





# Polyethyleneimine (PEI) as a Polymer-Based Co-Delivery System for Breast Cancer Therapy

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**Abstract:** Cancer has become one of the leading causes of morbidity and mortality worldwide. This disease is classified broadly by tissue, organ, and system; different cancer types and subtypes require different treatments. Drug bioavailability, selectivity, and high dosage, as well as extended treatment, are significantly associated with the development of resistance – a complex problem in cancer therapy. It is expected that the combination of anticancer drugs and drug delivery systems, using polymers to increase the access of such agents to their site of action, will improve the efficacy of therapy. Polyethyleneimine (PEI) is a polymer used as a co-delivery system for anticancer drugs and gene therapy. PEI is also useful for other purposes, such as transfection and bio-adsorbent agents. In co-delivery, PEI can promote drug internalization. However, PEI with a high molecular weight is linked to higher cytotoxicity, thus requiring further evaluation of clinical safety. This review focuses on the utilization of PEI as a co-delivery system for anticancer therapy, as well as its potential to overcome resistance, particularly in the treatment of specific subtypes (eg, breast cancer). In conclusion, PEI has promising applications and is improvable for the development of anticancer drugs.

**Keywords:** polyethyleneimine, polymer cationic, co-delivery system, cancer, breast cancer, therapy

## Introduction

Cancer is a disease that causes abnormal and uncontrolled cell growth with the potential to invade or metastasize to other parts of the body. It has become a major public health problem and a leading cause of mortality worldwide.<sup>1</sup> In women, the most prevalent type of cancer is breast cancer (a histopathologically and molecularly heterogeneous type). The classification of breast cancer into subtypes is based on the clinical stage, histopathologic features, hormone indicator, growth factor expression, biomarkers, and molecular profiling.<sup>2</sup> Despite research and technological developments, unfortunately, the variability of cancer poses a significant challenge to accurate diagnosis and treatment efficacy.<sup>3,4</sup>

There are numerous reports on the occurrence of drug resistance in cancer therapy.<sup>5</sup> Some anticancer drugs are characterized by low bioavailability, low selectivity, and the need for high dosage to reach their target, and are associated with the development of multi-drug resistance.<sup>6</sup> Several factors cause this resistance; for example, intracellular adenosine triphosphates (ATPs) play a significant role in promoting resistance to anticancer drugs. Also, extracellular ATPs can induce drug resistance. The avoidance or blockage of ATP internalization by combinational therapy may increase the cellular uptake of anticancer drugs.<sup>7,8</sup> Nevertheless, combining two or more anticancer therapeutics leads to an increase in toxicity on healthy cells.

The use of nanoparticle-based (NP-based) drug delivery systems may reduce the occurrence of drug resistance. Different types of NPs (eg, organic, inorganic, and hybrid) have been used in anticancer therapy. Organic NPs have been investigated for an extended period of time and involve many materials. This type includes liposome-based, polymer-based, and

dendrimer NPs.<sup>9</sup> Liposome-based NPs were the first nanomedicine to be approved for clinical use.<sup>10</sup> Polymer-based NPs are considerably more specific for drug delivery on resistant cancer cells than liposome-based NPs.<sup>11</sup> Various polymer-based NPs have been used in breast cancer therapy, including lipid combinations (eg, cholesterol) to form lipo-polymeric NPs.<sup>12</sup>

Polyethyleneimine (PEI) is a cationic polymer containing repeating units of primary amino groups and ethylene ( $\text{CH}_2\text{CH}_2$ ). PEI is commonly used as a transfection reagent and nanocarrier for drug delivery systems to enhance the efficacy of targeted drugs and gene therapy.<sup>13</sup> A Phase I clinical trial of PEI has been conducted for a DNA vaccine administered as a polymeric complex.<sup>14</sup> However, there is a lack of clinical studies on the use of PEI in the treatment of cancer. The safety of PEI has always been an important consideration for clinical use. PEI is one of the polymers used to coat the combination of anticancer drugs and gene therapy. Differences in its characteristics can affect the ability of PEI for drug delivery. The use of polymer-based nanocarriers as co-delivery systems for anticancer drugs and gene-targeted therapy has been reported. Nevertheless, there is a limited number of studies using PEI for co-delivery in cancer, particularly in the treatment of specific subtypes such as breast cancer.<sup>15–17</sup> This review focuses on the utilization of PEI as a co-delivery system for anticancer drugs and gene therapy, as well as its potential to overcome resistance.

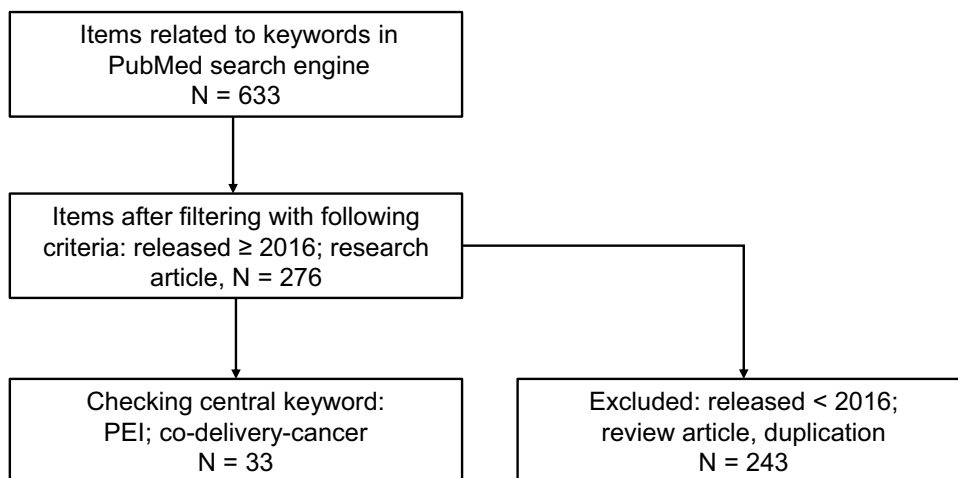
## Methods

Relevant literature was collected from the PubMed database using the following terms: “co-delivery,” “cancer,” and “breast cancer”; only articles published in English were screened. The relevant research articles published from 2016 to 2021 were included according to the criteria original research articles, namely investigations on PEI combined with anticancer drugs for co-delivery cancer with or without other supportive components. Related articles on PEI as a co-delivery system for the treatment of cancer were selected, and review articles and non-cancer research publications were excluded. Figure 1 shows the flowchart of the methodology.

## PEI Structure

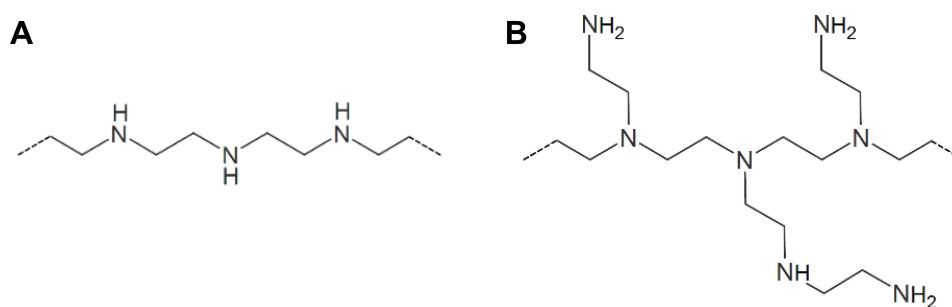
PEI is also termed polyfunctional aziridine (polyaziridine) due to its origin (ie, polymerization process from the ring-opening of aziridine). The protonated amino nitrogen chain on every third atom renders this polymer as the highest positively charged density potential.<sup>18</sup> It was reported that PEI has two different structures:  $\text{H}(\text{NHCH}_2\text{CH}_2)_n\text{NH}_2$  for branched PEI and  $(\text{CH}_2\text{CH}_2\text{NH})_n$  for linear PEI (Figure 2).<sup>19–21</sup>

The different PEI compositions provide various properties and reactivities to particular structures.<sup>21</sup> In addition, the molecular weight of PEI varies broadly, and high-molecular-weight-PEI is associated with increased cytotoxicity. Also, the effects of PEI on DNA damage are considered based on the polymer/DNA (C/P) ratios. Thus, PEI with different



**Figure 1** Flow chart of review methods.

**Abbreviation:** PEI, polyethyleneimine.



**Figure 2** Structure of linear PEI (A) and branched PEI (B).

**Note:** Reproduced from: Polymerdatabase. Available from: <https://polymerdatabase.com/PolymerBrands/Polyethylenimine.html>. Accessed March 23, 2022.<sup>21</sup> © 2015–2022 polymerdatabase.com.

**Abbreviation:** PEI, polyethylenimine.

structures and molecular weights in different C/P ratios will exert variable DNA-damaging effects, thereby causing cell death.<sup>22</sup> Previously, Wightman et al reported that, among all PEI/DNA complexes, linear-PEI exhibits better transfection efficiency than branch-PEI under salt conditions.<sup>23</sup> A subsequent report showed that branch-PEI is more toxic than linear-PEI; additionally, it is more effective for drug delivery because its structure exhibits better interaction with subcellular moieties.<sup>24</sup> However, the study of diverse aspects of PEI use is currently in progress.

## PEI as a Drug Co-Delivery System

PEI has been utilized for several purposes and is one of the preferable options due to its efficiency and cost-effectiveness. It is used to transfect nucleic acids to the nucleus, and numerous reports showed that PEI is a potential co-delivery system for various types of combinations (eg, amine-functionalized biochar modified with PEI and PEI-functionalized magnetic Fe<sub>3</sub>O<sub>4</sub> system). Both systems enhanced drug release and function.<sup>25,26</sup> Moreover, PEI and its modification may be used for gene delivery using N-acetyl-L-leucine (N-Ac-L-Leu-PEI) to effectively deliver CpG oligodeoxynucleotides (CpG-ODN). This is a crucial component in resisting inflammation, it is involved in the bone resorption process, and enhances cell apoptosis.<sup>27</sup>

Yoshitomi et al discovered an increase in the accumulation of astaxanthin in *Haematococcus pluvialis* cells following the addition of PEI. The polymer caused excessive production of reactive oxygen species and increased oxidative stress, thereby leading to the increment of astaxanthin accumulation.<sup>28</sup> The application of PEI is broad and versatile. Many research studies use PEI as a co-delivery system for anticancer drugs, cancer gene therapy, and distinct functions to enhance drug adsorption or accumulation.

## PEI as a Co-Delivery System for Anticancer Drugs

The combination of anticancer drugs has been used to overcome drug resistance in cancer therapy. The goal of the combination of two anticancer drugs is to increase efficacy without inducing more adverse effects. Furthermore, polymers are used as drug delivery systems to promote the entry of combinations of anticancer drugs to their site of action, mainly in the cell nucleus. PEI is a cationic polymer that can enter the nucleus. Some research studies reported employing PEI as a co-delivery system for breast cancer therapy (Table 1) and the treatment of other types of cancer (Table 2). It has been shown that this system increases the anticancer effects of agents by enhancing drug uptake, selectivity, stability, and biocompatibility.

Li et al, Zhao et al, and Sharma et al used branch PEI as the co-delivery system for anticancer drugs, such as paclitaxel (PTX).<sup>29–31</sup> These co-delivery systems increased therapeutic efficacy without causing more adverse reactions. The cytotoxicity of PTX and epirubicin was increased, and the presence of PEI in NPs enhanced drug uptake. Moreover, treatment of human embryonic kidney cell lines with NPs did not lead to significant cytotoxicity, highlighting the selectivity of NPs.

Another study combining poly(2-ethyl-2-oxazoline)-co-poly(ethyleneimine) (PEtOx-co-PEI) with poly(E-caprolactone) (PCL) as PEtOx-co-PEI-b-PCL block copolymers showed high stability and effective delivery of

**Table I** PEI as Co-Delivery System for Cancer Therapy

No	PEI Type	Anticancer Drug	Gene-Therapy	Combination	Cancer Type	In vitro	In vivo	Reference
1.	Branched	PTX	-	Ag-PEI-PTX Functionalized AgNPs, PEI, and PTX	Liver cancer	HepG2	-	[29]
2.	Branched, 10 kDa	PTX	-	(PEI-SA) HA/PTX PEI grafted with SA, modified with HA, and complexed with PTX	Ovarian cancer	SKOV3-TR30 (MDR)	Tumor-bearing nude mice	[30]
3.	Branched, 25 kDa	EPI and PTX	-	PLGA-PEI-EPI or -PTX PGLA conjugated with PEI, and loaded with EPI and PTX	Lung cancer	A549	-	[31]
4.	PEI coated with ZnO-SiO <sub>2</sub>	Ru	-	Ru-CA-PEI-ZnO-SiO <sub>2</sub> CSNPs Ru grafted with CA; co-delivery of PEI coated with ZnO-SiO <sub>2</sub>	Cervical cancer	HeLa	Female athymic nude mice	[33]
5.	2 kDa	-	pEGFP-N2	Pluronic-PEI-DR5-TAT/pEGFP-N2 DNA DR5-TAT-modified Pluronic-PEI loaded with pEGFP-N2 DNA.	DR5-positive (cervical cancer, liver cancer, prostate cancer)	HeLa, HepG2, and NIH 3T	-	[34]
6.	Branched, 25 kDa	-	pEGFP, Bcl-2 AS ODN	PEI-CA/pEGFP and Bcl-2 AS ODN PEI-CA complexed with Bcl-2 AS-ODN and pEGFP plasmid	Cervical cancer	HeLa	-	[35]
7.	Linear, 22, 87, and 217 kDa Branched, 25 kDa	-	pZsGreenI-NI	PEI/pDNA	Bovine fetal fibroblast, kidney cancer, liver cancer	BFF, HEK293, and HepG2	-	[36]
8.	Branched, 25 kDa	-	MRPI-siRNA	PEI copped-pSiNPs-MRPI-siRNA pSiNPs complex with PEI loaded with MRPI-siRNA	Glioblastoma	U87	CD-1 nude mice	[37]
9.	Branched, 25 kDa	-	Human DKK-1 and murine PD-L1 cDNA subcloned to pcDNA3.1 plasmid	PLGA/PEI-pPD-L1/pDDK-1 vaccine PLGA/PEI complexed with DKK-1 and PD-L1	Multiple myeloma/ Plasma cell cancer	-	Tumor-bearing Female BALB/c mice	[38]

10.	Branched, 25 kDa	-	Anti-PLK1 (PLK1-homo-581) siRNA	PEG-GO-PEI-FA/anti-PLK1 (PLK1-homo-581) siRNA PEG and PEI complexed with functionalized GO and FA, loaded with anti-PLK1 (PLK1-homo-581) siRNA	Ovarian cancer	SKOV3	-	[39]
11.	Branched, 25 kDa	DTX	pDNA	TAT-DTX/pDNA LPNs modified TAT, DTX, and pDNA loaded to CPPs modified LPNs	Prostate cancer	PC3	Female BALB/c mice weighing	[52]
12.	Branched	DTX	p44/42 MAPK siRNA	DTX/BSA-PEILBL/siRNAP PSMAab-conjugated fluorescent BSA-branched PEILBL) loaded with DTX and p44/42 MAPK siRNA	Prostate cancer	PZ-HPV-7, CWR22R, PC3, LNCaP, DU145, HEK293, MGH-U1, and MGH-U1R	Mice	[53]
13.	Branched, 25 kDa	DOX	pEGFP	HDDN-DOX-HBA-PEI (DHP) complexed with HA and pDNA	Liver cancer	HepG2	-	[46]
14.	PEI-g-Pleu	DOX	pGL3, Bcl-2-shRNA	PEI-g-Pleu PEI grafted by one step ROP with L-leucine N-carboxy anhydride, and complexed with DNA and shRNA	Melanoma	BI6F10	-	[48]
15.	1.8 kDa	DOX	pEGFP-C1	PEI/DNA-DNPs DNPs condensed with PEI/DNA nano complex	Cervical cancer	HeLa, L929	Male BALB/c nude mice	[49]
16.	Branched (0.8, 1.3, 25 kDa) Linear (2.5, 8 kDa)	MTX	pcDNA3-FLAG-p53	PEI/p53 encoding pDNA/MTX PEI/pDNA nanoparticles loaded with MTX	Cervical cancer	HeLa	-	[55]
17.	Branched, 1.8 kDa	DOX	Bcl-2 siRNA	Bcl-2 siRNA/DOX/GH-DPP DSPE-PEG-PEI complexed with GA-HA as liver-targeted nanoparticles (GH-DPP), loaded with Bcl-2 siRNA and DOX	Liver cancer	HepG2	H22-bearing mice	[51]
18.	Branched, 25 kDa	MTX	Cy5-labeled survivin siRNA	M-MTX/Cy-5 labeled survivin siRNA Functionalized MTX conjugated with LA-modified branched PEI (MTX-bPEI-LA) to form mixed micelles (M-MTX), and loaded with Cy5-labeled survivin siRNA	Cervical cancer	HeLa	Tumor-bearing nude mice	[56]

(Continued)

Table I (Continued).

No	PEI Type	Anticancer Drug	Gene-Therapy	Combination	Cancer Type	In vitro	In vivo	Reference
19.	Branched	RA	miR-29b	PI03-PEI-RA/miR-29b Pluronic PI03 linked to PEI, complexed with RA and miR-29b	Lung cancer	A549 and H1299	-	[58]
20.	1.8 kDa	DOX	siPgp	PAPD nanoparticles Hypoxia-sensitive PAPD loaded with DOX and anti-siPgp	Ovarian cancer	A2780 Adriamycin resistant (ADR), MCF7	-	[61]
21.	Branched, 25 kDa	PFD	siScr	F-PEI-PFD/siScr F-PEI and PFD emulsions, complexed with siScr	Melanoma	B16F10	C57BL/6 mice	[65]

**Abbreviations:** Ag-NPs, silver nanoparticles; AS-ODN, antisense oligodeoxyribonucleotide; Bcl-2, B cell lymphoma-2; BSA, bovine serum albumin; CA, cholic acid; CPPs, cell-penetrating peptides; DKK-1, dickkopf-1; DTX, docetaxel; DOX, doxorubicin; DNPs, doxorubicin nanoparticles; DSPE, 1,2-distearoyl-sn glycerol-3-phosphoethanolamine; EPI, epirubicin; FA, folic acid; F-PEI, fluorinated polyethyleneimine; GA-HA, glycyrrhetic acid-modified hyaluronic acid; GO, graphene oxide; HA, hyaluronic acid; HBA, hydrazinobenzoic acid; HDDN, hyaluronic-acid DHP/DNA nanoparticles; LA, linoleic acid; LPNs, lipid-PEI hybrid nanoparticles; MAPK, mitogen-activated protein kinase; MRPI, multi-drug resistance-associated protein 1; MTX, methotrexate; PAPD, PEG-azobenzene-PEI-DOPE; pDNA, plasmid DNA; PD-L1, programmed death-ligand-1; PEG, polyethylene glycol; pEGFP, enhanced green fluorescent protein plasmid; PEI, polyethyleneimine; PEI-g-PLeu, PEI-g-poly-L-leucine; PEILBL, PEI layer by layer; PFD, perfluoro decalin; pSiNPs, porous silicon nanoparticles; PLGA, poly-(lactic-co-glycolic) acid; PLKI, polo like kinase 1; PSMAab, prostate-specific membrane antigen-antibody; PTX, paclitaxel; RA, retinoic acid; ROP, ring-opening polymerization; Ru, ruthenium pro-drug; SA, salicylic acid; shRNA, short hairpin RNA; siRNA, small interfering RNA; siPgp, P-gp siRNA; siScr, scrambled siRNA; TAT, RKKRRQRRR peptide.

**Table 2** PEI as Co-Delivery System for Breast Cancer Therapy

No	PEI Type	Anticancer Drugs	Gene Therapy	Combination	In vitro	In vivo	Reference
1.	PEtOx-co-PEI	DOX		DOX-loaded PEtOx-co-PEI-b-PCL PEtOx-co-PEI complexed with PCL, and loaded with DOX	MCF-7	-	[32]
2.	25 kDa	-	siEGFR1, siEGFR2, siEGFR3, siBRD41, siBRD42, siBRD43	GALA-PEG-SS-PEI and CREKA-PEG-SS-PEI GALA- and CREKA-modified with PEG; SS; PEI (PEG-SS-PEI)	MDA-MB-231 and HUVEC	-	[40]
3.	Branched, 1.2 kDa	-	siRNA, pDNA	pDNA/siRNA/lipopolymer Lipid-grafted PEI loaded with pTRAIL and silencing BCL2L12 and SOD1 siRNA	MCF-7, MDA-MB-231	MDA-MB-231 xenografted mice	[57]
4.	1.3 kDa	DOX	pArg	DOX-loaded CA-PEI-pArg PMs pH-responsive PMs contain CA-PEI-pArg copolymers, loaded with DOX	MCF-7	-	[43]
5.	Linear, 5 kDa	GA	pTRAIL	GA/pTRAIL-HA/PP Adding HA to neutralize the excess positive charge	MDA-MB-231 and MCF-7	Tumor-bearing nude BALB/c mice (5-week-old, 18–20 g, female)	[54]
6.	Branched, 10 kDa	DOX	Bcl-xL shRNA	PLGA-DOX-Alkyl-PEI NPs/shRNA DOX encapsulated by PLGA, complexed with PEI modified with alkyl chain, and loaded with Bcl-xL shRNA.	MCF-7	-	[45]
7.	Branched, 1 kDa	DOX	p53	PDP/DOX/p53 Grafted lipophilic deoxycholic acid and low molecular weight (1 kDa) branched PEI to pullulan, and complexed with DOX and p53	MCF-7	6–8-week-old female BALB/C nude mice	[47]
8.	Branched, 25 kDa	DOX	pORF-hTRAIL	PCL-PEI-PEG-T7/DOX/pORF-hTRAIL PEG-PEI-PCL-S-S-PCL-PEI-PEG loaded with pORF-hTRAIL and DOX	MCF-7	6-week-old BALB/c female nude mice	[50]
9.	Branched, 1.8 kDa	EMB	pTRAIL	EMB/TRAIL-HA/PBAE-PEI EMB and pTRAIL delivered with amphiphilic polymers PEI- PBAE. Tumor targeting mediated by HA	MDA-MB-231	-	[59]

(Continued)

Table 2 (Continued).

No	PEI Type	Anticancer Drugs	Gene Therapy	Combination	In vitro	In vivo	Reference
10.	Branched, 1.8 kDa	GemC18	NF-κB siRNA	GemC18-containing PMs/anti NF-κB siRNA PCL-PEI-PEG functionals PMs, loaded with 4-(N)-stearoyl GemC18 and NF-κB siRNA	AsPC-1 and MCF7	Balb/c mice bearing 4T1 xenograft breast tumor	[60]
11.	JetPEI	DOX	miR-142-3p	Jet PEI/CD44-specific-siRNA/DOX Jet PEI complexed with CD44-specific-siRNA and DOX	MDA-MB-468	-	[62]

**Abbreviations:** Bcl-Xl, B-cell lymphoma-extra-large; BCL2L12, Bcl2-like12; CA, cholic acid; DOX, doxorubicin; EMB, embelin; GemC18, gemcitabine pro-drug; HA, hyaluronic acid; hTRAIL, human tumor necrosis factor-related apoptosis-inducing ligand; NF-κB, nuclear factor-κB; pArg, poly-L-arginine; PBAE, poly[(1,6-hexanediol)-diacrylate-β-5-hydroxyamylamine]; PCL, poly(E-caprolactone); pDNA, plasmid DNA; PEG, polyethylene glycol; PEI, polyethyleneimine; PetOx-co-PEI, poly(2-ethyl-2-oxazoline)-co-poly(ethyleneimine); PLGA, poly-(lactic-co-glycolic) acid; PMs, polymeric micelles; pORF, plasmid open reading frame; pTRAIL, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) plasmid; siRNA, small interfering RNA; SOD1, superoxide dismutase 1; SS, disulfide.

doxorubicin (DOX). These effects occurred because PEI as copolymers decreased the metabolic activity of breast cancer cell lines.<sup>32</sup> Zhou reported that the combination of grafted ruthenium pro-drug (Ru) with cholic acid (CA) and PEI-ZnO-SiO<sub>2</sub> to form core-shell nanoparticles (CSNPs) (Ru-CA-PEI-ZnO-SiO<sub>2</sub> CSNPs) demonstrated antitumor activity against HeLa cells.<sup>33</sup> The co-delivery system for anticancer drugs (ie, PTX, DOX, and Ru) resulted in excellent drug biocompatibility and may be a promising system for drug delivery in the treatment of cancer.

## PEI as a Co-Delivery System for Gene Therapy

Recently, the use of gene-targeted therapy for the treatment of cancer has also been accepted. PEI is an established transfection reagent that can deliver nucleic acids to the nucleus. The utilization of PEI as a delivery system for gene-targeted therapy is the most coherent approach and expected to provide substantial scientific evidence. In study by Weecharangsan et al, PEI was used as a co-delivery system for a plasmid constructed with gene-targeted therapy or small interfering RNA (siRNA).<sup>35</sup> The findings indicated that the use of this system could reduce toxicity in normal cells, increase the efficiency of gene transfection, and enhance the inhibition of cancer cells. In vitro analysis showed that the combination of PEI with siRNA could increase the efficiency of gene transfection and reduce toxicity in normal cells.<sup>34,35</sup> Furthermore, another research study showed high transfection rates in BFF, HEK293, and HepG2 cells following treatment with PEI/plasmid DNA (PEI/pDNA) and PEI/siRNA complexes. Thus, PEI complexes with pDNA and siRNA may provide a new cost-effective strategy for transfection. PEI/siRNA complexes also induced a reduction in tumor volume, as a result of efficient drug delivery.<sup>36,37</sup> PEI is also useful for a DNA vaccination complex system, consisting of a tumor-specific antigen termed Dickkopf-1 (DKK-1) and the immune checkpoint termed programmed death-ligand 1 (PD-L1) complexed with poly-(lactic-co-glycolic) acid/ PEI (PLGA/PEI). The treatment led to significant inhibition of tumor growth and induction of a transient inflammatory reaction, as well as maintenance of stable body weight and health. These findings confirmed that this tumor vaccine was effective with limited toxicity.<sup>38</sup>

Subsequent research studies used polyethylene glycol (PEG)- and PEI-complexed siRNA and marker peptides. The data showed that the combination could escape from lysosome activity and successfully release the gene. This combination could suppress tumor growth in folate receptor-positive ovarian cancer or similar cancer cells.<sup>39,40</sup> GALA belongs to a group of pH-sensitive peptides that can penetrate cells and protect the drug from endocytosis by endosomes.<sup>41</sup> CREKA is a pentapeptide linear cysteine-arginine glutamic acid-lysine-alanine, utilized as a tumor-homing peptide. Using the nano-delivery system, CREKA showed high selectivity for triple-negative breast cancer (TNBC).<sup>42</sup> GALA- and CREKA-modified PEI with siRNA complexes showed excellent transfection efficiency with good biocompatibility and profiled redox-sensitive release property that is suitable for siRNA delivery system in tumor condition. Moreover, siRNA was protected from RNA enzymatic degradation by the complex. The complexes showed significant

cytotoxicity against MDA-MB-231 cells via the BDR4-c-Myc and EGFR/PI3K/Akt pathway. Collectively, the available evidence indicates that this combination provides efficient delivery of siRNA for the treatment of TNBC.

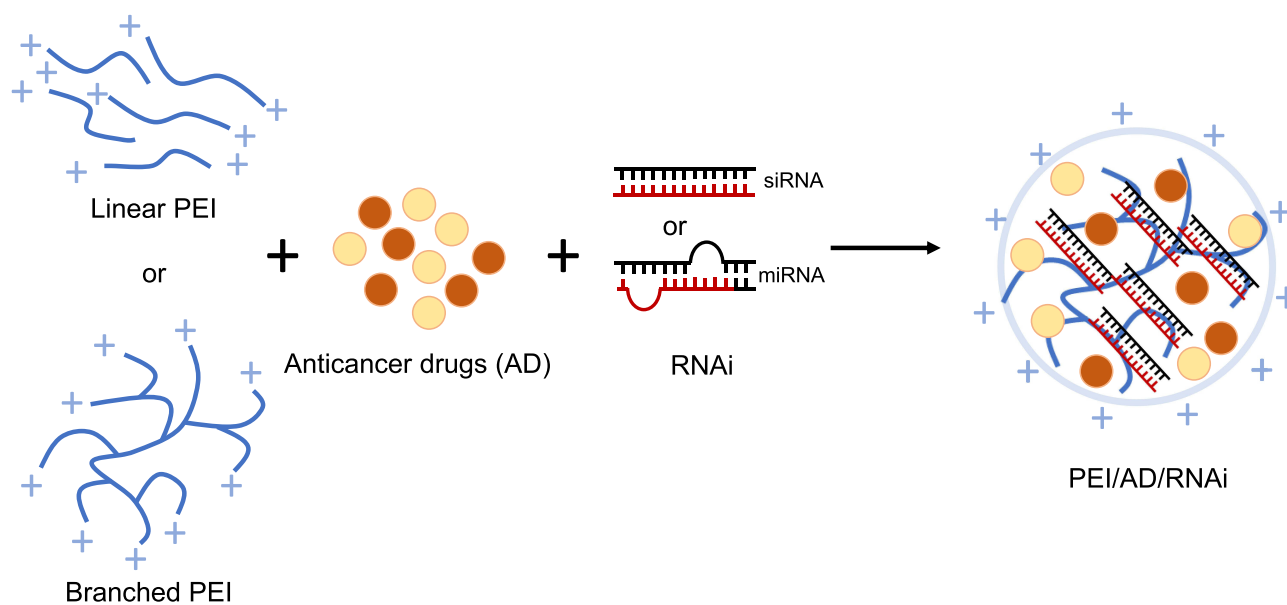
## PEI as a Co-Delivery System for Anticancer Drug and Gene Therapy

Recently, research studies combined anticancer drugs and gene therapy to overcome significant efficacy. It is expected that the use of a co-delivery system that effectively delivers the agent to the target will enhance the combined effect of drugs and gene-targeted therapy. PEI is a robust co-delivery system due to its ability to lead the anticancer drug into the cells and transfect nucleic acid-based therapy to the nucleus. Figure 3 illustrates PEI nanoparticle complexes with anticancer drugs and RNA interference (RNAi).

The formation of a co-polymer system by loading DOX into PEI along with pDNA, short hairpin RNA (shRNA), and siRNA showed higher cytotoxicity than treatment with DOX only. Drug release was examined under several pH conditions, and pH adjustment to 5.0 showed that >80% of DOX was released. The PEI complex has exhibited excellent biocompatibility, reliable delivery of DOX and DNA, and protection of DNA from degradation by DNase I.<sup>43–51</sup> It is established that the intracellular uptake of PEI nanoparticles is dominated by clathrin- and caveolae-independent endocytosis and an energy-dependent process, as shown in Figure 4.<sup>49</sup> The PEI co-delivery system has shown a good capability for the delivery of genes, and the transfection efficiency improved following the increase in weight ratios. Thus, the combination of DNA and DOX exerted synergistic apoptotic effects, and the PEI co-delivery system significantly reduced tumor volume in vivo.<sup>50</sup>

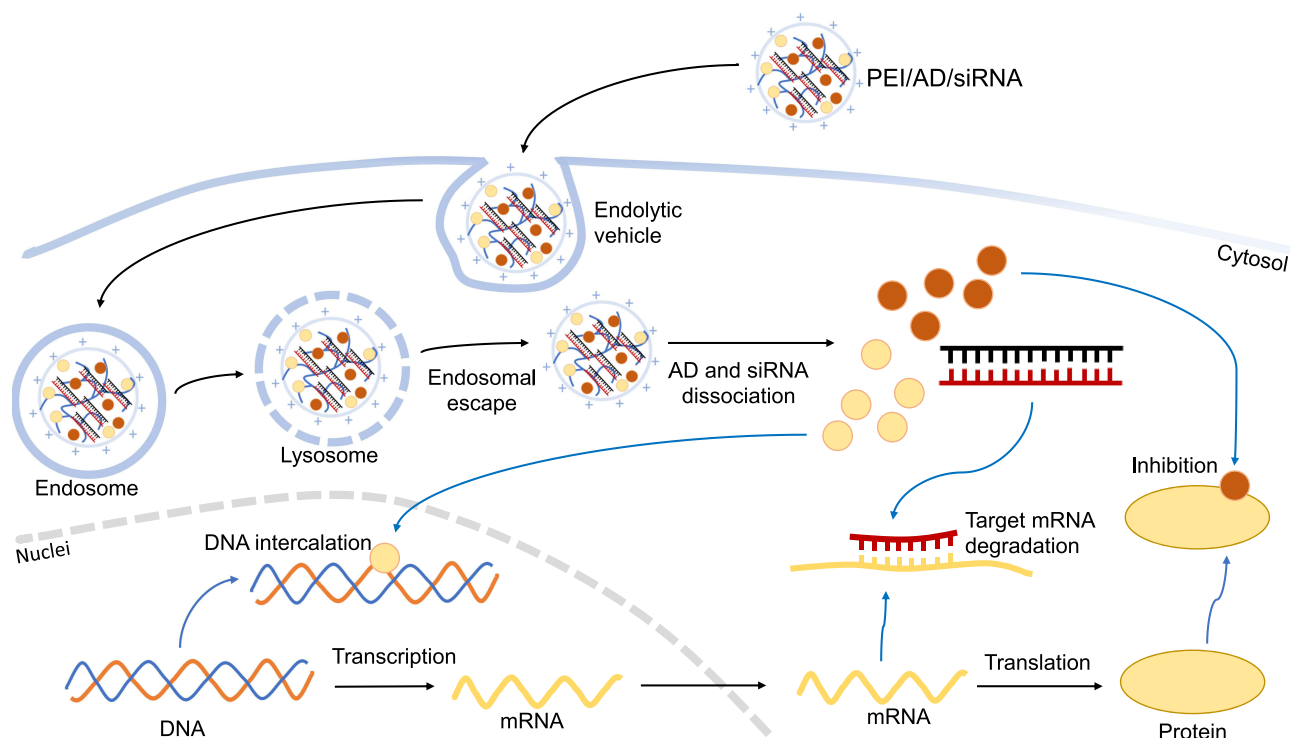
Research using a complex composed of docetaxel combined with PEI and pDNA yielded similar results. In vivo transfection analysis in tumor-bearing mice showed that this system significantly improved the transfection efficiency.<sup>52,53</sup>

Wang et al revealed that the co-loading of tumor necrosis factor-related apoptosis-inducing ligand plasmid (pTRAIL) and gambogic acid (GA) significantly enhanced cytotoxicity against MCF-7 and MDA-MB-231 cells.<sup>54</sup> The GA encapsulated into PEI-PLGA NPs (GA/pTRAIL-HA/PPNPs) showed high cytotoxicity, indicating the effectiveness of the combination that may lead to the selective accumulation of CD44 in MDA-MB-231 cells. In vivo observation of tumor-bearing nude BALB/c mice showed that both GA and pTRAIL separately loaded in NPs could significantly enhance tumor inhibition. Moreover, the combination of GA and pTRAIL yielded more significant results. The hyaluronic acid-PPNP co-delivery system for GA and pTRAIL represents a potential strategy for the treatment of



**Figure 3** Scheme of nanoparticles containing PEI as drug and RNAi delivery.

**Abbreviations:** miRNA, micro-RNA; PEI, polyethyleneimine; RNAi, RNA interference; siRNA, small interfering RNA.



**Figure 4** Mechanism of action of PEI for drug and nucleic acid internalization via endocytosis.

**Abbreviations:** AD, anticancer drugs; mRNA, messenger RNA; PEI, polyethyleneimine; RNAi, RNA interference; siRNA, small interfering RNA.

TNBC through the combination of anticancer drugs and therapeutic DNA. Methotrexate (MTX) has been loaded to PEI NPs in combination with pDNA and siRNA.<sup>55,56</sup> The cytotoxic effects of both PEI with pDNA and siRNA polyplexes on HeLa cells was observed. Linear-PEI did not show cytotoxicity, but demonstrated significant biocompatibility. Branch-PEI/pDNA complexes are less biocompatible, but induce more cytotoxicity than linear PEI.<sup>56</sup> Furthermore, PEI with siRNA polyplexes showed higher cytotoxicity and protein inhibition in HeLa cells versus PEI with pDNA. In vivo analysis showed that mice treated with M-MTX/siRNA had lower tumor weight, indicating that the delivery system is a more effective anticancer agent versus free MTX.

Other research studies have used PEI to deliver anticancer drugs along with pDNA and/or siRNA, as well as increase therapeutic responses. Drugs that have been used include retinoic acid, embelin, 4-(N)-stearoyl gemcitabine (GemC18), and DOX.<sup>57–62</sup> The complex increased apoptosis in cancer cells, but had a minimal effect on nonmalignant cells. In vivo analysis of this system in tumor-bearing mice showed markedly low tumor volume and high therapeutic response.<sup>57</sup>

The results of a tumor accumulation assay showed that PEG shells effectively protects siRNA from cell interaction and recognition by polymeric micelles in the reticuloendothelial system.<sup>60</sup> Delivery of DOX with CD44-specific-siRNA transfection led to the downregulation of CD44, matrix metalloproteinase 9 (MMP9), vascular endothelial growth factor, and C-X-C motif chemokine receptor 4 (CXCR4), thereby reducing the inhibition of apoptosis by CD44.<sup>62</sup> Taken together, the evidence shows that PEI can be used as a drug co-delivery system and gene-transfection reagent to overcome resistance in the treatment of cancer.

## PEI as a Co-Delivery System for Breast Cancer Therapy

Breast cancer is one of the leading causes of cancer-related death among women. Previously, all patients with breast cancer were receiving the same treatment. More recently, research revealed the presence of different types of breast cancer that led to various clinical findings and the development of specific treatments. The classification of breast cancer is based on histological and molecular characteristics. The relevant molecular subtypes reported in breast cancer is triple-negative breast cancer (TNBC), that has specific treatment response behavior among all subtypes of breast cancer.

TNBC is negative for the typical receptors in breast cancer: estrogen receptors; progesterone receptors; and excess human epidermal growth factor receptor-2 (HER2).<sup>63,64</sup> This type of breast cancer is more aggressive and associated with a poor prognosis versus other types of the disease. Owing to its specific features, TNBC does not respond to hormonal therapy or HER2-targeted medicine; hence, its treatment involves a combination of surgery, radiation therapy, and chemotherapy.

The development of drug resistance poses challenges to the treatment of breast cancer. Moreover, the development of high-efficiency/low-toxicity agents is crucial. Therefore, developing a suitable co-delivery system for breast cancer is also essential to enhance bioavailability and cellular uptake. The development of a potent anticancer agent with a multifunctional molecule characterized by high specificity and capability for delivery is challenging.

Based on the reviewed literature, PEI has been used in various research fields of cancer therapy as a polymeric complex along with anticancer drugs and/or genetic substances. Numerous types of cancer cells respond well to treatment with an NP complex composed of PEI. Thus, this approach could potentially improve cancer therapy.

Recently, PEI was utilized as a co-delivery system for the anticancer drug DOX, and in vitro study on MCF-7 cells confirmed an improvement in the anticancer effect.<sup>32</sup> As a gene therapy co-delivery system, PEI can efficiently deliver siRNA to the target, and induce an increase in cytotoxicity and tumor growth suppression.<sup>40,57</sup> Moreover, as the co-delivery system for the combination of drug and gene therapy, PEI has improved the cytotoxic effect on breast cancer cell lines and reduced tumor volume in a xenograft model.<sup>43,45,47,50,54,59,60,62</sup> Taken together, PEI shows excellent ability as a co-delivery system in breast cancer therapy. Further investigation is warranted to improve cancer therapy, particularly for breast cancer.

## Conclusion

PEI is a cationic polymer utilized as a co-delivery system for drugs and gene therapy. The PEI co-delivery system improves the anticancer effect by enhancing drug selectivity for malignant cells, uptake, stability, and biocompatibility. PEI has been used for the delivery of anticancer drugs, such as DOX, PTX, epirubicin, Ru, docetaxel, MTX, retinoic acid, GemC18, perfluorodecalin, GA, and embelin. PEI co-delivery of a plasmid constructed with gene-targeted therapy, pDNA, or siRNA reduced toxicity in normal cells, increased the efficiency of gene transfection, and improved the inhibition of cancer cells. For the treatment of breast cancer, the combination of drugs with PEI induced higher cytotoxicity against breast cancer cell lines versus treatment with the drugs only. Moreover, it significantly reduced the tumorigenic ability in a xenograft model. Thus, PEI has promising applications and is improvable for the development of anticancer drugs.

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## Disclosure

The authors report no conflicts of interest in this work.

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