>> [22 August 2025]

Frangou, E.A., Bertsias, G., and Boumpas, D.T. (2016) 'Cytotoxic-Immunosuppressive Drug Treatment'. *Systemic Lupus Erythematosus: Basic, Applied and Clinical Aspects* 533–541

Gabizon, A.A., Lyass, O., Berry, G.J., and Wildgust, M. (2004) 'Cardiac Safety of Pegylated Liposomal Doxorubicin (Doxil®/Caelyx®) Demonstrated by Endomyocardial Biopsy in Patients with Advanced Malignancies'.
<https://doi-org.nottingham.idm.oclc.org/10.1081/CNV-200032899>
[online] 22 (5), 663–669. available from <<https://www-tandfonline-com.nottingham.idm.oclc.org/doi/abs/10.1081/CNV-200032899>> [26 November 2021]

Gaspar, R. and Duncan, R. (2009) 'Polymeric Carriers: Preclinical Safety and the Regulatory Implications for Design and Development of Polymer Therapeutics'. *Advanced Drug Delivery Reviews* 61 (13), 1220–1231

Gautam, S., Maurya, R., Vikal, A., Patel, P., Thakur, S., Singh, A., Gupta, G. Das, and Kurmi, B. Das (2025) 'Understanding Drug Resistance in Breast Cancer: Mechanisms and Emerging Therapeutic Strategies'. *Medicine in Drug Discovery* [online] 26, 100210. available from
<<https://www.sciencedirect.com/science/article/pii/S2590098625000077>> [29 August 2025]

- Gewirtz, D.A. (1999) 'A Critical Evaluation of the Mechanisms of Action Proposed for the Antitumor Effects of the Anthracycline Antibiotics Adriamycin and Daunorubicin'. *Biochemical Pharmacology* [online] 57 (7), 727–741. available from <<https://pubmed-ncbi-nlm-nih-gov.nottingham.idm.oclc.org/10075079/>> [26 November 2021]
- Grossen, P., Witzigmann, D., Sieber, S., and Huwyler, J. (2017) 'PEG-PCL-Based Nanomedicines: A Biodegradable Drug Delivery System and Its Application'. *Journal of Controlled Release* [online] 260, 46–60. available from <https://www.sciencedirect.com/science/article/abs/pii/S0168365917306004?utm_source=chatgpt.com> [16 August 2025]
- Guo, Q. and Jiang, C. (2020) 'Delivery Strategies for Macromolecular Drugs in Cancer Therapy'. *Acta Pharmaceutica Sinica B* 10 (6), 979–986
- Guo, X., Cheng, Y., Zhao, X., Luo, Y., Chen, J., and Yuan, W.E. (2018) 'Advances in Redox-Responsive Drug Delivery Systems of Tumor Microenvironment'. *Journal of Nanobiotechnology* [online] 16 (1), 1–10. available from <<https://jnanobiotechnology.biomedcentral.com/articles/10.1186/s12951-018-0398-2>> [18 November 2021]
- Hao, Q., Dai, L., Chang, L., Song, D., Liu, D., Ma, X., Wu, H., and Kang, H. (2024) 'Evaluation of Neoadjuvant Chemotherapy for Clinical T1 Triple-Negative Breast Cancer'. *Scientific Reports* [online] 14 (1), 1–11. available from

<<https://www.nature.com/articles/s41598-024-68719-7>> [29 August 2025]

Heskins, M. and Guillet, J.E. (2006) 'Solution Properties of Poly(N-Isopropylacrylamide)'. *https://doi.org/10.1080/10601326808051910* [online] 2 (8), 1441–1455. available from <<https://www.tandfonline.com/doi/abs/10.1080/10601326808051910>> [17 November 2021]

Ho, A.Y., Shiao, S., Kobald, S.A., Chen, J., Duda, D.G., Ly, A., Bossuyt, V., Cho, H.L., Arnold, B., Knott, S., Gupta, G.P., McAndrew, P., Karlan, S., Tighiouart, M., Muzikansky, A., Basho, R., and McArthur, H. (2024) 'PEARL: A Phase Ib/II Biomarker Study of Adding Radiation Therapy to Pembrolizumab before Neoadjuvant Chemotherapy in Human Epidermal Growth Factor Receptor 2-Negative Breast Cancer'. *Journal of Clinical Oncology* [online] 42 (36), 4282–4293. available from <<https://ascopubs.org/doi/10.1200/JCO.24.00003>> [29 August 2025]

Ho-Lun, W., Bendayan, R., Rauth, A.M., Hui, Y.X., Babakhanian, K., and Xiao, Y.W. (2006) 'A Mechanistic Study of Enhanced Doxorubicin Uptake and Retention in Multidrug Resistant Breast Cancer Cells Using a Polymer-Lipid Hybrid Nanoparticle System'. *The Journal of Pharmacology and Experimental Therapeutics* [online] 317 (3), 1372–1381. available from <<https://www.sciencedirect.com/science/article/abs/pii/S0022356524327429>> [22 August 2025]

- Imamura, Y., Mukohara, T., Shimon, Y., Funakoshi, Y., Chayahara, N., Toyoda, M., Kiyota, N., Takao, S., Kono, S., Nakatsura, T., and Minami, H. (2015) 'Comparison of 2D- and 3D-Culture Models as Drug-Testing Platforms in Breast Cancer'. *Oncology Reports* [online] 33 (4), 1837–1843. available from <<https://pubmed.ncbi.nlm.nih.gov/25634491/>> [23 July 2025]
- Jain, A., Barge, A., and Parris, C.N. (2024) 'Combination Strategies with PARP Inhibitors in BRCA-Mutated Triple-Negative Breast Cancer: Overcoming Resistance Mechanisms'. *Oncogene* [online] 44 (4), 193. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC11746151/>> [29 August 2025]
- Jeong, B., Bae, Y.H., and Kim, S.W. (2000) 'Drug Release from Biodegradable Injectable Thermosensitive Hydrogel of PEG–PLGA–PEG Triblock Copolymers'. *Journal of Controlled Release* 63 (1–2), 155–163
- Johnston, H.J., Semmler-Behnke, M., Brown, D.M., Kreyling, W., Tran, L., and Stone, V. (2010) 'Evaluating the Uptake and Intracellular Fate of Polystyrene Nanoparticles by Primary and Hepatocyte Cell Lines in Vitro'. *Toxicology and Applied Pharmacology* [online] 242 (1), 66–78. available from <<https://pubmed-ncbi-nlm-nih-gov.nottingham.idm.oclc.org/19799923/>> [22 November 2021]
- Juul, N., Szallasi, Z., Eklund, A.C., Li, Q., Burrell, R.A., Gerlinger, M., Valero, V., Andreopoulou, E., Esteva, F.J., Symmans, W.F., Desmedt, C., Haibe-Kains, B., Sotiriou, C., Pusztai, L., and Swanton, C. (2010) 'Assessment of an RNA Interference Screen-Derived Mitotic and Ceramide Pathway Metagene as

a Predictor of Response to Neoadjuvant Paclitaxel for Primary Triple-Negative Breast Cancer: A Retrospective Analysis of Five Clinical Trials'. *The Lancet. Oncology* [online] 11 (4), 358–365. available from <[https://pubmed-ncbi-nlm-nih.gov.nottingham.idm.oclc.org/20189874/](https://pubmed-ncbi-nlm-nih.gov/nottingham.idm.oclc.org/20189874/)> [25 November 2021]

Kaurav, M., Ruhi, S., Al-Goshae, H.A., Jeppu, A.K., Ramachandran, D., Sahu, R.K., Sarkar, A.K., Khan, J., and Ashif Ikbal, A.M. (2023) 'Dendrimer: An Update on Recent Developments and Future Opportunities for the Brain Tumors Diagnosis and Treatment'. *Frontiers in Pharmacology* [online] 14, 1159131. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC10060650/>> [28 August 2025]

Keller, F., Rudolf, R., and Hafner, M. (2019) 'Towards Optimized Breast Cancer 3D Spheroid Mono-and Co-Culture Models for Pharmacological Research and Screening'. *Journal of Cellular Biotechnology* 5 (2), 89–101

Khan, M.Z.I., Prebeg, Ž., and Kurjaković, N. (1999) 'A PH-Dependent Colon Targeted Oral Drug Delivery System Using Methacrylic Acid Copolymers: I. Manipulation of Drug Release Using Eudragit® L100-55 and Eudragit® S100 Combinations'. *Journal of Controlled Release* 58 (2), 215–222

Khandare, J. and Minko, T. (2006) 'Polymer–Drug Conjugates: Progress in Polymeric Prodrugs'. *Progress in Polymer Science* 31 (4), 359–397

- Kim, J., Harper, A., McCormack, V., Sung, H., Houssami, N., Morgan, E., Mutebi, M., Garvey, G., Soerjomataram, I., and Fidler-Benaoudia, M.M. (2025) 'Global Patterns and Trends in Breast Cancer Incidence and Mortality across 185 Countries'. *Nature Medicine* [online] 31 (4), 1154–1162. available from <<https://pubmed.ncbi.nlm.nih.gov/39994475/>> [29 August 2025]
- Kuo, Y.M. and Barrett, J.S. (2024) 'Consideration of the Root Causes in Candidate Attrition During Oncology Drug Development'. *Clinical Pharmacology in Drug Development* [online] 13 (9), 952–960. available from <[doi/pdf/10.1002/cpdd.1464](https://doi.org/10.1002/cpdd.1464)> [22 August 2025]
- Kuppusamy, P., Li, H., Ilangoan, G., Cardounel, A.J., Zweier, J.L., Yamada, K., Krishna, M.C., and Mitchell, J.B. (2002) 'Noninvasive Imaging of Tumor Redox Status and Its Modification by Tissue Glutathione Levels'. *Cancer Research* 62 (1)
- Lai, J.I., Tseng, Y.J., Chen, M.H., Huang, C.Y.F., and Chang, P.M.H. (2020) 'Clinical Perspective of FDA Approved Drugs With P-Glycoprotein Inhibition Activities for Potential Cancer Therapeutics'. *Frontiers in Oncology* [online] 10, 561936. available from <www.frontiersin.org> [5 July 2025]
- Lee, C.M. and Tannock, I.F. (2006) 'Inhibition of Endosomal Sequestration of Basic Anticancer Drugs: Influence on Cytotoxicity and Tissue Penetration'. *British Journal of Cancer* [online] 94 (6), 863. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC2361369/>> [3 July 2025]

Lehmann, B.D., Bauer, J.A., Chen, X., Sanders, M.E., Chakravarthy, A.B., Shyr, Y., and PiTENpol, J.A. (2011a) 'Identification of Human Triple-Negative Breast Cancer Subtypes and Preclinical Models for Selection of Targeted Therapies'. *Journal of Clinical Investigation* [online] 121 (7), 2750–2767. available from <<https://pubmed.ncbi.nlm.nih.gov/21633166/>> [29 August 2025]

Lehmann, B.D., Bauer, J.A., Chen, X., Sanders, M.E., Chakravarthy, A.B., Shyr, Y., and PiTENpol, J.A. (2011b) 'Identification of Human Triple-Negative Breast Cancer Subtypes and Preclinical Models for Selection of Targeted Therapies'. *Journal of Clinical Investigation* 121 (7), 2750–2767

Lehmann, B.D., Colaprico, A., Silva, T.C., Chen, J., An, H., Ban, Y., Huang, H., Wang, L., James, J.L., Balko, J.M., Gonzalez-Ericsson, P.I., Sanders, M.E., Zhang, B., PiTENpol, J.A., and Chen, X.S. (2021) 'Multi-Omics Analysis Identifies Therapeutic Vulnerabilities in Triple-Negative Breast Cancer Subtypes'. *Nature Communications* 2021 12:1 [online] 12 (1), 1–18. available from <<https://www.nature.com/articles/s41467-021-26502-6>> [29 August 2025]

Lehmann, B.D., Jovanović, B., Chen, X., Estrada, M. V., Johnson, K.N., Shyr, Y., Moses, H.L., Sanders, M.E., and PiTENpol, J.A. (2016) 'Refinement of Triple-Negative Breast Cancer Molecular Subtypes: Implications for Neoadjuvant Chemotherapy Selection'. *PLoS ONE* [online] 11 (6). available from <<https://pubmed.ncbi.nlm.nih.gov/27310713/>> [25 August 2025]

- Li, Qiao, Li, Qing, Zhang, P., Yuan, P., Wang, J., Ma, F., Luo, Y., Fan, Y., Cai, R., and Xu, B. (2015) 'A Phase II Study of Capecitabine plus Cisplatin in Metastatic Triple-Negative Breast Cancer Patients Pretreated with Anthracyclines and Taxanes'. *Cancer Biology & Therapy* [online] 16 (12), 1746–1753. available from <<https://pubmed-ncbi-nlm-nih.gov/nottingham.idm.oclc.org/26466918/>> [26 November 2021]
- Lv, D., Hu, Z., Lu, L., Lu, H., and Xu, X. (2017) 'Three-Dimensional Cell Culture: A Powerful Tool in Tumor Research and Drug Discovery'. *Oncology Letters* [online] 14 (6), 6999–7010. available from <<https://pubmed.ncbi.nlm.nih.gov/29344128/>> [23 July 2025]
- Madaan, K., Kumar, S., Poonia, N., Lather, V., and Pandita, D. (2014) 'Dendrimers in Drug Delivery and Targeting: Drug-Dendrimer Interactions and Toxicity Issues'. *Journal of Pharmacy and Bioallied Sciences* [online] 6 (3), 139–150. available from <https://journals.lww.com/jpbs/fulltext/2014/06030/dendrimers_in_drug_delivery_and_targeting_1.aspx> [28 August 2025]
- Mahmoud, R., Ordóñez-Morán, P., and Allegrucci, C. (2022a) 'Challenges for Triple Negative Breast Cancer Treatment: Defeating Heterogeneity and Cancer Stemness'. *Cancers* [online] 14 (17). available from <<https://pubmed.ncbi.nlm.nih.gov/36077812/>> [29 August 2025]
- Mahmoud, R., Ordóñez-Morán, P., and Allegrucci, C. (2022b) 'Challenges for Triple Negative Breast Cancer Treatment: Defeating Heterogeneity and

Cancer Stemness'. *Cancers* [online] 14 (17). available from
<<https://pubmed.ncbi.nlm.nih.gov/36077812/>> [29 August 2025]

Mahmoud, R., Ordóñez-Morán, P., and Allegrucci, C. (2022c) 'Challenges for Triple Negative Breast Cancer Treatment: Defeating Heterogeneity and Cancer Stemness'. *Cancers* [online] 14 (17). available from
<<https://pubmed.ncbi.nlm.nih.gov/36077812/>> [29 August 2025]

Mahmoud, R., Ordóñez-Morán, P., and Allegrucci, C. (2022d) 'Challenges for Triple Negative Breast Cancer Treatment: Defeating Heterogeneity and Cancer Stemness'. *Cancers* [online] 14 (17). available from
<<https://pubmed.ncbi.nlm.nih.gov/36077812/>> [29 August 2025]

Mansilla, E., Nunez, L., Marin, G.H., Rivera, L., Macor, P., and Luengas, S.P. (2019) 'New Advances in Chronic Lymphocytic Leukemia Treatment: Biodegradable ZnO Hybrid Cluster Nanoparticle as Antineoplastic Agents'. *Biomedical Applications of Nanoparticles* 409–430

Matanović, M.R., Kristl, J., and Grabnar, P.A. (2014) 'Thermoresponsive Polymers: Insights into Decisive Hydrogel Characteristics, Mechanisms of Gelation, and Promising Biomedical Applications'. *International Journal of Pharmaceutics* 472 (1–2), 262–275

Mcgowan, J. V, Chung, R., Maulik, A., Piotrowska, I., Malcolm Walker, & J., and Yellon, D.M. (2017) *Anthracycline Chemotherapy and Cardiotoxicity*.

Meng, F., Cheng, R., Deng, C., and Zhong, Z. (2012) 'Intracellular Drug Release Nanosystems'. *Materials Today* 15 (10), 436–442

- Meng, F., Hennink, W.E., and Zhong, Z. (2009) 'Reduction-Sensitive Polymers and Bioconjugates for Biomedical Applications'. *Biomaterials* 30 (12), 2180–2198
- Mi, P. (2020) 'Stimuli-Responsive Nanocarriers for Drug Delivery, Tumor Imaging, Therapy and Theranostics'. *Theranostics* [online] 10 (10), 4557. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC7150471/>> [28 August 2025]
- von Minckwitz, G., Schneeweiss, A., Loibl, S., Salat, C., Denkert, C., Rezai, M., Blohmer, J.U., Jackisch, C., Paepke, S., Gerber, B., Zahm, D.M., Kümmel, S., Eidtmann, H., Klare, P., Huober, J., Costa, S., Tesch, H., Hanusch, C., Hilfrich, J., Khandan, F., Fasching, P.A., Sinn, B. v., Engels, K., Mehta, K., Nekljudova, V., and Untch, M. (2014) 'Neoadjuvant Carboplatin in Patients with Triple-Negative and HER2-Positive Early Breast Cancer (GeparSixto; GBG 66): A Randomised Phase 2 Trial'. *The Lancet. Oncology* [online] 15 (7), 747–756. available from <<https://pubmed-ncbi-nlm-nih-gov.nottingham.idm.oclc.org/24794243/>> [25 November 2021]
- Mirzaei, S., Gholami, M.H., Hashemi, F., Zabolian, A., Farahani, M.V., Hushmandi, K., Zarrabi, A., Goldman, A., Ashrafizadeh, M., and Orive, G. (2022) 'Advances in Understanding the Role of P-Gp in Doxorubicin Resistance: Molecular Pathways, Therapeutic Strategies, and Prospects'. *Drug Discovery Today* [online] 27 (2), 436–455. available from <https://www.sciencedirect.com/science/article/pii/S1359644621004281?utm_source=chatgpt.com> [16 August 2025]

- Mu, X., Gan, S., Wang, Y., Li, H., and Zhou, G. (2019) 'Stimulus-Responsive Vesicular Polymer Nano-Integrators for Drug and Gene Delivery.' *International Journal of Nanomedicine* [online] 14, 5415–5434. available from <<https://europepmc.org/articles/PMC6645615>> [8 November 2021]
- Mura, S., Nicolas, J., and Couvreur, P. (2013) 'Stimuli-Responsive Nanocarriers for Drug Delivery'. *Nature Materials* 2013 12:11 [online] 12 (11), 991–1003. available from <<https://www.nature.com/articles/nmat3776>> [18 November 2021]
- Nakatsukasa, K., Koyama, H., Oouchi, Y., Imanishi, S., Mizuta, N., Sakaguchi, K., Fujita, Y., Fujiwara, I., Kotani, T., Matsuda, T., Fukuda, K., Morita, M., Kawakami, S., Kadotani, Y., Konishi, E., Yanagisawa, A., and Taguchi, T. (2017) 'Docetaxel and Cyclophosphamide as Neoadjuvant Chemotherapy in HER2-Negative Primary Breast Cancer'. *Breast Cancer (Tokyo, Japan)* [online] 24 (1), 63–68. available from <<https://pubmed-ncbi-nlm-nih-gov.nottingham.idm.oclc.org/26754092/>> [25 November 2021]
- Nakayama, M., Okano, T., Miyazaki, T., Kohori, F., Sakai, K., and Yokoyama, M. (2006) 'Molecular Design of Biodegradable Polymeric Micelles for Temperature-Responsive Drug Release'. *Journal of Controlled Release* 115 (1), 46–56
- Nath, S. and Devi, G.R. (2016) 'Three-Dimensional Culture Systems in Cancer Research: Focus on Tumor Spheroid Model'. *Pharmacology and*

Therapeutics [online] 163, 94–108. available from
<<https://pubmed.ncbi.nlm.nih.gov/27063403/>> [23 July 2025]

Nayanathara, U., Rossi Herling, B., Ansari, N., Zhang, C., Logan, S.R., Beach, M.A., Smith, S.A., Boase, N.R.B., Johnston, A.P.R., and Such, G.K. (2023) 'Impact of the Core Architecture of Dual PH-Responsive Polymer Nanoparticles on Intracellular Delivery of Doxorubicin'. *ACS Applied Nano Materials* [online] 6 (12), 10015–10022. available from
<doi/pdf/10.1021/acsanm.3c01598?ref=article_openPDF> [17 August 2025]

Nedeljković, M. and Damjanović, A. (2019a) 'Mechanisms of Chemotherapy Resistance in Triple-Negative Breast Cancer-How We Can Rise to the Challenge'. *Cells* [online] 8 (9). available from
<<https://pubmed.ncbi.nlm.nih.gov/31443516/>> [29 August 2025]

Nedeljković, M. and Damjanović, A. (2019b) 'Mechanisms of Chemotherapy Resistance in Triple-Negative Breast Cancer—How We Can Rise to the Challenge'. *Cells 2019, Vol. 8, Page 957* [online] 8 (9), 957. available from
<<https://www.mdpi.com/2073-4409/8/9/957/htm>> [29 August 2025]

Nedeljković, M. and Damjanović, A. (2019c) 'Mechanisms of Chemotherapy Resistance in Triple-Negative Breast Cancer—How We Can Rise to the Challenge'. *Cells 2019, Vol. 8, Page 957* [online] 8 (9), 957. available from
<<https://www.mdpi.com/2073-4409/8/9/957/htm>> [29 August 2025]

- Nedeljković, M. and Damjanović, A. (2019d) 'Mechanisms of Chemotherapy Resistance in Triple-Negative Breast Cancer—How We Can Rise to the Challenge'. *Cells* [online] 8 (9), 957. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC6770896/>> [3 July 2025]
- Nogueira, J., Soares, S.F., Amorim, C.O., Amaral, J.S., Silva, C., Martel, F., Trindade, T., and Daniel-Da-Silva, A.L. (2020) 'Magnetic Driven Nanocarriers for PH-Responsive Doxorubicin Release in Cancer Therapy'. *Molecules* [online] 25 (2), 333. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC7024164/>> [22 August 2025]
- Noriega-Luna, B., Godínez, L.A., Rodríguez, F.J., Rodríguez, A., Zaldívar-Lelo De Larrea, G., Sosa-Ferreira, C.F., Mercado-Curiel, R.F., Manríquez, J., and Bustos, E. (2014) 'Applications of Dendrimers in Drug Delivery Agents, Diagnosis, Therapy, and Detection'. *Journal of Nanomaterials* 2014
- Nostrum, C.F. van (2011) 'Covalently Cross-Linked Amphiphilic Block Copolymer Micelles'. *Soft Matter* [online] 7 (7), 3246–3259. available from <<https://pubs-rsc.org/nottingham.idm.oclc.org/en/content/articlehtml/2011/sm/c0sm00999g>> [27 September 2021]
- Ochiai, T., Nishimura, K., Noguchi, H., Kitajima, M., Tsukada, A., Watanabe, E., Nagaoka, I., and Futagawa, S. (2006) 'Prognostic Impact of Orotate Phosphoribosyl Transferase among 5-Fluorouracil Metabolic Enzymes in Resectable Colorectal Cancers Treated by Oral 5-Fluorouracil-Based Adjuvant Chemotherapy'. *International Journal of Cancer* [online] 118

(12), 3084–3088. available from <<https://onlinelibrary-wiley-com.nottingham.idm.oclc.org/doi/full/10.1002/ijc.21779>> [25 November 2021]

Oliveira, M.S., Aryasomayajula, B., Pattni, B., Mussi, S. V., Ferreira, L.A.M., and Torchilin, V.P. (2016) 'Solid Lipid Nanoparticles Co-Loaded with Doxorubicin and α -Tocopherol Succinate Are Effective against Drug-Resistant Cancer Cells in Monolayer and 3-D Spheroid Cancer Cell Models'. *International Journal of Pharmaceutics* [online] 512 (1), 292–300. available from <<https://pubmed.ncbi.nlm.nih.gov/27568499/>> [22 August 2025]

Ossovskaya, V., Wang, Y., Budoff, A., Xu, Q., Lituev, A., Potapova, O., Vansant, G., Monforte, J., and Daraselia, N. (2011) 'Exploring Molecular Pathways of Triple-Negative Breast Cancer'. *Genes and Cancer* [online] 2 (9), 870–879. available from <https://scholar.google.com/scholar_url?url=https://journals.sagepub.com/doi/pdf/10.1177/1947601911432496&hl=en&sa=T&oi=ucasa&ct=ufr&ei=6dxnaOvfKMzM6rQP_8mNwQs&scisig=AAZF9b929BBJ6cDWoqYGRqJ1OZBO> [4 July 2025]

Oun, R., Moussa, Y.E., and Wheate, N.J. (2018) 'The Side Effects of Platinum-Based Chemotherapy Drugs: A Review for Chemists'. *Dalton Transactions (Cambridge, England : 2003)* [online] 47 (19), 6645–6653. available from <<https://pubmed-ncbi-nlm-nih-gov.nottingham.idm.oclc.org/29632935/>> [25 November 2021]

Paramanantham, A., Jung, E.J., Kim, H.J., Jeong, B.K., Jung, J.M., Kim, G.S., Chan, H.S., and Lee, W.S. (2021a) 'Doxorubicin-Resistant TNBC Cells Exhibit Rapid Growth with Cancer Stem Cell-like Properties and EMT Phenotype, Which Can Be Transferred to Parental Cells through Autocrine Signaling'. *International Journal of Molecular Sciences* 2021, Vol. 22, Page 12438 [online] 22 (22), 12438. available from <<https://www.mdpi.com/1422-0067/22/22/12438/htm>> [12 August 2025]

Paramanantham, A., Jung, E.J., Kim, H.J., Jeong, B.K., Jung, J.M., Kim, G.S., Chan, H.S., and Lee, W.S. (2021b) 'Doxorubicin-Resistant TNBC Cells Exhibit Rapid Growth with Cancer Stem Cell-like Properties and EMT Phenotype, Which Can Be Transferred to Parental Cells through Autocrine Signaling'. *International Journal of Molecular Sciences* 2021, Vol. 22, Page 12438 [online] 22 (22), 12438. available from <<https://www.mdpi.com/1422-0067/22/22/12438/htm>> [17 July 2024]

Pareja, F., Bhargava, R., Borges, V.F., Brogi, E., Canas Marques, R., Cardoso, F., Desmedt, C., Harigopal, M., Lakhani, S.R., Lee, A., Leone, J.P., Linden, H., Lord, C.J., Marchio, C., Merajver, S.D., Rakha, E., Reis-Filho, J.S., Richardson, A., Sawyer, E., Schedin, P., Schwartz, C.J., Tutt, A., Ueno, N.T., Vincent-Salomon, A., Weigelt, B., Wen, Y.H., Schnitt, S.J., and Oesterreich, S. (2025) 'Unraveling Complexity and Leveraging Opportunities in Uncommon Breast Cancer Subtypes'. *Npj Breast Cancer* 2025 11:1 [online] 11 (1), 1–13. available from

<<https://www.nature.com/articles/s41523-025-00719-w>> [29 August 2025]

- Patra, J.K., Das, G., Fraceto, L.F., Campos, E.V.R., Rodriguez-Torres, M.D.P., Acosta-Torres, L.S., Diaz-Torres, L.A., Grillo, R., Swamy, M.K., Sharma, S., Habtemariam, S., and Shin, H.S. (2018) 'Nano Based Drug Delivery Systems: Recent Developments and Future Prospects'. in *Journal of Nanobiotechnology*. vol. 16 (1). BioMed Central Ltd.
- Pearce, A.K., Anane-Adjei, A.B., Cavanagh, R.J., Monteiro, P.F., Bennett, T.M., Taresco, V., Clarke, P.A., Ritchie, A.A., Alexander, M.R., Grabowska, A.M., and Alexander, C. (2020) 'Effects of Polymer 3D Architecture, Size, and Chemistry on Biological Transport and Drug Delivery In Vitro and in Orthotopic Triple Negative Breast Cancer Models'. *Advanced Healthcare Materials* 9 (22)
- Pont, M., Marqués, M., and Sorolla, A. (2024) 'Latest Therapeutical Approaches for Triple-Negative Breast Cancer: From Preclinical to Clinical Research'. *International Journal of Molecular Sciences* 2024, Vol. 25, Page 13518 [online] 25 (24), 13518. available from <<https://www.mdpi.com/1422-0067/25/24/13518/htm>> [29 August 2025]
- Raman, R., Debata, S., Govindarajan, T., and Kumar, P. (2025a) 'Targeting Triple-Negative Breast Cancer: Resistance Mechanisms and Therapeutic Advancements'. *Cancer Medicine* [online] 14 (9). available from <<https://pubmed.ncbi.nlm.nih.gov/40318146/>> [3 July 2025]

- Raman, R., Debata, S., Govindarajan, T., and Kumar, P. (2025b) 'Targeting Triple-Negative Breast Cancer: Resistance Mechanisms and Therapeutic Advancements'. *Cancer Medicine* [online] 14 (9), e70803. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC12048392/>> [4 July 2025]
- Salata, G.C. and Lopes, L.B. (2022a) 'Phosphatidylcholine-Based Nanoemulsions for Paclitaxel and a P-Glycoprotein Inhibitor Delivery and Breast Cancer Intraductal Treatment'. *Pharmaceuticals* [online] 15 (9), 1110. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC9503599/>> [22 August 2025]
- Salata, G.C. and Lopes, L.B. (2022b) 'Phosphatidylcholine-Based Nanoemulsions for Paclitaxel and a P-Glycoprotein Inhibitor Delivery and Breast Cancer Intraductal Treatment'. *Pharmaceuticals* [online] 15 (9), 1110. available from <<https://www.mdpi.com/1424-8247/15/9/1110/htm>> [22 August 2025]
- dos Santos, T., Varela, J., Lynch, I., Salvati, A., and Dawson, K.A. (2011) 'Effects of Transport Inhibitors on the Cellular Uptake of Carboxylated Polystyrene Nanoparticles in Different Cell Lines'. *PLOS ONE* [online] 6 (9), e24438. available from <<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0024438>> [22 November 2021]
- Schiff, P.B., Fant, J., and Horwitz, S.B. (1979) 'Promotion of Microtubule Assembly in Vitro by Taxol'. *Nature* 1979 277:5698 [online] 277 (5698),

665–667. available from <<https://www-nature-com.nottingham.idm.oclc.org/articles/277665a0>> [24 November 2021]

Schneider, M., Windbergs, M., Daum, N., Loretz, B., Collnot, E.M., Hansen, S., Schaefer, U.F., and Lehr, C.M. (2013) 'Crossing Biological Barriers for Advanced Drug Delivery'. *European Journal of Pharmaceutics and Biopharmaceutics* 84 (2), 239–241

Sherje, A.P., Jadhav, M., Dravyakar, B.R., and Kadam, D. (2018) 'Dendrimers: A Versatile Nanocarrier for Drug Delivery and Targeting'. *International Journal of Pharmaceutics* 548 (1), 707–720

Shi, J., Gao, P., Song, Y., Chen, X., Li, Y., Zhang, C., Wang, H., and Wang, Z. (2017) 'Efficacy and Safety of Taxane-Based Systemic Chemotherapy of Advanced Gastric Cancer: A Systematic Review and Meta-Analysis'. *Scientific Reports* 2017 7:1 [online] 7 (1), 1–10. available from <<https://www-nature-com.nottingham.idm.oclc.org/articles/s41598-017-05464-0>> [24 November 2021]

Shi, M., Li, Z., Shen, G., Wang, T., Li, J., Wang, M., Liu, Z., Zhao, F., Ren, D., and Zhao, J. (2024) 'Efficacy and Safety of First-Line Treatment for Metastatic Triple-Negative Breast Cancer: A Network Meta-Analysis'. *Cancer Pathogenesis and Therapy* [online] 2 (2), 81–90. available from <<https://www.sciencedirect.com/science/article/pii/S2949713223000290>> [29 August 2025]

- Soo Choi, H., Liu, W., Misra, P., Tanaka, E., Zimmer, J.P., Itty Ipe, B., Bawendi, M.G., and Frangioni, J. v. (2007) 'Renal Clearance of Quantum Dots'. *Nature Biotechnology* [online] 25 (10), 1165–1170. available from <[https://pubmed.ncbi.nlm.nih.gov.nottingham.idm.oclc.org/17891134/](https://pubmed.ncbi.nlm.nih.gov/nottingham.idm.oclc.org/17891134/)> [9 November 2021]
- Sørli, T., Perou, C.M., Tibshirani, R., Aas, T., Geisler, S., Johnsen, H., Hastie, T., Eisen, M.B., Van De Rijn, M., Jeffrey, S.S., Thorsen, T., Quist, H., Matese, J.C., Brown, P.O., Botstein, D., Lønning, P.E., and Børresen-Dale, A.L. (2001) 'Gene Expression Patterns of Breast Carcinomas Distinguish Tumor Subclasses with Clinical Implications'. *Proceedings of the National Academy of Sciences of the United States of America* [online] 98 (19), 10869–10874. available from <<https://pubmed.ncbi.nlm.nih.gov/11553815/>> [29 August 2025]
- Sousa De Almeida, M., Susnik, E., Drasler, B., Taladriz-Blanco, P., Petri-Fink Ab, A., and Rothen-Rutishauser, B. (2021) 'Understanding Nanoparticle Endocytosis to Improve Targeting Strategies in Nanomedicine'. *Chem. Soc. Rev* 50, 5397
- Stasiak, P., Sopel, J., Lipowicz, J.M., Rawłuszko-Wieczorek, A.A., Korbecki, J., and Januchowski, R. (2025) 'The Role of Elacridar, a P-Gp Inhibitor, in the Re-Sensitization of PAC-Resistant Ovarian Cancer Cell Lines to Cytotoxic Drugs in 2D and 3D Cell Culture Models'. *International Journal of Molecular Sciences* 2025, Vol. 26, Page 1124 [online] 26 (3), 1124.

available from <<https://www.mdpi.com/1422-0067/26/3/1124/htm>> [17 August 2025]

Su, J., Chen, F., Cryns, V.L., and Messersmith, P.B. (2011) 'Catechol Polymers for PH-Responsive, Targeted Drug Delivery to Cancer Cells'. *Journal of the American Chemical Society* [online] 133 (31), 11850–11853. available from <<https://pubs.acs.org/doi/full/10.1021/ja203077x>> [17 November 2021]

Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., and Bray, F. (2021) 'Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries'. *CA: A Cancer Journal for Clinicians* 71 (3), 209–249

Takemura, G. and Fujiwara, H. (2007) 'Doxorubicin-Induced Cardiomyopathy: From the Cardiotoxic Mechanisms to Management'. *Progress in Cardiovascular Diseases* 49 (5), 330–352

Tan, P.H., Ellis, I., Allison, K., Brogi, E., Fox, S.B., Lakhani, S., Lazar, A.J., Morris, E.A., Sahin, A., Salgado, R., Sapino, A., Sasano, H., Schnitt, S., Sotiriou, C., van Diest, P., White, V.A., Lokuhetty, D., and Cree, I.A. (2020) 'The 2019 World Health Organization Classification of Tumours of the Breast'. *Histopathology* [online] 77 (2), 181–185. available from </doi/pdf/10.1111/his.14091> [29 August 2025]

Tchoryk, A., Taresco, V., Argent, R.H., Ashford, M., Gellert, P.R., Stolnik, S., Grabowska, A., and Garnett, M.C. (2019) 'Penetration and Uptake of

Nanoparticles in 3D Tumor Spheroids'. *Bioconjugate Chemistry* 30 (5), 1371–1384

Thorn, C.F., Oshiro, C., Marsh, S., Hernandez-Boussard, T., McLeod, H., Klein, T.E., and Altman, R.B. (2011) 'Doxorubicin Pathways: Pharmacodynamics and Adverse Effects'. *Pharmacogenetics and Genomics* [online] 21 (7), 440. available from </pmc/articles/PMC3116111/> [26 November 2021]

Tian, H., Ma, D., Tan, X., Yan, W., Wu, X., He, C., Zhong, L., Zhang, Yan, Yu, B., Zhang, Yi, and Qi, X. (2021) 'Platinum and Taxane Based Adjuvant and Neoadjuvant Chemotherapy in Early Triple-Negative Breast Cancer: A Narrative Review'. *Frontiers in Pharmacology* [online] 12, 770663. available from <www.frontiersin.org> [29 August 2025]

Tian, Y., Yongrong, L.E.I., Wang, Y., Jiejuan, L.A.I., Wang, J., and Feng, X.I.A. (2023) 'Mechanism of Multidrug Resistance to Chemotherapy Mediated by P-Glycoprotein (Review)'. *International Journal of Oncology* [online] 63 (5), 119. available from <https://pmc.ncbi.nlm.nih.gov/articles/PMC10546381/> [20 August 2025]

Venditti, I. (2019) 'Morphologies and Functionalities of Polymeric Nanocarriers as Chemical Tools for Drug Delivery: A Review'. *Journal of King Saud University - Science* 31 (3), 398–411

Vinci, M., Gowan, S., Boxall, F., Patterson, L., Zimmermann, M., Court, W., Lomas, C., Mendiola, M., Hardisson, D., and Eccles, S.A. (2012) 'Advances

in Establishment and Analysis of Three-Dimensional Tumor Spheroid-Based Functional Assays for Target Validation and Drug Evaluation'. *BMC Biology* [online] 10 (1), 1–21. available from <<https://bmcbiol.biomedcentral.com/articles/10.1186/1741-7007-10-29>> [21 August 2025]

Wang, B., Soltero, R.A., and Saihaan, T. (2005) *Drug Delivery: Principles and Applications* - Binghe Wang, Teruna J. Siahaan, Richard A. Soltero - Google Books [online] available from <[https://books.google.co.uk/books?hl=en&lr=&id=HbSeX8BpVmkC&oi=fnd&pg=PA1&dq=Wang,++B.,++Siahaan,++T.++%26++Soltero,++R.++A.+Drug++delivery+Principles++and++applications.++1st++edition+edn,++\(John+Wiley%26Sons,+2005\).&ots=TJhk8Akg7s&sig=9PTaEWIPf6nedXFPZYGHJ0NaHII&redir_esc=y#v=onepage&q&f=false](https://books.google.co.uk/books?hl=en&lr=&id=HbSeX8BpVmkC&oi=fnd&pg=PA1&dq=Wang,++B.,++Siahaan,++T.++%26++Soltero,++R.++A.+Drug++delivery+Principles++and++applications.++1st++edition+edn,++(John+Wiley%26Sons,+2005).&ots=TJhk8Akg7s&sig=9PTaEWIPf6nedXFPZYGHJ0NaHII&redir_esc=y#v=onepage&q&f=false)> [27 September 2021]

Wang, C., Qi, P., Lu, Y., Liu, L., Zhang, Y., Sheng, Q., Wang, T., Zhang, M., Wang, R., and Song, S. (2020) 'Bicomponent Polymeric Micelles for PH-Controlled Delivery of Doxorubicin'. *Drug Delivery* [online] 27 (1), 344–357. available from <<https://pubmed.ncbi.nlm.nih.gov/32090637/>> [17 August 2025]

Wei, L., Kim, S.H., Armaly, A.M., Aubé, J., Xu, L., and Wu, X. (2024) 'HuR Inhibition Overcomes CFLIP-Mediated Doxorubicin Resistance in Triple-Negative Breast Cancer'. *Npj Precision Oncology* 2024 8:1 [online] 8 (1), 1–12. available from <<https://www.nature.com/articles/s41698-024-00780-x>> [4 July 2025]

- Wu, J., Lin, N., Li, F., Zhang, G., He, S., Zhu, Y., Ou, R., Li, N., Liu, S., Feng, L., Liu, L., Liu, Z., and Lu, L. (2016) 'Induction of P-Glycoprotein Expression and Activity by Aconitum Alkaloids: Implication for Clinical Drug-Drug Interactions'. *Scientific Reports* [online] 6. available from <<https://pubmed.ncbi.nlm.nih.gov/27139035/>> [17 August 2025]
- Xiong, N., Wu, H., and Yu, Z. (2024) 'Advancements and Challenges in Triple-Negative Breast Cancer: A Comprehensive Review of Therapeutic and Diagnostic Strategies'. *Frontiers in Oncology* 14, 1405491
- Xuan, L., Ju, Z., Skonieczna, M., Zhou, P.K., and Huang, R. (2023) 'Nanoparticles-induced Potential Toxicity on Human Health: Applications, Toxicity Mechanisms, and Evaluation Models'. *MedComm* [online] 4 (4), e327. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC10349198/>> [16 August 2025]
- Yan, J., Ye, Z., Chen, M., Liu, Z., Xiao, Y., Zhang, Y., Zhou, Y., Tan, W., and Lang, M. (2011) 'Fine Tuning Micellar Core-Forming Block of Poly(Ethylene Glycol)-Block-Poly(ϵ -Caprolactone) Amphiphilic Copolymers Based on Chemical Modification for the Solubilization and Delivery of Doxorubicin'. *Biomacromolecules* [online] 12 (7), 2562–2572. available from <<https://pubs-acsc.org.nottingham.idm.oclc.org/doi/full/10.1021/bm200375x>> [7 November 2021]

- Yersal, O. and Barutca, S. (2014) 'Biological Subtypes of Breast Cancer: Prognostic and Therapeutic Implications'. *World Journal of Clinical Oncology* [online] 5 (3), 412–424. available from <<https://pubmed.ncbi.nlm.nih.gov/25114856/>> [25 August 2025]
- Yin, L., Duan, J.J., Bian, X.W., and Yu, S.C. (2020) 'Triple-Negative Breast Cancer Molecular Subtyping and Treatment Progress'. in *Breast Cancer Research*. vol. 22 (1). BioMed Central Ltd
- Yingyuad, P., Mével, M., Prata, C., Kontogiorgis, C., Thanou, M., and Miller, A.D. (2014) 'Enzyme-Triggered PEGylated SiRNA-Nanoparticles for Controlled Release of SiRNA'. *Journal of RNAi and Gene Silencing : An International Journal of RNA and Gene Targeting Research* [online] 10, 490. available from <pmc/articles/PMC3983657/> [27 September 2021]
- Yvon, A.M.C., Wadsworth, P., and Jordan, M.A. (1999) 'Taxol Suppresses Dynamics of Individual Microtubules in Living Human Tumor Cells'. *Molecular Biology of the Cell* [online] 10 (4), 947–959. available from <<https://www-molbiolcell-org.nottingham.idm.oclc.org/doi/abs/10.1091/mbc.10.4.947>> [24 November 2021]
- van der Zanden, S.Y., Qiao, X., and Neefjes, J. (2021) 'New Insights into the Activities and Toxicities of the Old Anticancer Drug Doxorubicin'. *FEBS Journal* [online] 288 (21), 6095–6111. available from <doi/pdf/10.1111/febs.15583> [29 August 2025]

- Zauner, W., Farrow, N.A., and Haines, A.M.R. (2001) 'In Vitro Uptake of Polystyrene Microspheres: Effect of Particle Size, Cell Line and Cell Density'. *Journal of Controlled Release : Official Journal of the Controlled Release Society* [online] 71 (1), 39–51. available from <<https://pubmed-ncbi-nlm-nih-gov.nottingham.idm.oclc.org/11245907/>> [22 November 2021]
- Zeng, W., Luo, Y., Gan, D., Zhang, Y., Deng, H., and Liu, G. (2023) 'Advances in Doxorubicin-Based Nano-Drug Delivery System in Triple Negative Breast Cancer'. *Frontiers in Bioengineering and Biotechnology* 11, 1271420
- Zhai, Y., Zhou, X., Zhang, Z., Zhang, L., Wang, D., Wang, X., and Sun, W. (2018) *Polymers Design, Synthesis, and Characterization of Schiff Base Bond-Linked PH-Responsive Doxorubicin Prodrug Based on Functionalized MPEG-PCL for Targeted Cancer Therapy*. [online] available from <www.mdpi.com/journal/polymers> [22 April 2025]
- Zhang, J., Wang, Z., Hu, X., Wang, B., Wang, L., Yang, W., Liu, Y., Liu, G., Di, G., Hu, Z., Wu, J., and Shao, Z. (2015) 'Cisplatin and Gemcitabine as the First Line Therapy in Metastatic Triple Negative Breast Cancer'. *International Journal of Cancer* [online] 136 (1), 204–211. available from <<https://pubmed-ncbi-nlm-nih-gov.nottingham.idm.oclc.org/24824628/>> [25 November 2021]
- Zhang, N., Yin, Y., Xu, S.J., and Chen, W.S. (2008) '5-Fluorouracil: Mechanisms of Resistance and Reversal Strategies'. *Molecules* [online] 13 (8), 1551. available from <[pmc/articles/PMC6244944/](https://pubmed-ncbi-nlm-nih-gov.nottingham.idm.oclc.org/186244944/)> [25 November 2021]

Zhang, Z., Wang, J., Nie, X., Wen, T., Ji, Y., Wu, X., Zhao, Y., and Chen, C. (2014)

‘Near Infrared Laser-Induced Targeted Cancer Therapy Using Thermoresponsive Polymer Encapsulated Gold Nanorods’. *Journal of the American Chemical Society* [online] 136 (20), 7317–7326. available from <<https://pubs-acsc.org.nottingham.idm.oclc.org/doi/abs/10.1021/ja412735p>> [16 November 2021]

Zhitomirsky, B., Assaraf, Y.G., Zhitomirsky, B., and Assaraf, Y.G. (2014)

‘Lysosomal Sequestration of Hydrophobic Weak Base Chemotherapeutics Triggers Lysosomal Biogenesis and Lysosome-Dependent Cancer Multidrug Resistance’. *Oncotarget* [online] 6 (2), 1143–1156. available from <<https://www.oncotarget.com/article/2732/text/>> [3 July 2025]

Zhou, Z., Li, L., Yang, Y., Xu, X., and Huang, Y. (2014) ‘Tumor Targeting by PH-

Sensitive, Biodegradable, Cross-Linked N-(2-Hydroxypropyl) Methacrylamide Copolymer Micelles’. *Biomaterials* 35 (24), 6622–6635

Abelman, R.O., McLaughlin, S., Fell, G.G., Garrido-Castro, A.C., Lynce, F.,

Trippa, L., Poorvu, P.D., Rosenstock, A.S., Medford, A.J., Wander, S.A.,

Comander, A.H., Isakoff, S.J., Moy, B., Tung, N.M., Mittendorf, E.A.,

Ellisen, L., Tolaney, S.M., Bardia, A., and Spring, L. (2025) ‘A Phase 2

Study of Response-Guided Neoadjuvant Sacituzumab Govitecan and

Pembrolizumab (SG/P) in Patients with Early-Stage Triple-Negative

Breast Cancer: Results from the NeoSTAR Trial.’ *Journal of Clinical*

Oncology [online] 43 (16_suppl), 511–511. available from
<https://ascopubs.org/doi/pdf/10.1200/JCO.2025.43.16_suppl.511> [29
August 2025]

Agha Zeeshan Mirza and Farhan Ahmed Siddiqui (2014) ‘Nanomedicine and
Drug Delivery: A Mini Review’. *International Nano Letter*

Ahmad, Z., Shah, A., Siddiq, M., and Kraatz, H.B. (2014) ‘Polymeric Micelles as
Drug Delivery Vehicles’. *RSC Advances* [online] 4 (33), 17028–17038.
available from <[https://pubs-rsc-
org.nottingham.idm.oclc.org/en/content/articlehtml/2014/ra/c3ra47370](https://pubs-rsc-org.nottingham.idm.oclc.org/en/content/articlehtml/2014/ra/c3ra47370)
h> [9 November 2021]

Ambudkar, S. V., Kimchi-Sarfaty, C., Sauna, Z.E., and Gottesman, M.M. (2003)
‘P-Glycoprotein: From Genomics to Mechanism’. *Oncogene* [online] 22
(47 REV. ISS. 6), 7468–7485. available from
<<https://pubmed.ncbi.nlm.nih.gov/14576852/>> [17 August 2025]

Anderson, N.M. and Simon, M.C. (2020) ‘The Tumor Microenvironment’.
Current Biology [online] 30 (16), R921–R925. available from
<<https://pubmed.ncbi.nlm.nih.gov/32810447/>> [25 August 2025]

Arora, A., Seenu, V., Parshad, R., Bansal, V.K., Dhar, A., Mathur, S., Tanwar, P.,
Mishra, P., Kataria, K., Suhani, Gogia, A., Kumar, B., Haresh, K.P., Mallick,
S., Saini, S.K., Mishra, A., Bansal, B., Sharma, J., Saikia, J., Rangarajan, K.,
Dhamija, E., Prasad, C.P., Shamin, S.A., Agastm, S., Kalra, K., Vishvam, D.,
Mani, K., Kumar, A., Bakhshi, S., and Batra, A. (2025) ‘A Phase II,

Randomized, Open-Labeled Study to Evaluate Low-Dose Pembrolizumab in Addition to Neoadjuvant Chemotherapy for Triple-Negative Breast Cancer (TNBC)'. *Trials* [online] 26 (1). available from <<https://pubmed.ncbi.nlm.nih.gov/40011935/>> [29 August 2025]

Arroyo-Crespo, J.J., Armiñán, A., Charbonnier, D., Deladriere, C., Palomino-Schätzlein, M., Lamas-Domingo, R., Forteza, J., Pineda-Lucena, A., and Vicent, M.J. (2019) 'Characterization of Triple-negative Breast Cancer Preclinical Models Provides Functional Evidence of Metastatic Progression'. *International Journal of Cancer* [online] 145 (8), 2267–2281. available from <<https://onlinelibrary.wiley.com/doi/abs/10.1002/ijc.32270>> [24 March 2021]

Arroyo-Crespo, J.J., Armiñán, A., Charbonnier, D., Deladriere, C., Palomino-Schätzlein, M., Lamas-Domingo, R., Forteza, J., Pineda-Lucena, A., and Vicent, M.J. (2019) 'Characterization of Triple-Negative Breast Cancer Preclinical Models Provides Functional Evidence of Metastatic Progression'. *International Journal of Cancer* 145 (8), 2267–2281

Ashique, S., Garg, A., Hussain, A., Farid, A., Kumar, P., and Taghizadeh-Hesary, F. (2023) 'Nanodelivery Systems: An Efficient and Target-Specific Approach for Drug-Resistant Cancers'. *Cancer Medicine* [online] 12 (18), 18797–18825. available from <[doi/pdf/10.1002/cam4.6502](https://doi.org/10.1002/cam4.6502)> [29 August 2025]

- Bai, X., Ni, J., Beretov, J., Graham, P., and Li, Y. (2021) 'Triple-Negative Breast Cancer Therapeutic Resistance: Where Is the Achilles' Heel?' *Cancer Letters* [online] 497, 100–111. available from <<https://www.sciencedirect.com/science/article/abs/pii/S0304383520305425>> [29 August 2025]
- Bajpai, A.K., Shukla, S.K., Bhanu, S., and Kankane, S. (2008) 'Responsive Polymers in Controlled Drug Delivery'. *Progress in Polymer Science* 33 (11), 1088–1118
- Bansal, K., Sasso, L., Makwana, H., Awwad, S., Brocchini, S., and Alexander, C. (2016) 'Nanopharmacy: Exploratory Methods for Polymeric Materials'. *Pharmaceutical Nanotechnology: Innovation and Production* [online] 231–270. available from <https://www.researchgate.net/publication/311661945_Nanopharmacy_Exploratory_Methods_for_Polymeric_Materials_Innovation_and_Production> [9 November 2021]
- Bao, L., Hazari, S., Mehra, S., Kaushal, D., Moroz, K., and Dash, S. (2012) 'Increased Expression of P-Glycoprotein and Doxorubicin Chemoresistance of Metastatic Breast Cancer Is Regulated by MiR-298'. *The American Journal of Pathology* [online] 180 (6), 2490. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC3378910/>> [16 August 2025]
- Bauer, J.A., Chakravarthy, A.B., Rosenbluth, J.M., Mi, D., Seeley, E.H., Granja-Ingram, N.D.M., Olivares, M.G., Kelley, M.C., Mayer, I.A., Meszoely, I.M., Means-Powell, J.A., Johnson, K.N., Tsai, C.J., Ayers, G.D., Sanders, M.E.,

- Schneider, R.J., Formenti, S.C., Caprioli, R.M., and Pietenpol, J.A. (2010) 'Identification of Markers of Taxane Sensitivity Using Proteomic and Genomic Analyses of Breast Tumors from Patients Receiving Neoadjuvant Paclitaxel and Radiation'. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research* [online] 16 (2), 681–690. available from <<https://pubmed-ncbi-nlm-nih-gov.nottingham.idm.oclc.org/20068102/>> [25 November 2021]
- Belfayol-Pisanté, L., Guillevin, L., Tod, M., and Fauvelle, F. (2000) 'Pharmacokinetics of Cyclophosphamide (CP) and 4-OH-CP/Aldophosphamide in Systemic Vasculitis'. *Fundamental & Clinical Pharmacology* [online] 14 (4), 415–421. available from <<https://pubmed-ncbi-nlm-nih-gov.nottingham.idm.oclc.org/11030450/>> [25 November 2021]
- Belger, C., Abrahams, C., Imamdin, A., and Lecour, S. (2023) 'Doxorubicin-Induced Cardiotoxicity and Risk Factors'. *International Journal of Cardiology. Heart & Vasculature* [online] 50, 101332. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC10784684/>> [28 August 2025]
- Bloom, H.J. and Richardson, W.W. (1957) 'Histological Grading and Prognosis in Breast Cancer a Study of 1409 Cases of Which 359 Have Been Followed for 15 Years'. *British Journal of Cancer* [online] 11 (3), 359–377. available from <<https://www.nature.com/articles/bjc195743>> [29 August 2025]

- Carrillo-Castillo, T.D., Castro-Carmona, J.S., Luna-Velasco, A., and Zaragoza-Contreras, E.A. (2020) 'PH-Responsive Polymer Micelles for Methotrexate Delivery at Tumor Microenvironments'. *E-Polymers* 20 (1), 624–635
- Carvalho, E., Canberk, S., Schmitt, F., and Vale, N. (2025) 'Molecular Subtypes and Mechanisms of Breast Cancer: Precision Medicine Approaches for Targeted Therapies'. *Cancers* 2025, Vol. 17, Page 1102 [online] 17 (7), 1102. available from <<https://www.mdpi.com/2072-6694/17/7/1102/htm>> [29 August 2025]
- Chalakur-Ramireddy, N.K.R. and Pakala, S.B. (2018) 'Combined Drug Therapeutic Strategies for the Effective Treatment of Triple Negative Breast Cancer'. *Bioscience Reports* [online] 38 (1), BSR20171357. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC5789156/>> [28 August 2025]
- Chanmugam, A., Langemo, D., Thomason, K., Haan, J., Altenburger, E.A., Tippet, A., Henderson, L., and Zortman, T.A. (2017) 'Relative Temperature Maximum in Wound Infection and Inflammation as Compared with a Control Subject Using Long-Wave Infrared Thermography'. *Advances in Skin and Wound Care* [online] 30 (9), 406–414. available from <https://journals-lww-com.nottingham.idm.oclc.org/aswcjournal/Fulltext/2017/09000/Relative_Temperature_Maximum_in_Wound_Infection.4.aspx> [16 November 2021]

- Chatterjee, K., Zhang, J., Honbo, N., and Karliner, J.S. (2010) 'Doxorubicin Cardiomyopathy'. *Cardiology* [online] 115 (2), 155. available from </pmc/articles/PMC2848530/> [26 November 2021]
- Che, J., Pan, L., Yang, X., Liu, Z., Huang, L., Wen, C., Lin, A., and Liu, H. (2017) 'Thymidine Phosphorylase Expression and Prognosis in Colorectal Cancer Treated with 5-Fluorouracil-Based Chemotherapy: A Meta-Analysis'. *Molecular and Clinical Oncology* [online] 7 (6), 943. available from </pmc/articles/PMC5740914/> [25 November 2021]
- Chen, H., Shien, K., Suzawa, K., Tsukuda, K., Tomida, S., Sato, H., Torigoe, H., Watanabe, M., Namba, K., Yamamoto, H., Soh, J., Asano, H., Miyoshi, S., and Toyooka, S. (2017) 'Elacridar, a Third-Generation ABCB1 Inhibitor, Overcomes Resistance to Docetaxel in Non-Small Cell Lung Cancer'. *Oncology Letters* [online] 14 (4), 4349. available from <https://pmc.ncbi.nlm.nih.gov/articles/PMC5607652/> [5 July 2025]
- Chen, H., Wu, J., Zhang, Z., Tang, Y., Li, Xiaoxuan, Liu, S., Cao, S., and Li, Xianzhu (2018) 'Association between BRCA Status and Triple-Negative Breast Cancer: A Meta-Analysis'. *Frontiers in Pharmacology* [online] 9 (AUG). available from <https://pubmed.ncbi.nlm.nih.gov/30186165/> [25 August 2025]
- Chen, W.L., Li, F., Tang, Y., Yang, S. Di, Li, J.Z., Yuan, Z.Q., Liu, Y., Zhou, X.F., Liu, C., and Zhang, X.N. (2017a) 'Stepwise PH-Responsive Nanoparticles for Enhanced Cellular Uptake and on-Demand Intracellular Release of Doxorubicin'. *International Journal of Nanomedicine* [online] 12, 4241.

available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC5473598/>>

[17 August 2025]

Chen, W.L., Li, F., Tang, Y., Yang, S. Di, Li, J.Z., Yuan, Z.Q., Liu, Y., Zhou, X.F., Liu, C., and Zhang, X.N. (2017b) 'Stepwise PH-Responsive Nanoparticles for Enhanced Cellular Uptake and on-Demand Intracellular Release of Doxorubicin'. *International Journal of Nanomedicine* [online] 12, 4241.

available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC5473598/>>

[28 August 2025]

Cheng, H., Liao, J., Ma, Y., Sarwar, M.T., and Yang, H. (2025) 'Advances in Targeted Therapy for Tumor with Nanocarriers: A Review'. *Materials Today Bio* [online] 31, 101583. available from <<https://www.sciencedirect.com/science/article/pii/S2590006425001413>> [13 August 2025]

Chintala, L., Vaka, S., Baranda, J., and Williamson, S.K. (2011) 'Capecitabine versus 5-Fluorouracil in Colorectal Cancer: Where Are We Now?' *Oncology Reviews* [online] 5 (2), 129–140. available from <<https://link-springer-com.nottingham.idm.oclc.org/article/10.1007/s12156-011-0074-3>> [26 November 2021]

Chittasupho, C., Anuchapreeda, S., and Sarisuta, N. (2017) 'CXCR4 Targeted Dendrimer for Anti-Cancer Drug Delivery and Breast Cancer Cell Migration Inhibition'. *European Journal of Pharmaceutics and Biopharmaceutics* 119, 310–321

- Cho, Y.S., Shin, C.H., and Han, S. (2016) 'Dispersion Polymerization of Polystyrene Particles Using Alcohol as Reaction Medium'. *Nanoscale Research Letters* [online] 11 (1), 1–9. available from <<https://nanoscalereslett.springeropen.com/articles/10.1186/s11671-016-1261-8>> [22 November 2021]
- Cserni, G. (2020) 'Histological Type and Typing of Breast Carcinomas and the WHO Classification Changes over Time'. *Pathologica* [online] 112 (1), 25. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC8138497/>> [29 August 2025]
- Cuong, N. Van, Jiang, J.L., Li, Y.L., Chen, J.R., Jwo, S.C., and Hsieh, M.F. (2010) 'Doxorubicin-Loaded PEG-PCL-PEG Micelle Using Xenograft Model of Nude Mice: Effect of Multiple Administration of Micelle on the Suppression of Human Breast Cancer'. *Cancers* [online] 3 (1), 61. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC3756349/>> [16 August 2025]
- Danhier, F., Ansorena, E., Silva, J.M., Coco, R., Le Breton, A., and Pr  at, V. (2012) 'PLGA-Based Nanoparticles: An Overview of Biomedical Applications'. *Journal of Controlled Release* [online] 161 (2), 505–522. available from <<https://www.sciencedirect.com/science/article/pii/S0168365912000752>> [26 August 2025]
- Dong, X., Mattingly, C.A., Tseng, M.T., Cho, M.J., Liu, Y., Adams, V.R., and Mumper, R.J. (2009) 'Doxorubicin and Paclitaxel-Loaded Lipid-Based

Nanoparticles Overcome Multi-Drug Resistance by Inhibiting P-Gp and Depleting ATP'. *Cancer Research* [online] 69 (9), 3918. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC2758164/>> [16 August 2025]

Duncan, R. and Gaspar, R. (2011) 'Nanomedicine(s) under the Microscope'. *Molecular Pharmaceutics* [online] 8 (6), 2101–2141. available from <<https://pubs-acsc.org.nottingham.idm.oclc.org/doi/abs/10.1021/mp200394t>> [27 September 2021]

Edmondson, R., Broglie, J.J., Adcock, A.F., and Yang, L. (2014) 'Three-Dimensional Cell Culture Systems and Their Applications in Drug Discovery and Cell-Based Biosensors'. *Https://Home.Liebertpub.Com/Adt* [online] 12 (4), 207–218. available from <doi/pdf/10.1089/adt.2014.573?download=true> [23 July 2025]

Engel, C., Rhiem, K., Hahnen, E., Loibl, S., Weber, K.E., Seiler, S., Zachariae, S., Hauke, J., Wappenschmidt, B., Waha, A., Blümcke, B., Kiechle, M., Meindl, A., Niederacher, D., Bartram, C.R., Speiser, D., Schlegelberger, B., Arnold, N., Wieacker, P., Leinert, E., Gehrig, A., Briest, S., Kast, K., Riess, O., Emons, G., Weber, B.H.F., Engel, J., and Schmutzler, R.K. (2018) 'Prevalence of Pathogenic BRCA1/2 Germline Mutations among 802 Women with Unilateral Triple-Negative Breast Cancer without Family Cancer History'. *BMC Cancer* [online] 18 (1). available from <<https://pubmed.ncbi.nlm.nih.gov/29514593/>> [25 August 2025]

- Estrella, V., Chen, T., Lloyd, M., Wojtkowiak, J., Cornnell, H.H., Ibrahim-Hashim, A., Bailey, K., Balagurunathan, Y., Rothberg, J.M., Sloane, B.F., Johnson, J., Gatenby, R.A., and Gillies, R.J. (2013) 'Acidity Generated by the Tumor Microenvironment Drives Local Invasion'. *Cancer Research* [online] 73 (5), 1524–1535. available from </cancerres/article/73/5/1524/586608/Acidity-Generated-by-the-Tumor-Microenvironment> [25 August 2025]
- Etrych, T., Chytil, P., Jelínková, M., Íhová, B.R.ˇ, and Ulbrich, K. (2002) 'Synthesis of HPMA Copolymers Containing Doxorubicin Bound via a Hydrazone Linkage. Effect of Spacer on Drug Release and in Vitro Cytotoxicity'. *Macromol. Biosci* 2, 43–52
- Famta, P., Shah, S., Chatterjee, E., Singh, H., Dey, B., Guru, S.K., Singh, S.B., and Srivastava, S. (2021) 'Exploring New Horizons in Overcoming P-Glycoprotein-Mediated Multidrug-Resistant Breast Cancer via Nanoscale Drug Delivery Platforms'. *Current Research in Pharmacology and Drug Discovery* [online] 2, 100054. available from <https://www.sciencedirect.com/science/article/pii/S2590257121000419> [22 August 2025]
- Frangou, E.A., Bertsias, G., and Boumpas, D.T. (2016) 'Cytotoxic-Immunosuppressive Drug Treatment'. *Systemic Lupus Erythematosus: Basic, Applied and Clinical Aspects* 533–541
- Gabizon, A.A., Lyass, O., Berry, G.J., and Wildgust, M. (2004) 'Cardiac Safety of Pegylated Liposomal Doxorubicin (Doxil®/Caelyx®) Demonstrated by

Endomyocardial Biopsy in Patients with Advanced Malignancies’.

Https://Doi-Org.Nottingham.Idm.Oclc.Org/10.1081/CNV-200032899

[online] 22 (5), 663–669. available from <<https://www-tandfonline-com.nottingham.idm.oclc.org/doi/abs/10.1081/CNV-200032899>> [26

November 2021]

Gaspar, R. and Duncan, R. (2009) ‘Polymeric Carriers: Preclinical Safety and the Regulatory Implications for Design and Development of Polymer Therapeutics’. *Advanced Drug Delivery Reviews* 61 (13), 1220–1231

Gautam, S., Maurya, R., Vikal, A., Patel, P., Thakur, S., Singh, A., Gupta, G. Das, and Kurmi, B. Das (2025) ‘Understanding Drug Resistance in Breast Cancer: Mechanisms and Emerging Therapeutic Strategies’. *Medicine in Drug Discovery* [online] 26, 100210. available from <<https://www.sciencedirect.com/science/article/pii/S2590098625000077>> [29 August 2025]

Gewirtz, D.A. (1999) ‘A Critical Evaluation of the Mechanisms of Action Proposed for the Antitumor Effects of the Anthracycline Antibiotics Adriamycin and Daunorubicin’. *Biochemical Pharmacology* [online] 57 (7), 727–741. available from <<https://pubmed-ncbi-nlm-nih-gov.nottingham.idm.oclc.org/10075079/>> [26 November 2021]

Grossen, P., Witzigmann, D., Sieber, S., and Huwyler, J. (2017) ‘PEG-PCL-Based Nanomedicines: A Biodegradable Drug Delivery System and Its Application’. *Journal of Controlled Release* [online] 260, 46–60. available from

<https://www.sciencedirect.com/science/article/abs/pii/S0168365917306004?utm_source=chatgpt.com> [16 August 2025]

Guo, Q. and Jiang, C. (2020) 'Delivery Strategies for Macromolecular Drugs in Cancer Therapy'. *Acta Pharmaceutica Sinica B* 10 (6), 979–986

Guo, X., Cheng, Y., Zhao, X., Luo, Y., Chen, J., and Yuan, W.E. (2018) 'Advances in Redox-Responsive Drug Delivery Systems of Tumor Microenvironment'. *Journal of Nanobiotechnology* [online] 16 (1), 1–10. available from

<<https://jnanobiotechnology.biomedcentral.com/articles/10.1186/s12951-018-0398-2>> [18 November 2021]

Hao, Q., Dai, L., Chang, L., Song, D., Liu, D., Ma, X., Wu, H., and Kang, H. (2024) 'Evaluation of Neoadjuvant Chemotherapy for Clinical T1 Triple-Negative Breast Cancer'. *Scientific Reports* [online] 14 (1), 1–11. available from <<https://www.nature.com/articles/s41598-024-68719-7>> [29 August 2025]

Heskins, M. and Guillet, J.E. (2006) 'Solution Properties of Poly(N-Isopropylacrylamide)'. *https://doi.org/10.1080/10601326808051910* [online] 2 (8), 1441–1455. available from <<https://www.tandfonline.com/doi/abs/10.1080/10601326808051910>> [17 November 2021]

Ho, A.Y., Shiao, S., Kobald, S.A., Chen, J., Duda, D.G., Ly, A., Bossuyt, V., Cho, H.L., Arnold, B., Knott, S., Gupta, G.P., McAndrew, P., Karlan, S.,

Tighiouart, M., Muzikansky, A., Basho, R., and McArthur, H. (2024)

‘PEARL: A Phase Ib/II Biomarker Study of Adding Radiation Therapy to Pembrolizumab before Neoadjuvant Chemotherapy in Human Epidermal Growth Factor Receptor 2-Negative Breast Cancer’. *Journal of Clinical Oncology* [online] 42 (36), 4282–4293. available from <<https://ascopubs.org/doi/10.1200/JCO.24.00003>> [29 August 2025]

Ho-Lun, W., Bendayan, R., Rauth, A.M., Hui, Y.X., Babakhanian, K., and Xiao,

Y.W. (2006) ‘A Mechanistic Study of Enhanced Doxorubicin Uptake and Retention in Multidrug Resistant Breast Cancer Cells Using a Polymer-Lipid Hybrid Nanoparticle System’. *The Journal of Pharmacology and Experimental Therapeutics* [online] 317 (3), 1372–1381. available from <<https://www.sciencedirect.com/science/article/abs/pii/S0022356524327429>> [22 August 2025]

Imamura, Y., Mukohara, T., Shimono, Y., Funakoshi, Y., Chayahara, N., Toyoda,

M., Kiyota, N., Takao, S., Kono, S., Nakatsura, T., and Minami, H. (2015) ‘Comparison of 2D- and 3D-Culture Models as Drug-Testing Platforms in Breast Cancer’. *Oncology Reports* [online] 33 (4), 1837–1843. available from <<https://pubmed.ncbi.nlm.nih.gov/25634491/>> [23 July 2025]

Jain, A., Barge, A., and Parris, C.N. (2024) ‘Combination Strategies with PARP

Inhibitors in BRCA-Mutated Triple-Negative Breast Cancer: Overcoming Resistance Mechanisms’. *Oncogene* [online] 44 (4), 193. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC11746151/>> [29 August 2025]

- Jeong, B., Bae, Y.H., and Kim, S.W. (2000) 'Drug Release from Biodegradable Injectable Thermosensitive Hydrogel of PEG–PLGA–PEG Triblock Copolymers'. *Journal of Controlled Release* 63 (1–2), 155–163
- Johnston, H.J., Semmler-Behnke, M., Brown, D.M., Kreyling, W., Tran, L., and Stone, V. (2010) 'Evaluating the Uptake and Intracellular Fate of Polystyrene Nanoparticles by Primary and Hepatocyte Cell Lines in Vitro'. *Toxicology and Applied Pharmacology* [online] 242 (1), 66–78. available from <<https://pubmed-ncbi-nlm-nih.gov/nottingham.idm.oclc.org/19799923/>> [22 November 2021]
- Juul, N., Szallasi, Z., Eklund, A.C., Li, Q., Burrell, R.A., Gerlinger, M., Valero, V., Andreopoulou, E., Esteva, F.J., Symmans, W.F., Desmedt, C., Haibe-Kains, B., Sotiriou, C., Pusztai, L., and Swanton, C. (2010) 'Assessment of an RNA Interference Screen-Derived Mitotic and Ceramide Pathway Metagene as a Predictor of Response to Neoadjuvant Paclitaxel for Primary Triple-Negative Breast Cancer: A Retrospective Analysis of Five Clinical Trials'. *The Lancet. Oncology* [online] 11 (4), 358–365. available from <<https://pubmed-ncbi-nlm-nih.gov/nottingham.idm.oclc.org/20189874/>> [25 November 2021]
- Kaurav, M., Ruhi, S., Al-Goshae, H.A., Jeppu, A.K., Ramachandran, D., Sahu, R.K., Sarkar, A.K., Khan, J., and Ashif Ikbal, A.M. (2023) 'Dendrimer: An Update on Recent Developments and Future Opportunities for the Brain Tumors Diagnosis and Treatment'. *Frontiers in Pharmacology* [online] 14, 1159131. available from

<<https://pmc.ncbi.nlm.nih.gov/articles/PMC10060650/>> [28 August 2025]

Keller, F., Rudolf, R., and Hafner, M. (2019) 'Towards Optimized Breast Cancer 3D Spheroid Mono-and Co-Culture Models for Pharmacological Research and Screening'. *Journal of Cellular Biotechnology* 5 (2), 89–101

Khan, M.Z.I., Prebeg, Ž., and Kurjaković, N. (1999) 'A PH-Dependent Colon Targeted Oral Drug Delivery System Using Methacrylic Acid Copolymers: I. Manipulation of Drug Release Using Eudragit® L100-55 and Eudragit® S100 Combinations'. *Journal of Controlled Release* 58 (2), 215–222

Khandare, J. and Minko, T. (2006) 'Polymer–Drug Conjugates: Progress in Polymeric Prodrugs'. *Progress in Polymer Science* 31 (4), 359–397

Kim, J., Harper, A., McCormack, V., Sung, H., Houssami, N., Morgan, E., Mutebi, M., Garvey, G., Soerjomataram, I., and Fidler-Benaoudia, M.M. (2025) 'Global Patterns and Trends in Breast Cancer Incidence and Mortality across 185 Countries'. *Nature Medicine* [online] 31 (4), 1154–1162. available from <<https://pubmed.ncbi.nlm.nih.gov/39994475/>> [29 August 2025]

Kuo, Y.M. and Barrett, J.S. (2024) 'Consideration of the Root Causes in Candidate Attrition During Oncology Drug Development'. *Clinical Pharmacology in Drug Development* [online] 13 (9), 952–960. available from </doi/pdf/10.1002/cpdd.1464> [22 August 2025]

- Kuppusamy, P., Li, H., Ilangoan, G., Cardounel, A.J., Zweier, J.L., Yamada, K., Krishna, M.C., and Mitchell, J.B. (2002) 'Noninvasive Imaging of Tumor Redox Status and Its Modification by Tissue Glutathione Levels'. *Cancer Research* 62 (1)
- Lai, J.I., Tseng, Y.J., Chen, M.H., Huang, C.Y.F., and Chang, P.M.H. (2020) 'Clinical Perspective of FDA Approved Drugs With P-Glycoprotein Inhibition Activities for Potential Cancer Therapeutics'. *Frontiers in Oncology* [online] 10, 561936. available from <www.frontiersin.org> [5 July 2025]
- Lee, C.M. and Tannock, I.F. (2006) 'Inhibition of Endosomal Sequestration of Basic Anticancer Drugs: Influence on Cytotoxicity and Tissue Penetration'. *British Journal of Cancer* [online] 94 (6), 863. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC2361369/>> [3 July 2025]
- Lehmann, B.D., Bauer, J.A., Chen, X., Sanders, M.E., Chakravarthy, A.B., Shyr, Y., and PiTENpol, J.A. (2011a) 'Identification of Human Triple-Negative Breast Cancer Subtypes and Preclinical Models for Selection of Targeted Therapies'. *Journal of Clinical Investigation* [online] 121 (7), 2750–2767. available from <<https://pubmed.ncbi.nlm.nih.gov/21633166/>> [29 August 2025]
- Lehmann, B.D., Bauer, J.A., Chen, X., Sanders, M.E., Chakravarthy, A.B., Shyr, Y., and PiTENpol, J.A. (2011b) 'Identification of Human Triple-Negative Breast Cancer Subtypes and Preclinical Models for Selection of Targeted Therapies'. *Journal of Clinical Investigation* 121 (7), 2750–2767

Lehmann, B.D., Colaprico, A., Silva, T.C., Chen, J., An, H., Ban, Y., Huang, H., Wang, L., James, J.L., Balko, J.M., Gonzalez-Ericsson, P.I., Sanders, M.E., Zhang, B., Pietenpol, J.A., and Chen, X.S. (2021) 'Multi-Omics Analysis Identifies Therapeutic Vulnerabilities in Triple-Negative Breast Cancer Subtypes'. *Nature Communications* 2021 12:1 [online] 12 (1), 1–18. available from <<https://www.nature.com/articles/s41467-021-26502-6>> [29 August 2025]

Lehmann, B.D., Jovanović, B., Chen, X., Estrada, M. V., Johnson, K.N., Shyr, Y., Moses, H.L., Sanders, M.E., and Pietenpol, J.A. (2016) 'Refinement of Triple-Negative Breast Cancer Molecular Subtypes: Implications for Neoadjuvant Chemotherapy Selection'. *PLoS ONE* [online] 11 (6). available from <<https://pubmed.ncbi.nlm.nih.gov/27310713/>> [25 August 2025]

Li, Qiao, Li, Qing, Zhang, P., Yuan, P., Wang, J., Ma, F., Luo, Y., Fan, Y., Cai, R., and Xu, B. (2015) 'A Phase II Study of Capecitabine plus Cisplatin in Metastatic Triple-Negative Breast Cancer Patients Pretreated with Anthracyclines and Taxanes'. *Cancer Biology & Therapy* [online] 16 (12), 1746–1753. available from <<https://pubmed-ncbi-nlm-nih-gov.nottingham.idm.oclc.org/26466918/>> [26 November 2021]

Lv, D., Hu, Z., Lu, L., Lu, H., and Xu, X. (2017) 'Three-Dimensional Cell Culture: A Powerful Tool in Tumor Research and Drug Discovery'. *Oncology Letters* [online] 14 (6), 6999–7010. available from <<https://pubmed.ncbi.nlm.nih.gov/29344128/>> [23 July 2025]

- Madaan, K., Kumar, S., Poonia, N., Lather, V., and Pandita, D. (2014)
'Dendrimers in Drug Delivery and Targeting: Drug-Dendrimer Interactions and Toxicity Issues'. *Journal of Pharmacy and Bioallied Sciences* [online] 6 (3), 139–150. available from
<https://journals.lww.com/jpbs/fulltext/2014/06030/dendrimers_in_drug_delivery_and_targeting_.1.aspx> [28 August 2025]
- Mahmoud, R., Ordóñez-Morán, P., and Allegrucci, C. (2022a) 'Challenges for Triple Negative Breast Cancer Treatment: Defeating Heterogeneity and Cancer Stemness'. *Cancers* [online] 14 (17). available from
<<https://pubmed.ncbi.nlm.nih.gov/36077812/>> [29 August 2025]
- Mahmoud, R., Ordóñez-Morán, P., and Allegrucci, C. (2022b) 'Challenges for Triple Negative Breast Cancer Treatment: Defeating Heterogeneity and Cancer Stemness'. *Cancers* [online] 14 (17). available from
<<https://pubmed.ncbi.nlm.nih.gov/36077812/>> [29 August 2025]
- Mahmoud, R., Ordóñez-Morán, P., and Allegrucci, C. (2022c) 'Challenges for Triple Negative Breast Cancer Treatment: Defeating Heterogeneity and Cancer Stemness'. *Cancers* [online] 14 (17). available from
<<https://pubmed.ncbi.nlm.nih.gov/36077812/>> [29 August 2025]
- Mahmoud, R., Ordóñez-Morán, P., and Allegrucci, C. (2022d) 'Challenges for Triple Negative Breast Cancer Treatment: Defeating Heterogeneity and Cancer Stemness'. *Cancers* [online] 14 (17). available from
<<https://pubmed.ncbi.nlm.nih.gov/36077812/>> [29 August 2025]

- Mansilla, E., Nunez, L., Marin, G.H., Rivera, L., Macor, P., and Luengas, S.P. (2019) 'New Advances in Chronic Lymphocytic Leukemia Treatment: Biodegradable ZnO Hybrid Cluster Nanoparticle as Antineoplastic Agents'. *Biomedical Applications of Nanoparticles* 409–430
- Matanović, M.R., Kristl, J., and Grabnar, P.A. (2014) 'Thermoresponsive Polymers: Insights into Decisive Hydrogel Characteristics, Mechanisms of Gelation, and Promising Biomedical Applications'. *International Journal of Pharmaceutics* 472 (1–2), 262–275
- Mcgowan, J. V, Chung, R., Maulik, A., Piotrowska, I., Malcolm Walker, & J., and Yellon, D.M. (2017) *Anthracycline Chemotherapy and Cardiotoxicity*.
- Meng, F., Cheng, R., Deng, C., and Zhong, Z. (2012) 'Intracellular Drug Release Nanosystems'. *Materials Today* 15 (10), 436–442
- Meng, F., Hennink, W.E., and Zhong, Z. (2009) 'Reduction-Sensitive Polymers and Bioconjugates for Biomedical Applications'. *Biomaterials* 30 (12), 2180–2198
- Mi, P. (2020) 'Stimuli-Responsive Nanocarriers for Drug Delivery, Tumor Imaging, Therapy and Theranostics'. *Theranostics* [online] 10 (10), 4557. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC7150471/>> [28 August 2025]
- von Minckwitz, G., Schneeweiss, A., Loibl, S., Salat, C., Denkert, C., Rezai, M., Blohmer, J.U., Jackisch, C., Paepke, S., Gerber, B., Zahm, D.M., Kümmel, S., Eidtmann, H., Klare, P., Huober, J., Costa, S., Tesch, H., Hanusch, C.,

Hilfrich, J., Khandan, F., Fasching, P.A., Sinn, B. v., Engels, K., Mehta, K., Nekljudova, V., and Untch, M. (2014) 'Neoadjuvant Carboplatin in Patients with Triple-Negative and HER2-Positive Early Breast Cancer (GeparSixto; GBG 66): A Randomised Phase 2 Trial'. *The Lancet. Oncology* [online] 15 (7), 747–756. available from <<https://pubmed-ncbi-nlm-nih-gov.nottingham.idm.oclc.org/24794243/>> [25 November 2021]

Mirzaei, S., Gholami, M.H., Hashemi, F., Zabolian, A., Farahani, M.V., Hushmandi, K., Zarrabi, A., Goldman, A., Ashrafizadeh, M., and Orive, G. (2022) 'Advances in Understanding the Role of P-Gp in Doxorubicin Resistance: Molecular Pathways, Therapeutic Strategies, and Prospects'. *Drug Discovery Today* [online] 27 (2), 436–455. available from <https://www.sciencedirect.com/science/article/pii/S1359644621004281?utm_source=chatgpt.com> [16 August 2025]

Mu, X., Gan, S., Wang, Y., Li, H., and Zhou, G. (2019) 'Stimulus-Responsive Vesicular Polymer Nano-Integrators for Drug and Gene Delivery.' *International Journal of Nanomedicine* [online] 14, 5415–5434. available from <<https://europepmc.org/articles/PMC6645615>> [8 November 2021]

Mura, S., Nicolas, J., and Couvreur, P. (2013) 'Stimuli-Responsive Nanocarriers for Drug Delivery'. *Nature Materials* 2013 12:11 [online] 12 (11), 991–1003. available from <<https://www.nature.com/articles/nmat3776>> [18 November 2021]

- Nakatsukasa, K., Koyama, H., Oouchi, Y., Imanishi, S., Mizuta, N., Sakaguchi, K., Fujita, Y., Fujiwara, I., Kotani, T., Matsuda, T., Fukuda, K., Morita, M., Kawakami, S., Kadotani, Y., Konishi, E., Yanagisawa, A., and Taguchi, T. (2017) 'Docetaxel and Cyclophosphamide as Neoadjuvant Chemotherapy in HER2-Negative Primary Breast Cancer'. *Breast Cancer (Tokyo, Japan)* [online] 24 (1), 63–68. available from <<https://pubmed-ncbi-nlm-nih.gov/nottingham.idm.oclc.org/26754092/>> [25 November 2021]
- Nakayama, M., Okano, T., Miyazaki, T., Kohori, F., Sakai, K., and Yokoyama, M. (2006) 'Molecular Design of Biodegradable Polymeric Micelles for Temperature-Responsive Drug Release'. *Journal of Controlled Release* 115 (1), 46–56
- Nath, S. and Devi, G.R. (2016) 'Three-Dimensional Culture Systems in Cancer Research: Focus on Tumor Spheroid Model'. *Pharmacology and Therapeutics* [online] 163, 94–108. available from <<https://pubmed.ncbi.nlm.nih.gov/27063403/>> [23 July 2025]
- Nayanathara, U., Rossi Herling, B., Ansari, N., Zhang, C., Logan, S.R., Beach, M.A., Smith, S.A., Boase, N.R.B., Johnston, A.P.R., and Such, G.K. (2023) 'Impact of the Core Architecture of Dual PH-Responsive Polymer Nanoparticles on Intracellular Delivery of Doxorubicin'. *ACS Applied Nano Materials* [online] 6 (12), 10015–10022. available from <doi/pdf/10.1021/acsanm.3c01598?ref=article_openPDF> [17 August 2025]

- Nedeljković, M. and Damjanović, A. (2019a) 'Mechanisms of Chemotherapy Resistance in Triple-Negative Breast Cancer-How We Can Rise to the Challenge'. *Cells* [online] 8 (9). available from <<https://pubmed.ncbi.nlm.nih.gov/31443516/>> [29 August 2025]
- Nedeljković, M. and Damjanović, A. (2019b) 'Mechanisms of Chemotherapy Resistance in Triple-Negative Breast Cancer—How We Can Rise to the Challenge'. *Cells* 2019, Vol. 8, Page 957 [online] 8 (9), 957. available from <<https://www.mdpi.com/2073-4409/8/9/957/htm>> [29 August 2025]
- Nedeljković, M. and Damjanović, A. (2019c) 'Mechanisms of Chemotherapy Resistance in Triple-Negative Breast Cancer—How We Can Rise to the Challenge'. *Cells* 2019, Vol. 8, Page 957 [online] 8 (9), 957. available from <<https://www.mdpi.com/2073-4409/8/9/957/htm>> [29 August 2025]
- Nedeljković, M. and Damjanović, A. (2019d) 'Mechanisms of Chemotherapy Resistance in Triple-Negative Breast Cancer—How We Can Rise to the Challenge'. *Cells* [online] 8 (9), 957. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC6770896/>> [3 July 2025]
- Nogueira, J., Soares, S.F., Amorim, C.O., Amaral, J.S., Silva, C., Martel, F., Trindade, T., and Daniel-Da-Silva, A.L. (2020) 'Magnetic Driven Nanocarriers for PH-Responsive Doxorubicin Release in Cancer Therapy'. *Molecules* [online] 25 (2), 333. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC7024164/>> [22 August 2025]

- Noriega-Luna, B., Godínez, L.A., Rodríguez, F.J., Rodríguez, A., Zaldívar-Lelo De Larrea, G., Sosa-Ferreya, C.F., Mercado-Curiel, R.F., Manríquez, J., and Bustos, E. (2014) 'Applications of Dendrimers in Drug Delivery Agents, Diagnosis, Therapy, and Detection'. *Journal of Nanomaterials* 2014
- Nostrum, C.F. van (2011) 'Covalently Cross-Linked Amphiphilic Block Copolymer Micelles'. *Soft Matter* [online] 7 (7), 3246–3259. available from <[https://pubs-rsc.org.nottingham.idm.oclc.org/en/content/articlehtml/2011/sm/c0sm00999g](https://pubs-rsc.org/nottingham.idm.oclc.org/en/content/articlehtml/2011/sm/c0sm00999g)> [27 September 2021]
- Ochiai, T., Nishimura, K., Noguchi, H., Kitajima, M., Tsukada, A., Watanabe, E., Nagaoka, I., and Futagawa, S. (2006) 'Prognostic Impact of Orotate Phosphoribosyl Transferase among 5-Fluorouracil Metabolic Enzymes in Resectable Colorectal Cancers Treated by Oral 5-Fluorouracil-Based Adjuvant Chemotherapy'. *International Journal of Cancer* [online] 118 (12), 3084–3088. available from <<https://onlinelibrary-wiley-com.nottingham.idm.oclc.org/doi/full/10.1002/ijc.21779>> [25 November 2021]
- Oliveira, M.S., Aryasomayajula, B., Pattni, B., Mussi, S. V., Ferreira, L.A.M., and Torchilin, V.P. (2016) 'Solid Lipid Nanoparticles Co-Loaded with Doxorubicin and α -Tocopherol Succinate Are Effective against Drug-Resistant Cancer Cells in Monolayer and 3-D Spheroid Cancer Cell Models'. *International Journal of Pharmaceutics* [online] 512 (1), 292–

300. available from <<https://pubmed.ncbi.nlm.nih.gov/27568499/>> [22 August 2025]

Ossovskaia, V., Wang, Y., Budoff, A., Xu, Q., Lituev, A., Potapova, O., Vansant, G., Monforte, J., and Daraselia, N. (2011) 'Exploring Molecular Pathways of Triple-Negative Breast Cancer'. *Genes and Cancer* [online] 2 (9), 870–879. available from <https://scholar.google.com/scholar_url?url=https://journals.sagepub.com/doi/pdf/10.1177/1947601911432496&hl=en&sa=T&oi=ucasa&ct=ufr&ei=6dxnaOvfKMzM6rQP_8mNwQs&scisig=AAZF9b929BBJ6cDWoqYGRqJ1OZBO> [4 July 2025]

Oun, R., Moussa, Y.E., and Wheate, N.J. (2018) 'The Side Effects of Platinum-Based Chemotherapy Drugs: A Review for Chemists'. *Dalton Transactions (Cambridge, England : 2003)* [online] 47 (19), 6645–6653. available from <<https://pubmed-ncbi-nlm-nih.gov/nottingham.idm.oclc.org/29632935/>> [25 November 2021]

Paramanantham, A., Jung, E.J., Kim, H.J., Jeong, B.K., Jung, J.M., Kim, G.S., Chan, H.S., and Lee, W.S. (2021a) 'Doxorubicin-Resistant TNBC Cells Exhibit Rapid Growth with Cancer Stem Cell-like Properties and EMT Phenotype, Which Can Be Transferred to Parental Cells through Autocrine Signaling'. *International Journal of Molecular Sciences* 2021, Vol. 22, Page 12438 [online] 22 (22), 12438. available from <<https://www.mdpi.com/1422-0067/22/22/12438/htm>> [12 August 2025]

- Paramanantham, A., Jung, E.J., Kim, H.J., Jeong, B.K., Jung, J.M., Kim, G.S., Chan, H.S., and Lee, W.S. (2021b) 'Doxorubicin-Resistant TNBC Cells Exhibit Rapid Growth with Cancer Stem Cell-like Properties and EMT Phenotype, Which Can Be Transferred to Parental Cells through Autocrine Signaling'. *International Journal of Molecular Sciences* 2021, Vol. 22, Page 12438 [online] 22 (22), 12438. available from <<https://www.mdpi.com/1422-0067/22/22/12438/htm>> [17 July 2024]
- Pareja, F., Bhargava, R., Borges, V.F., Brogi, E., Canas Marques, R., Cardoso, F., Desmedt, C., Harigopal, M., Lakhani, S.R., Lee, A., Leone, J.P., Linden, H., Lord, C.J., Marchio, C., Merajver, S.D., Rakha, E., Reis-Filho, J.S., Richardson, A., Sawyer, E., Schedin, P., Schwartz, C.J., Tutt, A., Ueno, N.T., Vincent-Salomon, A., Weigelt, B., Wen, Y.H., Schnitt, S.J., and Oesterreich, S. (2025) 'Unraveling Complexity and Leveraging Opportunities in Uncommon Breast Cancer Subtypes'. *Npj Breast Cancer* 2025 11:1 [online] 11 (1), 1–13. available from <<https://www.nature.com/articles/s41523-025-00719-w>> [29 August 2025]
- Patra, J.K., Das, G., Fraceto, L.F., Campos, E.V.R., Rodriguez-Torres, M.D.P., Acosta-Torres, L.S., Diaz-Torres, L.A., Grillo, R., Swamy, M.K., Sharma, S., Habtemariam, S., and Shin, H.S. (2018) 'Nano Based Drug Delivery Systems: Recent Developments and Future Prospects'. in *Journal of Nanobiotechnology*. vol. 16 (1). BioMed Central Ltd.

Pearce, A.K., Anane-Adjei, A.B., Cavanagh, R.J., Monteiro, P.F., Bennett, T.M., Taresco, V., Clarke, P.A., Ritchie, A.A., Alexander, M.R., Grabowska, A.M., and Alexander, C. (2020) 'Effects of Polymer 3D Architecture, Size, and Chemistry on Biological Transport and Drug Delivery In Vitro and in Orthotopic Triple Negative Breast Cancer Models'. *Advanced Healthcare Materials* 9 (22)

Pont, M., Marqués, M., and Sorolla, A. (2024) 'Latest Therapeutical Approaches for Triple-Negative Breast Cancer: From Preclinical to Clinical Research'. *International Journal of Molecular Sciences* 2024, Vol. 25, Page 13518 [online] 25 (24), 13518. available from <<https://www.mdpi.com/1422-0067/25/24/13518/htm>> [29 August 2025]

Raman, R., Debata, S., Govindarajan, T., and Kumar, P. (2025a) 'Targeting Triple-Negative Breast Cancer: Resistance Mechanisms and Therapeutic Advancements'. *Cancer Medicine* [online] 14 (9). available from <<https://pubmed.ncbi.nlm.nih.gov/40318146/>> [3 July 2025]

Raman, R., Debata, S., Govindarajan, T., and Kumar, P. (2025b) 'Targeting Triple-Negative Breast Cancer: Resistance Mechanisms and Therapeutic Advancements'. *Cancer Medicine* [online] 14 (9), e70803. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC12048392/>> [4 July 2025]

Salata, G.C. and Lopes, L.B. (2022a) 'Phosphatidylcholine-Based Nanoemulsions for Paclitaxel and a P-Glycoprotein Inhibitor Delivery and Breast Cancer Intraductal Treatment'. *Pharmaceuticals* [online] 15 (9),

1110. available from

<<https://pmc.ncbi.nlm.nih.gov/articles/PMC9503599/>> [22 August 2025]

Salata, G.C. and Lopes, L.B. (2022b) 'Phosphatidylcholine-Based

Nanoemulsions for Paclitaxel and a P-Glycoprotein Inhibitor Delivery and

Breast Cancer Intraductal Treatment'. *Pharmaceuticals* [online] 15 (9),

1110. available from <[https://www.mdpi.com/1424-](https://www.mdpi.com/1424-8247/15/9/1110/htm)

8247/15/9/1110/htm> [22 August 2025]

dos Santos, T., Varela, J., Lynch, I., Salvati, A., and Dawson, K.A. (2011) 'Effects

of Transport Inhibitors on the Cellular Uptake of Carboxylated

Polystyrene Nanoparticles in Different Cell Lines'. *PLOS ONE* [online] 6

(9), e24438. available from

<[https://journals.plos.org/plosone/article?id=10.1371/journal.pone.002](https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0024438)

4438> [22 November 2021]

Schiff, P.B., Fant, J., and Horwitz, S.B. (1979) 'Promotion of Microtubule

Assembly in Vitro by Taxol'. *Nature* 1979 277:5698 [online] 277 (5698),

665–667. available from <[https://www-nature-](https://www-nature-com.nottingham.idm.oclc.org/articles/277665a0)

com.nottingham.idm.oclc.org/articles/277665a0> [24 November 2021]

Schneider, M., Windbergs, M., Daum, N., Loretz, B., Collnot, E.M., Hansen, S.,

Schaefer, U.F., and Lehr, C.M. (2013) 'Crossing Biological Barriers for

Advanced Drug Delivery'. *European Journal of Pharmaceutics and*

Biopharmaceutics 84 (2), 239–241

- Sherje, A.P., Jadhav, M., Dravyakar, B.R., and Kadam, D. (2018) 'Dendrimers: A Versatile Nanocarrier for Drug Delivery and Targeting'. *International Journal of Pharmaceutics* 548 (1), 707–720
- Shi, J., Gao, P., Song, Y., Chen, X., Li, Y., Zhang, C., Wang, H., and Wang, Z. (2017) 'Efficacy and Safety of Taxane-Based Systemic Chemotherapy of Advanced Gastric Cancer: A Systematic Review and Meta-Analysis'. *Scientific Reports* 2017 7:1 [online] 7 (1), 1–10. available from <<https://www-nature-com.nottingham.idm.oclc.org/articles/s41598-017-05464-0>> [24 November 2021]
- Shi, M., Li, Z., Shen, G., Wang, T., Li, J., Wang, M., Liu, Z., Zhao, F., Ren, D., and Zhao, J. (2024) 'Efficacy and Safety of First-Line Treatment for Metastatic Triple-Negative Breast Cancer: A Network Meta-Analysis'. *Cancer Pathogenesis and Therapy* [online] 2 (2), 81–90. available from <<https://www.sciencedirect.com/science/article/pii/S2949713223000290>> [29 August 2025]
- Soo Choi, H., Liu, W., Misra, P., Tanaka, E., Zimmer, J.P., Itty Ipe, B., Bawendi, M.G., and Frangioni, J. v. (2007) 'Renal Clearance of Quantum Dots'. *Nature Biotechnology* [online] 25 (10), 1165–1170. available from <<https://pubmed-ncbi-nlm-nih-gov.nottingham.idm.oclc.org/17891134/>> [9 November 2021]
- Sørli, T., Perou, C.M., Tibshirani, R., Aas, T., Geisler, S., Johnsen, H., Hastie, T., Eisen, M.B., Van De Rijn, M., Jeffrey, S.S., Thorsen, T., Quist, H., Matese, J.C., Brown, P.O., Botstein, D., Lønning, P.E., and Børresen-Dale, A.L.

(2001) 'Gene Expression Patterns of Breast Carcinomas Distinguish Tumor Subclasses with Clinical Implications'. *Proceedings of the National Academy of Sciences of the United States of America* [online] 98 (19), 10869–10874. available from <<https://pubmed.ncbi.nlm.nih.gov/11553815/>> [29 August 2025]

Sousa De Almeida, M., Susnik, E., Drasler, B., Taladriz-Blanco, P., Petri-Fink Ab, A., and Rothen-Rutishauser, B. (2021) 'Understanding Nanoparticle Endocytosis to Improve Targeting Strategies in Nanomedicine'. *Chem. Soc. Rev* 50, 5397

Stasiak, P., Sopel, J., Lipowicz, J.M., Rawłuszko-Wieczorek, A.A., Korbecki, J., and Januchowski, R. (2025) 'The Role of Elacridar, a P-Gp Inhibitor, in the Re-Sensitization of PAC-Resistant Ovarian Cancer Cell Lines to Cytotoxic Drugs in 2D and 3D Cell Culture Models'. *International Journal of Molecular Sciences* 2025, Vol. 26, Page 1124 [online] 26 (3), 1124. available from <<https://www.mdpi.com/1422-0067/26/3/1124/htm>> [17 August 2025]

Su, J., Chen, F., Cryns, V.L., and Messersmith, P.B. (2011) 'Catechol Polymers for PH-Responsive, Targeted Drug Delivery to Cancer Cells'. *Journal of the American Chemical Society* [online] 133 (31), 11850–11853. available from <<https://pubs.acs.org/doi/full/10.1021/ja203077x>> [17 November 2021]

Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., and Bray, F. (2021) 'Global Cancer Statistics 2020: GLOBOCAN Estimates

of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries’.

CA: A Cancer Journal for Clinicians 71 (3), 209–249

Takemura, G. and Fujiwara, H. (2007) ‘Doxorubicin-Induced Cardiomyopathy:

From the Cardiotoxic Mechanisms to Management’. *Progress in*

Cardiovascular Diseases 49 (5), 330–352

Tan, P.H., Ellis, I., Allison, K., Brogi, E., Fox, S.B., Lakhani, S., Lazar, A.J., Morris,

E.A., Sahin, A., Salgado, R., Sapino, A., Sasano, H., Schnitt, S., Sotiriou, C.,

van Diest, P., White, V.A., Lokuhetty, D., and Cree, I.A. (2020) ‘The 2019

World Health Organization Classification of Tumours of the Breast’.

Histopathology [online] 77 (2), 181–185. available from

</doi/pdf/10.1111/his.14091> [29 August 2025]

Tchoryk, A., Taresco, V., Argent, R.H., Ashford, M., Gellert, P.R., Stolnik, S.,

Grabowska, A., and Garnett, M.C. (2019) ‘Penetration and Uptake of

Nanoparticles in 3D Tumor Spheroids’. *Bioconjugate Chemistry* 30 (5),

1371–1384

Thorn, C.F., Oshiro, C., Marsh, S., Hernandez-Boussard, T., McLeod, H., Klein,

T.E., and Altman, R.B. (2011) ‘Doxorubicin Pathways: Pharmacodynamics

and Adverse Effects’. *Pharmacogenetics and Genomics* [online] 21 (7),

440. available from </pmc/articles/PMC3116111/> [26 November 2021]

Tian, H., Ma, D., Tan, X., Yan, W., Wu, X., He, C., Zhong, L., Zhang, Yan, Yu, B.,

Zhang, Yi, and Qi, X. (2021) ‘Platinum and Taxane Based Adjuvant and

Neoadjuvant Chemotherapy in Early Triple-Negative Breast Cancer: A

Narrative Review'. *Frontiers in Pharmacology* [online] 12, 770663.

available from <www.frontiersin.org> [29 August 2025]

Tian, Y., Yongrong, L.E.I., Wang, Y., Jiejuan, L.A.I., Wang, J., and Feng, X.I.A.

(2023) 'Mechanism of Multidrug Resistance to Chemotherapy Mediated

by P-Glycoprotein (Review)'. *International Journal of Oncology* [online]

63 (5), 119. available from

<<https://pmc.ncbi.nlm.nih.gov/articles/PMC10546381/>> [20 August

2025]

Venditti, I. (2019) 'Morphologies and Functionalities of Polymeric

Nanocarriers as Chemical Tools for Drug Delivery: A Review'. *Journal of*

King Saud University - Science 31 (3), 398–411

Vinci, M., Gowan, S., Boxall, F., Patterson, L., Zimmermann, M., Court, W.,

Lomas, C., Mendiola, M., Hardisson, D., and Eccles, S.A. (2012) 'Advances

in Establishment and Analysis of Three-Dimensional Tumor Spheroid-

Based Functional Assays for Target Validation and Drug Evaluation'. *BMC*

Biology [online] 10 (1), 1–21. available from

<[https://bmcbiol.biomedcentral.com/articles/10.1186/1741-7007-10-](https://bmcbiol.biomedcentral.com/articles/10.1186/1741-7007-10-29)

29> [21 August 2025]

Wang, B., Soltero, R.A., and Saihaan, T. (2005) *Drug Delivery: Principles and*

Applications - Binghe Wang, Teruna J. Siahaan, Richard A. Soltero -

Google Books [online] available from

<[https://books.google.co.uk/books?hl=en&lr=&id=HbSeX8BpVmkC&oi=f](https://books.google.co.uk/books?hl=en&lr=&id=HbSeX8BpVmkC&oi=fnd&pg=PA1&dq=Wang,++B.,++Siahaan,++T.++%26++Soltero,++R.++A.++D)

[nd&pg=PA1&dq=Wang,++B.,++Siahaan,++T.++%26++Soltero,++R.++A.++D](https://books.google.co.uk/books?hl=en&lr=&id=HbSeX8BpVmkC&oi=fnd&pg=PA1&dq=Wang,++B.,++Siahaan,++T.++%26++Soltero,++R.++A.++D)

rug++delivery+Principles++and++applications.++1st++edition+edn,++(John+Wiley%26Sons,+2005).&ots=TJhk8Akg7s&sig=9PTaEWIPf6nedXFPZYG
HJ0NaHII&redir_esc=y#v=onepage&q&f=false> [27 September 2021]

Wang, C., Qi, P., Lu, Y., Liu, L., Zhang, Y., Sheng, Q., Wang, T., Zhang, M.,
Wang, R., and Song, S. (2020) 'Bicomponent Polymeric Micelles for PH-
Controlled Delivery of Doxorubicin'. *Drug Delivery* [online] 27 (1), 344–
357. available from <<https://pubmed.ncbi.nlm.nih.gov/32090637/>> [17
August 2025]

Wei, L., Kim, S.H., Armaly, A.M., Aubé, J., Xu, L., and Wu, X. (2024) 'HuR
Inhibition Overcomes CFLIP-Mediated Doxorubicin Resistance in Triple-
Negative Breast Cancer'. *Npj Precision Oncology* 2024 8:1 [online] 8 (1),
1–12. available from <<https://www.nature.com/articles/s41698-024-00780-x>> [4 July 2025]

Wu, J., Lin, N., Li, F., Zhang, G., He, S., Zhu, Y., Ou, R., Li, N., Liu, S., Feng, L.,
Liu, L., Liu, Z., and Lu, L. (2016) 'Induction of P-Glycoprotein Expression
and Activity by Aconitum Alkaloids: Implication for Clinical Drug-Drug
Interactions'. *Scientific Reports* [online] 6. available from
<<https://pubmed.ncbi.nlm.nih.gov/27139035/>> [17 August 2025]

Xiong, N., Wu, H., and Yu, Z. (2024) 'Advancements and Challenges in Triple-
Negative Breast Cancer: A Comprehensive Review of Therapeutic and
Diagnostic Strategies'. *Frontiers in Oncology* 14, 1405491

- Xuan, L., Ju, Z., Skonieczna, M., Zhou, P.K., and Huang, R. (2023) 'Nanoparticles-induced Potential Toxicity on Human Health: Applications, Toxicity Mechanisms, and Evaluation Models'. *MedComm* [online] 4 (4), e327. available from <<https://pmc.ncbi.nlm.nih.gov/articles/PMC10349198/>> [16 August 2025]
- Yan, J., Ye, Z., Chen, M., Liu, Z., Xiao, Y., Zhang, Y., Zhou, Y., Tan, W., and Lang, M. (2011) 'Fine Tuning Micellar Core-Forming Block of Poly(Ethylene Glycol)-Block-Poly(ϵ -Caprolactone) Amphiphilic Copolymers Based on Chemical Modification for the Solubilization and Delivery of Doxorubicin'. *Biomacromolecules* [online] 12 (7), 2562–2572. available from <<https://pubs-acsonline.sagepub.com/doi/full/10.1021/bm200375x>> [7 November 2021]
- Yersal, O. and Barutca, S. (2014) 'Biological Subtypes of Breast Cancer: Prognostic and Therapeutic Implications'. *World Journal of Clinical Oncology* [online] 5 (3), 412–424. available from <<https://pubmed.ncbi.nlm.nih.gov/25114856/>> [25 August 2025]
- Yin, L., Duan, J.J., Bian, X.W., and Yu, S.C. (2020) 'Triple-Negative Breast Cancer Molecular Subtyping and Treatment Progress'. in *Breast Cancer Research*. vol. 22 (1). BioMed Central Ltd
- Yingyuad, P., Mével, M., Prata, C., Kontogiorgis, C., Thanou, M., and Miller, A.D. (2014) 'Enzyme-Triggered PEGylated SiRNA-Nanoparticles for

Controlled Release of SiRNA'. *Journal of RNAi and Gene Silencing : An International Journal of RNA and Gene Targeting Research* [online] 10, 490. available from </pmc/articles/PMC3983657/> [27 September 2021]

Yvon, A.M.C., Wadsworth, P., and Jordan, M.A. (1999) 'Taxol Suppresses Dynamics of Individual Microtubules in Living Human Tumor Cells'. *Molecular Biology of the Cell* [online] 10 (4), 947–959. available from <https://www-molbiolcell-org.nottingham.idm.oclc.org/doi/abs/10.1091/mbc.10.4.947> [24 November 2021]

van der Zanden, S.Y., Qiao, X., and Neefjes, J. (2021) 'New Insights into the Activities and Toxicities of the Old Anticancer Drug Doxorubicin'. *The FEBS Journal* [online] 288 (21), 6095–6111. available from </doi/pdf/10.1111/febs.15583> [29 August 2025]

Zauner, W., Farrow, N.A., and Haines, A.M.R. (2001) 'In Vitro Uptake of Polystyrene Microspheres: Effect of Particle Size, Cell Line and Cell Density'. *Journal of Controlled Release : Official Journal of the Controlled Release Society* [online] 71 (1), 39–51. available from <https://pubmed-ncbi-nlm-nih-gov.nottingham.idm.oclc.org/11245907/> [22 November 2021]

Zeng, W., Luo, Y., Gan, D., Zhang, Y., Deng, H., and Liu, G. (2023) 'Advances in Doxorubicin-Based Nano-Drug Delivery System in Triple Negative Breast Cancer'. *Frontiers in Bioengineering and Biotechnology* 11, 1271420

Zhai, Y., Zhou, X., Zhang, Z., Zhang, L., Wang, D., Wang, X., and Sun, W. (2018)

Polymers Design, Synthesis, and Characterization of Schiff Base Bond-Linked PH-Responsive Doxorubicin Prodrug Based on Functionalized MPEG-PCL for Targeted Cancer Therapy. [online] available from <www.mdpi.com/journal/polymers> [22 April 2025]

Zhang, J., Wang, Z., Hu, X., Wang, B., Wang, L., Yang, W., Liu, Y., Liu, G., Di, G.,

Hu, Z., Wu, J., and Shao, Z. (2015) 'Cisplatin and Gemcitabine as the First Line Therapy in Metastatic Triple Negative Breast Cancer'. *International Journal of Cancer* [online] 136 (1), 204–211. available from <https://pubmed-ncbi-nlm-nih.gov.nottingham.idm.oclc.org/24824628/> [25 November 2021]

Zhang, N., Yin, Y., Xu, S.J., and Chen, W.S. (2008) '5-Fluorouracil: Mechanisms

of Resistance and Reversal Strategies'. *Molecules* [online] 13 (8), 1551. available from </pmc/articles/PMC6244944/> [25 November 2021]

Zhang, Z., Wang, J., Nie, X., Wen, T., Ji, Y., Wu, X., Zhao, Y., and Chen, C. (2014)

'Near Infrared Laser-Induced Targeted Cancer Therapy Using Thermoresponsive Polymer Encapsulated Gold Nanorods'. *Journal of the American Chemical Society* [online] 136 (20), 7317–7326. available from <https://pubs-acsc.org.nottingham.idm.oclc.org/doi/abs/10.1021/ja412735p> [16 November 2021]

Zhitomirsky, B., Assaraf, Y.G., Zhitomirsky, B., and Assaraf, Y.G. (2014)

'Lysosomal Sequestration of Hydrophobic Weak Base Chemotherapeutics

Triggers Lysosomal Biogenesis and Lysosome-Dependent Cancer Multidrug Resistance'. *Oncotarget* [online] 6 (2), 1143–1156. available from <<https://www.oncotarget.com/article/2732/text/>> [3 July 2025]

Zhou, Z., Li, L., Yang, Y., Xu, X., and Huang, Y. (2014) 'Tumor Targeting by PH-Sensitive, Biodegradable, Cross-Linked N-(2-Hydroxypropyl) Methacrylamide Copolymer Micelles'. *Biomaterials* 35 (24), 6622–6635