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An updated review of folate-functionalized nanocarriers: A promising ligand in cancer

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Teaser: This review highlights the potential of folate-conjugated nanocarriers for the diagnosis and treatment of cancer. Such nanotherapeutics have been shown to have excellent targeting efficiency due to their promising specificity for folate receptors overexpressed in many tumor types.

Abstract

The uncontrolled release of drugs in conventional drug delivery systems has led to the introduction of new nanotechnology-based drug delivery systems and the use of targeted nanocarriers for cancer treatment. These targeted nanocarriers which consist of intelligent nanoparticles modified with targeting ligands can deliver drugs to specified locations at the right time and reduce the dose of drugs to prevent side effects. Folate is a suitable targeting ligand for folate receptors overexpressed on cancer cells and has shown promising results in the diagnosis and treatment of cancer. In this review, we highlight the latest developments on the use of folate-conjugated nanoparticles in cancer diagnosis and treatment. Moreover, the toxicity, biocompatibility, and efficacy of these nanocarriers are discussed.

Keywords: Folic acid; Cancer therapy; Nanoparticles; Targeted delivery; Diagnosis

Introduction

Cancer

The word cancer is originally from *Latin* and means crab. In fact, the word is known because of the similarity of cancer cells to the crab [1]. Cancer cells have logarithmic and uncontrolled growth and rapidly disrupt the function of cells, tissues, organs, and can, unfortunately, lead to patient death. Cancer is one of the serious health problems of today's society, and as such many efforts have been made to combat it. It is the second leading cause of death in the world and is on the rise [2]. Common cancer treatments include surgery, chemotherapy, and radiation. These treatments however have failed in the control of cancer due to the many side effects they present, mainly due to the lack of a targeted strategy. In many cases, cancer cells can also eventually counteract the therapeutic strategies offered, and even at times with chemotherapy resistance, take advantage of the treatments used to grow the tumor faster. It is therefore absolutely necessary to find alternative methods to kill cancer cells [3,4]. In recent research, some strategies including synthesizing the new chemical entities which are more efficacious than the previously approved drugs or encapsulation of drugs in the novel carriers have proved useful. One of the best ways for this smart defence is to target the neoplastic cell's weaknesses and use it to make drugs that will not give the cancer cells a chance to fight. This is known as "targeted cancer treatment" [5,6]. This approach usually involves two different approaches: (a) using special drugs to target the weaknesses of the cancer cells, and (b) ways to transfer the drug directly to abnormal cells and prevent further damage to patients. The ultimate goal of this is to provide each patient with personalized treatment based on the underlying cause of illness and brings medical science into the field of "person-centered medicine" [7].

In the current review article, the goals that are used for targeted treatment are firstly introduced, along with the rationale for their selection and the drugs they provide followed by a discussion on the intelligent methods of drug delivery by folate to target cancer cells.

Novel drug delivery systems (DDSs)

Historical documents show that man has made great efforts to fight diseases as civilization progresses. For example, the selection of materials by trial and error from nature and the surrounding environment and experimentally using them to treat illness and relieve pain. As science and technology have progressed, the active ingredients of these compounds have been identified, isolated, and their mechanism of action explored [8]. Today, there is an addition of new drugs with increased efficacy to fight against diseases. It should be noted that the properties of the drugs differ. Even those with similar therapeutic effects differ in chemical composition, molecule size, hydrophilicity, and potency. In fact, a series of factors determine whether a drug has any structural or specific effects [9]. Therefore, with increasing knowledge in various disciplines such as cell biology at the molecular level, human genome identification and the development of technologies in this field, more applications especially in peptides and nucleic acids in gene delivery are being discovered [10]. Since the activity of each drug is the result of molecular interactions within the target cells, it can, therefore, be concluded that following drug administration (oral, injectable, topical, transdermal, etc.) and for effective efficacy, the drug should reach its targeted site with sufficient concentration. This is known as drug delivery [11,12].

These important goals are not achieved by choosing a simple, traditional treatment in multiple doses, and even if suitable treatment is chosen, the side effects and ultimately patient dissatisfaction with the treatment process persist. Rapid breakthroughs in drug discovery methods have led to exponential growth in new drugs, and today there are tremendous amounts of recombinant peptide and protein drugs and analogues of hormones, most of which are engineered by genetic engineering technologies. Most of these drugs are used to treat important and life-threatening diseases such as cancer, diabetes and autoimmune diseases [13].

Due to the increases in the growth of these sensitive drugs as a result of their diverse physical and chemical properties, it is essential to change the treatment process by improving drug carriers as a new approach in DDS. Other important reasons include; reducing the resources needed for treatment, increasing the therapeutic index of drugs, avoiding the multiplicity of doses, reducing dissatisfaction and the cost of treatment. In the routine administration of drugs (either oral or intravenous), the drug is distributed throughout the body and the whole body is affected hence side effects from the drug occur [14,15]. A large amount of medicine also needs to be consumed to achieve a high concentration of the drug next to the site of action. In traditional delivery systems, there is no control over the timing, location and speed of drug release, as well as the concentration of drug in the plasma. The fluctuations are in the form of valleys and peaks and may be out of treatment and may result in lower efficacy and greater side effects. Modern DDSs, also called controlled release DDSs, can control and determine the three main parameters namely drug release rate, time, and location [16]. DDSs range from electronic devices that are implanted *in vivo* to include polymer chains that must be compatible with *in vivo* processes. DDS alters the drug tissue distribution and pharmacokinetics of the drug, due to the dose-dependency of the drug used at the time of administration and the accumulation of the drug in different parts of the body [17].

Also, reasons such as the decrease in drug solubility, destructive factors (e.g., enzymatic or environmental factors), rapid clearance, lack of specificity of toxicity, inability to cross biological barriers, are only a few of the many reasons to reduce the effectiveness of the anticancer drugs. Generally, the best way to increase the effectiveness of drugs and reduce their side effects is to design and engineer DDSs. Overall, modern DDSs are a set of methods that are deliberately applied by a pharmaceutical formulation manufacturer to cause temporal or spatial changes in the process of drug release in the body. Designing new drugs can be costly

and time-consuming with regards to the length of time it takes to enter the market whereas modern DDSs are less expensive since existing drugs are used, meaning less time is needed to bring them to the market [18].

Although the use of conventional anticancer drugs has been somewhat successful in the control of cancer, the treatment is usually not very effective due to insufficient absorption of the drug. This means low bioavailability, an increased dose thereby increased side effects, low therapeutic indexes, increased resistance to multiple drugs and non-selective targeting. The important goal in the development of drug carriers is to successfully eliminate these challenges associated with the transportation and delivery of drugs to the intended sites of treatment whilst reducing the side effects. To achieve an efficient DDS, features such as biodegradability and biocompatibility are crucial and ought to be considered [19]. Recently, nanotechnology-based DDSs have shown promising results in cancer treatment. There are different kinds of nanocarriers, which fall into two main categories of inorganic nanoparticles (e.g., iron oxide [20], silica [21], gold [22], carbon nanotubes [23], quantum dots [24]) and organic nanoparticles (e.g., liposomes [25], polymers [26], micelles [27], dendrimers [28]).

The most commonly available form of nanocarriers in clinical use is liposomal formulations. The first liposomal formulation was approved by the United States Food and Drug Administration (FDA) and was marketed in 1996. It encapsulated the antitumor drug doxorubicin (Doxil/ Caelyx [Janssen Pharmaceutica NV, Beerse, Belgium] or Myocet [GP Pharm SA, Barcelona, Spain/Teva Pharmaceutical Industries Ltd., Krakow, Poland]) [29,30]. The first indication for this liposome-based product was HIV-associated Kaposi's sarcoma. Moreover, it was approved for ovarian and recurrent breast cancers [31]. In comparison with free doxorubicin, reduced cardiotoxicity, improved tumor-specific delivery, and increased half-life

have been achieved with this formulation [32]. Other delivery systems which have been extensively investigated for cancer treatment are polymeric or micelle formulations. For instance, there are various paclitaxel or docetaxel micelles currently in clinical trial studies.

Passive and active targeted DDSs in cancer

In passive targeting, physical dissimilarities between healthy and cancer cells are crucial for specific drug delivery. The vessels around the cancer cells show a clear deformation that distinguishes them from healthy cells. These systems release the drug directly into a specific area or tissue of the body where the drug works through the enhanced permeability and retention (EPR) effect [33].

Several recent studies have shown that the application of molecules such as folic acid [34], biotin [35], hyaluronic acid [36] and glucuronic acid [37], and macromolecules like monoclonal antibodies [38] that have specific receptors on the surface of cancer cells as active targeting moieties can target cancer cells. These ligands bind to specific receptors on the surfaces of the target cells, so they can effectively increase the concentration of chemotherapeutics in the tumor area, enhance cellular uptake of drug-loaded nanocarriers, greatly reduce the side effects of the drug, and thereby improve therapeutic efficacy [39]. Compared to singular ligands, increased ligand density can be beneficial for improving binding and cellular uptake via multivalent interactions [40]. In general, there are two ways for the functionalization of nanoparticles with these ligands. One is the physical adsorption or chemical conjugation of ligands onto the surface of nanoparticles after the formation of nanoparticles, and the other is the introduction of the ligands to nanoparticles before formation [41].

Folic acid properties

The application of naturally occurring materials against various disorders has been reported in research [42,43]. The B vitamins family has attracted many researchers due to its importance and essential role in the body, especially vitamin B9 (a water-soluble vitamin) which is one of the most abundant compounds in foods and is routinely recognized as folate or folic acid. Folate is the natural form of vitamin B9 and is found in foods and plants, whilst folic acid is known as the synthetic form of folate and is added to foods [44,45].

Folate acts as an enzyme cofactor in the synthesis of DNA and RNA and remarkably helps in rapid cell division and growth, especially in infancy and pregnancy. It is also a precursor to make, repair, and methylate DNA. The presence of folate is necessary for adrenal function, support, calm and keeps the nervous system healthy. In addition, folate has a wide spectrum of pharmacological activities such as cellular metabolic processes, RNA synthesis and amino acid biosynthesis. Deficiency of dietary folic acid may cause various health issues, including walking difficulty, skeletal muscle development, carcinogenesis, cardiovascular disease, elevated plasma homocysteine, macrocytic anemia, cervical dysplasia, memory loss, depression, pregnancy loss, low birth weight and birth defects [46,47].

Folic acid deficiency affects skeletal muscle development by inhibiting cell proliferation and induction of cell cycle breaking as well as cellular senescence in C2C12 myoblasts. In addition, folic acid deficiency prevents differentiation of C2C12 myoblasts and persuades deregulation of cell cycle-related genes. It also inhibits the expression of muscle-specific marker MyHC and myogenic regulatory factor (myogenin) [48].

Furthermore, folic acid-deficient C2C12 cells cultured in differentiating media indicated more DNA damage that was evaluated by immunocytochemistry and Western blot. It is interesting to note that folic acid re-supplementation reversed cellular senescence in folic acid-deficient C2C12 myoblasts, however, it does not reverse the differentiation of C2C12 cells. These findings prove that folic acid has a key role in the normal development of skeletal muscle cells [48].

Folic acid is also an essential vitamin for cell proliferation. Therefore, the surface modification of nanoparticles with folic acid may suggest a considerable potential to develop a new approach for progressing the efficiency of cancer diagnosis and therapy (Figure 1). These recent developments in the design and biological administration of different folate-decorated nanoparticles are reviewed herein.

Transportation of folate in mammalian cells and tissues is chiefly achieved by two transporters: reduced folate carrier (RFC) and folate receptors (FRs). RFC is ubiquitously expressed in normal and neoplastic tissues and is one of the major proteins that mediate folate transport. Loss of RFC expression or function may have potential physiological, developmental and pharmacologic consequences. For antifolate cancer drugs like pemetrexed and methotrexate, loss of RFC transcripts or synthesis of mutant RFCs results in antifolate resistance because of incomplete inhibition of cellular enzyme targets and inadequate substrate for polyglutamate synthesis. Although RFC has a low affinity (Km= 1-10 μM) for folic acid, it is a high-capacity transporter. On the contrary, FRs have high affinity but low capacity transporters. FRs are found in the kidneys, lungs, and placenta [49,50].

FRs are cysteine-rich cell-surface glycoproteins and are divided into three isoforms namely FRα, FRβ, and FRγ. These receptors, especially FRα is a membrane-bound protein with most extensively expressed at low to negligible levels in normal cells, but overexpressed in specific malignant tumors such as lung, renal, colon, breast, mesothelioma, brain, pediatric ependymal, ovarian tumors, and head and neck carcinomas [51].

FRs mediate the cellular uptake of natural folates, antifolates, and folate-drug conjugates with published results indicating that this can have impressive therapeutic applications. It is also believed that FRs can be beneficial biological targets for disease management because the tissue expression profile of FRs appear to be limited to tissues that are responsible for the whole-body retention of folates (e.g., kidney, choroid plexus, and the placenta) or certain pathologic tissues like chronic inflammation sites and tumors [49,52,53].

It has also been reportedthat a folate-targeted drug could be a novel approach against tumor xenografts with decreasing the unpleasant toxicities which are seen in non-targeted drug regimens [54]. As FRs are over-expressed on the surface of different types of human cancer cells and have a high affinity for folate as a ligand, drug conjugates with folate can firmly bind to the FR and activate cellular uptake via endocytosis. Although FRα keeps itself away from direct contact with folate in circulation as it is commonly expressed at the luminal surface of epithelial cells, tumor cells alter the status by changing the transmembrane domain and transcriptional/translational level of FRα. In the preliminary stage of oncogenesis in FRα-positive tumors, the increase in the level of FRα expression raises folate uptake leading to trigger cellular DNA repair. This extending FRα expression supports tumor progression that is related to the uncontrolled growth of tumors [55].

Therefore, one of the useful strategies in targeted cancer therapy is the application of folate. Folic acid has been extensively immobilized as a targeting moiety on the surface of carriers for the delivery of various anticancer drugs. Although many efforts have been made to introduce FR-targeted nanomedicines into clinics, a limited number including folic acid-drug conjugates, FR-targeted imaging and diagnosis, FR-targeted immunotherapies, and FR-targeted nanoparticles have passed clinical trials [56-58].

FRα can be used as a suitable target for designing passive and active cancer immunotherapies. Passive immune therapy involves the administration of monoclonal antibodies for recognition of FRα and inhibition of its signaling pathways, which results in malignant cell death. Active immune strategies include: 1) Chimeric antigen receptor T cells, which allow for the recognition of FRα, thus resulting in tumor cell killing; 2) Folate-hapten immunotherapy: immune effector cells that bear Fc receptors bind to the folate-hapten and FRα complex, and cause antibody-dependent cell cytotoxic activity and phagocytosis; 3) Vaccines targeting FRα: dendritic cells engineered with FRα mRNA elicit anti-FRα immunogenic responses mediated by T cells [59].

Structure and biochemical analysis of folic acid and its receptors

Folic acid is odorless orange-yellow needles or platelets crystals. It darkens and chars from approximately 250 °C. The chemical formula of folic acid is $C_{19}H_{19}N_7O_6$ and its molar mass is 441.404 g mol^{-1}. The chemical structure of folic acid is comprised of three major parts, including glutamic acid, para-aminobenzoic acid (PABA), and a heterocyclic pteridine ring (Figure 2) [60-62]. Folic acid and 5-methyltetrahydrofolate (5-MTHF) are two active forms of vitamin B that bind to FRα, but folic acid shows a considerably greater affinity to FRα in comparison with 5-MTHF [63]. Studies on the structure of FRα revealed the mechanism of folate

binding to the FRα receptor. FRα is spherical and has four long alpha-helices, two short alpha-helices, four short beta-strands and loop regions. The three-dimensional structure of FRα is stabilized by eight disulfide bonds formed by sixteen strongly conserved cysteine residues. FRα contains three predicted glycosylation sites at N47, N139 and N179. Research shows that folic acid binds to the α1, α2 and α3 of FRα structures in a long groove (Figure 3) [61]. Folic acid also binds to the FRα pethidine ring with hydrophobic interactions and strong hydrogen bonds. It has been reported that the N and O atoms (N1, N2, N3, N5 and O4) in the hydrophilic portion of the pterin rings are attached to the carboxyl, hydroxyl and guanidinium groups in the receptor structure. The major reason for folic acid's tendency to FRα is the strong binding of the aspartate carboxylic oxygen to the N1 and N2 peptide nitrogens of folic acid. It has also been shown that the glutamate group exits the pocket inlet and can bind to carboxylate groups of drugs, contrast agents or nanoparticles [64,65].

The high affinity of folate to a variety of tumors has caused its application for the treatment and diagnosis of cancer as folate conjugated drugs and toxins, folate-based imaging agents, high-affinity antifolates, and anti-FRα antibodies [66]. To determine the mechanism of folate binding to its receptors, the crystalline structure of human FRα in the presence of folic acid has been investigated. It has been found that FRα has a spherical structure and has eight disulfide bonds and also has a deep open binding site for folate. In this case, the petroleum portion of the folate is incorporated into the receptor structure, but the glutamate portion adheres to the pocket inlet and attaches to the drug without interruption [61]. In fact, multiple interactions and high affinity between the folate receptor and folate can be a viable strategy for designing a targeting DDS to the receptor [67].

Expression of folate receptors in narmal and cancerous cells

There are only low levels of FRα (the most extensively studied isoforms of FR) on normal cells. FRα has been reported to be expressed in the normal placenta, choroid plexus, breast, lung, kidney, and fallopian tube, but, it is highly expressed on several epithelial tumor cells, especially the colon, kidney, brain, endometrium, uterus, ovary, head and neck, and mesothelium [68]. This difference between normal and tumor cells has been confirmed by measuring the binding of 3H-folic acid to crude plasma membrane preparations [69]. It is also reported that the levels of FRα in epithelial ovarian tumor cells correspond with a higher histologic grade and progressing stage of cancer. This may show the correlation between fast tumor growth and high folate level [70]. In addition, the correlation between the degree of FR level and resistance to anti-cancer drugs has been reported [71].

In contrast, FRβ is expressed in hematopoietic cells and some malignancies of nonepithelial origin, such as sarcomas and myelogenous leukemias, in an inactive form that shows no affinity for folates. Actually, FRβ is detected on activated macrophages which are seen in inflammatory diseases, containing systemic lupus erythematosus, Crohn's disease, psoriasis, and rheumatoid arthritis [72]. FRγ and its truncated version (FRγ′) are particularly expressed only at very low levels in hematopoietic tissues, especially lymphoid cells [73].

Therefore, FRs have been considered as targets for both diagnostics and therapy in different diseases. In addition, the level of FR in serum can be considered as a potential marker for hematopoietic cancers. As high levels of FR may be found in the kidney and brain tissues, applied FR-targeted drugs on these tissues might cause damage. According to immunohistochemical studies, however, there is no evidence that FR-targeted macromolecular agents can cause toxic effects on these tissues with elevated levels of FR expression [52,74].

Surface modification of nanoparticles by folic acid

Different ligands as targeting moieties have been applied on the surface of nanocarriers for the active delivery of agents against tumor cells via receptor-mediated endocytosis. One of these molecules, which has been highly effective for targeting chemotherapeutic drugs, is folate [75]. In comparison with other molecules, particularly antibody-based targeting moieties, folate indicates various potential privileges as a targeting moiety such as the small size of ligand, low molecular weight, natural resource and availability, high biocompatibility, non-immunogenicity that permits for repeated administration, comparatively high structural stability, low cost; fairly easy and definite conjugation chemistry, and high receptor affinity in specific tumor tissue [76]. For these advantages, folic acid (folate) has been covalently conjugated to antineoplastic agents, liposomes, niosomes, carbon nanotubes, dendrimers and polymers for tumor targeting [77]. For example, folate-modified polyethylene glycol polymer-based DDS containing oxaliplatin was synthesized for targeting colorectal cancer cells [78]. The oxaliplatin loaded-PLGA-PEG-FA nanoparticles indicated enhanced cellular uptake and induced more cell death than the untargeted particles and free drug. Moreover, *in vivo* experiments showed that the oxaliplatin loaded-PLGA-PEG-FA reduced the tumor size and enhanced the antitumoral effect in comparison to the untargeted particles.

Folate-decorated nanomaterials for tumor targeted therapy

It is reported that decoration of folate in polymers such as PLGA, PLGA-PEG conjugate, vitamin E TPGS conjugate, liposomes, micelles, SLN, niosomes and carbon nanotubes for the delivery of doxorubicin indicated a higher cellular uptake and cell cytotoxicity in comparison with its free drug counterpart against tumor cells [79]. This section describes the latest findings in the application of folic acid in nanocarriers as a promising strategy for cancer treatment.

Table 1 shows the summary of folate decorated nanoparticles that have been employed in cancer treatment.

Folate-modified polymer-based nanoparticles

It has been shown that high concentrations of methotrexate in HT29 colon carcinoma cells is due to an overexpression of the folate binding protein [80]. Ebrahimnejad et al. synthesized PLGA-PEG-FA nanocarriers for the targeted delivery of SN-38, a drug of choice in lung, ovarian, and colon cancer therapy. In the study, SN38-loaded PLGA-PEG-FA nanoparticles were prepared and the cytotoxicity of the nanoparticles against HT29 cells was evaluated and reported. The obtained results demonstrated higher uptake and cytotoxicity of SN38 in HT29 cell lines [81,82].

The ZnS quantum dots (QDs) were encapsulated in folate-functionalized chitosan nanoparticles for simultaneous cellular imaging and targeted drug delivery. The physicochemical characterization of the obtained nanocomposites was acceptable for drug delivery purposes with the emission of a red-orange fluorescence of about 600 nm and stability at a wide range of pH. The MTT assay showed that the synthesized nanocomposite had no toxicity against MCF-10 (non-cancer cell line) and breast cancer cell lines (MDA-MB-231 and MCF-7). The cellular uptake of the nanoparticles was examined using confocal laser scanning microscopy. The report exhibited that the presence of folic acid in the chitosan-encapsulated QDs increased the binding of nanoparticles to folate receptor cells and thereby increased intracellular entry [83].

Nanomicelles have also been manufactured using folic acid-bound poly (styrene-co-maleic anhydride) copolymer and applied for the targeted delivery of curcumin-difluoride to FRα. These nanoparticles were tested in the FRs expressing cervical and ovarian cancer cells with

the results showing that the nanoparticles have high anticancer activities. This caused a remarkable eradication of cancer cells due to tumor apoptosis, phosphatase inhibition and homologous tensin, and inhibition of the nuclear factor kappa-B (NFκB), demonstrating a greater targeting ability by folate formulations [84].

In another study, poly(l-lysine)-grafted folic acid-conjugated poly(2-methyl-2-oxazoline) (PLL-PMOXA-FA) was prepared and applied against folate overexpressing tumors. PLL-PMOXA-FA was shown to adhere to FR positive cancer cells (JEG-3, HeLa) while being non-adherent to FR negative cells (HepG2, MCF-7) at 3 h. It was shown that the amount of folic acid on the substrate had a noticeable effect on the adhesion of folate to HeLa cells. The experiments indicated that the affinity of substrates to cells significantly decreased with decreasing the ratio of folic acid ($P < 0.01$) [85].

Polymeric micelles prepared by polyamphiphilic acid (styrene-co-maleic acid), conjugated with folic acid and loaded with curcumin-difluorinated was used for targeted delivery. The results of this reported study revealed that the solubility of the drug increased with nanomicelles obtained from polymer synthesis, with the entrapment efficiency being more than 85%. The nanocomposite containing the drug showed no negative cytotoxicity on human retinal pigment epithelial cells (ARPE-19), which confirmed the safety of the nanoparticles. However, it demonstrated highly lethal activity on both retinoblastoma WERI-RB and Y-79 cell lines, indicating its potent effects on cancer cells [86].

Folic acid-modified amphiphilic alternating copolymer poly(styrene-alt-maleic anhydride) (FA-SMA) as a pH-sensitive polymer was synthesized to actively deliver curcumin to cancer cells. This polymer forms amphiphilic nanostructures at pH 7 and allows hydrophobic drugs to

be encapsulated in its structure. In addition, this carrier is only stable at neutral pH and destroys the acidic environment of the tumor and releases the drugs in situ from its nucleus [87]. Fluorescence microscopy showed an increase in the uptake of the curcumin-loaded nanocarrier in human pancreatic cancer cells compared to non-folate polymers. These results clearly demonstrate that folate-conjugated polymers have the potential of an anticancer DDS for targeted delivery into tumor cells and the ability to internalize them after they individually bind to tumors [87].

The ability of the folic acid-conjugated polymers to kill breast cancer spheroids and monolayer pancreatic cancer cells when different anti-cancer drugs such as curcumin, paclitaxel and 5-fluorouracil (they do have different molecular weights hence different sizes) are attached to FA-SMA nanoparticles were investigated using WST-1 cell proliferation assay. As shown in Figure 4, the smart tumor-targeting nanoparticles effectively indicated high penetration and internalizing in these cancer cells and decreased their amounts in a dose- and time-dependent scale [88].

In another study a modular nanotransporters (MNTs) conjugated with maleimide-polyethylene glycol (PEG)-folic acid as a multifunctional system was synthesized to transport active agents (eg, the Auger electron emitter ^{111}In) into the nuclei of FR-positive cells employing the polypeptide domains, sequential endosomal escape, nuclear translocation flow cytometry, and confocal laser scanning microscopy of cells. Folate-MNT labelled with ^{111}In against FR-positive HeLa cancer cells proved the power of the cell nuclei targeting and enhancement of the cell destruction. *In vivo* study also confirmed the sustained release of ^{111}In and inhibition of tumor growth up to 80% by folate-MNT against HeLa xenograft model [89].

Folate-modified lipid-based nanoparticles

There are several lipid nanoparticles systems including nanostructured lipid carriers (NLCs), solid lipid nanoparticles (SLNs), and liposomes which are commonly applied as a carrier for drug delivery. Specific drug delivery with lipid nanoparticles is a new approach for the treatment of cancer [90]. There have been several reports on the use of lipids in the treatment of cancer patients especially liposomal formulations where their surfaces have been decorated with PEG and folate [91-93].

Surface-decorated nanostructured lipid carriers (NLCs) by folate were used to target letrozole to MCF-7 breast cancer cells [94]. In this study, the obtained nanoparticles had desirable features such as an average particle size below 100 nm with a low polydispersity index (PDI). The apoptotic percentage changed from 24.6% to 42.2% by folate-NLC-letrozole in comparison with its free drug counterpart. Flow cytometric assays also showed a noticeable accumulation of apoptotic cells in the subG1 phase by folate-NLC-letrozole [94].

Folate-modified casein micelles were synthesized for actively targeted co-delivery of resveratrol as plant medicine and monascus as yellow pigments. For passive-targeting, PEGylated resveratrol-phospholipid nanocomplex bilayer enveloping casein-loaded micelles were prepared. Both folate and PEGylated nanocarriers as evergreen nanomicelles indicated low polydispersity, extended-release and acceptable hemocompatibility. MTT assay revealed that free drugs had inferior cytotoxicity compared to folate and PEGylated micelles against MCF-7 breast tumor cells. In a murine model of breast tumor, both nanomicelles exhibited higher efficacy than their free drugs counterpart [27].

The folic acid-coupled PEGylated liposome containing paclitaxel were prepared and evaluated for cytotoxicity in SKOV3/TAX cell line and a peritoneal xenograft model of paclitaxel-resistant ovarian cancer. The uptake of the folic acid-liposome reached the highest point at 4 h and was superior to the PEGylated liposome. The folic acid-liposome significantly inhibited the enlargement of ovarian cancer cells ($P < 0.01$) and caused a two-fold enhancement in the doubling time. The cytotoxicity of the folic acid-liposome was superior to the PEGylated liposome in both SKOV3 and SKOV3/TAX cell lines. The folic acid-liposome induced more G2-M cell cycle arrest and apoptosis in ovarian cancer cells compared to PEGylated liposome or free paclitaxel [95].

Lipid nanoparticles with folic acid containing vincristine (VCR) were prepared to target the B-cell lymphoma cell lines which expressed the folate receptor to treat the lymphoma. *In vitro* anti-tumor analysis and an *in vivo* study in murine-bearing lymphoma xenografts were applied to investigate the effects of folic acid-nanolipid structure formulations. Folic acid-decorated lipid nanoparticles demonstrated a targeted effect on drug delivery to B-cell lymphoma cells and caused the highest anti-tumor effect in lymphoma with reduced side effects [96].

FA-PEG-NLCs containing cisplatin was synthesized and prepared to target cervical cancer cells. The optimized NLCs formulations showed a surface charge of +25.7 mV and particle size of 143.2 nm. Folate decorated NLCs indicated more potent anti-cancer activities against cervical cancer cells both *in vitro* and *in vivo* compared to its free drug counterpart and non-decorated folate NLCs [97].

Folate-modified dendrimers

Dendrimers are three-dimensional polymers that have many branches like a tree and are shaped like a ball. The dendrimer has a central hydrophobic or hydrophilic core, a branched dendron, and surface-active groups, which assigns its biomedical applications. As the branches of the dendrimers and surface groups increase, the generation number of dendrimers increases accordingly. Dendrimers are one of the most important nanostructures that facilitate drug delivery through endocytosis into the cell [98].

Folate-conjugated polyamidoamine dendrimer G4 (FA-G4) nanoparticles were prepared and utilized for the targeted delivery of genes to head and neck tumor cells [99]. The Fluorescence imaging demonstrated that FA-G4 nanoparticles facilitated the cellular uptake of DNA plasmids in an FR-dependent manner and selectively delivered plasmids to FR-positive cells. The *in vitro* gene transfection efficiency of FA-G4 was investigated by HN12 cells. The flow cytometry results revealed that G4-FA showed superior gene expression compared to untargeted nanoparticles.

In another study, folate-decorated dendrimer-functionalized gold nanoparticles were fabricated for luciferase gene silencing [100]. The results showed that siRNA can be delivered safely and efficiently. The study also showed that folic acid conjugation increased cellular uptake by the mechanism of receptor-mediated endocytosis and the results corroborated the key role of dendrimer and Au the in enhancement of transgene silencing.

Folate-modified Au nanoparticles

Metal nanoparticles are important nanocarriers due to their specific properties. These features include adjustable size, specific shape, simple synthesis, particular optical effects, thermal

removal of tumors, and simple surface modifications. Among them, gold nanocarriers have shown unique properties against cancer cells. Gold nanoparticles have the potential for loading various biological molecules like drug molecules, proteins, and genes. In addition, gold nanoparticle shows a phenomenon called surface plasmon resonance which converts light to heat and destroys tumors [101].

Gold nanoparticles however have limited stability and solubility without surface modifications or ligands. To enhance these properties, gold nanoparticles have been PEGylated. In many studies, various ligands, such as folate or transferrin for targeted drug delivery, have been introduced on the surface of Au nanoparticles. These modified nanoparticles have shown superior efficacy against tumors in comparison to simple Au nanoparticles [102].

In another study, doxorubicin-loaded zeolitic imidazolate framework (ZIF-8) nanoparticles, a subclass of metal-organic frameworks (MOFs), were prepared with folate conjugate as a DDS for the treatment of liver cancer. The obtained nanoparticles had desirable physicochemical properties and high drug loading capacity and showed efficient drug release and pH-responsivity at the tumor site. Comparison of the results of free doxorubicin and doxorubicin-loaded ZIF-8 nanoparticles with doxorubicin-loaded ZIF-8-FA nanoparticles showed much more effective anti-cancer properties for the folate-conjugated nanoparticles in HepG2 cells. This demonstrated that folic acid-modified nanoparticles have higher drug delivery efficiency at the tumor site and treatment of cancer [103].

Folate-modified iron oxide nanoparticles (IO nanoparticles)

Cancer therapy with iron oxide nanoparticles is a promising treatment strategy [104]. Shetty et al. fabricated magnetic nanoparticles functionalized with folic acid, loaded with cinnamaldehyde

and, used it for active targeting of breast cancer cells [105]. Conjugation of Folic acid moieties improved cell uptake of nanoparticles in breast cancer cells with their localization in both nucleus and cytoplasm compartments. Moreover, drug-loaded folate-functionalized nanoparticles induced apoptosis in tumor cells via increasing the expression of caspases. *In vivo* studies demonstrated that cinnamaldehyde-loaded targeted nanoparticles reduced the tumor burden in the mouse breast cancer model in comparison with those treated with free cinnamaldehyde and nanoparticles without folate moieties.

In another research, multi-functional nanocarrier based on IO nanoparticles conjugated with doxorubicin (by monomeric methyl adipate (MMA) as a linker), PEG, and folic acid (IO-MMA-DOX-PEG-OCH3/FA) was fabricated for targeted cancer treatment (Figure 5) [106]. The size of the folic acid-modified nanoparticles was 40 nm and saturation magnetization and transverse relaxivity values were reported to be 28.62 Am^2/kg and 133 $mM^{-1}s^{-1}$, respectively. Because of the hydrazone bond between drug and IO nanoparticles, the percentage of drugs released from folic acid-modified nanoparticles in an acidic environment (pH 5.6) was higher than neutral environment (pH 7.4). Folic acid-modified nanoparticles indicated higher cellular uptake and cytotoxicity against apoptotic HeLa cells than folic acid-free nanoparticles [106].

Folate-modified carbon nanotubes (CNTs)

CNTs are from the fullerene family and a group of carbon allotropes. They are hollow spheres, ellipses, tubes and other shapes. The CNT is a cylindrical tube formed by the rotation of a graphene sheet into a seamless one. They have two shapes, single-walled (SWCNT) and multi-walled (MWCNT). MWCNTs are capable of crossing various cellular barriers and PEGylated SWCNTs are capable of localization in specific cellular segments. SWCNTs are more effective in drug delivery than MWCNTs because of the better wall formation in SWCNTs. Also,

structural defects are relatively higher in MWCNTs [107]. Functional groups attach to their surfaces to improve the performance of CNTs in drug delivery. One of these factors is PEG, which increases solubility, prevents apnea and reduces the toxicity of CNTs [108]. The use of folate to attach to the surface of CNTs also enhances the specificity of active agents in the diagnosis and treatment of cancer. CNTs are used in gene delivery to transfer plasmid DNA, siRNA, disinfectant oligonucleotides and aptamers as well as thermal ablation of tumors [109,110].

A Folic acid-conjugated CNT-based DDS was fabricated by Jawahar et al. for targeted delivery of raloxifene hydrochloride to breast cancer cells [111]. Cytotoxicity study obviously demonstrated the efficacy of the folate-conjugated CNTs with affectivity induces apoptosis in the cancerous cells. The fluorescence imaging study revealed that the cellular uptake of raloxifene hydrochloride-loaded nanoparticles was higher than that of free drug and untargeted CNTs.

Folate-modified Quantum dots (QDs)

QDs are fluorescent semiconductor nanocarriers, which consist of three parts (from inside to outside); a core, shell, and coating. The nucleus is a semiconductor material with a size in diameter ranging 2–10 nm such as CdSe. The shell is made of another semiconductor such as ZnS that surrounds the core. The outer coating that encloses the following layers can be of different materials such as polymers [112]. QDs have various applications in drug delivery due to their very small size which makes them effective in tracking DDSs, the possibility of chemical changes on the QD surface, and their specific photophysical properties that precisely examine drug delivery pathways and drug release processes. QDs are prepared in both top-down and bottom-up approaches [113].

QDs, like other new nanocarriers, can be functionalized to act as smart nanoparticles. The surface modifications of QDs by PEG can be kept out from the RES uptake, which is a good solution for their non-specific action. To target the tumor site, different ligands such as peptides, folates, hyaluronic acid, biotin and monoclonal antibodies can bind to the QDs surface [12,114]. In the literature, it has been shown that graphene QDs-based DDS can be used for the targeted delivery of doxorubicin to tumor cells. Highly dispersed and water-soluble graphene QD were prepared through acid oxidation and exfoliation of MWCNTs, and covalently attached to the biotin, which is capable of effectively binding to the biotin receptor that is highly expressed on the cancer cells [35].

Another study reported a carboxylated graphene quantum dots (cGQDs) nanosystem modified by PEG and folic acid to be successfully synthesized and then loaded with mitoxantrone (MTN) (FA-PEGcGQDs-MTN). Loading capacity and entrapment efficiency of the carrier were found to be 40.1% and 97.5%, respectively. Cell imaging showed that the nanocarrier was mainly internalized by human cervical cancer cells via the macropinocytosis-dependent pathway. Moreover, *in vivo* testing revealed that the sizes of the tumors treated with FA-PEG-cGQDs-MTN were the smallest of all experimental samples and FA-PEG-cGQDs-MTN inhibited tumor growth with a TGI rate of 99.68%. Also, programmed cell necrosis was observed in most tumor tissues (Figure 6) [115].

Coupling a photosensitizer with folic acid

Photodynamic therapy (PDT) has been of great interest in research due to the increasing targeting of chemotherapeutics and photosensitive agents into the tumors. The binding of photosensitive agents to folic acid could bring about higher targeting into the folate-positive cancer cells [116].

Folic acid has been reported to be attached to various photosynthetic materials such as small oligo (ethylene glycol), 2,2- (ethylene deoxy) diethylamine, porphyrin, and Ce6. Physicochemical characterization and full evaluation of these folic acid-targeted photosensitizing agents were performed with all indicating suitable effects with Ce6 reported to be the most suitable [117].

Folate-modified micelles

Micelles are defined as nanosized core/shell structures with particle sizes of 5-100 nm. Micelles have an inner hydrophobic core, enabling them to encapsulate poorly water-soluble drugs in order to improve their bioavailability and stability. The hydrophilic shell of micelles can prolong their blood circulation time and prevent their uptake via the mononuclear phagocyte system [118].

Among all nanocarriers, micelles have the privilege of possessing a very small size, which plays a pivotal role in passive targeting to solid tumors, especially poorly vascularized tumors. Micelles have various morphologies including lamellae, tubules, vesicles, rods, and spheres. Such characteristics lead to a profound impact on the pharmacokinetics of micellar systems. These drug delivery platforms can act as reservoirs and can provide a high amount of chemotherapeutics at the tumor site. Micelles are mostly used for drug targeting, controlled drug release, and drug solubilization [119].

A crucial barrier to achieve the efficient result of paclitaxel in the treatment of cancer is its non-specific function against tumor cells. In the literature, folic acid-conjugated deoxycholic acid-O-carboxymethylated chitosan (FA-DOMC) nanoparticles were synthesized to prepare micelles containing paclitaxel. The physicochemical characteristics of paclitaxel loaded

micelles and *in vitro* cell culture examinations were performed against MCF-7 human breast cancer cells, with high folate receptors on them [120].

This study showed the increased uptake in folate-conjugated micelles in MCF-7 cells in comparison with simple micelles. The main cause of the increased uptake of the folate micelles was attributed to the efficacious process of FR-mediated endocytosis. The obtained results indicated that folate-conjugated micelles increased cell death and cytotoxicity compared to non-folate micelles or a commercial injection of paclitaxel (Taxol) against cancer cells. This study demonstrates that folate-conjugated micelles containing paclitaxel are an efficacious target delivery system for cancer therapy [120].

Folate-dendrimer-like star polymers were synthesized and applied in the preparation of micelles containing doxorubicin to target tumors. The size of the nanoparticles was 15 nm and drug loading was 4%. The release of the drug from the micellar structure was found to be pH-dependent and gradually started from the hydrophobic shell to the hydrophobic core. Flow cytometry and confocal microscopy showed that the binding of folate micelles against human KB cells to dispersed FRs was approximately twice that of folic acid-free micelles. Cytotoxicity tests showed that drug-free folate-bound micelles had acceptable biocompatibility with KB cells, whereas micelles containing the drug and the free drug showed similar cytotoxicity against KB cells. The report thus proved that folate-bound micelles could be a suitable carrier for targeting anticancer drugs [121].

Another study reports the synthesizes, fabrication and complete evaluation of folic acid-modified stealthy conjugate micelles containing doxorubicin. Cytotoxicity determination and cellular internalization were performed using different cancer cell lines such as HeLa, KB, and

MCF-7/ADR (FR-positive) and A549 (FR-negative). The results indicated a significant reduction in IC50 for FR-positive cells, whereas only a small reduction for FR-negative cells was reported ($P < 0.05$). Folic acid-conjugated micelles showed enhanced intracellular delivery of doxorubicin in MCF-7/ADR cells. In addition, the increase in the cellular uptake of the folic acid conjugate micelles was seen in the FR-positive cell lines. Tumor growth evaluation in mice exhibited superior effectiveness with inferior toxicity for the folic acid stealthy targeted micelles to its free drug counterpart [122].

Folate-modified albumin-bound nanoparticles

Albumin-based colloidal nanoparticles have also received great attention in drug delivery. Various kinds of albumin (human, bovine, and egg) and different techniques in fabricating and transporting albumin-bound nanoparticles have been reported in several published papers [123-125]. In one study, curcumin difluorinated (CDF), as a powerful anticancer analogue of curcumin was incorporated in folate-conjugated albumin to obtain biocompatible nanoparticles. This novel nanoformulation showed higher cellular uptake of folate-conjugated albumin by SKOV3 ovarian cancer cells, which was due to the receptor-mediated endocytosis [126].

Folate-modified mesoporous silica nanoparticles

Mesoporous silica nanoparticles (MSNs) are inorganic materials that contain a lot of empty mesopores channels arranged in a 2D hexagonal structure. MSNs have displayed specific properties in the delivery of active compounds due to their unique structure which leads to high loading and surface modification capabilities [127]. MSNs with folic acid (MSN-FA) or methionine (MSN-Met) containing docetaxel were synthesized. The obtained nanoparticles indicated suitable size, high drug loading and pH-dependent release. The cytotoxicity study of the folate formulation against MCF-7 cells exhibited better efficacy and cell apoptosis and

higher cellular uptake in comparison with conventional nanoparticles and free drug. In addition, *in vivo* fluorescence imaging in healthy mice showed that the majority of MSN-FA or MSN-Met were accumulated in the kidney whilst untargeted MSN-NH_2 were mainly concentrated in the liver. In tumor-induced BALB/c mice, the fluorescence intensity in tumor tissue was higher than that in other tissues. Furthermore, tumor tissues exhibited a relatively high fluorescence intensity in MSN-FA samples whereas the MSN-Met accumulation in tumor tissue was relatively low [128].

Natural agents such as curcumin, quercetin, and colchicine have also been incorporated in two different kinds of MSN; Fibrous Nano-Silica (KCC-1) and Mobil Composition of Matter 41 (MCM-41) that were first synthesized by Mobil's researchers in 1992. The mean size of KCC-1 was 324 nm and the mean pore diameter was 3.4 nm, whereas the mean size of MCM-41 was 197 and pore diameter was 2 nm. These were evaluated against HepG2 and HeLa cancer cell lines. Folic acid-modified KCC-1 and MCM-41containing curcumin showed superior cytotoxicity, cellular uptake, apoptosis, antioxidant and anticancer effects compared to non-conjugated MSNs. The KCC-1 type MSNs containing curcumin exhibited the greatest anticancer effect [129].

Folate-decorated nanomaterials for tumor diagnostics

Nanotechnology has been widely used to improve tumor imaging and diagnostics. There are various medical imaging techniques for cancer diagnosis including near-infrared (NIR) fluorescence imaging, magnetic resonance imaging (MRI), computed tomography (CT), and ultrasonic imaging [50]. Besides the delivery of therapeutic molecules, folic acid has been extensively applied for the specific delivery of imaging contrast agents.

QDs are suitable materials for cancer imaging because of their inherent nature. For instance, folate-conjugated nitrogen-doped graphene QD nanoprobe was prepared as a fluorescent diagnostic tool for breast tumor cells [130]. The laser confocal scanning microscopy results revealed that the enhanced folic acid binding facilitated the recognition of and internalization into cancer cells. This made the labeled cells emit a stronger fluorescence intensity thereby making the tumor cells better detected.

Folic acid-modified trypsin-stabilized gold nanoclusters (FA-try-Au) with NIR fluorescence was prepared by Liu et al. for the *in vivo* cancer bioimaging of FR-overexpressing tumors [131]. The fluorescence signal was detected right after intrathecal injection of the FA-try-Au nanoprobe in Hela tumor-bearing nude mice. The signal in the tumor remained for up to 12 h.

In addition, rare-earth nanoparticles have been extensively applied in tumor diagnosis, bioimaging, and tumor therapy [132]. Jain et al. fabricated folic acid functionalized $Gd_2O_3:Eu^{3+}$ nanoparticles for the detection of breast cancer cells [133]. Cellular uptake experiments showed that folate-modified rare-earth nanoparticles had a higher degree of cellular uptake in T-47D (high FR expression) cells than MDA-MB-231 (low FR expression) breast cancer cells. *In vivo* confocal and CT imaging investigations demonstrated that folate-modified $Gd_2O_3:Eu^{3+}$ nanoparticles showed a greater accumulation in T-47D tumor xenograft in comparison to the MDA-MB-231 tumor.

Lin et al. reported a multifunctional nanocomposite based on NaYF4:Yb,Er@ NaYF4: Yb@NaYF4:Yb,Nd nanoparticles for bioimaging and tumor targeting therapy with NIR excitation (793 nm) and NIR emission (980 nm) [134]. Mesoporous silica and polyallylamine (PAH) were coated on the surface of the nanoparticles to enable the loading of the IR806

photothermal sensitizer. The modified nanoparticles were then conjugated with FA-PEG to obtain tumor targeting. *In vitro* experiments proved the uptake of the nanocomposite by the MDA-MB-231 cells using NIR fluorescence imaging for targeted photothermal therapy.

F127-Folate coated superparamagnetic iron oxide (SPIO) nanoparticles were fabricated to target tumors efficiently and providing imaging contrast in MRI [135]. *In vitro* studies revealed that the folate-conjugated nanoparticles specifically targeted the KB cells through FRs. The *in vivo* MRI imaging showed negative contrast enhancement from the F127-Folate coated SPIO nanoparticles in tumor-bearing mice.

Khademi et al. synthesized cysteamine-folic acid conjugated gold (FA-Cys-Au) nanoparticles as a nanoprobe for X-ray CT of cancer cells [136]. FA-Cys-Au nanoparticles exhibited higher X-ray attenuation intensity than an iodinated contrast agent. The CT values of targeted cells were calculated to be two times higher than that of untargeted cells.

Folate-conjugated liposome-based nanoparticles were prepared as an ultrasound contrast agent for the targeted imaging of cancer cells [137]. The *in vitro* results indicated that the folate modified nanoparticles were clearly uptaken into the cytoplasm of SW620 and Bel7402 cells (FR-overexpressing cancer cells), whereas the A549 cells, which expressed low levels of FR just bound with few nanoparticles. The folate modified nanoparticles also attained a greater enhancement of tumor ultrasound imaging and had a longer enhanced duration in FR-overexpressing tumors in *in vivo* experiments.

Biodistribution and nephrotoxicity of folic acid-decorated nanoparticles

According to the literature, the most important problem of folate-decorated nanoparticles is the rapid excretion of these nanoparticles through the renal system. Tissue distribution evaluation shows high concentrations of folate-targeted nanoparticles in the liver and kidneys [50].

Depending on their size, nanoparticles may accumulate in organs such as the liver, spleen, and lungs. In general, nanoparticles can increase in the liver due to discontinuous endothelium with sinusoidal vascular beds. The nanoparticles larger than 200 nm have been shown to accumulate in the spleen due to the 200-500 nm size range of interendothelial cell slits. Nanoparticles with the size of 50-100 nm can accumulate in the liver due to noncontinuous endothelia with vascular fenestrations. Nanoparticles smaller than 5 nm can traverse tight endothelial junctions and may be rapidly filtered out via the glomeruli of the kidneys. Microparticles with the size of 2-5 μm have been shown to accumulate readily in lungs capillaries [138].

Prednisolone-conjugated folate was synthesized for targeted and controlled release drug delivery to the kidney. The results indicate the importance of folate-mediated renal-targeted delivery and the enhancement of the effect of folate modification for the treatment of renal ischemia/reperfusion injury. In spite of the high expression of folate in proximal tubular cells, no renal toxicity was reported for vintafolide in the clinics. The absence of nephrotoxicity could be due to various processes of folate internalization. In other words, folate receptors conjugate folic acid and produce primary endosomes in tumors. With the formation of early endosomes, a potent reduction of the microenvironment is observed, which breaks the disulfide bond to release anticancer drugs such as vinblastine into the cytoplasm, demonstrating its cytotoxicity and efficacy. In addition, folate receptors help to rescue folates from fresh urine, which are transmitted through the transcytosis in the epithelium of the kidneys to the blood. This

phenomenon reduces the release of vinblastine from the receptor and causes a low amount of drug to be present in the proximal tubule cells, thereby decreasing its cytotoxicity and nephrotoxicity [139].

Scale-up of folate-modified nanoparticles

Clinical translation and integration depend on the consistency and reproducibility of products. Apart from some exceptions, nanoparticles utilized in the preclinical phase are almost exclusively fabricated in small batches and as such, it may not always be possible to scale up for large quantities, even for the clinical phase. So, having a consistent and highly reproducible formulation before the clinical research phase is necessary due to the complexity of human diseases. The effects of scale-up have mostly been observed on the stability, particle size distribution, and drug loading and encapsulation efficiency [140]. A comparative study of gefitinib-loaded folic acid-modified dextran-based nanoparticles reported that the nanoparticles from the flash nanoprecipitation method had a hydrodynamic radius of about 78 nm, a narrow size distribution with PDI value of about 0.24 [141]. The size and size distribution hardly changed for more than fifteen days. This fact demonstrated that the prepared nanoparticles had good stability. In contrast, in the traditional precipitation method, the size of the nanoparticles increased, a broad size distribution observed, and nanoparticles precipitated on the second day. Furthermore, the drug loading content and encapsulation efficiency for flash nanoprecipitation method were 5.4% and 5.1%, respectively, wheras for the traditional precipitation method, these values were calculated to be 4.9% and 4.7% respectively.

Clinical trials and patents

Some of the folic acid-tagged formulations have been the objects of clinical trials. The therapeutic agents were conjugated with folate moieties by linkers such as PEGs, proteins, or

polysaccharides that separate the drug molecule from folic acid avoiding interference from steric interactions. The clinical trials of folic-acid tagged formulations developed for the diagnosis and treatment of different types of cancer have been summarized in Table 2.

In addition, some of the patented folate-conjugated nanocarriers utilized for tumor diagnosis and therapy have been presented in Table 3.

Conclusion and outlooks

Folic acid is an attractive molecule due to its use for different goals. The most consequential benefit of folic acid is its broad utilization in cancer and its ability to act as a targeting moiety in drug delivery. There has been an enormous development in using folate conjugated with carrier-drugs to maximize the anticancer effect of anticancer drugs and to reduce their side effect. Accordingly, further investigations in clinics are expected for the formulation of novel drugs constructed on folic acid. It is hoped that this review will be of interest to both researchers and patients with the former looking for new research guidance in this field to provide extensive benefits for patients and the latter gaining an understanding of how these systems work.

References

1. David AR, Zimmerman MR. Cancer: an old disease, a new disease or something in between? *Nat Rev Cancer*. 2010;10:728-733.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71:209-249.
3. Arruebo M, Vilaboa N, Sáez-Gutierrez B, Lambea J, Tres A, Valladares M, et al. Assessment of the evolution of cancer treatment therapies. *Cancers*. 2011;3:3279-3330.
4. Indoria S, Singh V, Hsieh MF. Recent advances in theranostic polymeric nanoparticles for cancer treatment: A review. *Int J Pharm.* 2020; 582: 119314.
5. Pérez-Herrero E, Fernández-Medarde A. Advanced targeted therapies in cancer: drug nanocarriers, the future of chemotherapy. *Eur J Pharm Biopharm*. 2015;93:52-79.
6. Boix-Montesinos P, Soriano-Teruel PM, de Benito AA, Orzáez M, Vicent MJ. The Past, Present, and Future of Breast Cancer Models for Nanomedicine Development. *Adv Drug Deliv Rev*. 2021;173:306-330.
7. Firer MA, Gellerman G. Targeted drug delivery for cancer therapy: the other side of antibodies. *J Hematol Oncol*. 2012;5:1-16.

8. Swamy MK, Sinniah UR. Patchouli (Pogostemon cablin Benth.): botany, agrotechnology and biotechnological aspects. *Ind Crops Prod*. 2016;87:161-176.
9. Orive G, Gascon AR, Hernández RM, Domínguez-Gil A, Pedraz JL. Techniques: new approaches to the delivery of biopharmaceuticals. *Trends Pharmacol Sci*. 2004;25:382-387.
10. Ding N, Lee S, Lieber-Kotz M, Yang J, Gao X. Advances in genome editing for genetic hearing loss. *Adv Drug Deliv Rev*. 2021;168:118-133.
11. Martinho N, Damgé C, Reis CP. Recent advances in drug delivery systems. *J Biomater Nanobiotechnol*. 2011;2:510-526.
12. Monge P, Søgaard AB, Andersen DG, Chandrawati R, Zelikin AN. Synthetic chemical ligands and cognate antibodies for biorthogonal drug targeting and cell engineering. *Adv Drug Deliv Rev*. 2021;170:281-293.
13. Li C, Wang J, Wang Y, Gao H, Wei G, Huang Y, et al. Recent progress in drug delivery. *Acta Pharm Sin B.* 2019;9:1145-1162.
14. Hoffman AS. The origins and evolution of "controlled" drug delivery systems. *J Control Release*. 2008;132:153-163.
15. Sadeghi-Ghadi Z, Ebrahimnejad P, Talebpour Amiri F, Nokhodchi A. Improved oral delivery of quercetin with hyaluronic acid containing niosomes as a promising formulation. *J Drug Target*. 2021;29:225-234.
16. Park K. Controlled drug delivery systems: past forward and future back. *J Control Release*. 2014;190:3-8.
17. Wen H, Jung H, Li X. Drug delivery approaches in addressing clinical pharmacology-related issues: opportunities and challenges. *AAPS J*. 2015;17:1327-1340.
18. Lombardo D, Kiselev MA, Caccamo MT. Smart nanoparticles for drug delivery application: development of versatile nanocarrier platforms in biotechnology and nanomedicine. *J Nanomater*. 2019;2019:1-26.
19. Patra JK, Das G, Fraceto LF, et al. Nano based drug delivery systems: recent developments and future prospects. *J Nanobiotechnology*. 2018;16:1-33.
20. El-Boubbou K. Magnetic iron oxide nanoparticles as drug carriers: preparation, conjugation and delivery. *Nanomedicine*. 2018;13:929-952.
21. Sodagar Taleghani A, Ebrahimnejad P, Heydarinasab A, Akbarzadeh A. Adsorption and controlled release of iron-chelating drug from the amino-terminated PAMAM/ordered mesoporous silica hybrid materials. *J Drug Deliv Sci Technol*. 2020; 56: 1-8.
22. Chauhan G, Chopra V, Tyagi A, Rath G, Sharma RK, Goyal AK. "Gold nanoparticles composite-folic acid conjugated graphene oxide nanohybrids" for targeted chemo-thermal cancer ablation: In vitro screening and in vivo studies. *Eur J Pharm Sci*. 2017;96:351-361.
23. Jafari M, Heidari D, Ebrahimnejad P. Synthesizing and characterizing functionalized short multiwall carbon nanotubes with folate, magnetite and polyethylene glycol as multi-targeted nanocarrier of anti-cancer drugs. *Iran J Pharm Res.* 2016;15:449-456.
24. Zhang L, Yang X-Q, An J, Zhao S-D, Zhao T-Y, Tan F, et al. In vivo tumor active cancer targeting and CT-fluorescence dual-modal imaging with nanoprobe based on gold nanorods and InP/ZnS quantum dots. *J Mater Chem B*. 2018;6:2574-2583.
25. Saadat E, Dinarvand R, Ebrahimnejad P. Encapsulation of nystatin in nanoliposomal formulation: characterization, stability study and antifungal activity against Candida albicans. *Pharm Biomed Res*. 2016;2:44-54.
26. Mohammady H, Dinarvand R, Esfandyari Manesh M, Ebrahimnejad P. Encapsulation of irinotecan in polymeric nanoparticles: Characterization, release kinetic and cytotoxicity evaluation. *Nanomed J*. 2016;3:159-168.
27. El-Far SW, Helmy MW, Khattab SN, Bekhit AA, Hussein AA, Elzoghby AO. Folate conjugated vs PEGylated phytosomal casein nanocarriers for codelivery of fungal-and herbal-derived anticancer drugs. *Nanomedicine*. 2018;13:1463-1480.
28. Xu L, Yang H. Folate-Decorated Polyamidoamine Dendrimer Nanoparticles for Head and Neck Cancer Gene Therapy. *RNA Interference and Cancer Therapy*. Springer; 2019:393-408.
29. Barenholz YCJJocr. Doxil®—the first FDA-approved nano-drug: lessons learned. *J control Release*. 2012;160:117-134.

30. Chang H-I, Yeh M-K. Clinical development of liposome-based drugs: formulation, characterization, and therapeutic efficacy. *Int J Nanomedicine*. 2012;7:49-60.
31. Tejada-Berges T, Granai C, Gordinier M, Gajewski W. Caelyx/Doxil for the treatment of metastatic ovarian and breast cancer. *Expert Rev Anticancer Ther*. 2002;2:143-150.
32. Bulbake U, Doppalapudi S, Kommineni N, Khan W. Liposomal formulations in clinical use: an updated review. *Pharmaceutics.* 2017;9:1-33.
33. Attia MF, Anton N, Wallyn J, Omran Z, Vandamme TF. An overview of active and passive targeting strategies to improve the nanocarriers efficiency to tumour sites. *J Pharm Pharmacol*. 2019;71:1185-1198.
34. Mir M, Ebrahimnejad P. Preparation and characterization of bifunctional nanoparticles of vitamin E TPGS-emulsified PLGA-PEG-FOL containing deferasirox. *Nanosci Nanotechnol Asia*. 2014;4:80-87.
35. Iannazzo D, Pistone A, Salamò M, Galvagno S, Romeo R, Giofré SV, et al. Graphene quantum dots for cancer targeted drug delivery. *Int J Pharm.* 2017;518:185-192.
36. Li J, Yang X, Yang P, Gao F. Hyaluronic acid–conjugated silica nanoparticles for breast cancer therapy. *Inorg Nano-Met Chem.* 2017;47:777-782.
37. Sodagar Taleghani A, Ebrahimnejad P, Heidarinasab A, Akbarzadeh A. Sugar-conjugated dendritic mesoporous silica nanoparticles as pH-responsive nanocarriers for tumor targeting and controlled release of deferasirox. *Mater Sci Eng C*. 2019;98:358-368.
38. Julien DC, Behnke S, Wang G, Murdoch GK, Hill RA. Utilization of monoclonal antibody-targeted nanomaterials in the treatment of cancer. *MAbs*. 2011;3:467-478.
39. Sharifi F, Jahangiri M, Ebrahimnejad P. Synthesis of novel polymeric nanoparticles (methoxy-polyethylene glycol-chitosan/hyaluronic acid) containing 7-ethyl-10-hydroxycamptothecin for colon cancer therapy: in vitro, ex vivo and in vivo investigation. *Artif Cells Nanomed Biotechnol*. 2021;49:367-380.
40. Montet X, Funovics M, Montet-Abou K, Weissleder R, Josephson L. Multivalent effects of RGD peptides obtained by nanoparticle display. *J Med Chem*. 2006;49:6087-6093.
41. Bahrami B, Hojjat-Farsangi M, Mohammadi H, Anvari E, Ghalamfarsa G, Yousefi M, et al. Nanoparticles and targeted drug delivery in cancer therapy. *Immunol Lett*. 2017;190:64-83.
42. Yi SS, Hwang E, Baek HK, Kim T-H, Lee H-H, Jun HS, et al. Application of bioactive natural materials-based products on five women's diseases. *J Menopausal Med.* 2015;21:121-125.
43. Cheuka PM, Mayoka G, Mutai P, Chibale K. The role of natural products in drug discovery and development against neglected tropical diseases. *Molecules*. 2017;22:1-41.
44. Bailey LB, Gregory III JF. Folate metabolism and requirements. *J nutr*. 1999;129:779-782.
45. van der Windt M, Schoenmakers S, van Rijn B, Galjaard S, Steegers-Theunissen R, van Rossem L. Epidemiology and (Patho) physiology of folic acid supplement use in obese women before and during pregnancy. *Nutrients*. 2021;13:1-16.
46. Bailey LB, Stover PJ, McNulty H, Fenech MF, Gregory III JF, Mills JL, et al. Biomarkers of nutrition for development—folate review. *J nutr*. 2015;145:1636S-1680S.
47. Harlan De Crescenzo A, Panoutsopoulos AA, Tat L, Schaaf Z, Racherla S, Henderson L, et al. Deficient or excess folic acid supply during pregnancy alter cortical neurodevelopment in mouse offspring. *Cereb Cortex*. 2021;31:635-649.
48. Hwang SY, Kang YJ, Sung B, Jang JY, Hwang NL, Oh HJ, et al. Folic acid is necessary for proliferation and differentiation of C2C12 myoblasts. *J cell physiol.* 2018;233:736-747.
49. Leamon CP. Folate-targeted drug strategies for the treatment of cancer. *Curr Opin Investig Drugs.* 2008;9:1277-1286.
50. Xu L, Bai Q, Zhang X, Yang H. Folate-mediated chemotherapy and diagnostics: an updated review and outlook. *J Control Release*. 2017;252:73-82.
51. Fernández M, Javaid F, Chudasama V. Advances in targeting the folate receptor in the treatment/imaging of cancers. *Chem Sci*. 2018;9:790-810.
52. Lu Y, Low PS. Folate-mediated delivery of macromolecular anticancer therapeutic agents. *Adv Drug Deliv Rev*. 2002;54:675-693.

53. Wang D, Li H, Chen W, Yang H, Liu Y, You B, et al. Efficient tumor-targeting delivery of siRNA via folate-receptor mediated biomimetic albumin nanoparticles enhanced by all-trans retinoic acid. *Mater Sci Eng C*. 2021;119:111583.
54. Alkilany AM, Zhu L, Weller H, Mews A, Parak WJ, Barz M, et al. Ligand density on nanoparticles: A parameter with critical impact on nanomedicine. *Adv Drug Deliv Rev*. 2019;143:22-36.
55. Ledermann J, Canevari S, Thigpen T. Targeting the folate receptor: diagnostic and therapeutic approaches to personalize cancer treatments. *Ann Oncol*. 2015;26:2034-2043.
56. Low PS, Kularatne SA. Folate-targeted therapeutic and imaging agents for cancer. *Curr opin chem biol*. 2009;13:256-262.
57. Ghalehkhondabi V, Soleymani M, Fazlali A. Folate-targeted nanomicelles containing silibinin as an active drug delivery system for liver cancer therapy. *J Drug Deliv Sci Technol*. 2021;61:102157.
58. Guzik P, Fang H-Y, Deberle LM, Benesova M, Cohrs S, Boss SD, et al. Identification of a PET Radiotracer for Imaging of the Folate Receptor-alpha–A Potential Tool to Select Patients for Targeted Tumor Therapy. *J Nucl Med*. 2021;62:57-60.
59. Farran B, Pavitra E, Kasa P, Peela S, Raju, GSR, Nagaraju, GP. Folate-targeted immunotherapies: passive and active strategies for cancer. *Cytokine Growth Factor Rev.* 2019;45:45-52.
60. Della-Longa S, Arcovito A. Structural and functional insights on folate receptor α (FRα) by homology modeling, ligand docking and molecular dynamics. *J Mol Graph Model*. 2013;44:197-207.
61. Chen C, Ke J, Zhou XE, Yi W, Brunzelle JS, Li J, et al. Structural basis for molecular recognition of folic acid by folate receptors. *Nature*. 2013;500:486-489.
62. Boss SD, Ametamey SMJC. Development of Folate Receptor−Targeted PET Radiopharmaceuticals for Tumor Imaging—A Bench-to-Bedside Journey. *Cancers.* 2020;12:1-21.
63. Obeid R, Holzgreve W, Pietrzik K. Is 5-methyltetrahydrofolate an alternative to folic acid for the prevention of neural tube defects? *J perinat med*. 2013;41:469-483.
64. Cheung A, Bax HJ, Josephs DH, Ilieva KM, Pellizzari G, Opzoomer J, et al. Targeting folate receptor alpha for cancer treatment. *Oncotarget*. 2016;7:52553-52574.
65. Al-Thiabat MG, Saqallah FG, Gazzali AM, Mohtar N, Yap BK, Choong YS, et al. Heterocyclic Substitutions Greatly Improve Affinity and Stability of Folic Acid towards FRα. an In Silico Insight. *Molecules*. 2021;26:1079-1084.
66. Zhao X, Li H, Lee RJ. Targeted drug delivery via folate receptors. *Expert Opin Drug Deliv*. 2008;5:309-319.
67. Gangopadhyay S, Nikam RR, Gore KR. Folate Receptor-Mediated Small Interfering RNA Delivery: Recent Developments and Future Directions for RNA Interference Therapeutics. *Nucleic Acid Ther*. 2021;3:1-26.
68. O'Shannessy DJ, Somers EB, Wang L-C, Wang H, Hsu R. Expression of folate receptors alpha and beta in normal and cancerous gynecologic tissues: correlation of expression of the beta isoform with macrophage markers. *J Ovarian Res.* 2015;8:1-9.
69. Ross JF, Chaudhuri PK, Ratnam M. Differential regulation of folate receptor isoforms in normal and malignant tissues in vivo and in established cell lines. Physiologic and clinical implications. *Cancers*. 1994;73:2432-2443.
70. Kalli KR, Oberg AL, Keeney GL, Christianson TJ, Low PS, Knutson KL, et al. Folate receptor alpha as a tumor target in epithelial ovarian cancer. *Gynecol Oncol*. 2008;108:619-626.
71. Patel NR, Piroyan A, Ganta S, Morse AB, Candiloro KM, Solon AL, et al. In Vitro and In Vivo evaluation of a novel folate-targeted theranostic nanoemulsion of docetaxel for imaging and improved anticancer activity against ovarian cancers. *Cancer biol*. 2018;19:554-564.
72. Chandrupatla DM, Molthoff CF, Lammertsma AA, van der Laken CJ, Jansen G. The folate receptor β as a macrophage-mediated imaging and therapeutic target in rheumatoid arthritis. *Drug Deliv Transl Res*. 2019;9:366-378.
73. Shen F, Ross J, Wang X, Ratnam M. Identification of a novel folate receptor, a truncated receptor, and receptor type. beta. in hematopoietic cells: cDNA cloning, expression, immunoreactivity, and tissue specificity. *Biochemistry*. 1994;33:1209-1215.

74. Unnikrishnan B, Sen A, Preethi G, Joseph MM, Maya S, Shiji R, et al. Folic acid-appended galactoxyloglucan-capped iron oxide nanoparticles as a biocompatible nanotheranostic agent for tumor-targeted delivery of doxorubicin. *Int J Biol Macromol*. 2021;168:130-142.
75. Farran B, Montenegro RC, Kasa P, Pavitra E, Huh YS, Han Y-K, et al. Folate-conjugated nanovehicles: Strategies for cancer therapy. *Mater Sci Eng C*. 2020;107:110341.
76. Bahrami B, Mohammadnia-Afrouzi M, Bakhshaei P, Yazdani Y, Ghalamfarsa G, Yousefi M, et al. Folate-conjugated nanoparticles as a potent therapeutic approach in targeted cancer therapy. *Tumor Biol*. 2015;36:5727-5742.
77. Narmani A, Rezvani M, Farhood B, Darkhor P, Mohammadnejad J, Amini B, et al. Folic acid functionalized nanoparticles as pharmaceutical carriers in drug delivery systems. *Drug Dev Res.* 2019;80:404-424.
78. de SL Oliveira ALC, Zerillo L, Cruz LJ, Schomann T, Chan AB, de Carvalho TG, et al. Maximizing the potency of oxaliplatin coated nanoparticles with folic acid for modulating tumor progression in colorectal cancer. *Mater Sci Eng C Mater Biol Appl.* 2021;120:111678.
79. Singh AP, Biswas A, Shukla A, Maiti P. Targeted therapy in chronic diseases using nanomaterial-based drug delivery vehicles. *Signal Transduct Target Ther.* 2019;4:1-21.
80. de Nonancourt-Didion M, Guéant J-L, Adjalla C, Chéry C, Hatier R, Namour F. Overexpression of folate binding protein α is one of the mechanism explaining the adaptation of HT29 cells to high concentration of methotrexate. *Cancer lett*. 2001;171:139-145.
81. Ebrahimnejad P, Dinarvand R, Sajadi A, Jaafari MR, Nomani AR, Azizi E, et al. Preparation and in vitro evaluation of actively targetable nanoparticles for SN-38 delivery against HT-29 cell lines. *Nanomedicine.* 2010;6:478-485.
82. Ebrahimnejad P, Dinarvand R, Sajadi A, Jafari MR, Movaghari F, Atyabi F. Development and Validation of an Ion-pair HPLC Chromatography for Simultaneous Determination of Lactone and Carboxylate Forms of SN-38 in Nanoparticles. *J Food Drug Anal* 2009; 17: 246-256.
83. Bwatanglang IB, Mohammad F, Yusof NA, Abdullah J, Hussein M Z, Alitheen NB, et al. Folic acid targeted Mn: ZnS quantum dots for theranostic applications of cancer cell imaging and therapy. *Int J Nanomedicine*. 2016;11:413-428.
84. Alsaab H, Alzhrani RM, Kesharwani P, Sau S, Boddu SH, Iyer AK. Folate Decorated Nanomicelles Loaded with a Potent Curcumin Analogue for Targeting Retinoblastoma. *Pharmaceutics*. 2017;9:1-12
85. Chen Y, Cao W, Zhou J, et al. Poly (l-lysine)-graft-folic acid-coupled poly (2-methyl-2-oxazoline)(PLL-g-PMOXA-c-FA): A Bioactive Copolymer for Specific Targeting to Folate Receptor-Positive Cancer Cells. *ACS Appl Mater Interfaces.* 2015;7:2919-2930.
86. Luong D, Kesharwani P, Alsaab HO, Sau S, Padhye S, Sarkar FH, et al. Folic acid conjugated polymeric micelles loaded with a curcumin difluorinated analog for targeting cervical and ovarian cancers. *Colloids Surf B.* 2017;157:490-502.
87. Li X, Szewczuk MR, Malardier-Jugroot C. Folic acid-conjugated amphiphilic alternating copolymer as a new active tumor targeting drug delivery platform. *Drug Des Devel Ther*. 2016;10:4101-4110.
88. Li X, Sambi M, DeCarlo A, Burov SV, Akasov R, Markvicheva E, et al. Functionalized Folic Acid-Conjugated Amphiphilic Alternating Copolymer Actively Targets 3D Multicellular Tumour Spheroids and Delivers the Hydrophobic Drug to the Inner Core. *Nanomaterials*. 2018;8:1-21.
89. Slastnikova TA, Rosenkranz AA, Khramtsov YV, Karyagina TS, Ovechko SA, Sobolev AS. Development and evaluation of a new modular nanotransporter for drug delivery into nuclei of pathological cells expressing folate receptors. *Drug Des Devel Ther*. 2017; 11: 1315-1334.
90. Puri A, Loomis K, Smith B, Lee J-H, Yavlovich A, Heldman E, et al. Lipid-based nanoparticles as pharmaceutical drug carriers: from concepts to clinic. *Crit Rev Ther Drug Carrier Syst.* 2009; 26: 523-580.
91. Hattori Y, Shimizu S, Ozaki K-i, Onishi H. Effect of cationic lipid type in folate-PEG-modified cationic liposomes on folate receptor-mediated siRNA transfection in tumor cells. *Pharmaceutics.* 2019;11:1-22.

92. de Oliveira Silva J, Fernandes RS, Oda CMR, Ferreira TH, Botelho AFM, Melo MM, et al. Folate-coated, long-circulating and pH-sensitive liposomes enhance doxorubicin antitumor effect in a breast cancer animal model. *Biomed Pharmacother*. 2019;118: 1-30.
93. Wang X, Li Y, Wang D, Wang X, Yuan W, Zhao W, et al. Evaluation of antitumor efficacy of folate-poly (2-ethyl-2-oxazoline)-distearoyl phosphatidyl ethanolamine based liposome. *Pharm Dev Technol*. 2021;26:110-118.
94. Sabzichi M, Mohammadian J, Khosroushahi AY, Bazzaz R, Hamishehkar H. Folate-targeted nanostructured lipid carriers (NLCs) enhance (Letrozol) efficacy in MCF-7 breast cancer cells. *Asian Pac J Cancer Prev*. 2016;17:5185-5188.
95. Tong L, Chen W, Wu J, Li H. Folic acid-coupled nano-paclitaxel liposome reverses drug resistance in SKOV3/TAX ovarian cancer cells. *Anticancer Drugs*. 2014;25:244-254.
96. Qiu L, Dong C, Kan X. Lymphoma-targeted treatment using a folic acid-decorated vincristine-loaded drug delivery system. *Drug Des Devel Ther*. 2018; 12: 863-872.
97. Zhang G, Liu F, Jia E, Jia L, Zhang Y. Folate-modified, cisplatin-loaded lipid carriers for cervical cancer chemotherapy. *Drug delivery*. 2016;23:1393-1397.
98. Noriega-Luna B, Godínez LA, Rodríguez FJ, Rodríguez A, Zaldívar-Lelo de Larrea G, Sosa-Ferreyra C, et al. Applications of dendrimers in drug delivery agents, diagnosis, therapy, and detection. *J Nanomater*. 2014;2014:1-19.
99. Xu L, Kittrell S, Yeudall WA, Yang H. Folic acid-decorated polyamidoamine dendrimer mediates selective uptake and high expression of genes in head and neck cancer cells. *Nanomedicine*. 2016;11:2959-2973.
100. Mbatha LS, Maiyo FC, Singh M. Dendrimer functionalized folate-targeted gold nanoparticles for luciferase gene silencing in vitro: A proof of principle study. *Acta Pharm*. 2019;69:49-61.
101. Daraee H, Eatemadi A, Abbasi E, Fekri Aval S, Kouhi M, Akbarzadeh A. Application of gold nanoparticles in biomedical and drug delivery. *Artif cells nanomed biotechnol*. 2016;44:410-422.
102. Kong F-Y, Zhang J-W, Li R-F, Wang Z-X, Wang W-J, Wang W. Unique roles of gold nanoparticles in drug delivery, targeting and imaging applications. *Molecules*. 2017;22:1-13.
103. Bi J, Lu Y, Dong Y, Gao P. Synthesis of Folic Acid-Modified DOX@ ZIF-8 Nanoparticles for Targeted Therapy of Liver Cancer. *J Nanomater*. 2018;2018:1-5
104. Soetaert F, Korangath P, Serantes D, Fiering S, Ivkov R. Cancer therapy with iron oxide nanoparticles: Agents of thermal and immune therapies. *Adv Drug Deliv Rev*. 2020;163:65-83.
105. Shetty V, Jakhade A, Shinde K, Chikate R, Kaul-Ghanekar R. Folate mediated targeted delivery of cinnamaldehyde loaded and FITC functionalized magnetic nanoparticles in breast cancer: in vitro, in vivo and pharmacokinetic studies. *New J Chem*. 2021;45:1500-1515.
106. Rajkumar S, Prabaharan M. Multi-functional nanocarriers based on iron oxide nanoparticles conjugated with doxorubicin, poly (ethylene glycol) and folic acid as theranostics for cancer therapy. *Colloids Surf B*. 2018;170:529-537.
107. Bianco A, Kostarelos K, Prato M. Applications of carbon nanotubes in drug delivery. *Curr opin chem biol*. 2005;9:674-679.
108. Sharma S, Mehra NK, Jain K, Jain NK. Effect of functionalization on drug delivery potential of carbon nanotubes. *Artif cells nanomed biotechnol*. 2016;44:1851-1860.
109. Karimi M, Solati N, Ghasemi A, Estiar MA, Hashemkhani M, Kiani P, et al. Carbon nanotubes part II: a remarkable carrier for drug and gene delivery. *Expert Opin Drug Deliv*. 2015;12:1089-1105.
110. Liu X, Tao H, Yang K, Zhang S, Lee S-T, Liu Z. Optimization of surface chemistry on single-walled carbon nanotubes for in vivo photothermal ablation of tumors. *Biomaterials*. 2011;32:144-151.
111. Jawahar N, De A, Jubee S, Reddy ES. Folic acid-conjugated raloxifene hydrochloride carbon nanotube for targeting breast cancer cells. *Drug Dev Res*. 2020;81:305-314.
112. Matea CT, Mocan T, Tabaran F, Pop T, Mosteanu O, Puia C, et al. Quantum dots in imaging, drug delivery and sensor applications. *Int J Nanomed*. 2017;12:5421-5431.
113. Zhao M-X, Zhu B-J. The research and applications of quantum dots as nano-carriers for targeted drug delivery and cancer therapy. *Nanoscale res lett* 2016;11:1-9.
114. Sadeghi Ghadi Z, Ebrahimnejad P. Curcumin entrapped hyaluronan containing niosome: Preparation, characterization and in vitro/in vivo evaluation. *J microencapsul*. 2019;36:169-179.

115. Li Z, Fan J, Tong C, Zhou H, Wang W, Li B, et al. A smart drug-delivery nanosystem based on carboxylated graphene quantum dots for tumor-targeted chemotherapy. *Nanomedicine*. 2019;14:2011-2025.
116. Bugaj AM. Targeted photodynamic therapy–a promising strategy of tumor treatment. *Photochem Photobiol Sci*. 2011;10:1097-1109.
117. Stallivieri A, Colombeau L, Jetpisbayeva G, Moussaron A, Myrzakhmetov B, Arnoux P, et al. Folic acid conjugates with photosensitizers for cancer targeting in photodynamic therapy: Synthesis and photophysical properties. *Bioorg med chem*. 2017;25:1-10.
118. Ghezzi M, Pescina S, Padula C, Santi P, Del Favero E, Cantù L, et al. Polymeric micelles in drug delivery: An insight of the techniques for their characterization and assessment in biorelevant conditions. *J Control Release*. 2021;332:312-336.
119. Amirmahani N, Mahmoodi NO, Galangash MM, Ghavidast A. Advances in nanomicelles for sustained drug delivery. *J Ind Eng Chem*. 2017;55:21-34.
120. Wang F, Chen Y, Zhang D, Zhang Q, Zheng D, Hao L, et al. Folate-mediated targeted and intracellular delivery of paclitaxel using a novel deoxycholic acid-O-carboxymethylated chitosan-folic acid micelles. *Int J Nanomedicine*. 2012;7:325-337.
121. Cao W, Zhou J, Mann A, Wang Y, Zhu L. Folate-functionalized unimolecular micelles based on a degradable amphiphilic dendrimer-like star polymer for cancer cell-targeted drug delivery. *Biomacromolecules*. 2011;12:2697-2707.
122. Qiu LY, Yan L, Zhang L, Jin YM, Zhao QH. Folate-modified poly(2-ethyl-2-oxazoline) as hydrophilic corona in polymeric micelles for enhanced intracellular doxorubicin delivery. *Int J Pharm*. 2013;456:315-324.
123. Karami E, Behdani M, Kazemi-Lomedasht F. Albumin nanoparticles as nanocarriers for drug delivery: Focusing on antibody and nanobody delivery and albumin-based drugs. *J Drug Deliv Sci*. 2020;55:101471.
124. Hoogenboezem EN, Duvall CL. Harnessing albumin as a carrier for cancer therapies. *Adv Drug Deliv Rev*. 2018;130:73-89.
125. Gharbavi M, Danafar H, Sharafi A. Microemulsion and bovine serum albumin nanoparticles as a novel hybrid nanocarrier system for efficient multifunctional drug delivery. *J Biomed Mater Res A*. 2020;108:1688-1702.
126. Gawde KA, Kesharwani P, Sau S, Sarkar FH, Padhye S, Kashaw SK, et al. Synthesis and characterization of folate decorated albumin bio-conjugate nanoparticles loaded with a synthetic curcumin difluorinated analogue. *J colloid interface sci*. 2017;496:290-299.
127. Sodagar Taleghani A, Nakhjiri AT, Khakzad MJ, Rezayat SM, Ebrahimnejad P, Heydarinasab A, et al. Mesoporous silica nanoparticles as a versatile nanocarrier for cancer treatment: A review. *J Mol Liq*. 2021;328:1-18.
128. Khosravian P, Shafiee Ardestani M, Khoobi M, Ostad SN, Dorkoosh FA, Akbari Javar H, et al. Mesoporous silica nanoparticles functionalized with folic acid/methionine for active targeted delivery of docetaxel. *Onco Targets Ther*. 2016;9:7315-7330.
129. AbouAitah K, Swiderska-Sroda A, Farghali AA, Wojnarowicz J, Stefanek A, Gierlotka S, et al. Folic acid–conjugated mesoporous silica particles as nanocarriers of natural prodrugs for cancer targeting and antioxidant action. *Oncotarget*. 2018;9:26466-26490.
130. Feng S, Pan J, Li C, Zheng Y. Folic acid-conjugated nitrogen-doped graphene quantum dots as a fluorescent diagnostic material for MCF-7 cells. *Nanotechnology*. 2020;31:135701.
131. Liu J-M, Chen J-T, Yan X-P. Near infrared fluorescent trypsin stabilized gold nanoclusters as surface plasmon enhanced energy transfer biosensor and in vivo cancer imaging bioprobe. *Anal Chem*. 2013;85:3238-3245.
132. Pavitra E, Raju GSR, Nagaraju GP, Nagaraju G, Han YK, Huh YS, et al. TPAOH assisted size-tunable Gd_2O_3@ mSi core–shell nanostructures for multifunctional biomedical applications. *Chem Commun*. 2018;54:747-750.
133. Jain A, Fournier PG, Mendoza-Lavaniegos V, Sengar P, Guerra-Olvera FM, Iñiguez E, et al. Functionalized rare earth-doped nanoparticles for breast cancer nanodiagnostic using fluorescence and CT imaging. *Nanobiotechnology*. 2018;16:1-18.

134. Lin S-L, Chen Z-R, Chang CAJN. Nd3+ sensitized core-shell-shell nanocomposites loaded with IR806 dye for photothermal therapy and up-conversion luminescence imaging by a single wavelength NIR light irradiation. *Nanotheranostics*. 2018;2:243-257.
135. Vu-Quang H, Vinding MS, Nielsen T, Ullisch MG, Nielsen NC, Nguyen DT, et al. Pluronic F127-folate coated super paramagenic iron oxide nanoparticles as contrast agent for cancer diagnosis in magnetic resonance imaging. *Polymers*. 2019;11:1-14.
136. Khademi S, Sarkar S, Shakeri-Zadeh A, Attaran N, Kharrazi S, Ay MR, et al. Folic acid-cysteamine modified gold nanoparticle as a nanoprobe for targeted computed tomography imaging of cancer cells. *Mater Sci Eng C.* 2018;89:182-193.
137. Li K, Liu Y, Zhang S, Xu Y, Jiang J, Yin F, et al. Folate receptor-targeted ultrasonic PFOB nanoparticles: Synthesis, characterization and application in tumor-targeted imaging. *Int J Mol Med*. 2017;39:1505-1515.
138. Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat Biotechnol.* 2015;33:941-951.
139. Samodelov SL, Gai Z, Kullak-Ublick GA, Visentin M. Renal Reabsorption of Folates: Pharmacological and Toxicological Snapshots. *Nutrients*. 2019;11:1-16.
140. Paliwal R, Babu RJ, Palakurthi SJAP. Nanomedicine scale-up technologies: feasibilities and challenges. *Aaps Pharmscitech.* 2014;15:1527-1534.
141. Bian W, Wang M, Ahsan B, Lin S, Ren Z, Huang JA, et al. Gefitinib-loaded Nanoparticles with Folic Acid-modified Dextran Surface Prepared by Flash Nanoprecipitation. *Chem Lett*. 2018;47:1405-1408.
142. Koirala N, Das D, Fayazzadeh E, Sen S, McClain A, Puskas JE, et al. Folic acid conjugated polymeric drug delivery vehicle for targeted cancer detection in hepatocellular carcinoma. *J Biomed Mater Res A.* 2019;107:2522-2535.
143. Peng F, Qiu L, Chai R, Meng F, Yan C, Chen Y, et al. Conjugated Polymer-Based Nanoparticles for Cancer Cell-Targeted and Image-Guided Photodynamic Therapy. *Macromol Chem Phys*. 2018; 219: 1700440.
144. Amreddy N, Babu A, Panneerselvam J, Srivastava A, Muralidharan R, Chen A, et al. Chemo-biologic combinatorial drug delivery using folate receptor-targeted dendrimer nanoparticles for lung cancer treatment. *Nanomedicine*. 2018;14:373-384.
145. Narmani A, Kamali M, Amini B, Salimi A, Panahi Y. Targeting delivery of oxaliplatin with smart PEG-modified PAMAM G4 to colorectal cell line: In vitro studies. *Process Biochem*. 2018;69:178-187.
146. Agabeigi R, Rasta SH, Rahmati-Yamchi M, Salehi R, Alizadeh E. Novel Chemo-Photothermal Therapy in Breast Cancer Using Methotrexate-Loaded Folic Acid Conjugated Au@ SiO_2 Nanoparticles. *Nanoscale res lett*. 2020;15:1-14.
147. Majidi FS, Mohammadi E, Mehravi B, Nouri S, Ashtari K, Neshasteh-Riz A. Investigating the effect of near infrared photo thermal therapy folic acid conjugated gold nano shell on melanoma cancer cell line A375. *Artif Cells Nanomed Biotechnol*. 2019;47:2161-2170.
148. Yücel O, Şengelen A, Emik S, Önay-Uçar E, Arda N, Gürdağ G. Folic acid-modified methotrexate-conjugated gold nanoparticles as nano-sized trojans for drug delivery to folate receptor-positive cancer cells. *Nanotechnology*. 2020;31:355101.
149. Ghaznavi H, Hosseini-Nami S, Kamrava SK, Irajirad R, Maleki S, Shakeri-Zadeh A, et al. Folic acid conjugated PEG coated gold–iron oxide core–shell nanocomplex as a potential agent for targeted photothermal therapy of cancer. *Artif Cells Nanomed Biotechnol*. 2018;46:1594-1604.
150. Angelopoulou A, Kolokithas-Ntoukas A, Fytas C, Avgoustakis K. Folic acid-functionalized, condensed magnetic nanoparticles for targeted delivery of doxorubicin to tumor cancer cells overexpressing the folate receptor. *ACS omega*. 2019;4:22214-22227.
151. Singh RP, Sharma G, Singh S, Bharti S, Pandey BL, Koch B, et al. Chitosan-folate decorated carbon nanotubes for site specific lung cancer delivery. *Mater Sci Eng C*. 2017;77:446-458.
152. Uttekar PS, Lakade SH, Beldar VK, Harde MT. Facile synthesis of multi-walled carbon nanotube via folic acid grafted nanoparticle for precise delivery of doxorubicin. *IET nanobiotechnol* 2019;13:688-696.

153. Ruzycka-Ayoush M, Kowalik P, Kowalczyk A, Bujak P, Nowicka AM, Wojewodzka M, et al. Quantum dots as targeted doxorubicin drug delivery nanosystems. *Cancer Nanotechnol.* 2021;12:1-27.
154. Hashemkhani M, Muti A, Sennaroğlu A, Acar HY. Multimodal image-guided folic acid targeted Ag-based quantum dots for the combination of selective methotrexate delivery and photothermal therapy. *J Photochem Photobiol B.* 2020;213:112082.
155. Sun Z, Luo M, Li J, Wang A, Sun X, Wu Q, et al. Folic Acid Functionalized Chlorin e6-Superparamagnetic Iron Oxide Nanocarriers as a Theranostic Agent for MRI-Guided Photodynamic Therapy. *J Biomed Nanotechnol*. 2021;17:205-215.
156. Zhang X, Liang N, Gong X, Kawashima Y, Cui F, Sun S. Tumor-targeting micelles based on folic acid and α-tocopherol succinate conjugated hyaluronic acid for paclitaxel delivery. *Colloids Surf B.* 2019;177:11-18.
157. Pawar A, Singh S, Rajalakshmi S, Shaikh K, Bothiraja C. Development of fisetin-loaded folate functionalized pluronic micelles for breast cancer targeting. *Artif Cells Nanomed Biotechnol*. 2018;46:347-361.
158. Li J, Yao S, Wang K, Lu Z, Su X, Li L, et al. Hypocrellin B-loaded, folate-conjugated polymeric micelle for intraperitoneal targeting of ovarian cancer in vitro and in vivo. *Cancer sci*. 2018;109:1958-1969.
159. Nosrati H, Abbasi R, Charmi J, Rakhshbahar A, Aliakbarzadeh F, Danafar H, et al. Folic acid conjugated bovine serum albumin: An efficient smart and tumor targeted biomacromolecule for inhibition folate receptor positive cancer cells. *Int J biol macromol*. 2018;117:1125-1132.
160. Mishra S, Manna K, Kayal U, Saha M, Chatterjee S, Chandra D, et al. Folic acid-conjugated magnetic mesoporous silica nanoparticles loaded with quercetin: a theranostic approach for cancer management. *RSC Adv.* 2020;10:23148-23164.
161. Qu W, Meng B, Yu Y, Wang S. Folic acid-conjugated mesoporous silica nanoparticles for enhanced therapeutic efficacy of topotecan in retina cancers. *Int J Nanomedicine*. 2018;13:4379-4389.
162. Song Y, Zhou B, Du X, Wang Y, Zhang J, Ai Y, et al. Folic acid (FA)-conjugated mesoporous silica nanoparticles combined with MRP-1 siRNA improves the suppressive effects of myricetin on non-small cell lung cancer (NSCLC). *Biomed Pharmacother.* 2020;125:109561.
163. Wang Y-M, Lin K-T, Chen T-J, Liu G-C. Folate-receptor-targeting iron oxide nanoparticles coated with poly (ethylene glycol). US Patents 20080081891A1, 2009.
164. Nah JW, Jung TR, Jang MK, Kim DG, Heo SH. Low-molecular weight, water-soluble chitosan nanoparticle for gene delivery with folic acid conjugaed thereto as target ligand and preparation method thereof. US Patents 20100040694A1, 2010.
165. Bae YH, Na K, Lee ES. PH-sensitive polymeric micelles for drug delivery. US Patents 7659314B2, 2010.
166. Bazile D, Couvreur P, Lakkireddy HR, Mackiewicz N, Nicolas J. Functional PLA-PEG copolymers, the nanoparticles thereof, their preparation and use for targeted drug delivery and imaging. US Patents 9364444B2, 2016.
167. Smith B, Lusk J. Folic acid functionalized copper sulfide nanoparticles for the detection of ovarian cancer cells in flow. US Patents 20200271655A1, 2020.

Figure Captions:

Figure 1. (A) Different kinds of nanoparticles that have shown the most promising results in cancer. (B) Accumulation of drug in the tumor area after injection of drug-loaded nanoparticles. (C) Mechanism of receptor-mediated endocytosis for folate-modified nanoparticles.

Figure 2. Chemical structure of folic acid.

Figure 3. Two views of the structure of FRα-folic acid complex. FRα is shown in green, folic acid is shown in grey, and disulfide bonds are depicted as yellow sticks. The N terminus and C terminus are labeled. Reproduced from [61].

Figure 4. Fluorescent images of MDA-MB-231 spheroids incubated with curcumin-loaded SMA and curcumin-loaded FA-SMA which demonstrates the difference between tumor spheroid uptake of drug-laoded nanoparticles after 0.5 h and 6 h exposure. **(A)** Untreated MDA-MB-231 tumor spheroids. **(B)** MDA-MB-231 spheroids treated with curcumin-loaded SMA and FA-SMA for 0.5 h. **(C)** MDA-MB-231 spheroids treated with curcumin-loaded SMA and FA-SMA for 6 h. Reproduced from [88].

Figure 5. Schematic illustration of doxorubicin-loaded IO-MMA-DOX-PEG-OCH_3/FA. Reproduced from [106].

Figure 6. *In vivo* study of FA-PEG-cGQDs-MTN antitumor therapy. (A) The weight curves of cervical tumor-bearing nude mice. (B) Tumor growth curves of different groups with different treatments. The data represent the mean ± standard deviation (SD) (n = 3 mice per group). (C) Representative images of cervical tumor-bearing nude mice at day 20. The tumors as marked by dashed circles were excised after treatment for 20 days. (D) Hematoxylin and eosin stained images of the excised tumor tissue with different treatments. Reproduced from [115].

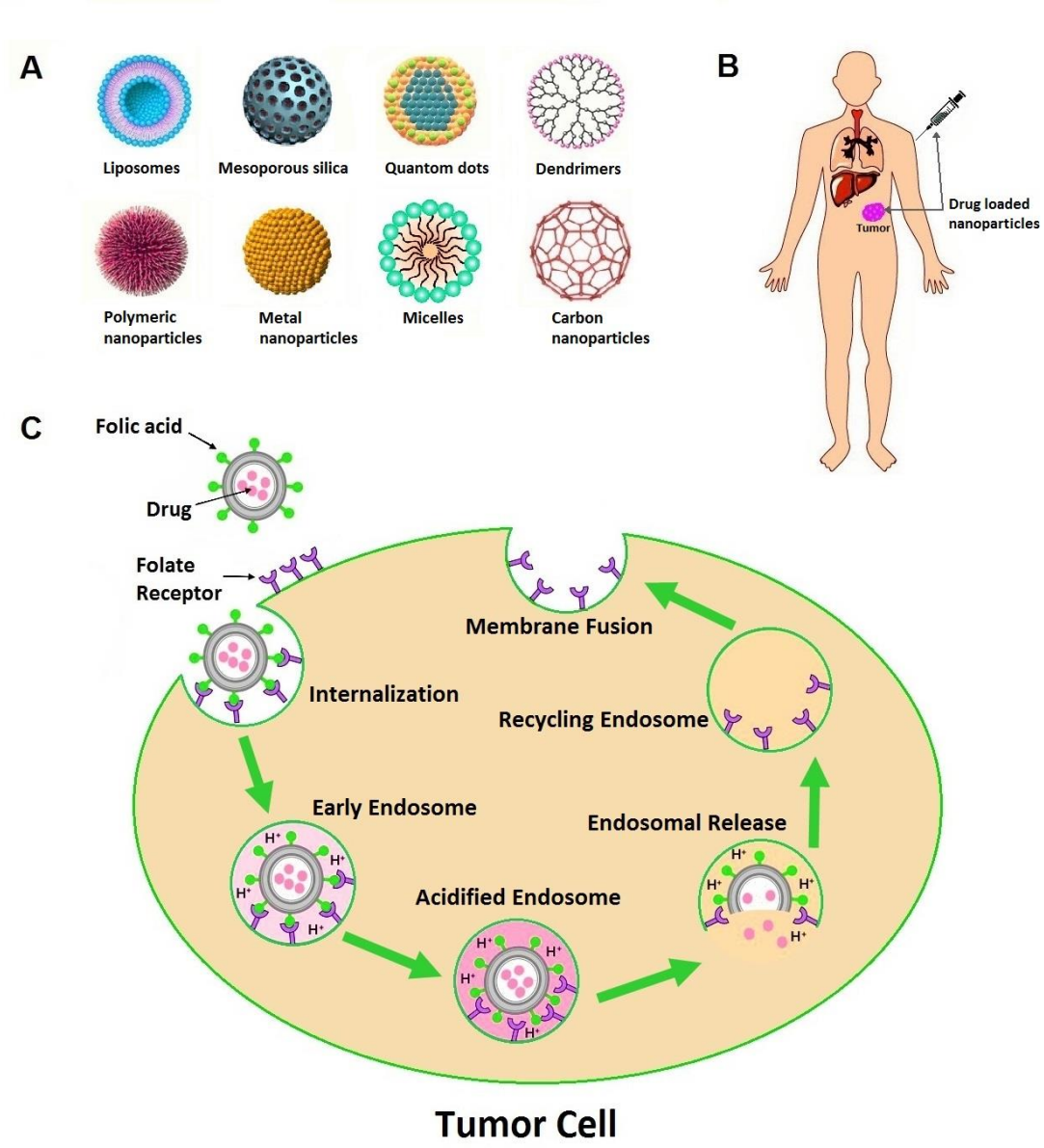

Figure 1

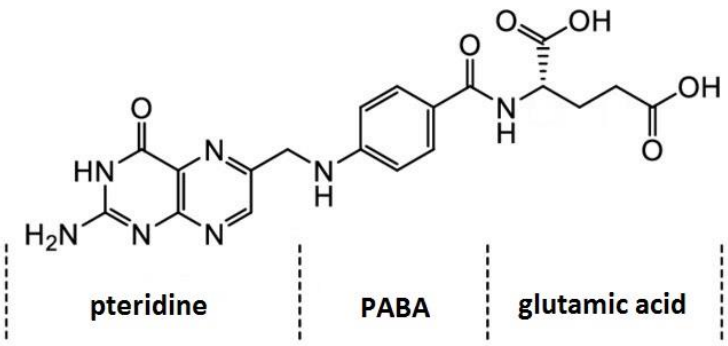

Figure 2

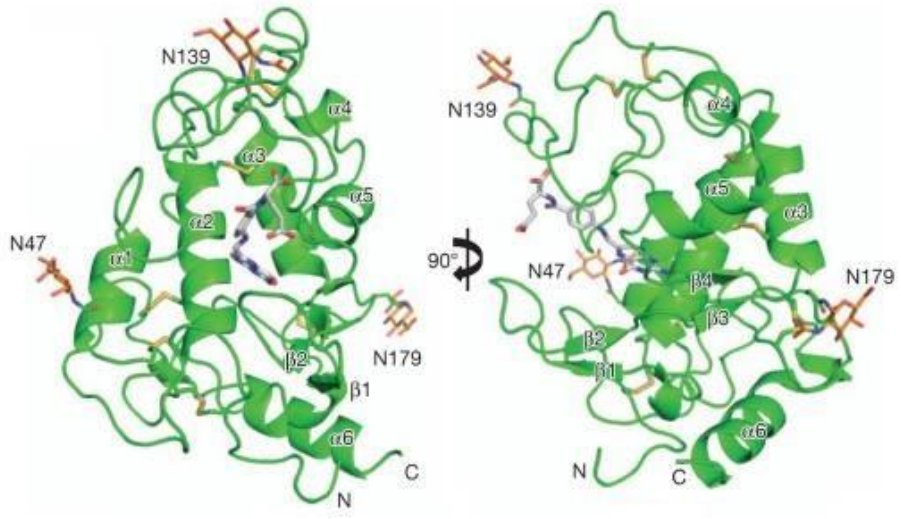

Figure 3

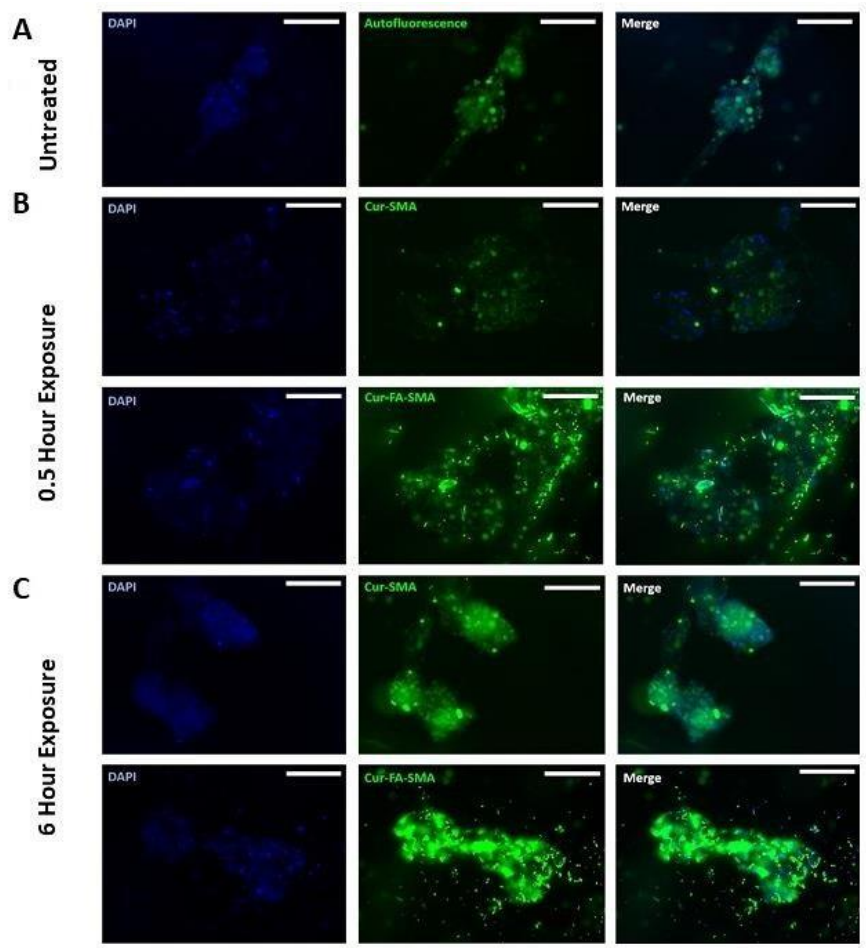

Figure 4

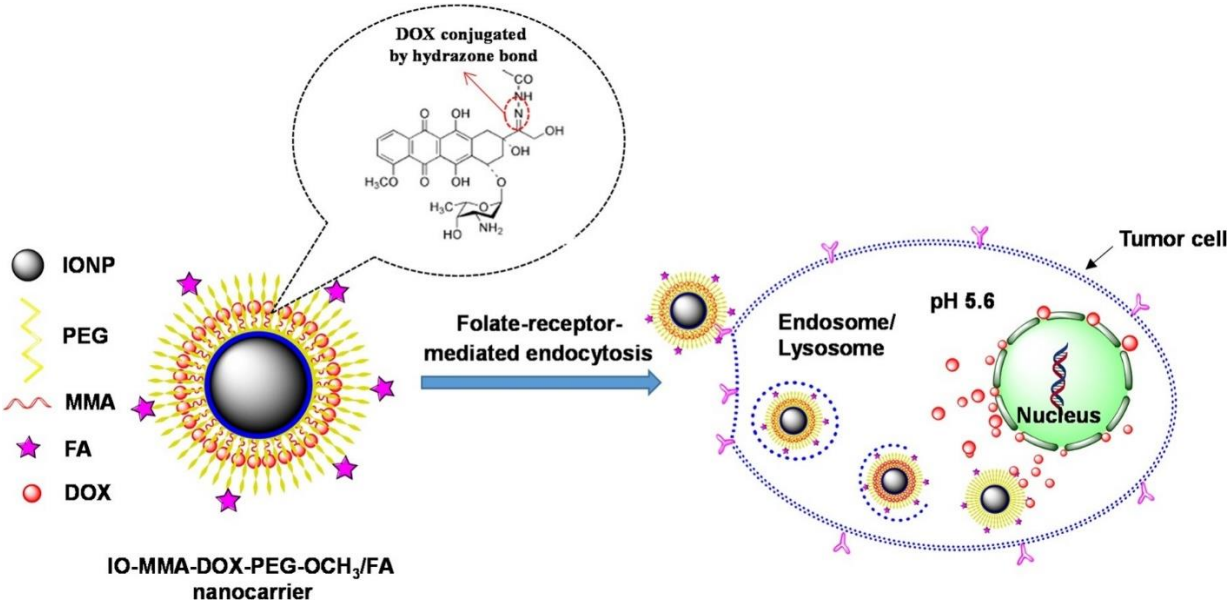
DOX conjugated
by hydrazone bond
IONP
PEG
MMA
FA
DOX
IO-MMA-DOX-PEG-OCH3/FA
nanocarrier
Folate-receptor-
mediated endocytosis
Tumor cell
Endosome/
Lysosome
pH 5.6
Nucleus

Figure 5

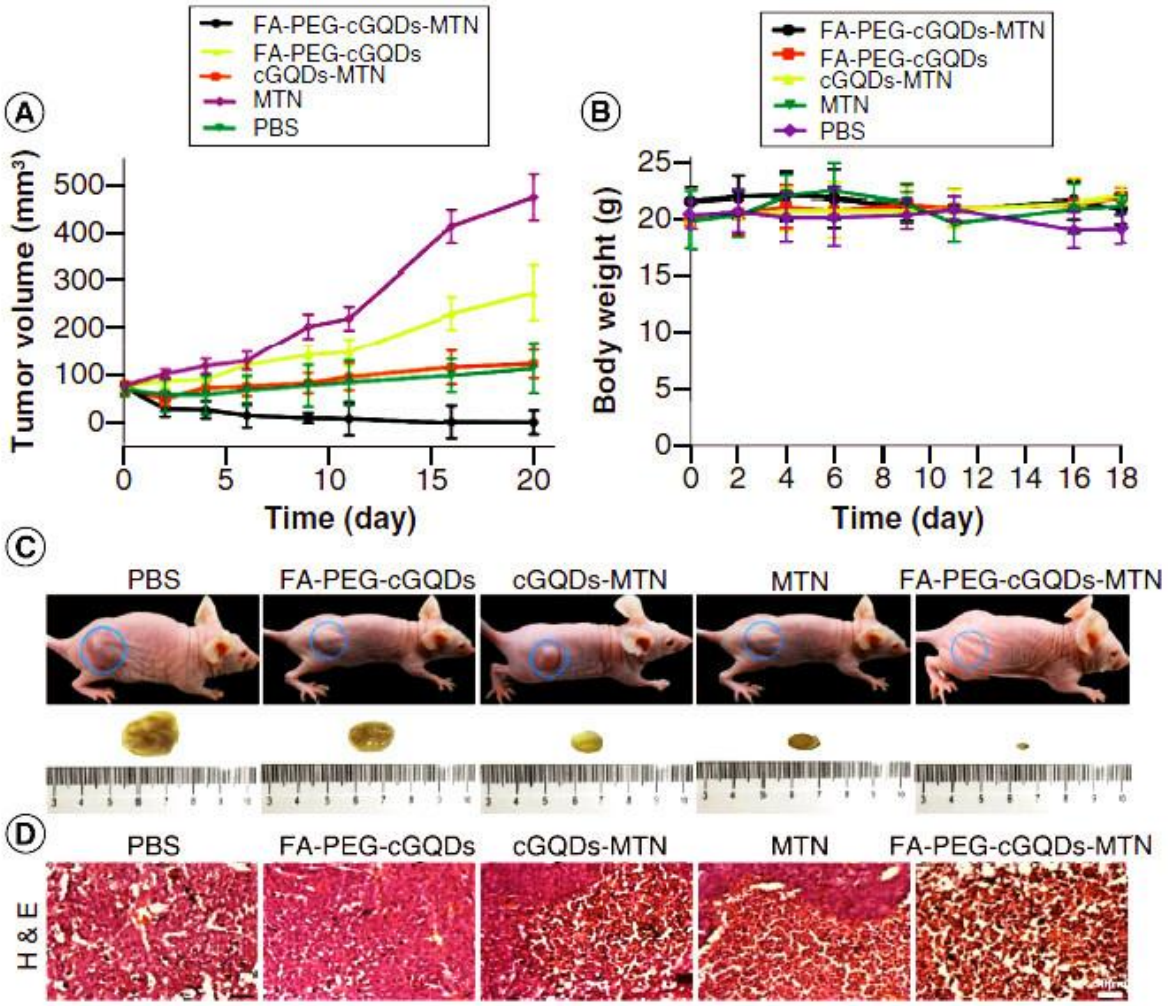
A
FA-PEG-cGQDs-MTN
FA-PEG-cGQDs
cGQDs-MTN
MTN
PBS
Tumor volume (mm³)
500
400
300
200
100
0
0
5
10
15
20
Time (day)
B
FA-PEG-cGQDs-MTN
FA-PEG-cGQDs
cGQDs-MTN
MTN
PBS
Body weight (g)
25
20
15
10
5
0
0 2 4 6 8 10 12 14 16 18
Time (day)
C
PBS
FA-PEG-cGQDs
cGQDs-MTN
MTN
FA-PEG-cGQDs-MTN
D
PBS
FA-PEG-cGQDs
cGQDs-MTN
MTN
FA-PEG-cGQDs-MTN
H & E

Figure 6

Table 1. Summary of folate modified nanoparticles for cancer therapy

Folate-modified nanocarriers	**Drug**	**Cell line/Model**	**Major findings**	**Reference**
Folate-modified polymer based nanoparticles				
PLGA-PEG-FA	SN-38	HT-29 cells	Folate-modified PLGA-PEG nanoparticles containing SN-38 showed superior cytotoxic effect than non-targeted nanoparticles and free SN-38 against HT-29 cells, *in vitro*.	81
FA-poly (styrene-co-maleic anhydride)	Curcumin-difluorinated	WERI-RB1, Y-79, and ARPE-19 cells	Folate-conjugated nanoparticles could markedly kill both WERI-RB and Y-79 retinoblastoma tumor cells.	84
FA-SMA	Curcumin	PANC-1 and RAW-Blue cells	The pH-sensitive nanocarrier could release the entrapped cargo efficiently into the cytoplasm and nucleus, and caused cell death.	87
FA-FI-PEG-FI-FA	Doxorubicin	U937and N1S1 cells HCC tumor-bearing rat	DOX-loaded polymeric nanoparticles indicated potent anti-tumoral activities both *in vitro* and *in vivo*.	142
FA-PEG-NH_2-conjugated polymer (CP)- poly (styrene-co-maleic anhydride)	——	HeLa and NIH-3T3 cells	FA-modified conjugated polymer nanoparticles showed *phototherapeutic* effect, *in vitro*.	143
Folate-modified lipid based nanoparticles				
Folate nanostructured lipid carrier (NLC)	Letrozole	MCF-7 cells	Folate-modified Letrozole-loaded NLC promoted apoptosis in FR-expressing cancer cells.	94
FA- PEGylated liposome	Paclitaxel	SKOV3 and SKOV3/TAX cells SKOV3/TAX tumor-bearing Balb/c nude mice	Paclitaxel-loaded liposomal nanoparticles showed high inhibition of tumor growth and reversed drug resistance in SKOV3/TAX cells both *in vitro* and *in vivo*.	95

Folate-modified dendrimers				
Folate-decorated dendrimer targeted gold nanoparticles	siRNA	HeLa-Tat-*Luc* cells	The siRNA nanocomplex evoked excellent gene silencing in FR expressing tumor cells.	[100]
Folic acid-conjugated polyamidoamine dendrimer-based nanoparticles	HuR siRNA, *cis*-diamine platinum	H1299 cells	FRα-targeted dendritic nanoparticles induced more DNA damage and cell death by apoptosis in comparison with non-targeted nanoparticles, *in vitro*.	[144]
FA-PAMAM G4-PEG nanoparticles	Oxaliplatin	SW480 cells	The oxaliplatin-loaded FA-PAMAM G4-PEG nanocomplex demonstrated a superior cellular uptake in FR^+ SW480 cells than MSC normal cells, *in vitro*.	[145]
Folate-modified Au nanoparticles				
Graphene oxide (GO) nanoparticles conjugated with folate-composite gold nanoparticles (FA-GO@Au)	Doxorubicin	MCF-7 and HeLa cells Ehrlich ascites tumor (EAT) bearing Balb/c mice	Doxorubicin-loaded FA-GO@Au nanoparticles exhibited remarkable efficacy against the cancer cells after NIR exposure, both *in vitro* and *in vivo*.	[22]
Folate-conjugated silica coated gold ($Au@SiO_2$) nanoparticles	Methotrexate	MDA-MB-231 and MCF-7 cells	MTX-loaded FA-$Au@SiO_2$ nanoparticles in combination with Low level laser therapy exerted apoptotic effect in *either* of the *two* breast cancer cell lines especially in MDA-MB-231 cells.	[146]
FA-SiO_2@Au nanoparticles	———	A-375 cells	Folic acid-conjugated silica-gold core-shell nanoplatform *could induce cancer cell* death *through* the hyperthermia *effect*, *in vitro*.	[147]
GSH-coated FA-modified Au nanoparticles	Methotrexate	U-87 MG and HeLa cells	MTX-loaded GSH-Au-FA nanoparticles exerted high cytotoxic activity against FR-positive HeLa and U-87 MG tumor cells.	[148]

Folate-modified iron oxide nanoparticles				
FA-PEG-Au@IO nanoparticles	———	KB and MCF-7 cells	FA-PEG-Au@IO nanocomplex selectively targeted FR-expressing cancer cells, *in vitro*.	149
Folic acid-functionalized PEGylated alginate-coated magnetic nanoparticles	Doxorubicin	MDA-MB-231 and MCF-7 cells	DOX-loaded FA-PEG-Alg-Mag nanoparticles caused increased apoptotic effect and cytotoxic activity against FR expressing MDA-MB-231 cancer cells in the presence of an external magnetic field.	150
Folate-modified carbon nanotubes				
Chitosan-folate conjugated MWCNTs	Docetaxel	A549 cells A549 tumor bearing Balb/c mice	DTX-loaded nanoparticles remarkably killed FR-expressing tumor cells both *in vitro* and *in vivo*.	151
FA-ethylene diamine (EDA)-MWCNTs	Doxorubicin	MCF-7 cells	DOX-loaded FA-EDA-MWCNTs inhibited tumor growth *in vitro*.	152
Folate-modified quantum dots				
Folate-decorated mercaptoundecanoic acid (MUA) modified QDs (FA-MUA-QDs)	Doxorubicin	A549 cells	Doxorubicin-loaded FA-MUA-QDs nanoparticles induced significantly more DNA breaks than untreated particles.	153
Folate-modified GSH-coated Ag-QDs	Methotrexate	HeLa, HT29, and A549 cells	MTX-loaded FA-tagged naocomplex killed the FR-positive HeLa cells under laser irradiation, *in vitro*.	154
Coupling a photosensitizer with folic acid				
FA-modified Ce6-conjugated SPIO nanoparticles	———	RM-1 cells RM-1 tumor-bearing mice	**FA-Ce6-SPIO nanocarrier** could be efficacious for selective PDT of cancer cells both *in vitro* and *in vivo*.	155
Folate-modified micelles				
Folic acid and α-tocopherol succinate conjugated hyaluronic acid micelles	Paclitaxel	MCF-7 cells H22 tumor-bearing Kunming mice	Paclitaxel-loaded micelles were proved to be efficacious in inhibiting tumor burden both *in vitro* and *in vivo*.	156

Folic acid-conjugated Pluronic127 (PF) micelles	Fisetin	MCF-7 cells	Fisetin-loaded FA-PF micelles inhibited the growth of overexpressed human breast cancer MCF-7 cells, *in vitro*.	[157]
FA-PEG-poly (lactic acid) (PLA) micelles	Hypocrellin B	SKOV3 and A2780 cells SKOV3 tumor-bearing female athymic nude mice	Hypocrellin B-loaded PEG-PLA-FA micelles killed FR^+ SKOV3 human ovarian tumor cells both *in vitro* and *in vivo*.	[158]
Folate-modified albumin-bound nanoparticles				
Folate-targeted bovine serum albumin (BSA) nanoparticles	Chrysin	MCF-7 cells	Chrysin-loaded FA-BSA nanoparticles suppressed tumor growth, *in vitro*.	[159]
Folate-modified mesoporous silica nanoparticles				
Folic acid-conjugated magnetic MSNs	Quercetin	HCT-116 cells	Quercetin-loaded FA-FE-SBA15 nanoparticles induced apoptosis through the downregulation of Bcl-2 and overexpression of Bax, p53, and cytochrome *C*.	[160]
Folic acid-conjugated MSN	Topotecan	Y79 cells Y79 tumor-bearing nude mice	Topotecan-loaded MSN-FA selectively killed retinoblastoma tumor cells both *in vitro* and *in vivo*.	[161]
FA-conjugated MSN	Myricetin, MRP-1	A549 and H1299 cells	MRP-1 and Myricetin loaded nanoformulation significantly inhibited the tumor growth by upregulation of cleaved PARP and Caspase-3.	[162]

Table 2. Summary of clinical trials of folic acid-tagged formulations

Title	**Drug/Agent**	**Indication**	**ClinicalTrials.gov identifier (phase)**

A study to evaluate ELU001 in patients with solid tumors that overexpress folate receptor alpha (FRα)	ELU001	Ovarian cancer Endometrial cancer Colorectal cancer Gastric cancer Gastroesophageal junction cancer Triple-negative breast cancer Non-small cell lung cancer Cholangiocarcinoma	NCT05001282 (phase I/II)
OTL38 injection for intraoperative imaging of folate receptor positive lung nodules	OTL38	Lung neoplasms Lung cancer	NCT02872701 (phase II)
Elucidate: enabling lung cancer identification using folate receptor targeting	OTL38	Lung neoplasms Lung cancer	NCT04241315 (phase III)
A pilot, single dose, open-label study of OTL38 injection (OTL38) for intra-operative imaging of folate receptor positive ovarian cancer-comparing camera imaging systems	OTL38	Ovarian cancer	NCT04941378 (phase I)
Phase 2 study of OTL38 for intra-operative imaging of folate receptor-alpha positive ovarian cancer	OTL38	Ovarian cancer	NCT02317705 (phase II)
OTL38 for intra-operative imaging of folate receptor positive ovarian cancer	OTL38	Ovarian cancer	NCT03180307 (phase III)
Intraoperative imagery of renal nodules with folate-fluorescein conjugate (EC17)	EC17	Renal cell carcinoma	NCT01778933 (early phase I)
EC17 for intraoperative imaging in occult ovarian cancer	EC17	Ovarian cancer	NCT02000778 (phase I)
Intra-op detection of occult ovarian carcinoma using a folate-alpha receptor specific fluorescent ligand	EC17	Ovarian cancer	NCT01511055 (phase II)
Pilot and feasibility study of the imaging potential of EC17: Intraoperative folate-fluorescein conjugate (EC17) lung cancer (CA)	EC17	Lung adenocarcinoma	NCT01778920 (phase I)
Phase 2 study of EC145 alone versus EC145+docetaxel versus docetaxel alone in participants with FR (++) 2nd Line Non Small Cell Lung Cancer	EC145 EC145+Docetaxel Docetaxel EC20	Non Small Cell Lung Cancer	NCT01577654 (phase II)
Study Using FolateScan to Identify Subjects With Folate Receptor-Positive Metastatic Renal Cell Carcinoma	EC20	Metastatic renal cell carcinoma	NCT01689766 (phase II)
Safety and efficacy of falatescan (Technetium Tc 99m EC20) in patients with known suspected recurrent or metastatic cancer from a solid tumor	EC20	Recurrent or metastatic cancer Head and neck cancer Pancreas cancer Bladder cancer	NCT01684098 (phase II)

		Testicular cancer	
Safety and efficacy of folatescan (Technetium Tc 99m EC20) in patients with suspected ovarian carcinoma or recurrent endometrial carcinoma	EC20	Ovarian carcinoma Recurrent endometrial carcinoma	NCT01689714 (phase II)

Table 3. Patented folate-conjugated nanomaterials for cancer diagnosis and treatment

Patent number	**Type of delivery system/nanomaterial**	**Size (nm)**	**Cancer cell line**	**Type of tumor**	**Year**	**Reference**
US20080081891 A1	SPIO-PEG-FA	3.8	KB	Epidermoid	2009	[163]
US20100040694 A1	Folic acid-conjugated, low-molecular weight, water-soluble chitosan nanoparticles	110	HEK-293	Kidney	2010	[164]
US7659314B2	FA-polyHis/PEG and FA-PLLA/PEG mixed micelles	114	MCF-7	Breast	2010	[165]
US9364444B2	FA-PLA-PEG-nanoparticles	118	KB-3-1	Cervical	2016	[166]
US20200271655 A1	Folic acid-capped copper sulfide nanoparticles	73.6	SKOV-3	Ovarian	2020	[167]