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Folate-Targeted Therapies for Cancer

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1. Introduction

1.1. General Strategies in the Design of Tumor Targeted Therapeutics. Today's quest for developing tumor-targeted therapies has naturally followed two nonoverlapping strategies. The first strategy involves pursuing agents that can selectively block novel pathways or proteins that emerge or become overexpressed in malignant cells. These pathways are generally critical for tumor cell survival but are either not present or not needed to a similar extent by normal cells. Examples of this type of targeted therapy include Gleevec (imatinib), a kinase inhibitor that targets the fusion protein bcr/abl, which arises solely from a chromosomal translocation during tumorigenesis,[1,2] and Avastin (bevacizumab), a monoclonal antibody that suppresses neoangiogenesis required to nourish tumors for proliferation.[3,4] The pharmaceutical industry is currently focusing its efforts almost exclusively on this type of targeted therapeutic agent.

The second strategy involves the use of a homing ligand that binds specifically to a receptor that is expressed primarily on malignant cells. When linked to a therapeutic drug, this ligand can be exploited to carry the nonselective drug specifically into the cancer cell. If the drug is released only after internalization by the diseased cell, unwanted collateral damage to receptor-negative tissues can be avoided. Thus, wherever appropriately specific ligands can be identified, they can be exploited to convert nonspecific cytotoxic drugs into finely tuned tumor-specific warheads. Ligands that have been exploited for this approach to tumor targeting include monoclonal antibodies[5−7] and low molecular weight receptor-binding molecules such as peptide hormones, receptor antagonists and agonists, oligosaccharides, oligopeptides, and vitamins.[8−13] This article will review the use of folic acid as a ligand to target therapeutic cargos of many sizes, shapes, and mechanisms of action to tumor cells both in vitro and in vivo. For reviews of the use of folic acid to deliver attached imaging agents to malignant masses, the reader is referred to other published articles.[14]

1.2. Pros and Cons of Ligand-Targeted Therapies. Ligand-targeted therapies offer several advantages over the aforementioned functionality-targeted therapies, the most notable being the former's remarkable flexibility and adaptability. Almost any potent drug can be targeted to a tumor tissue if it can be linked reversibly to a targeting ligand with specificity for a pathologic cell type. A second advantage is that a cognate imaging agent can almost always be synthesized using the same targeting ligand, and this targeted imaging agent can then be employed to select for patients whose tumors overexpress the ligand's receptor.[11,15] Third, ligand-targeted therapies are generally preferred for delivery of membrane-impermeable drugs because a good targeting ligand can convey its attached cargo into the target cell by receptor-mediated endocytosis, rendering an otherwise membrane impermeable drug more efficacious.[16,17] And finally, because overexpression of a receptor on cancer cells is usually a more common event than over-reliance on an enzyme unique to cancer cells (e.g., bcr/abl), more development potential may exist for ligand-targeted therapies than functionality-targeted therapies.

Development of ligand-targeted therapies also poses several challenges that are absent from functionality-targeted and non-targeted therapies. First, because most endocytic pathways transport relatively few molecules into a cell, the ligand-targeted drug must be effective at low concentrations. This can be problematic for diseases for which few highly potent drugs have been identified. Second, delivery to the correct site does not necessarily guarantee therapeutic efficacy; rather, the drug generally not only must be internalized by the cell but also must be released within the cell. Because only a few chemical functionalities allow for facile release of a covalently attached drug after targeted cell uptake, drugs must contain at least one of a limited number of chemical moieties (−SH, −COOH, −OH, or $-NH_2$) that can be adapted for intracellular release.[18,19] Third, an efficient drug release mechanism must be designed into the conjugate: one that is inert during transit to the pathologic lesion but is activated rapidly after target cell binding and internalization, enabling release of the therapeutic cargo only at the site of disease. Not all ligand-targeted conjugates lend themselves to such mechanisms. And finally, for some membrane-impermeable drugs, an endosome escape strategy must be designed. Thus, after receptor-mediated endocytosis, the drug will generally still need to pass through the endosomal membrane to reach its target within the pathologic cell. While much progress has been made in developing these endosomal escape mechanisms, more improvements are needed before the full potential of ligand-targeted macromolecular drugs (such as siRNAs,[a] proteins, and nanoparticles) can be realized.[16]

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[a]Abbreviations: CHEMS, cholesteryl hemisuccinate; CTL, cytotoxic T lymphocytes; DM1, maytansine; DOPE, dioleoylphosphatidylethanolamine; DSPE, distearoylphosphatidylethanolamine; EGFR, epidermal growth factor receptor; Fab, the antigen-binding fragment of an antibody; FITC, fluorescein; FR, folate receptor; FRET, fluorescence resonance energy transfer; HSV-1, herpes simplex virus type 1; LPD, liposome/protamine/DNA complex; MRI, magnetic resonance imaging; IC_{50}, the half maximal inhibitory concentration; IFN-α, interferon-α; IgG, immunoglobulin G; IL-2, interleukin 2; ODN, oligodeoxynucleotide; PE, phosphatidylethanolamine; PEG, polyethylene glycol; PEI, polyethylenimine; PHSM, pH-sensitive polymeric mixed micelles; PLLA, poly-L-lactic acid; pLys, poly-L-lysine; RES, the reticuloendothelial system; scFv, the single chain variable fragment of an antibody; siRNA, small interfering RNA; TCR, T cell receptor, TK, thymidine kinase.

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Table 1. Folate Receptor Expression in Selected Solid Tumors Determined by Either Immunohistochemistry or Imaging with EC20,[15] a Folate-Targeted ^{99m}Tc-Based Radioimaging Agent[22] (Personal Observations)

cancer	% FR$^+$	number of cases examined
ovarian	82	488
lung (NSCLC: non-small-cell carcinoma)	66	511
endometrial	64	148
renal cell carcinoma (RCC)	64	310
colorectal	34	314
breast	29	380
nonfunctional pituitary adenoma (NFPA)	100	20
thyroid	48	23
cervical	30	23
lung metastases	30	23
lung (SCLC: small-cell lung carcinoma)	25	24
mesothelioma	21	38

1.3. Folate Receptor Expression Patterns. Folic acid is a vitamin that is essential for the proliferation and maintenance of all cells. Because most mammalian cells obtain their normal folate requirement via a low affinity reduced folate carrier[20] or proton-coupled folate transporter,[21] accessible FRs are normally expressed in significant numbers only on cancer cells,[22−24] activated macrophages,[25−28] and the proximal tubule cells of the kidney.[29] Although folate conjugates display no affinity for the reduced folate carrier or proton-coupled folate transporter, they bind to folate receptors (FRs) on these cell types with high affinity ($K_d \approx 10^{-9}$ M) and enter FR-expressing cells by receptor-mediated endocytosis.[30,31]

Overexpression of FRs on cancer cells may have evolved as a consequence of their increased requirement for folic acid that is essential in the synthesis of nucleotide bases needed in cell proliferation. Because folate is often a limiting nutrient in human serum, up-regulation of a high affinity FR on cancer cells may enable malignant cells to compete more aggressively for the vitamin. Many, but not all, cancers express either the α or β isoform of FR.[22,23] Those cancers that most aggressively up-regulate the folate receptor include cancers of the ovary, lung, kidney, endometrium, breast, brain, colon, and myeloid cells of hematopoetic lineage (Table 1). While other tumors, such as sarcomas, lymphomas, pancreatic cancer, testicular cancer, and cancers of the bladder, prostate, liver, head/neck, and skin, do not commonly up-regulate FR[22] (and personal observations), these malignant FR-negative cells may, in contrast, up-regulate the reduced folate carrier or proton-coupled folate transporter to satisfy their heightened folate needs.[20]

Expression of FR on nonmalignant hematopoietic cells (primarily the β isoform) appears to be limited to activated macrophages and their precursors.[25−28] Because activated macrophages accumulate at sites of inflammation, their uptake of folate conjugates is seen primarily in patients with inflammatory or autoimmune diseases.[25,27] Folate's utility in diagnosing and treating these diseases has been reviewed elsewhere.[11,32,33]

FR expression in the kidney is limited to the apical surface of the proximal tubule where the receptor captures the vitamin (or a folate−drug conjugate) from the nascent urine and transcytoses it back across the kidney epithelium for release into the blood.[34] In this process, FR serves as a salvage receptor to prevent loss of folates in the urine. Because most folate conjugates are not retained in the kidneys, no renal toxicity has ever been observed in animals or humans treated with folate-chemotherapeutic agent conjugates.[35,36]

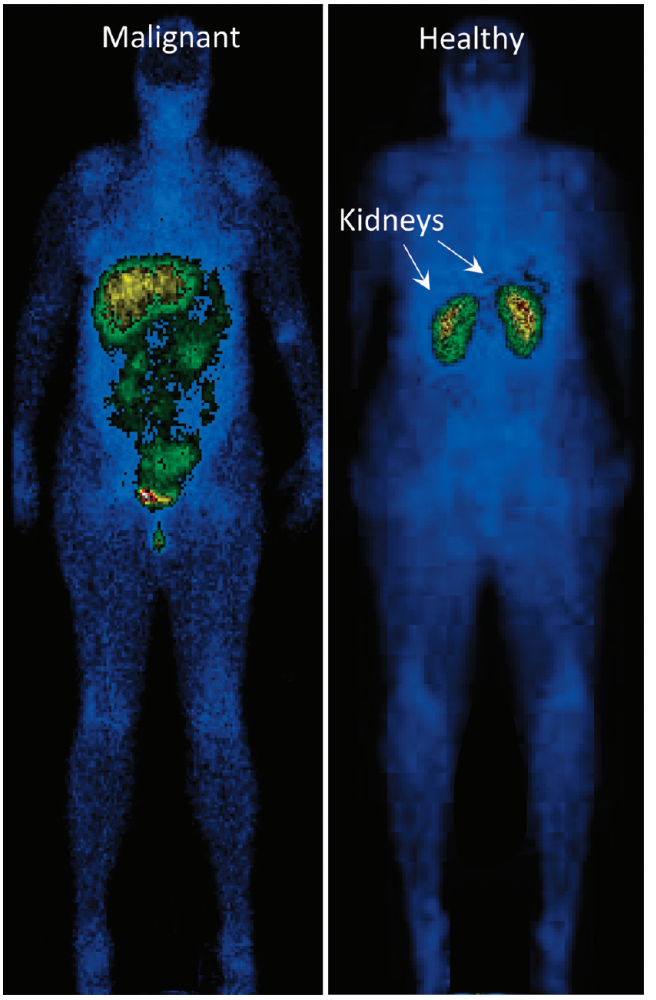

Figure 1. Comparison of [^{111}In]DTPA−folate uptake in a patient with stage III ovarian cancer (left) and a healthy volunteer (right).

A few other normal epithelial cells may also express FR,[29,37] especially alveolar epithelial cells,[38] but these FR are inaccessible to folate and folate conjugates because of their exclusive localization on the apical surfaces of polarized epithelia (i.e., the side facing the lumen or opening). Consequently, in healthy patients that do not have malignant masses or an inflammatory disease, measurable folate conjugate uptake is limited to the kidneys (Figure 1).

2. Folate-Targeted Cancer Therapeutics

Significant up-regulation of the folate receptor on tumor tissue has led us to hypothesize that folate-linked therapeutic agents might display reduced off-site toxicity and enhanced potency against tumor cells compared to nontargeted drugs.[17,22] The sections that follow describe the many applications of folic acid for targeting of a variety of therapeutic agents for treatment of malignant diseases. Although many types of folate conjugates have been evaluated in animal models of human cancers, only six folate-linked drugs have been tested in the clinic to date.[15,39−42]

2.1. Folate-Targeted Protein Toxins. Initial efforts to build folate-linked cytotoxic drugs focused on folate-linked protein

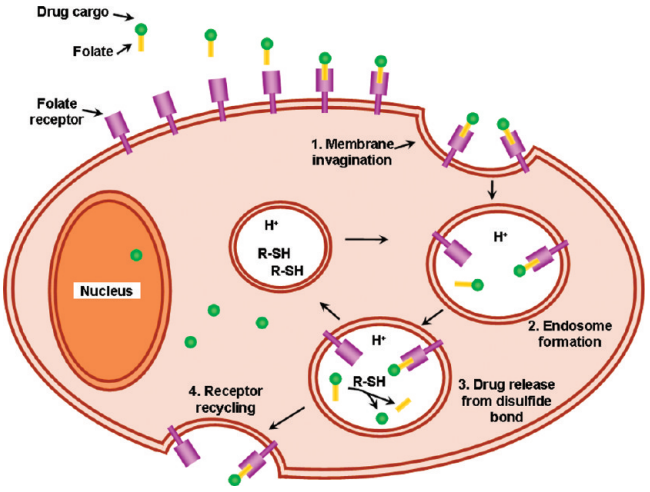

Figure 2. FR-mediated endocytosis of folate−drug conjugates. Exogenously added folate−drug conjugates bind specifically to FR with high affinity. The plasma membrane invaginates around the FR/folate conjugate complex to form an endosome. As the lumen of the maturing endosome acidifies, the receptor changes conformation and releases the conjugate, and the disulfide bond between folate and its drug cargo is reduced to release the parent drug cargo. Because the pH of FR-containing endosomes is only mildly acidic, acid-labile linkers do not release the attached drug as efficiently (see discussion in section 2.2).

toxins. A folate−*Pseudomonas* exotoxin (folate−PE38) conjugate was found to kill FR^+ cancer cells with an IC_{50} of $\sim 10^{-11}$ M. Interestingly, folate−PE38 demonstrated 100-fold greater inhibition of protein synthesis and similarly increased cytotoxicity toward cancer cells compared to folate−momordin, probably because of the former protein's built-in mechanism of endosomal escape following FR-mediated endocytosis.[43,44] To confirm that this difference in potency was indeed a consequence of a difference in endosomal escape, the mechanism of endosomal escape was selectively inhibited in folate−PE38 by blocking a critical cysteine residue. The resulting folate−toxin conjugate displayed a decrease in potency of 4 orders of magnitude, confirming the importance of endosomal release in the toxin's mechanism of action.[43] Similar requirements for endosomal escape have been observed for other macromolecular folate conjugates (personal observations).

2.2. Folate-Targeted Chemotherapeutic Agents. Because of its high affinity for FR, folate has often been observed to remain attached to FR as the receptor internalizes and then recycles back to the cell surface (Figure 2); i.e., the oxidized form of the vitamin (folic acid) may not normally dissociate from the receptor during FR trafficking.[31,45,46] A similar fate has been observed to compromise the potency of folate-targeted chemotherapeutic agents when the linker between the vitamin and its attached therapeutic warhead is not cleaved during FR recycling. Therefore, in an effort to ensure intracellular drug release, multiple strategies for unloading an attached therapeutic cargo within the target cell have been explored. The most prominent method to trigger intracellular drug release has exploited the large difference in reducing power between the extra- and intracellular environments. In this approach, a self-immolative disulfide bridge is introduced between the drug and folic acid with the anticipation that an unmodified drug will be discharged once the conjugate has entered a reducing endosome.[31,43] This approach was directly imaged by microscopy when folate was linked via a disulfide bond to two fluorescent dyes that engaged in fluorescence resonance energy transfer while covalently linked in the same folate conjugate[31] (Figure 3). Loss of fluorescence resonance energy transfer (FRET) without loss of the fluorescence of either dye was taken as evidence that the disulfide bond linking one of the dyes to folate had been reduced, allowing separation of the fluorescence donor and acceptor pair. Importantly, FRET was observed to decline immediately upon internalization of the conjugate by FR-mediated endocytosis, and energy transfer was seen to completely disappear before any folate conjugates could recycle to the cell surface.[31] These data demonstrated that disulfide bond reduction begins early during endosomal trafficking and continues rapidly until all disulfide bonds are cleaved. Assuming that the released therapeutic cargo is membrane permeable, the discharged drug was hypothesized to rapidly diffuse out of the endosome and into the cytoplasm where it could perform its function. This hypothesis was supported by observations demonstrating that disulfide-linked drugs, such as folate−maytansine DM1 and folate−camptothecin,[47] could kill a panel of FR^+ cancer cells with IC_{50} values between 10^{-11} and 10^{-10} M, suggesting efficient drug release following FR endocytosis.[47]

The second approach to trigger endosomal release of an active drug from its folate conjugate was designed to exploit the more acidic pH values commonly encountered in late endosomes and lysosomes.[48] In an early study, a paclitaxel-7-polyethylene glycol−folate conjugate was designed with a pH-sensitive ester connecting the taxane to the polyethylene glycol−folate in the hope that the low endosomal pH experienced during intracellular trafficking would release the paclitaxel.[49] However, the folate conjugate was found to be 50-fold less cytotoxic to FR^+ KB cells than the free paclitaxel despite the fact that the conjugate retained high affinity for the FR.[49] Subsequent experiments examined the cause of this unexpected inactivity as well as the poor potencies of other pH sensitive folate−drug conjugates. Surprisingly, analyses of the pH in FR-containing endosomal compartments using folate-linked pH indicator dyes revealed that these endosomes never reached pH below 6.2, suggesting that the pH was never sufficiently acidic to rapidly hydrolyze ester bridges ($t_{1/2} \approx 197$ h).[50] More detailed studies further revealed that monovalent folate−drug conjugates recycle through endosomes with median pH of 6.8 and never encounter strongly acidic compartments (Figure 4). In contrast, multivalent folate conjugates (e.g., liposomes with multiple folates attached) were found to traffic to lysosomes where median pH values were near pH 5.[48] Consequently, acid catalyzed drug release mechanisms will only be useful for delivery of multivalent folate-targeted constructs (e.g., nanoparticles and liposomes).

The above differences between disulfide and pH-sensitive linkers have been confirmed on multiple folate conjugates designed to differ only in the chemistries of their linkers. One well studied example involves a matched pair of folate−desacetylvinblastine monohydrazide conjugates: **1** (EC140) with an acylhydrazone (pH-sensitive) linker and **2** (EC145) with a disulfide linker (Figure 5). As anticipated, in vivo tests have demonstrated **2** to be dramatically more potent than **1**.[39,51,52]

Optimization of linker design requires not only that the bridging chemistry allow facile release of an unmodified drug following uptake by its target cell but also that the folate−drug conjugate remain intact during its brief transit in the vasculature from its site of injection to the tumor mass.

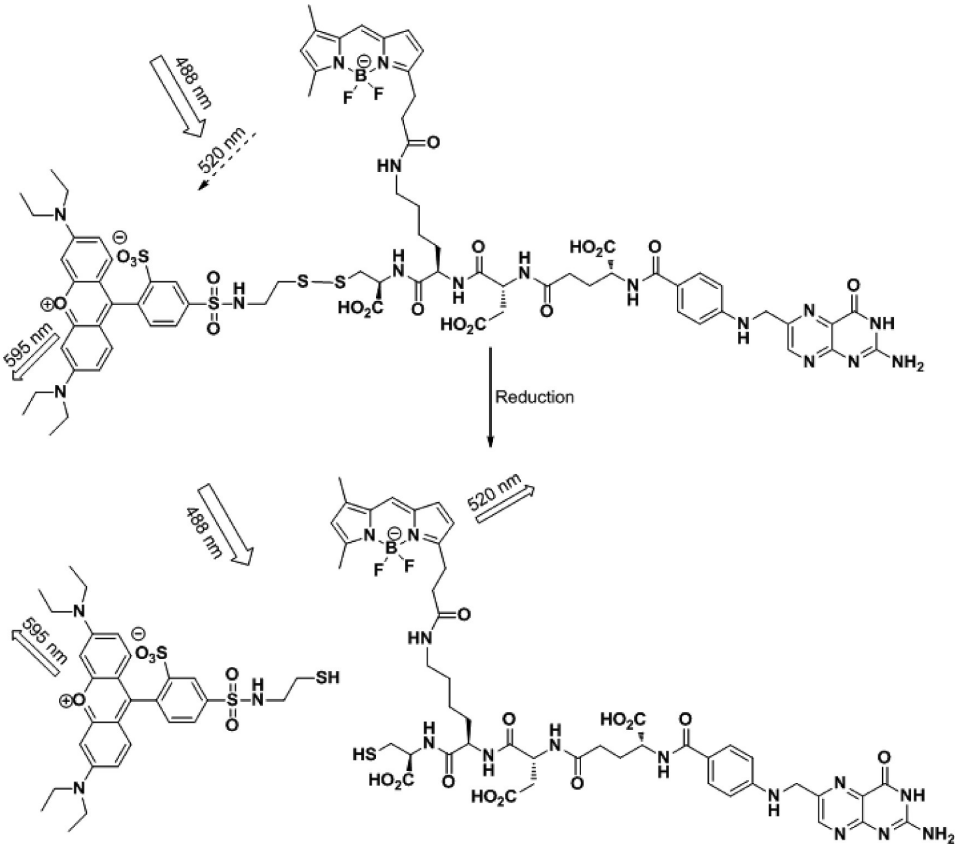

Figure 3. The folate−FRET reporter changes fluorescence from red to green upon disulfide reduction. In the nonreduced folate−FRET reporter (upper), excitation of BODIPY (488 nm) leads to rhodamine (red, 595 nm) emission due to FRET from BODIPY to rhodamine. Upon disulfide reduction (lower), rhodamine is released from the folate−BODIPY backbone, leading to loss of FRET signal and concurrent recovery of BODIPY (green, 520 nm) fluorescence.

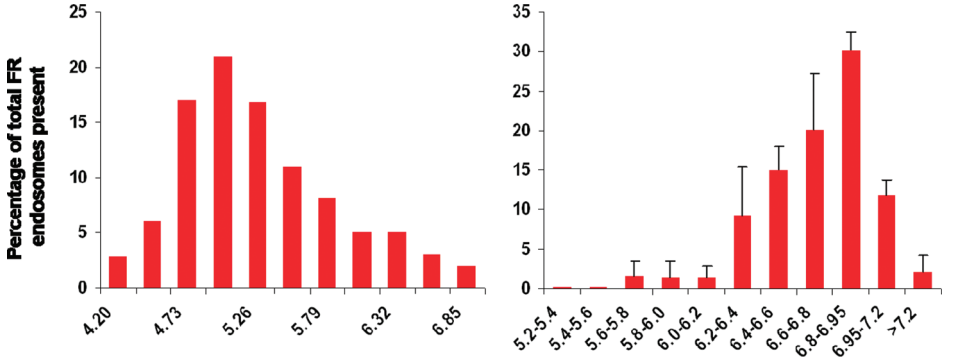

Figure 4. Comparison of endosomal compartment pH values encountered by monovalent and multivalent folate conjugates following their uptake by folate receptor-mediated endocytosis. Multivalent conjugates (left) bind simultaneously to multiple FR and traffic to acidic lysosomes following endocytosis.[48] In contrast, monovalent conjugates traffic to a mildly acidic recycling center where disulfide-linked drugs are rapidly released by disulfide reduction.[50].

Importantly, because the sizes of most folate−chemotherapeutic agents are small, they have been found to perfuse solid tissues very rapidly, leading to saturation of all FR in solid tumors in < 5 min in mice[53] and probably < 15 min in humans following intravenous injection. Since clearance of water-soluble folate conjugates from the bloodstream is $> 99\%$ complete in less than 20 min, the cleavable linker connecting folate to its attached drug need only be stable in the bloodstream for ~15−20 min. Pharmacokinetic studies in both rodents and humans have revealed that this stability requirement is readily satisfied, at least for disulfide linked folate conjugates.[35] Considered together with the previously established efficient release of disulfide bridged conjugates in early endosomes, our lab has decided that future monovalent folate-targeted chemotherapeutic agents should be tethered via a disulfide rather than a pH labile linker to its targeting ligand. Moreover, all future folate-targeted drugs will be linked via the γ-carboxyl rather than α-carboxyl because the former exhibits slightly higher affinity for FR than the latter.[54] Representative examples of such γ-carboxyl derivatized disulfide-linked cytotoxic drugs (Figure 6) include **2** ($IC_{50} \approx 9$ nM),[39,52] folate−maytansine DM1 (EC131, $IC_{50} \approx 16-25$ nM),[55] folate−tubulysin B (EC0305, $IC_{50} \approx 1-10$ nM),[56] folate−mitomycin C (EC72, $IC_{50} \approx 5$ nM),[57] folate−camptothecin ($IC_{50} \approx 10$ nM),[58] and a

dual warhead folate−mitomycin C/desacetylvinblastine conjugate (EC0225, $IC_{50} \approx 5.4$ nM).[40] Because the tumor delivery capacity of the FR endocytic pathway is limited primarily by the number of FR expressed on the cancer cell (usually $(1-3) \times 10^6$/cell), only highly potent warheads have eventually succeeded as effective folate-targeted therapeutics. As noted in the cited articles, each of the aforementioned folate-targeted cytotoxic drugs has demonstrated remarkable activity in animal tumor models with greatly reduced off-target toxicity to healthy cells. **2** will soon be entering phase 3 clinical trials, whereas several other folate−drug conjugates are currently undergoing testing in either phase 1 or phase 2 clinical trials.

2.3. Folate-Targeted Immunotherapy. One problem with the effectiveness of traditional cancer treatments is that most tumors evolve mechanisms to evade their chemotherapies by selection of mutations that lead to drug resistance. Because most immunotherapies are designed to educate the immune system to recognize mutations that arise during tumor progression, activation of humoral and/or cellular immunities against tumor cells can result in tumor cell killing and extension of patient lifespan. As with chemotherapy strategies, immunotherapies can also be improved by targeting the immune stimulant to the folate receptor. To date, protocols for targeting immunotherapeutic agents to FR^+ cancers have included (1) administration of unconjugated or conjugated anti-FR antibodies (MOv18, MOv19),[59−62] (2) the use of folate/anti-T cell receptor (anti-TCR IgG) bispecific antibodies,[63−65] (3) transduction of T lymphocytes with FR peptides for production of cellular anti-FR immunity,[66] and (4) administration of folate-targeted haptens to "paint" tumor cell surfaces with foreign/immunogenic molecules.[67,68]

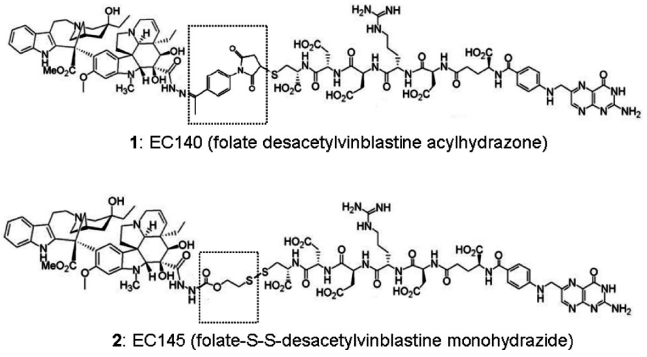

Figure 5. Chemical structures of two folate−desacetylvinblastine monohydrazide conjugates.[39]

Initial immunotherapy efforts focused on the use of two murine monoclonal antibodies, MOv18 (IgG1κ) and MOv19 (IgG2aκ), that exhibited high affinity for ovarian cancer cells ($K_A = 10^8-10^9$ M^{-1}).[62,69,70] MOv18 was initially exploited to deliver the therapeutic radionuclide ^{131}I or to redirect the cytotoxicity of activated autologous immune cells to FR^+ ovarian carcinoma cells in vitro.[59,60] Similarly, the single-chain Fv (scFv) of MOv19 was used to direct conjugated interleukin-2 (IL-2) to tumors with the hope that the IL-2 would stimulate activation of the immune system within the tumor.[61] Prolonged release of IL-2/MOv19 scFv protected 60% of mice from developing lung metastases caused by FR^+ tumors. Moreover, treatment with IL-2/MOv19 scFv (but not with nontargeted recombinant IL-2) significantly reduced the volume of subcutaneous FR^+ tumors in mice.[61] Although early human trials with these monoclonal antibodies failed, clinical trials using improved versions of anti-folate receptor monoclonal antibodies are currently underway and the results are very promising.[62,71] In order to overcome potential tumor penetration barriers that were thought to compromise the potencies of the larger intact IgGs, completely humanized antibody fragments (Fabs) against FR have been assembled using phage display and

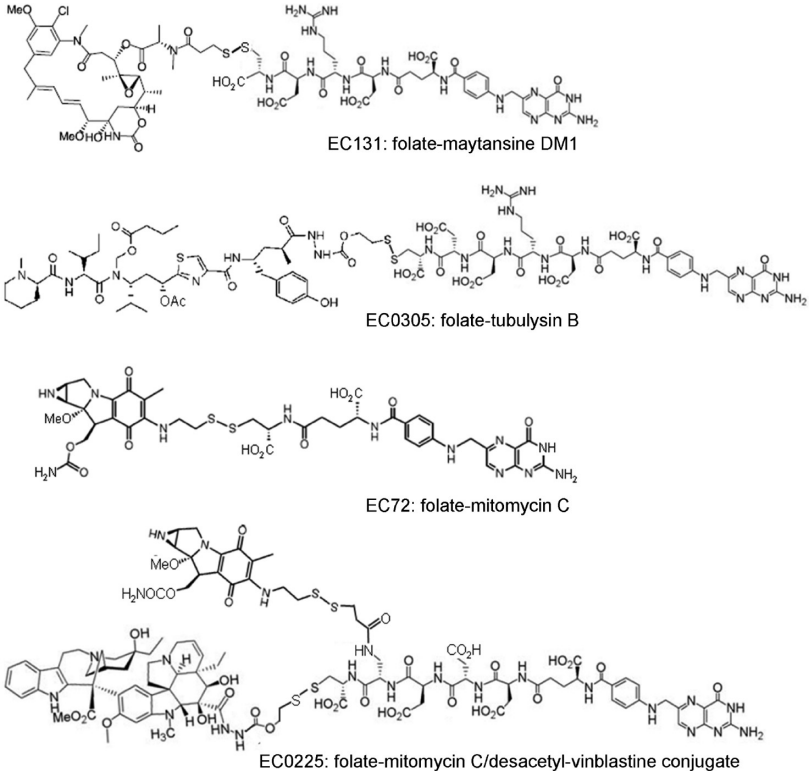

Figure 6. Chemical structures of folate−γ-carboxyl derivatized disulfide-linked cytotoxic drugs.[40,55−57]

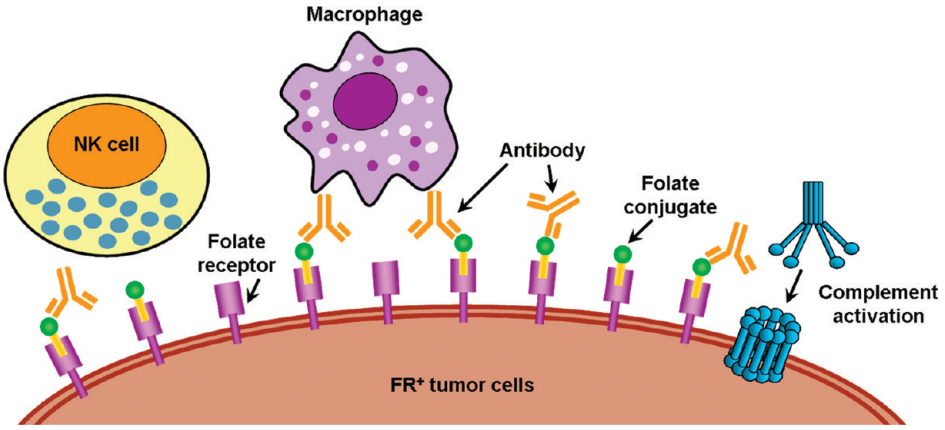

Figure 7. Illustration of folate−hapten mediated immunotherapy. Mice previously immunized against a hapten (e.g., fluorescein) are treated with a folate−hapten conjugate (such as folate−fluorescein) that can bind to cell surface FR. After binding of antifluorescein antibodies induced during the immunization, the cancer cell is recognized by Fc receptor-expressing immune cells, leading to cancer cell destruction by antibody-dependent cell cytotoxicity (ADCC).

epitope imprinting selection methodologies.[72,73] For example, a smaller Fab dimer was demonstrated with high affinity for FR and significantly improved tumor-to-blood ratios. Future development of this antibody construct for clinical applications is apparently planned.[74]

Instead of using an antifolate receptor antibody to deliver an attached cargo, Kranz et al.[63] used a folate-derivatized antibody against the T cell receptor (anti-TCR IgG) to force T cells (recognized by anti-TCR IgG) to dock with FR^+ cancer cells (recognized by the folic acid). With an average of five folates per IgG, this folate conjugate effectively mediated lysis of FR^+ tumor cells at concentrations as low as 1 pM. Importantly, the extent of tumor cell lysis was found to correlate with its level of FR expression, and cell killing was found to be completely inhibited by free folic acid.[63] For unknown reasons, this promising strategy has never been tested in the clinic. In a follow-up study, a "smaller" folate conjugate to a single-chain antibody fragment (scFv) of the anti-TCR IgG was constructed. Similar to IgG−folate, the scFv−folate was found to mediate lysis of FR^+ tumor cells by cytotoxic T lymphocytes (CTLs) (with $EC_{50} \approx 40$ pM) in vitro.[64] However, in vivo preclinical testing of the folate−antibody/scFv bispecific construct against human tumors was limited to immunodeficient mice that received the bispecific agent plus activated human effector T cells. Unfortunately, such a model does not accurately reflect a clinical scenario. Therefore, the same group developed a transgenic mouse model that would grow FR^+ human KB cell tumors and induced mobilization of endogenous CTLs with a synthetic antigenic peptide recognized by the CTLs.[65] Importantly, the transgenic mice rejected the human tumors after treatment with the activating peptide and the scFv−folate. However, since the T cells in this model were derived from a single clone, it can be cogently argued that such an immunotherapy strategy would best be tested in a syngeneic mouse model with a normal heterogeneous immune system before advancing the methodology into the clinic.

As an alternative to direct targeting of a CTL or inflammatory cytokine to a cancer, folate has also been exploited to deliver a highly immunogenic hapten to the surface of FR^+ tumors as a method of marking the tumor as "foreign." This "immunogenic flag" was hypothesized to render a malignant mass that had somehow escaped recognition by the immune system highly visible to immune cells (Figure 7). To test this hypothesis, tumor-bearing mice were vaccinated against fluorescein before intraperitoneal implantation of FR^+ M109 tumor cells. After an adequate anti-FITC antibody titer had been induced, a combination of folate−FITC, IL-2, and interferon-α (IFN-α) was administered to simultaneously decorate the FR-expressing tumor cells with FITC and stimulate the immune system with the cytokines.[41] Healthy FR-negative cells were found to be spared from immune attack, whereas FR^+ malignant cells were observed to be rapidly destroyed by a massive influx of immune cells.[75] More importantly, this folate−hapten therapy was seen to confer long-term immunity against the same cancer. Thus, when challenged with fresh tumor cells on multiple occasions at later dates, the same mice rejected the newly implanted cells without needing any further therapy.[41,75] Subsequent analysis of the mechanism of this immune memory demonstrated that the sustained memory resided in the T cell population. This cancer-specific immune memory probably constitutes the chief merit of folate-targeted hapten therapy, since it prevents recurrence of the disease in patients that would otherwise relapse. Given these encouraging results, the folate-targeted hapten therapy now is in early clinical trials.

2.4. Folate-Targeted Gene Therapy. Although a few reports exist regarding FR-targeted viral vectors for gene therapy,[76] obstacles including the immunogenicity of viral proteins and potential generation of infectious viral particles have redirected most of these efforts to folate-linked nonviral biocompatible polymeric gene carriers, such as cationic polymers, cationic peptides, cationic lipids, and so on.[77] However, because nonviral formulations lack the transfection machinery inherent in many viral vectors, improved circulation times, strategies for endosomal escape, and methods for nuclear trafficking have had to be designed into the folate-targeted nonviral gene therapy vectors to render them efficacious.

Cationic polymers have been utilized widely for folate-targeted gene delivery because of their (1) physiochemical versatility, (2) ease of manipulation, and (3) ability to condense DNA into compact complexes. Early efforts by Gottschalk et al.[78] and Reddy et al.[79,80] using folate-linked poly-L-lysine (pLys) and pLys-polyethylene glycol (PEG) complexes, respectively, yielded only low levels of reporter activity despite good gene delivery to FR^+ cells. These early

studies indicated that efficient DNA delivery and gene expression required an endosomal disruption mechanism to release that DNA from its intracellular compartment.

Unlike pLys, polyethylenimine (PEI) was known to possess inherent endosomal lytic activity, serving as both a DNA-condensing agent and a proton sponge that could osmotically lyse its entrapping endosome;[81] however, PEI also suffers from nonspecific uptake in the lungs and liver that can lead to unwanted toxicity. Thus, to achieve targeted gene delivery with minimal collateral toxicity, Benns et al.[82] linked folate to both ends of a monofunctionalized PEG and then grafted it to PEI (FPF-g-PEI). The complex of FPF-g-PEI and a luciferase reporter gene achieved transfection in FR^+ cancer cells in a FR-dependent manner.[82] However, because the barriers to in vivo delivery are much greater than those encountered in vitro, it will now be important to prove the utility of these complexes in tumor-bearing mice in vivo.

Several cationic liposomal formulations that incorporate a lipophilic folate derivative as a targeting moiety have also been studied. In pioneering studies, Hofland et al.[83] performed the first in vivo folate-targeted gene transfer in a subcutaneous mouse tumor model using a folate−PEG−lipid/DNA complex. While a biodistribution analysis of the ^{111}In-labeled folate-targeted liposomes showed no improvement in tumor-specific accumulation over nontargeted PEG−lipid/DNA complexes, attachment of folate−PEG to the cationic lipid/chloramphenicol acetyl transferase (CAT) reporter gene complex was found to significantly increase gene transfer activity in FR^+ tumors while decreasing nonspecific expression in the lungs 50- to 100-fold.[83] Although nonspecific gene transfer needs to be minimized further to generate an ideal DNA delivery formulation, the approach nonetheless provided the first in vivo proof of concept of systemically targeted gene delivery to tumors.

Novel liposome/protamine/DNA formulations (LPD) have also been linked to folate to improve tumor-specific gene transfer. In an in vivo ascites tumor model, Reddy et al.[84] reported an 8- to 10-fold increase in tumor-associated gene transfer compared to a corresponding nontargeted LPD formulation.[84] Moreover, using an LPD−PEG−folate formulation to deliver the herpes simplex virus type-1 (HSV-1) thymidine kinase (TK) gene into murine breast cancer cells, Bruckheimer et al.[85] demonstrated a greater reduction in mean tumor volume compared to either nontargeted LPD−PEG/TK, the vehicle alone, or an untreated control group.

Significant efforts have also been devoted to optimizing folate-targeted constructs for improved gene delivery, from titrating the use of PEG, which can reduce nonspecific uptake and increase transfection efficiency, to minimization of vector size, which can improve extravasation of the vector into the tumor. Continued efforts toward these objectives should yield improved folate-targeted gene therapy vectors, as should research into enhancement of the efficiencies of endosomal escape and gene trafficking to the nucleus.

2.5. Folate-Targeted Antisense Oligodeoxyribonucleotides (ODN) and Small Interfering RNAs (siRNA). Using ODN or siRNA to ablate or modulate gene expression shows great promise as a method to arrest tumor cell growth and induce apoptosis largely because of the potential specificity of oligonucleotides for their complementary target mRNAs. Unfortunately, nonspecific activation of the immune system,[86] inadequate permeability of naked oligonucleotides to cell membranes,[87] indiscriminate uptake of cationic complexes of oligonucleotides by nonpathologic cells,[88,89] and insufficient release of oligonucleotide complexes from endosomal compartments following uptake by endocytosis[88,90] have all compromised the potential of this important therapeutic strategy. While encapsulating/complexing oligonucleotides into liposomes, dendrimers, micelles, or nanoparticles has improved their pharmacokinetic properties, the same nanocomplexes have generally been handicapped by reduced target cell binding and tumor specificity.[88,89] Thus, the major obstacle to developing antisense ODN and siRNA for use in therapeutic applications can be summarized as a problem in oligonucleotide delivery. As will be seen below, while attachment of folate as a targeting ligand can improve tumor-specific oligonucleotide delivery and associated gene knockdown, endosomal escape strategies will have to be improved to achieve the full potential of ODN and siRNA therapies.

The simplest approach to folate-targeted delivery of siRNA and ODN to solid tumors has involved direct attachment of one strand of the oligonucleotide to the γ-carboxyl of folic acid via some type of cleavable or releasable bridge. In one study, folate was covalently attached to a single stranded oligonucleotide that was complementary to a single-stranded overhang added to the end of the siRNA.[91] Upon base-pairing with the single stranded oligonucleotide extension, the folate-linked oligonucleotide was found to facilitate delivery of the attached siRNA into any cell expressing FR. The utility of this universal linker strategy was most clearly demonstrated in vitro by showing its ability to suppress expression of an αV integrin gene by 80% in an FR^+ cancer cell line.[91]

In vivo demonstration of the ability of folate to target an attached oligonucleotide to solid tumors was achieved using a related but much simpler construct, where folate was attached to either the 3′- or 5′-end of the sense strand of an siRNA or ODN via a disulfide bond and the complementary antisense strand was labeled with a fluorescent dye (e.g., Dylight 647 or Cy5).[16] Four hours after injecting the naked oligonucleotide into the tail vein of KB tumor-bearing mice, the mice were imaged and found to contain the fluorescent marker almost exclusively in the solid tumors (Figure 8). Since the targeting ligand (i.e., folate) was not present on the strand labeled with the fluorescent dye, localization of fluorescence to the tumor demonstrated that the naked nucleotide duplex was delivered to the solid tumor intact.

More sophisticated efforts to target siRNA/ODN to tumors in vivo with folate have focused on the use a cationic polymer to condense the ODN or siRNA prior to folate-targeted delivery. Citro et al.[92] covalently linked folate to a pLys chain and delivered c-myb sense and antisense ODNs to HL60 leukemia cells in vitro, reporting a down-regulation of c-myb expression and inhibition of proliferation in HL60 cells.[92] In similar fashion, Kim et al.[93] utilized a folate-targeted PEI-based cationic polymer for ODN/siRNA delivery, which demonstrated enhanced cell uptake and more efficient green fluorescent protein (GFP) expression inhibition in FR^+ KB cells than nontargeted PEI. Importantly, the inhibitory difference between PEI−PEG−folate and nontargeted PEG-PEI disappeared when similar studies were performed in FR-negative A549 cells, suggesting an FR requirement for gene knockdown.[93] More recently, with the optimization of polymer architecture, a novel hydrophilic-block-cationic copolymer conjugated to folate with improved stability in circulation as well as fewer nonspecific side effects has been developed.[94] In vivo comparisons will

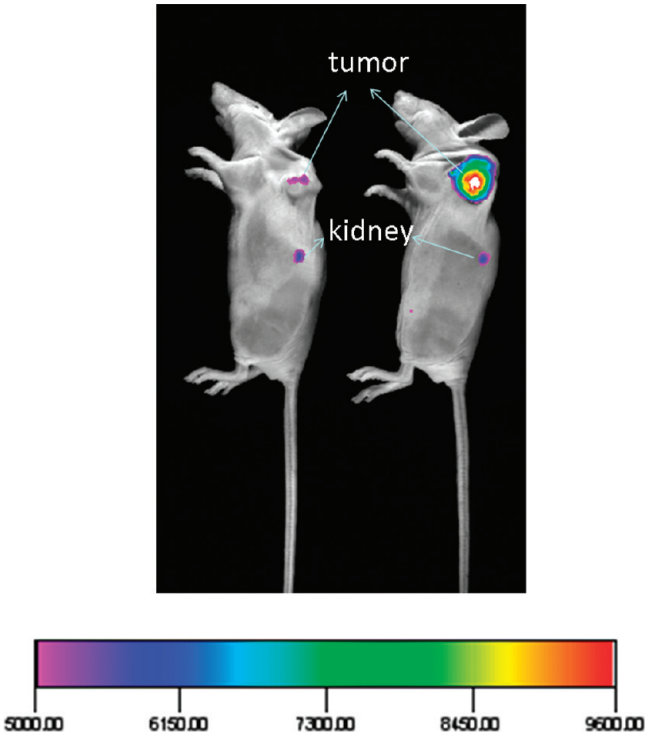

Figure 8. Folate-mediated targeting of fluorescent oligonucleotides to solid tumors in vivo, as visualized using a Kodak imaging station.[16] The mouse on the left was injected with nontargeted Dylight 647-labeled siRNA, while the mouse on the right was injected with a folate-targeted version of the same siRNA.

obviously now be required to assess the full potentials of these and other copolymer blends for siRNA delivery.

Cationic liposome-based formulations have also been investigated for improved tumor targeting via folate receptors. Initially, Wang et al.[95] constructed a folate−PEG−liposome formulation to deliver an ODN against epidermal growth factor receptor (EGFR) into KB cells. The folate−PEG−liposome complex was found to eliminate EGFR expression, alter cell morphology, and halt tumor cell growth.[95] Rait et al.[96] further showed that FR-targeted cationic liposomes (without PEG) were more efficient than the nonselective commercially available transfection reagent, Lipofectin, in delivering anti-HER2 ODN into breast cancer cells both in vitro and in vivo. Notably, treatment with folate−liposome−ODN resulted in chemosensitization of the tumor cells, and inhibition of tumor growth was sustained for >3 weeks after treatment.[96] This important publication constituted the first report of in vivo efficacy against tumors using a folate-targeted liposome delivery system for systemic ODN/siRNA therapy. Using a folate−PEG−liposome formulation, Leamon et al.[97] reported that nearly 100 000 ODN-loaded folate-targeted liposomes were taken up per KB cell in vitro during 1 h of incubation, and maximal FR loading was achieved with PEG linkers as low as 1000 Da. However, tumor-specific accumulation of the folate-targeted liposomes in vivo was unexpectedly no greater than that of their nontargeted counterparts.[97]

Somewhat related folate-linked lipid-based nanoparticles complexed with DNA have also been shown to display efficient delivery of synthetic siRNAs to tumors.[98] When HER2 siRNA was used as the test siRNA, the folate targeted formulation was found to inhibit KB tumor xenograft growth and selectively suppress HER2 protein expression significantly more than any comparable nontargeted formulation.[98]

Folate-derivatized polyelectrolyte (PEC) micelles, whose assembly is driven by ionic interactions between a folate−PEG−ODN and PEI, have also been used for delivery of ODN to tumors. Biodistribution studies have revealed that these micellar ODN also concentrate in FR^+ solid tumors following systemic administration. Although uptake was also high in several healthy tissues, probably due to macrophage scavenging, the data nonetheless suggest that folate-targeted micelles also exhibit potential for ODN delivery to cancer cells in vivo.[99]

Most platforms employed to deliver siRNA to tumors rely on some intrinsic ability of the internalized polymer/nanoparticle to escape its entrapping endosome and release its siRNA cargo into the cytoplasm. Although in vitro and in vivo studies with folate-targeted siRNAs have already demonstrated suppression of specific genes, the extent of gene knockdown to date has generally been modest compared to the knockdown achieved with similar quantities of siRNA complexed with a cell permeabilizing agent such as lipofectamine. This and related comparisons suggest that most internalized oligonucleotides do not escape their endosomal compartments and enter the cytoplasm where they must function. While it is possible that optimization of nanoparticle size, solubility, nonspecific adsorption, and particle shape could lead to enhanced oligonucleotide potency in vivo, we believe that the greatest improvement in gene suppression will eventually be achieved when an efficient endosomal escape strategy can be developed. In our opinion, this goal should be the primary focus of future efforts in formulating siRNA and ODN delivery vehicles.

2.6. Design of Folate-Targeted Nanoparticles. Nanoparticles can also be developed into effective delivery vehicles for chemotherapeutic agents, in part because they can be designed to carry large quantities of therapeutic cargos in compartments characterized by a diversity of sizes, shapes, rheologic properties, and chemistries.[100] Moreover, since the vasculature is often incompletely/abnormally formed in solid tumors, often displaying large gaps of ~300−800 nm between adjacent endothelial cells, nanoparticle extravasation and tumor accumulation can be greatly enhanced at these sites.[101] This passive accumulation of nanoparticles in solid tumors, together with the poor drainage of the same particles via a commonly flawed lymphatic system, can lead to significant accumulation of nanoparticles at malignant sites.

How then might folate targeting be exploited to improve intratumoral delivery of nanoparticles? In general, attachment of folate to nanoparticles, including liposomes, dendrimers, micelles, nanospheres, nanocapsules, nanowires, polyplexes, and lipoplexes, etc., cannot enhance entry of the particle into a solid tumor, since the density of tumor blood vessels, the porosity of the tumor vasculature, and the compactness of the extracellular matrix will largely control perfusion pharmacokinetics of nanoparticles into tumor masses. Indeed, "binding site barrier effects" due to high affinity docking of the initial folate-labeled particles to exit a vasculature onto FR^+ cancer cells directly adjacent to the blood vessels can actually reduce penetration of subsequent folate-targeted particles to sites further from the vasculature.[53] However, once a folate-linked nanoparticle arrives at an FR^+ tumor cell, ligation of the particle to folate can only enhance its therapeutic efficacy, since folate will not only increase retention of the nanoparticle in the tumor mass

but also facilitate uptake of the particle by an FR-mediated endocytosis. A few examples of folate-targeted nanoparticle therapeutics will now be provided below to illustrate their potential.

2.6.1. Folate-Targeted Liposomes. Liposomes are unilamellar or multilamellar lipid assemblies that can entrap large quantities of hydrophilic molecules within their aqueous interiors or hydrophobic drugs within their hydrocarbon bilayers. Because of this diverse "carrying capacity", as well as their desirable biocompatibility,[102] a number of liposomal drug formulations are in development, currently undergoing clinical trials, or already on the market. While no folate-targeted liposomes have yet achieved FDA approval, many have been employed successfully to deliver compounds to tumor cells in model systems with highly promising results.[79,98,103−114]

The first folate-targeted liposome was prepared by attaching folate to the distal end of a PEG spacer that was in turn linked to the amino group of phosphatidylethanolamine (folate−PEG−PE) embedded in the liposome bilayer.[115] Initial binding studies with this formulation demonstrated that the length of the spacer between folate and the lipid anchor was critical, where a 250 Å PEG_{3350} spacer yielded ~37 times greater uptake than similar liposomes with a 23 Å maleiimidocaproyl−lysine−SH spacer. Unfortunately, these relatively "naked" liposomal formulations were found to suffer from short circulation times in vivo due to their nonspecific uptake by the reticuloendothelial system (RES). Such nonspecific uptake, however, was subsequently overcome by incorporating 4% nontargeted PEGylated lipid into the folate-targeted liposomes to mimic the commercially successful "stealth liposomes".[103] Further improvements then emerged when it was observed that better tumor targeting could be achieved if the predominant PEGylated lipid comprising the "stealth" coating contained a shorter PEG chain (e.g., PEG_{2000}) than the folate-derivatized lipid (e.g., PEG_{3400}, PEG_{5000}).[116,117] A mole fraction of 0.03−0.5% folate−PEG−DSPE was then found to be optimal for effective delivery of the targeted "stealth" liposomes into FR^+ cells. In fact, increasing the density of the folate ligand on the liposome surface above this mole fraction was observed to reduce net uptake of the liposome by the target cell.[84,103,116,117] Finally, in addition to folate−PEG−DSPE, two other lipophilic folate derivatives have been reported to facilitate good liposome targeting to FR^+ tumor cells both in vitro and in vivo. These are folate−PEG−cholesterol and folate−PEG−cholesteryl hemisuccinate (CHEMS).[118,119]

As with siRNA delivery, one obstacle found to limit the potencies of some folate-targeted liposomal drug formulations has been the poor efficiency of drug unloading following receptor-mediated uptake into FR^+ cells. Because multivalent nanoparticles (in contrast to monovalent folate−drug conjugates) encounter low pH endosomes during their intracellular trafficking,[48] this problem has been addressed by exploring a number of pH-triggered liposome disrupting mechanisms. In one strategy, a pH sensitive membrane-destabilizing peptide has been entrapped together with the desired drug in a folate-targeted liposome.[120,121] Since the encapsulated peptide is designed to become fusogenic only at the low pH found in intracellular endosomes, these liposomes have been observed to unload their cargos only following internalization and trafficking to an acidic intracellular compartment.

A second endosomal unloading strategy has been based on the propensity of vinyl ethers to hydrolyze at the low pH characteristic of late endosomes and lysosomes. By using such linkages to connect the fatty acyl component of a phospholipid to its glycerol backbone, Rui et al.[122] were able to generate diplasmenylcholine (Figure 9) that would hydrolyze to lysolipid detergents upon trafficking to lysosomes. Employing such lipids to encapsulate cytosine arabinoside was found to increase the potency of the corresponding folate-targeted liposomal preparation 60-fold compared to its pH-insensitive phosphatidylcholine counterpart.[80,122]

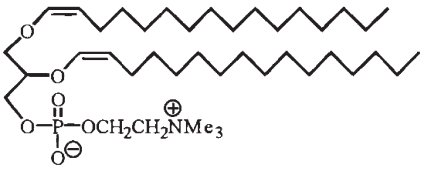

Figure 9. Chemical structure of diplasmenylcholine.

In a third strategy, folate-linked liposomes were supplemented with *N*-citraconyldioleoylphosphatidylethanolamine (C-DOPE), which was shown to hydrolyze rapidly at pH 5 to yield the highly fusogenic lipid, DOPE. When incorporated with folate−PEG−DOPE into liposomes, the resulting formulation was found to remain stable at a neutral pH but become fusogenic at pH 5, releasing an encapsulated plasmid into the target cell cytoplasm, thereby improving expression of the plasmid.[80] Finally, to improve liposome stability in the bloodstream, a novel serum-resistant pH-sensitive liposome formulation comprising egg phosphatidylcholine, CHEMS, oleyl alcohol, and Tween-80 has been designed to deliver cytosine-b-D-arabinofuranoside (Ara-C) to FR^+ KB cells. This interesting formulation has been reported to yield a 17-fold improvement in cancer cell death compared to its non-pH sensitive counterpart.[123]

Although each of the above mechanisms reports an improvement in endosomal drug release, the use of different therapeutic cargos, unrelated cell lines, and distinct therapeutic objectives has rendered a direct comparison of their efficacies impossible. Before considering any of these strategies for preclinical development, it will be important to compare their abilities to mediate each step of the delivery process directly in the same experimental system so that their relative strengths and weaknesses can be identified. Perhaps in this manner an optimal hybrid approach can eventually be designed that will efficiently deliver large quantities of therapeutic cargos into FR expressing cancer cells.

2.6.2. Folate-Targeted Micelles. Over the past decade, polymeric micelles consisting of a hydrophilic shell and a hydrophobic core have become an attractive alternative delivery vehicle for hydrophobic molecules, including many anticancer drugs.[124] While maintaining some similarities with liposomal delivery systems, polymeric micelles exhibit one distinct advantage over liposomes: their much smaller size. Typically less than 50 nm in diameter, polymeric micelles can both evade RES uptake and escape renal filtration while concurrently maintaining enhanced vascular permeability and improved tumor penetration. Not surprisingly, nontargeted micelles have been utilized extensively to deliver doxorubicin and paclitaxel to solid tumors through passive targeting,[125−127] but only recently have researchers explored the use of folate to improve the specificities of these formulations for tumor cells.

Lee et al.[128] prepared a novel pH-sensitive polymeric mixed micelle (PHSM) consisting of PEG_{2000}-poly-L-histidine (MW = 5K) and PEG_{2000}-poly-L-lactic acid (PLLA,

MW = 3K) block copolymers with or without folate conjugation. When loaded with doxorubicin, the PHSM−folate micelles showed >90% cytotoxicity to doxorubicin-resistant MCF-7 cells at 10 μg/mL doxorubicin.[129] In mice bearing subcutaneous doxorubicin-resistant MCF-7/doxorubicin xenografts, the accumulated doxorubicin level of PHSM−folate in the solid tumors was 20 times higher than that of free doxorubicin and 3 times higher than nontargeted PHSM. Accordingly, the tumor volumes of mice treated with targeted PHSM−folate were found to be significantly smaller than the volumes of either control group. On the basis of other observations, it was also suggested that FR-mediated internalization of the formulation by the tumor cell followed by polyhistidine-induced micelle destabilization and drug release endowed the drug carrier with the ability to bypass multidrug efflux pumps. Although biodistribution studies indicated that some PHSM−folate also accumulated in the liver and spleen, the results nevertheless suggests that the PHSM−folate formulation warrants further exploration as a possible means for treating drug-resistant FR^+ tumors.[129]

While the above studies augur well for the future of folate-targeted micelles, a number of studies suggest that in vivo stability problems must be addressed if the formulations are to eventually reach the clinic. FRET imaging studies of dual-labeled micelles comprising a block copolymer derivatized with a fluorescence donor and an entrapped fluorescent drug mimetic revealed a 50% decrease in FRET efficiency within 15 min of intravenous injection, implying that the micelle's cargo is released quickly into the blood.[130] A similar FRET study has similarly shown that another diblock copolymer micelle releases its hydrophobic cargo rapidly upon contact with the plasma membrane of KB cells, significantly reducing the amount of cargo that is eventually delivered inside the tumor cell.[131] These studies raise the question of whether current micelle formulations can retain their cargos long enough for in vivo tumor delivery, and they suggest that future improvements to in vivo stability may be required to render such micellar drug carriers promising agents for targeted cancer therapies.

2.6.3. Folate-Targeted Dendrimers. Dendrimers are synthetic, often biocompatible, nonimmunogenic, nanoscaled polymers that can be manufactured to specific sizes and reproducible surface characteristics. Because their surfaces can be densely functionalized, large amounts of diagnostic and/or therapeutic agents can be attached to these dendrimers, rendering them highly compact drug delivery vehicles.

The first folate-targeted dendrimers described in the literature consisted of an ammonium-core polyamidoamine-core modified with folate as a targeting molecule and FITC as a fluorescent reporter. This targeted dendrimeric construct was then capped with succinic anhydride to render the remaining surface groups negatively charged to avoid nonspecific cell adsorption.[132] When introduced to FR^+ erythroleukemia cells, the folate−dendrimer particle demonstrated biphasic uptake: rapid uptake within the first minutes (attributed to the dendrimer's initial binding to empty cell surface FR), followed by slower internalization attributed to endocytosis and recycling of FR back to the cell surface. Redesign of this folate−dendrimer construct to deliver an MRI contrast agent (gadolinium) provided good MRI contrast both in vitro and in vivo in FR^+ tumor xenografts.[132] Quintana et al.[133] then improved the folate−dendrimer design by capping the dendrimer surface amines with hydroxyl and acetamide groups, which enhanced projection of the targeting ligand away from the nanoparticle surface, permitting greater access to cell surface FR. These dendrimers demonstrated increased capture by KB cells and better drug delivery into the same cell type.[133] Importantly, neutralization of the dendrimer's amine surface charge was found to be essential to prevent toxicity and nonspecific uptake of the drug conjugate.

Building on the above improvements, Kukowska-Latallo and co-workers[134] next evaluated the ability of folate-targeted, acetylated dendrimers to deliver anticancer therapeutics and imaging agents to human KB tumor xenografts in immune compromised mice.[134] In contrast to the corresponding nontargeted polymer, folate-conjugated nanoparticles accumulated in the tumor and liver tissue over 4 days following intravenous administration. Additionally, folate-targeting was found to both increase methotrexate's antitumor activity and reduce its off-site toxicity. As proof of tumor targeting, tumor uptake of the dendrimer was shown to be attenuated by prior intravenous injection of free folic acid.[134] A later comparison of the release kinetics of a covalently conjugated methotrexate and a noncovalently complexed form of the same drug suggested that the former might be better suited for targeted drug delivery.[135] Despite their many positive properties (including improved in vivo stability over most polymeric micelles), these particles suffered the same shortcoming as previous nontargeted dendrimers, showing significant accumulation in the liver, spleen, and heart 4−7 days postinjection.[134] Perhaps some type of stealth coating can eventually be designed to reduce off-target uptake while retaining the desirable size and stability characteristics of these important nanomedicines.

2.6.4. Other Folate−Nanoparticle Drug Delivery Systems. Folate has also been conjugated to a host of other nanoparticle platforms, including superparamagnetic nanoparticles,[136,137] gold nanoparticles,[138,139] magnetite (Fe_3O_4) nanoparticles,[140] carbon nanotubes,[141,142] lipoprotein-based nanoplatforms,[143,144] thermoresponsive microgels,[145] bovine serum albumin nanoparticles,[146] and virus capsid proteins.[147,148] Because little data are available on the in vivo properties of these nanomedicines,[142,144,149] their potentials as novel drug carriers cannot be assessed at this juncture. However, in general, the design and application of such folate-targeted nanoparticles are similar to the folate-targeted nanomedicines described above in that the primary objective focuses on improved delivery of larger payloads and more hydrophobic drugs. Unfortunately, much like their better characterized counterparts, these particles may similarly suffer from compromised penetration into solid tumors and nonspecific uptake by macrophages in the liver, spleen, and other organs of the reticuloendothelial system. Use of PEGylation (or a related coating) to suppress macrophage recognition could conceivably reduce nonspecific uptake, but improvement of penetration into solid tumors will require creative strategies to digest or weaken the dense extracellular matrix that cements solid tumors together.[150] Further, because many nanoparticles carry their active components on their surfaces, an optimal ratio of PEG/PEG−folate/drug must still be determined to ensure adequate water solubility, good folate presentation, and maximal RES evasion. And in cases where the drug molecules are enclosed within the nanoparticle, they must still be released following target cell entry but not during transit to the tumor mass. Clearly, while tumor targeted nanoparticles offer attractive solutions to many problems plaguing small molecule delivery, they simultaneously introduce new obstacles

of their own that will require solutions before their full potentials can be realized.

3. Conclusion and Future Directions

The use of folate-conjugated drugs and drug-laden nanoformulations to treat solid tumors is poised to affect the management of many malignant diseases. While monovalent folate−drug conjugates will likely see clinical application in the very near future, similar targeting strategies may not prove successful for delivery of many biologic therapies, including peptides, siRNA, oligodeoxyribonucleotides, gene therapy vectors, and proteins. For efficient tumor targeting of these macromolecular medicines, nanoparticulate formulations may be required. However, for nanoformulations to reach their full potential, they may have to overcome problems associated with (1) poor tumor penetration, (2) unwanted accumulation in nontargeted tissues, and (3) inefficient release from endosomes. With improvements in these latter areas, use of folate to increase the selectivity and potency of nanoparticulate therapeutics can also rapidly become a reality.

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Biographies

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Philip S. Low is the Ralph C. Corley Distinguished Professor of Chemistry at Purdue University, IN, and Founder and Chief Science Officer of Endocyte Inc. Dr. Low has published over 300 refereed articles and has received both of Purdue University's awards for outstanding research, an NIH Merit Award, and several national and international research awards. He currently serves on six editorial boards and several external advisory boards for major institutions. His research into receptor-targeted therapeutic and imaging agents has led to >35 U.S. patents/patents pending, and development of these patents has yielded six targeted drugs that are currently undergoing human clinical trials for various cancers. One additional targeted drug from his research is in late preclinical development for treatment of inflammatory and autoimmune diseases.

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