Drug–Ligand Conjugates

Improved Chemical Strategies for the Targeted Therapy of Cancer**

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The selective targeting of a compound to tumors, or for that matter to any structure in the body, requires high stability of the compound in blood, biodistribution to the target site, adequate contact time with the target, adequate retention by the target, maintenance of drug potency, and adequate clearance of nontargeted compound. For over two decades, antibodies have been the scaffold of choice for delivering contrast agents and chemotherapeutics to tumor cells since they are stable in blood, typically have nanomolar affinities for their target, and, because binding and nonbinding domains are separated physically, tolerate substitution with contrast agents and chemotherapeutics.[1]

Although monoclonal antibodies (mAbs) are typically tolerant to the conjugation of chemotherapeutics, the agents themselves often lose potency in the conjugated form. Therefore, novel chemical strategies are required for releasing the cytotoxic agent, either after binding to the cancer cell surface or after endocytosis into the cell.[2] A compelling example of this comes from the antitumor antibiotic calicheamicin. When conjugated to a tumor-targeting mAb through an amide linkage, the conjugate is accumulated by the tumor, but has no appreciable cytotoxicity.[3] In contrast, when conjugated by using a pH-sensitive bifunctional linker that permits release of calicheamicin intracellularly[4] (Figure 1), the conjugate shows potent antitumor activity. Indeed, gemtuzumab ozogamicin ("gem-ozo", Mylotarg), a conjugate of a CD33-specific mAb and calicheamicin, which utilizes this bifunctional linker, is already approved for the treatment of certain acute myeloid leukemias.[4]

The taxanes are a class of potent chemotherapeutics that possess a complex chemical structure.[5] Recently, two different strategies have been employed to conjugate taxanes to tumor-targeting monoclonal antibodies, while retaining chemotherapeutic potency. In conjugate mAb-**1** (Scheme 1), an enzymatically cleavable glutarate ester bond[6] is placed between the amide linkage to

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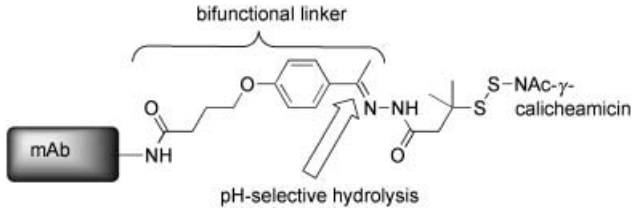
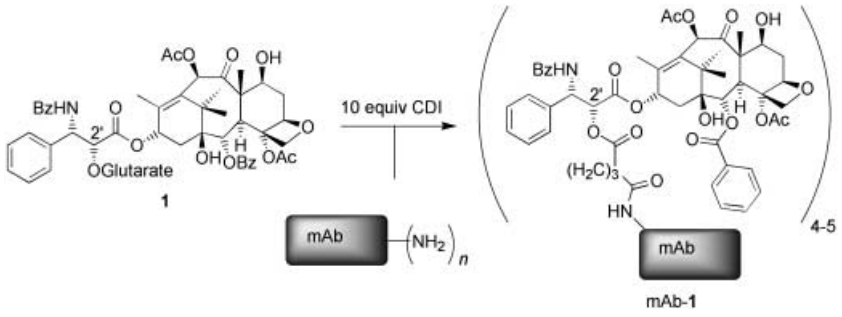


Figure 1. Cleavable bifunctional linker for the conjugation of calicheamicin to monoclonal antibodies.



Scheme 1. Conjugation of paclitaxel derivative **1** to monoclonal antibodies. CDI: *N*,*N*-carbonyldiimidazole.

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the mAb, and the 2′ position of a first-generation taxane such as paclitaxel (Taxol).[7] After endocytosis of the paclitaxel/mAb conjugate, nonspecific cellular esterases release free paclitaxel, which can diffuse out of endosomes, and into the cytosol, where it exerts its biological effect.[7]

As shown in Scheme 2, the second-generation taxane **2** (SB-T-110131, IDN5109; BAY59-8862) was conjugated at position 10, under mild conditions, by using a methyldisulfanyl alkanoyl (MDS) linker,[8] to mAbs specific for the epidermal growth factor (EGFR), which is known to be overexpressed in several human squamous cell cancers. The anti-EGFR mAbs were modified with *N*-succinimidyl-4-(2-pyridyldithio)-pentanoate to attach 4-pyridyldithio pentanoyl groups and subsequently coupled to taxoid **2** through a disulfide linkage. After endocytosis of the conjugate, the reducing environment of the cell would be expected to release the taxane from the antibody. Antitumor activities of two anti-EGFR mAb-**2** conjugates (KS61-**2** and KS77-**2**) were evaluated against human tumor xenografts in severe combined immune deficiency mice.[8] Both conjugates showed remarkable antitumor activity and it was demonstrated that the dose of mAb–taxoid conjugate used were nontoxic to the mice.

A very interesting and more general linker system based on enzymatic activation was recently reported by Shabat and co-workers.[9] The central core of this "chemical adapter" is a 4-hydroxymandelic acid unit with three functional groups that are suitable for conjugation to a targeting molecule, a cytotoxic moiety, and an enzyme substrate (Figure 2). The underlying concept is that the targeting molecule will guide the conjugate to the tumor site, where an enzymatic reaction with the enzyme substrate would trigger a spontaneous reaction that releases the cytotoxic molecule. The release mechanism proceeds by enzymatic cleavage of the enzyme substrate, subsequent elimination of a cyclic dimethylurea derivative, and formation of an intermediate quinone methide, which releases the cytotoxic molecule as a carbamic acid that decarboxylates spontaneously to yield the active drug (Figure 2). The concept has been proven for a conjugate consisting of a *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer[10] as a targeting molecule, etoposide as a cytotoxic molecule, and a substrate for a retro-aldol retro-Michael sequence that is catalyzed by the antibody 38C2 as the cleaving enzyme. However, it has not yet been tested in vivo.

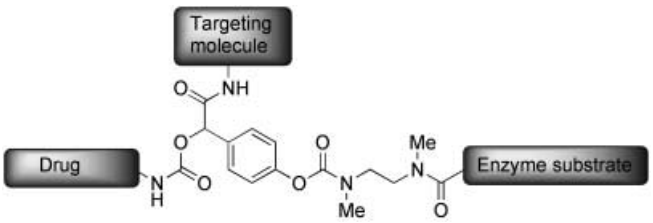
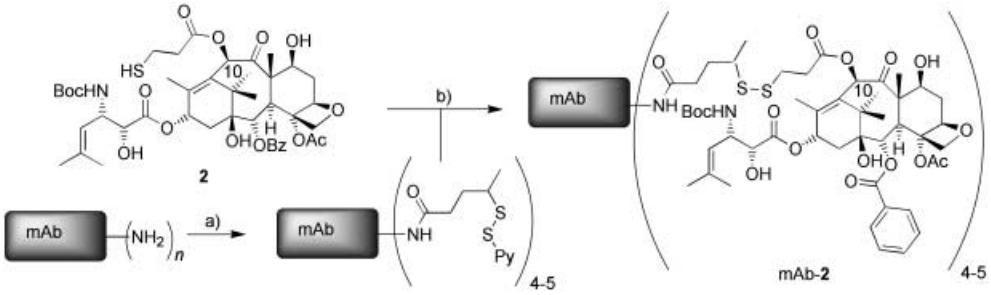


Figure 2. Chemical adapter system based on 4-hydroxymandelic acid.

Although the chemical strategies described above have been typically applied to mAbs, the advantages of mAbs come with a high cost in terms of biodistribution and pharmacokinetics. Immunoglobulin G (IgG) has a molecular weight (M_W) of about 150000 Da, corresponding to a hydrodynamic diameter (HD) of about 10.4 nm. Such macromolecules require over 24 h to reach equilibrium with the extracellular space, exhibit a half-life of two to three days, and typically require up to seven days for complete clearance from the body. Furthermore, the high hydrostatic pressure found in many solid tumors resists penetration of IgG.[11] Finally, although the apparent affinity of antibodies is high, this is achieved by divalency. Indeed, most single-chain antibodies do not possess an affinity high enough for effective targeting in vivo.

Recently, there has been a trend towards replacing the antibody scaffold with low molecular weight ligands. Conceptually, these ligands are similar to an antibody in that they possess one or more binding domains for tumor targeting, and a nonbinding domain for conjugation to functional molecules such as contrast agents and/or chemotherapeutics. Unlike mAbs, their low molecular weight and small hydrodynamic diameter permit rapid clearance, usually via the kidney and liver. The challenge is to engineer low molecular weight ligands such that they are "modular", that is, that they retain tumor binding even after substitution with a molecule that may be of similar, or larger, size.

Natural peptide ligands bind their receptors with high specificity, and with



Scheme 2. Conjugation of monoclonal antibodies to the second-generation taxane **2** (SB-T-110131; Boc = *tert*-butoxycarbonyl). a) *N*-succinimidyl-4-(2-pyridyldithio)pentanoate, 50 mM phosphate buffer, 50 mM NaCl, pH 6.5, 2 mM EDTA, 90 min; b) 50 mM phosphate buffer, 50 mM NaCl, pH 6.5, 2 mM EDTA, 1.7 equiv **2**, 24 h.

affinities often in the nanomolar or subnanomolar range. Since a number of peptide receptors are overexpressed on human cancer cells, they make attractive targets for therapy. Peptides such as somatostatin, substance P, gastrin, bombesin, α-melanocyte stimulating hormone (α-MSH), neurotensin, and vasoactive intestinal peptide (VIP), or analogues thereof,[12] are being studied as low molecular weight ligands in the preclinical and clinical phases.[13] In fact, radiolabeled derivatives of these peptides already constitute a large class of peptidic agents used for imaging.[14] Among them the ^{111}In-labeled somatostatin analogue OctreoScan is approved for clinical use and a large number of patients have been screened successfully with this analogue.[15]

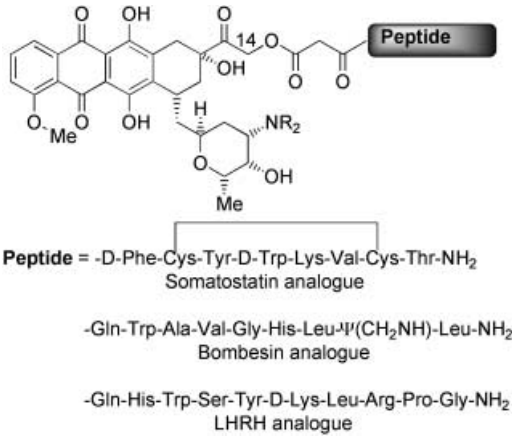


Figure 3. Conjugates of doxorubicin with different targeting peptides.

Conjugation of cytotoxic moieties to tumor-targeting low molecular weight ligands has also been successfully performed.[16] For example, a number of doxorubicin conjugates with analogues of somatostatin, bombesin, and luteneinizing hormone-releasing hormone (LHRH) as targeting molecules have been developed (Figure 3) and tested against various human cancers. Conjugation of the cytotoxic agent to the targeting peptide was done through the 14-*O*-glutaryl esters of doxorubicin, which have been previously reported to be effective antitumor agents.[17] Many of these peptides are also amenable to solid-phase synthesis. Certain LHRH conjugates were extensively investigated in preclinical studies and are planned to be tested in clinical trials soon.[16]

Selective targeting of malignant cells with monoclonal antibodies and low molecular weight ligands is rapidly gaining importance in cancer detection and treatment. For effective therapy, a chemical method for releasing the cytotoxic agent in a bioactive form, and at the appropriate subcellular location, is necessary. We have highlighted some of the newer strategies for effective chemical linkage between targeting molecule and cytotoxic agent, and have identified a trend toward designing more compact targeting molecules, with improved biodistribution and clearance. Such compounds hold great promise for cancer diagnosis and treatment in the future.

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