REVIEW

Antibody–Enzyme Conjugates for Cancer Therapy

*Roger G. Melton, Roger F. Sherwood**

The use of antibody–enzyme conjugates directed at tumor-associated antigens to achieve site-specific activation of prodrugs to potent cytotoxic species, termed "antibody-directed enzyme prodrug therapy" (ADEPT), has attracted considerable interest since the concept was first described in 1987. Prodrug forms of both clinically used anticancer agents and novel cytotoxic compounds have been developed to take advantage of potential prodrug-generating technology employing a variety of enzymes with widely differing substrate specificities. A particular advantage of the ADEPT approach is that it may allow the use of extremely potent agents such as nitrogen mustards and palytoxin, which are too toxic to be readily used in conventional chemotherapy. Preliminary studies using an antibody–enzyme conjugate constructed with a bacterial enzyme and a murine monoclonal antibody not only have established the value of the ADEPT technique, but also have highlighted the potential problem of immunogenicity of proteins of nonhuman origin. This problem has been tackled in the first instance by the use of immunosuppressive agents, but long-term solutions are being investigated in the development of second-generation ADEPT systems, including the development of human antibody–human enzyme fusion proteins and catalytic antibodies. Such improvements, coupled with further refinement of the prodrug–drug element of the system and the wide variety of antibody–enzyme–drug combinations available, should mean that ADEPT-based approaches will form an important element of the search for the anticancer drugs of the future. [J Natl Cancer Inst 1996;88:153-65]

The advent of monoclonal antibody technology in the 1970s was thought at the time to herald the fruition of the search for the "magic bullet" first proposed by Ehrlich in the early years of this century (*1*). In practice, however, this goal has remained elusive, inasmuch as many obstacles have presented themselves and have proved to be very difficult to overcome. To date, there have been numerous attempts to improve the cytotoxicity of the antibody "missiles" by attaching a variety of "warheads" to them—e.g., cytotoxic drugs such as doxorubicin or methotrexate, toxins such as ricin A-chain or *Pseudomonas* exotoxin, and radioisotopes (*2*). Recently, enzymes capable of activating prodrugs to active drugs have been the focus of considerable interest and form the subject of this review.

For any form of antibody-mediated targeting to be successful, it is axiomatic that there must be selective expression of the target antigen by the tumor cells. This provides the first barrier to overcome, for the only well-characterized tumor-specific antigens described to date are the idiotypic determinants on the surface immunoglobulins of B-cell lymphomas (*3*). Tissue-specific antigens are also known, e.g., prostate-specific antigen (PSA), but have yet to be exploited to any great extent in targeted immunotherapy regimens. Many antigens, e.g., oncofetal antigens such as carcinoembryonic antigen (CEA) or placental alkaline phosphatase, and other potential targets such as growth factor receptors are present in elevated levels in tumor tissue, but most, if not all, appear to be found to a greater or lesser extent in other tissues. These limitations have not prevented exploitation of the better characterized tumor-associated antigens (e.g., CEA) as targets for therapy, even though it is also well documented that tumor cells exhibit considerable antigenic heterogeneity (*4-6*). Tumor cells may express varying levels of antigen, and some cells may not express the target antigen at all, such that a single monoclonal antibody conjugate targeted at a tumor mass will not bind to all the cells present, although other antibodies may be used to target alternative antigens on such cells. This is a disadvantage for immunoconjugates constructed using cytotoxic moieties that need to bind to the target cells and be internalized to exert their effect; however, the problem may be overcome to some extent by the use of antibody "cocktails" in which a mixture of antibodies aimed at two or more discrete antigens is used in noncompetitive combination (*7*).

The ability of conjugates to gain access to the target cells is also essential, yet there is ample evidence that antibodies penetrate tumors poorly (*8,9*). Thus, the best access is at the tumor periphery, and cells located near the center of the tumor mass may be inaccessible to immunoconjugates, even though

**Affiliations of authors:* R. G. Melton, Centre for Applied Microbiology and Research, Porton Down, Salisbury, U.K.; R. F. Sherwood, Duramed Europe Ltd., Magdalen Centre, Oxford Science Park, Oxford, U.K.

Correspondence to: Roger G. Melton, Ph.D., Centre for Applied Microbiology and Research, Porton Down, Salisbury SP4 OJG, U.K.

See "Notes" section following "References."

they may express antigen. Cells in such inaccessible anoxic zones may be quiescent but possess the ability to regrow if exposed to a supply of oxygen and nutrients as a result of more vulnerable cells in oxic zones being killed. For this reason, radioimmunoconjugates have been extensively studied, because the emissions from the isotopes used clinically are capable of penetrating several cell layers, killing cells distal from where the antibody is localized. Although this approach is attractive, in practice the relatively low tumor uptake of the radioimmunoconjugate may require large patient exposures, giving rise to dose-limiting myelosuppression and necessitating the use of autologous bone marrow grafts (*10*).

Antibody-Directed Enzyme Prodrug Therapy as an Alternative Strategy

An alternative strategy that has recently been the subject of considerable interest is the use of antibodies as vectors for enzymes capable of activating a nontoxic drug precursor, termed a "prodrug," to a potently cytotoxic moiety. This approach, which has been termed "antibody-directed enzyme prodrug therapy" (ADEPT) (*11*), or "antibody-directed catalysis" (*12*), offers the potential to overcome most of the problems associated with the use of drug or toxin immunoconjugates described above. The system is shown by the diagram in Fig. 1. An antibody–enzyme conjugate is injected and allowed to localize at the tumor while clearing from the rest of the tissues. After a suitable interval, the prodrug is administered; while remaining innocuous to the normal tissues, it is converted to a cytotoxic form by enzyme localized within the tumor tissue. Being of low molecular weight, the active drug can diffuse to adjacent tissues, providing a bystander effect, although this may be of questionable benefit if the released drug is relatively stable and can diffuse back into the circulation. It is implicit in the ADEPT approach that the antigen to which the antibody–enzyme conjugate is targeted must remain extracellular; thus, secreted antigen that accumulates in the tumor interstitial spaces may assist in achieving high levels of conjugate at the tumor site. Although shed antigen present in the circulation will act as a competitor with the tumor site for binding of target antigen, there is evidence that tumor localization of conjugate can occur in the face of circulating antigen unless levels of the latter are particularly high (*11*). Another source of antigen may arise from the lysis of tumor cells following the active drug release from the prodrug by the action of the enzyme in the antibody–enzyme conjugate bound to cell surfaces. However, it is unlikely that there is any antibody–enzyme conjugate in the circulation at that time; thus, such antigen release may not be problematic except in situations where repeat cycle(s) of therapy are administered before this antigen is cleared from the body.

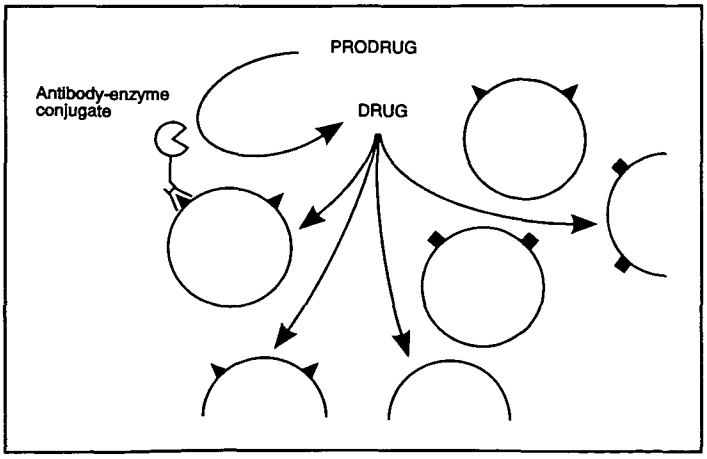

Fig. 1. Schematic representation of antibody-directed enzyme prodrug therapy.

A further advantage of ADEPT is that a single enzyme molecule has the potential to cleave many prodrug molecules—up to 800 mol/mol of enzyme per second in the case of the benzoic acid mustard substrates of carboxypeptidase G_2 (*13*)—providing an amplification effect giving high levels of drug localized at the tumor; this may be an important advantage in the clinic, in view of the typically low localization of immunoconjugates in humans (*14*). There is also some evidence that high levels of drug generated at the surface of tumor cells are more effective than equivalent concentrations of free drug (*15*). The prodrug is an integral component of ADEPT systems and requires careful design in its own right. An ideal prodrug would be one with a large differential in cytotoxicity between drug and prodrug, which is a good substrate for the enzyme under physiologic conditions and for which there is no mammalian homologue capable of performing the same reaction. The use of human enzymes requires qualification of the latter statement, as discussed below. Equal cytotoxicity of the released active drug toward proliferating and quiescent cells is also desirable if residual deposits of viable but nonproliferating cells with the potential for outgrowth are to be eradicated. Once formed, it would be desirable for the drug to have a very short half-life, limiting the possibility of escape of active drug back into the circulation and access to healthy tissue. Development of drug resistance may limit the effects of conventional active drugs produced from prodrugs; thus, the nitrogen mustard group of compounds has tended to be the most commonly used group of active drugs for ADEPT because these compounds are not cell cycle specific, can be hydrolyzed to nontoxic forms, can kill both well-oxygenated and hypoxic cells, and cells develop only low levels of resistance to them (*16*). The potential release of the active drug into the circulation is conceivable after tumor cell lysis, but most drugs proposed for ADEPT systems are potent alkylating or intercalating agents and are likely to remain bound irreversibly to intracellular targets.

In selecting an enzyme, one would look for activity under physiologic conditions, low immunogenicity, and little or no equivalent endogenous enzyme in humans. The enzyme needs to have a high specific activity, because the amounts that can be delivered by antibody vectors are limited; however, the definition of optimal enzyme kinetics is clear cut and, as we shall see, under certain circumstances, a low kcat (i.e., the turnover rate of enzyme, which is expressed as number of moles of substrate utilized per mole of enzyme per second) may be preferable to a higher kcat (*17*). If a human enzyme is selected in an attempt to develop a nonimmunogenic conjugate, it will probably need to be an intracellular enzyme so that endogenous enzyme does not give rise to activation of the prodrug in the blood. When coupled to an antibody, the enzyme must localize efficiently at the tumor, and the development of immune responses to non-

human enzymes is undesirable, since localization of the conjugate is likely to be drastically reduced upon readministration after the onset of an immune response (*18*) and there will be a risk of hypersensitive reactions. However, immunosuppressive drug regimens designed to extend the time window before the onset of the human immune response observed in patients (*19*) have been developed to counteract this problem (*20*). The gains obtained using such approaches in animal models have been modest, but they may be sufficient to allow two or three repeat courses of an ADEPT cycle.

An important factor in early stages of the development of an ADEPT system is the production of an antibody–enzyme conjugate without loss of antigen-binding and enzymatic activities. The techniques and heterobifunctional agents typically used are analogous to those developed for use in the construction of immunotoxins. An important requirement for conjugates prepared for use in ADEPT is that the linkage should be stable, since a period of up to or greater than 72 hours may elapse between the administration of conjugate and the administration of prodrug, in order to allow unbound conjugate to clear from the circulation. For this reason, conjugation chemistry based on thioether linkages has been virtually universally adopted.

A number of experimental ADEPT systems have been developed, with a range of different enzymes, and it is thus convenient to categorize them by class of enzyme (Table 1). Enzymes used for ADEPT systems to date include carboxypeptidases G_2 and A, alkaline phosphatase, glycosidases, penicillin amidase, β-lactamase, and cytosine deaminase. All are at a very early stage of development, with only the carboxypeptidase G_2 system having been tested clinically to date. Typically, these systems have been tested in nude mouse models with human xenograft targets. These represent probably the best models currently available, but they have clear limitations. For example, the subcutaneous tumor implant is nonmetastatic and, therefore, does not represent the typical clinical situation, where the treatment of advanced metastatic disease is likely to be the early target, with the eventual prospect of moving to minimal residual disease following surgery to debulk the tumor. Moreover, the levels of tumor localization achievable in mice far exceed those that have been found clinically with radiolabeled antibodies. The fact that nude mice are immunodeficient also prevents consideration of the problem of immunogenicity, an issue of considerable importance, as has been revealed by early clinical studies (*21,22*). Once a workable system has been established, there is likely to be a lengthy development stage while all aspects of the system are reevaluated and optimized. Typically, the necessary development work will include the synthesis of improved prodrugs, substitution of human enzymes for bacterial enzymes where possible, the adoption of chimeric or human antibodies and fragments thereof, the generation of nonimmunogenic clearing systems, and the develop-

Table 1. Some potential antibody-directed enzyme prodrug therapy (ADEPT) systems described in the literature

Enzyme	Antibody	Antigen	Prodrug	Active drug	In vitro cell line (in vivo tumor model)*	Reference No.
Carboxypeptidase G_2	W14	Human chorionic gonadotropin	*p-N*-bis (2-Chloroethyl)benzoyl glutamic acid	*p-N*-bis (2-Chloroethyl) amino baaenzoic acid	CC3 choriocarcinoma (CC3)	(*13,27*)
			4-[(2-Mesyloxyethyl)(2-chloroethyl) amino] benzoyl glutamic acid	4-[(2-Mesyloxyethyl)-(2-chloroethyl) amino] benzoic acid		(*13,19,26,27*)
Carboxypeptidase G_2	A5B7	Carcinoembryonic antigen	4-[(2-Mesyloxyethyl)(2-chloroethyl) amino] benzoyl glutamic acid	4-[(2-Mesyloxyethyl)-(2-chloroethyl) amino benzoic acid	LS174T colon carcinoma (LS174T)	(*19,21,28,30*)
Carboxypeptidase A	KS1/4	Lung adenocarcinoma-associated antigen	Methotrexate–α-alanine	Methotrexate	UCLA-P3 lung adenocarcinoma	(*15,34,37*)
Alkaline phosphatase	L6	Tumor-associated carbohydrate	Etoposide phosphate	Etoposide	H3347 colon carcinoma (H3347)	(*42,44*)
	L6		Mitomycin C phosphate	Mitomycin C	H2981 lung adenocarcinoma (H2981)	(*42*)
	L6		Doxorubicin phosphate	Doxorubicin	H2981 lung adenocarcinoma	(*45*)
	HRS-3/AP1	CD30 Hodgkin's lymphoma antigen	Mitomycin C phosphate	Mitomycin C	L540 Hodgkin's lymphoma	(*48*)
	L6		*N,N*-bis(2-Chloroethyl)aminophenyl phosphate	*N,N*-bis-(2-Chloroethyl)-4-hydroxyaniline	H2981 lung adenocarcinoma (H2981)	(*46*)
	BW431	Carcinoembryonic antigen	Etoposide phosphate	Etoposide	SW1398 colon carcinoma	(*47*)
Penicillin amidase	L6	Lung adenocarcinoma-associated antigen	*N*-Phenylacetamido doxorubicin	Doxorubicin	H2981 lung adenocarcinoma	(*58,59*)
			N-Phenylacetamido melphalan	Melphalan		
			N-(4′-Hydroxyphenylacetyl) palytoxin	Palytoxin	H2981 lung adenocarcinoma	(*61*)
β-Glucuronidase	RH1	Uncharacterized hepatocarcinoma antigen	*p*-bis-2-Chloroethylaminophenyl β-D-Glucopyranoside uronic acid (*tert*-butyl salt)	*N,N*-bis-(2-Chloroethyl)-4-hydroxyaniline	HepG2 hepatoma	(*49,50*)
	323/A3	Tumor-associated glycoprotein	Epirubicin–glucuronide	Epirubicin	MCF-7 breast cancer	(*54*)
	BW431	Carcinoembryonic antigen	Doxorubicin–glucuronide	Doxorubicin	OVCAR-3 ovarian cancer (LoVo, Mz-Sto-1) colon carcinoma	(*79*)
β-Lactamase	CEM2314	Carcinoembryonic antigen	Cephalosporin–vinca alkaloid	Desacetylvinblastine hydrazide	LS174 colon carcinoma (LS174T)	(*12,67,68*)
	L6		Cephalosporin mustard	Phenylene diamine mustard	H2981 lung adenocarcinoma	(*65,66*)
Cytosine deaminase	L6		5-Fluorocytosine	Fluorouracil	H2981 lung adenocarcinoma (H2981, HT29 colon carcinoma)	(*69-72*)

*All cell lines tested are of human origin.

ment of fusion proteins or bifunctional catalytic antibodies with suitable binding specificity for tumor-associated antigens. With such a range of parameters to be optimized, the development of ADEPT systems is clearly complex and likely to be time-consuming and expensive.

Enzymes and Prodrugs Used in Currently Available ADEPT Systems

Carboxypeptidase G_2

The first ADEPT system was proposed by Bagshawe (*23*) in 1987; it used carboxypeptidase G_2 (CPG_2), which was originally isolated as a methotrexate-degrading enzyme from *Pseudomonas* species and subsequently cloned in *Escherichia coli* (*24*), to cleave a deactivating glutamate moiety from a benzoic acid mustard (*25*). This remains probably the best characterized system and is the only system to date for which pilot clinical studies have commenced (*19,21,22*). The initial prodrug to be synthesized was 4-[*N*,*N*-bis(2-chloroethyl)amino]benzoyl-L-glutamic acid from which CPG_2 cleaves the glutamic acid to yield 4-[*N*,*N*-bis(2-chloroethyl)amino]benzoic acid (Fig. 2, A). Subsequently, modified versions were developed with one or both of the 2-chloroethyl arms replaced by more reactive 2-mesyloxyethyl moieties (Fig. 2, B) (*25*). The mono-(2-mesyloxyethyl) (MMCl) compound was more cytotoxic and a slightly better substrate for the enzyme than the bis(2-chloroethyl) compound, but the bis(2-mesyloxyethyl) form was too reactive and rapidly hydrolyzed in the absence of enzyme (*25,26*). The enzyme conjugate–prodrug combination was initially tested in vitro using the human chorionic gonadotropin (hCG)-expressing JAR choriocarcinoma cell line; it was found to give greater than 100-fold differential toxicity between drug or prodrug plus enzyme (IC_{50} [i.e., the concentration required to kill 50% of treated cells compared with untreated controls] = 20 μ*M*) and prodrug (IC_{50} = >800 μ*M*) (*27*). In antitumor studies using the hCG-expressing human CC3 choriocarcinoma xenograft model in nude mice, the combination of W14 (anti-hCG) $F(ab')_2$–CPG_2 conjugate followed 72 hours later by the MMCl prodrug resulted in complete eradication of established CC3 xenografts when prodrug was administered as a three-times-divided dose of 10 mg/kg (total dose, 30 mg/kg) at 16-hour intervals from 72 hours (*13,27*). In contrast, weekly intravenous injections of daily divided doses of methotrexate (5 mg/kg), hydroxyurea (50 mg/kg), dactinomycin (7.5 μg/kg), or cytarabine (20 mg/kg) failed to retard tumor growth, whereas the MMCl prodrug and the W14 $F(ab')_2$–CPG_2 conjugate produced a pronounced growth delay of more than 50 days (*27*).

When the same two-phase system was applied to the CEA-expressing human LS174T colon carcinoma model, using a conjugate constructed with the $F(ab')_2$ fragment of A5B7 anti-CEA antibody, it proved to be less effective in that minimal growth delays were seen (*28*). This was probably because of the much slower clearance of conjugate from the blood in this model compared with that in the choriocarcinoma. In the latter case, high levels of circulating antigen probably form immune complexes, leading to accelerated clearance; however, much lower levels of antigen are present in the circulation of CEA-expressing tumors (*29*), and the clearing effect of free antigen is thus not apparent. As a result, a second galactosylated clearing antibody, SB43, which enzymatically inactivated CPG_2, was used to remove unbound conjugate from the circulation, making the system a three-phase approach (*30*). The galactosylated antibody has a relatively short half-life in the circulation because it is rapidly cleared by hepatic galactose receptors; consequently, it can react with the CPG_2 conjugate in the circulation but is not extravasated sufficiently to reach the conjugate in the extravascular compartment. This situation should avoid the problem of

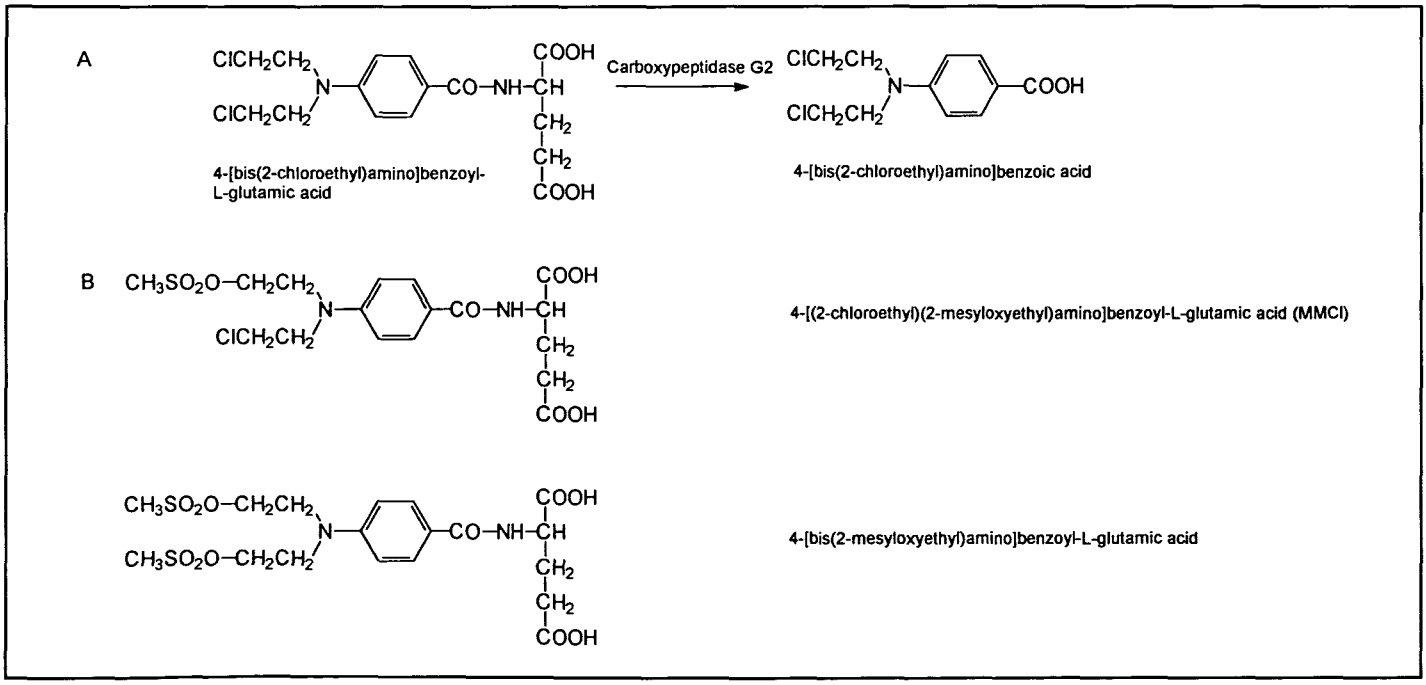

Fig. 2. Benzoic acid mustard prodrugs that are substrates for carboxypeptidase G_2 (CPG_2). A) Mechanism of cleavage of benzoic acid mustards by CPG_2. B) Alternative mustard functional groups.

clearing antibody reaching and inactivating the tumor-bound antibody–enzyme conjugate (*30*). When the three-phase system was tested against the same model, prolonged growth delays of up to 10-15 days, compared with untreated controls, were seen with the MMCl compound and, to a lesser extent, with the bischloro compound (*28*). Using a superficially identical model, Blakey et al. (*31*) were subsequently able to achieve growth delay of 10-15 days without recourse to clearing antibody. The principal difference between the experiments lay in the method of tumor implantation: Solid tumor fragments were used in the earlier work, whereas injection of a cell suspension was done in the later experiments.

In an alternative approach to the use of clearing antibody, Melton et al. (*32*) demonstrated enhanced tumor localization of antibody–enzyme conjugate when administered with tumor necrosis factor-α (TNF-α) in the murine E3 thymoma model. Coadministration of 1.5 μg TNF-α with conjugate led to a twofold increase in tumor localization and only transient increases in normal tissue localization. It is possible that this system could be used in combination with the galactosylated clearing antibody because the two approaches are complementary: TNF-α increases tumor localization of conjugate without significantly affecting blood levels, and galactosylated antibody markedly decreases blood levels without affecting tumor localization. Galactosylated antibody increases the ratios of tumor to normal tissue without affecting overall tumor uptake, whereas TNF-α provides a mechanism for achieving higher initial tumor uptake. Similar effects can be induced by the administration of drugs that modify blood flow, which essentially appear to act by inducing localized production of TNF-α (*33*).

The preclinical studies using the colon carcinoma xenograft model and the MMCl compound were deemed sufficiently encouraging to justify a limited clinical trial in patients with advanced metastatic, poorly differentiated colon or rectal cancer (*19,21,22*). Initial dose escalation studies using the monomesyl benzoic acid mustard prodrug showed that doses from 200 up to 2500 mg/m^2, given as six or 12 divided doses over a 3-day period, were well tolerated. A total of four male patients aged 55-70 years, all of whom had undergone extensive conventional chemotherapy (principally with fluorouracil), subsequently went on to receive 20 000 U/m^2 A5B7 (anti-CEA) F(ab′)$_2$–CPG$_2$ conjugate, a small proportion of which was ^{125}I labeled to facilitate confirmation of tumor localization by gamma camera imaging. SB43 clearing antibody was administered 36-48 hours later as a 3- to 5-hour infusion, typically followed by MMCl (1200-3000 mg/m^2) once measured serum enzyme levels were less than 0.02 U/mL. The serum levels of CEA and a second tumor antigen, 19-9, fell in all patients by 10-15 days after therapy; in two cases, there were objective responses in the reduction in size of liver metastases. A patient who had gross hepatomegaly and ascites became free of jaundice and ascites, although liver size was not reduced. Other subjective responses included relief of pain, and three patients gained weight and reported improved health. Adverse effects included the development of antibodies to both components of the conjugate, and the two patients receiving the highest doses of prodrug developed severe myelosuppression, although this was reversible once prodrug administration was ceased. The antibody responses developed within 10-12 days, limiting the treatment effectively to a single cycle; however, this time window may be extended by the use of the immunosuppressive drug cyclosporine (*20*). The development of myelosuppression appears to be due to the relatively long half-life of the active drug, which can diffuse out of the tumor and back into the circulation, indicating that more reactive drugs with shorter physiologic half-lives are desirable. The results of the pilot clinical study are encouraging, if one bears in mind the advanced state of the disease of the patients in the study, and provide ample justification for further development of the system.

Carboxypeptidase A

Huennekens and co-workers (*15,34-37*) described a system in which carboxypeptidase A (CPA) is used to cleave the alpha-carboxyl alanine residue from methotrexate–α-alanine. When human lung adenocarcinoma cells were exposed to conjugate consisting of CPA coupled to KS1/4 antibody, followed by washing and addition of prodrug, the IC_{50} of methotrexate–α-alanine decreased approximately sixfold from 8.9×10^{-6} *M* to 1.5×10^{-6} *M* after 48 hours of incubation. With prolonged incubation, the toxicity of methotrexate–α-alanine approached that of methotrexate (i.e., about 2×10^{-9} *M*) (*15*). It is thus clear that the enzyme turns over the substrate rather slowly, although the authors suggested that methotrexate generated in intimate contact with the cells in this way seems to be more cytotoxic than the equivalent concentration of free methotrexate. A study of the theoretical aspects of ADEPT by Yuan et al. (*17*) suggested that a low turnover rate may not be disadvantageous because the rate-limiting step is diffusion of drug from the capillaries to the extravascular tumor cells; however, this observation must be set against the need to generate sufficient drug to exert the desired cytotoxic effect. The most important factor in attaining tumor-specific activation may be the achievement of high tumor-to-normal tissue localization ratios and rapid reaction of the active drug to prevent its loss back into the circulation. If these issues can be resolved, then the use of an enzyme with a high turnover and affinity for substrate would seem to be ideal because the level of prodrug reaching the tumor is unlikely to match that present in blood. It seems likely, however, that the relatively poor kinetics of this enzyme with the first-generation prodrug are unlikely to lead to a workable system in vivo, and a number of groups (*38,39*) recently described the synthesis of potentially better substrates based on methotrexate. No animal studies have been reported for the CPA/methotrexate–α-alanine system, and the likelihood of activation of prodrug by endogenous CPA remains as a possible disadvantage of this approach. In view of the development of resistance to methotrexate and the high levels of drug needed to achieve maximal effect (*40*), it is questionable whether this is a suitable drug for ADEPT applications, particularly when the kinetics of the enzyme are relatively poor.

Aminopeptidase

Smal et al. (*41*) recently described the synthesis of a series of 2-aminoacyl methotrexate prodrugs designed to be substrates for pyroglutamate aminopeptidase or D-aminopeptidase. The cytotoxicity of the prodrug plus enzyme in vitro did not approach that of methotrexate alone and required high levels of enzyme to achieve any selective cell kill, suggesting that this is not a prac-

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.