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MONOGRAPH

BRENTUXIMAB VEDOTIN

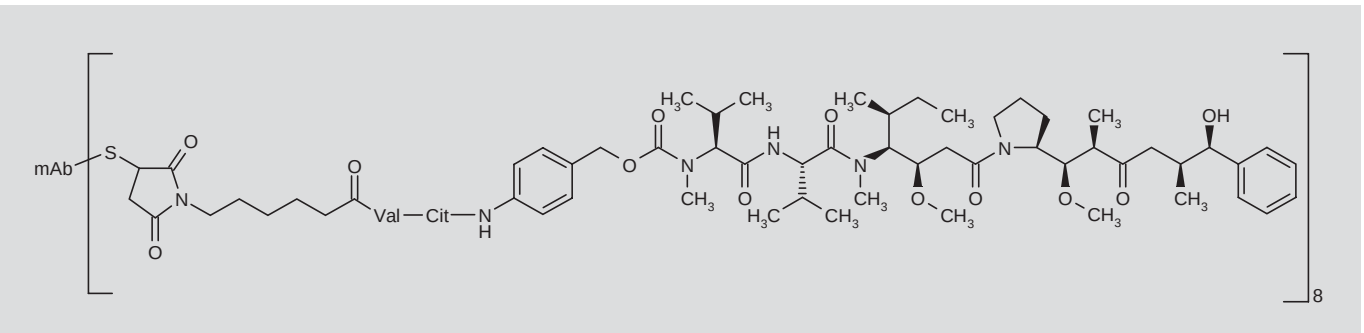
Prop INN; USAN

Anti-CD30 Antibody–Drug Conjugate
Oncolytic

SGN-35
cAC10-Val-Cit-MMAE
cAC10-vcMMAE

Immunoglobulin G_1, anti-(human CD30 (antigen)) (human-mouse monoclonal cAC10 γ1-chain), disulfide with human-mouse monoclonal cAC10 κ-chain, dimer, complex with *N*-[[[4-[[*N*-[6-(2,5-dihydro-2,5-dioxo-1*H*-pyrrol-1-yl)-1-oxohexyl]-L-valyl-N^5-(aminocarbonyl)-L-ornithyl]amino]phenyl]methoxy]carbonyl]-*N*-methyl-L-valyl-*N*-[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenylethyl]amino]-1-methoxy-2-methyl-3-oxopropyl]-1-pyrrolidinyl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4- oxobutyl]-*N*-methyl-L-valinamide

Immunoglobulin G_1, anti-(human tumor necrosis factor receptor superfamily member 8 (lymphocyte activation antigen CD30 or KI-1 antigen); chimeric (mouse variable domain/human constant domain) monoclonal cAC10 des-Lys$^{\omega}$($CH3^{107}$-K)-γ1 heavy chain (220-218')-disulfide with chimeric (mouse variable domain/human constant domain) monoclonal cAC10 κ light chain, dimer (226-226'':229-229'')-bisdisulfide complex with *N*-[[[4-[[*N*-[6-(2,5-dihydro-2,5-dioxo-1*H*-pyrrol-1-yl)-1-oxohexyl]-L-valyl-N^5-(aminocarbonyl)-L-ornithyl]amino]phenyl]methoxy]carbonyl]-*N*-methyl-L-valyl-*N*-[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[[(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenylethyl]amino]-1-methoxy-2-methyl-3-oxopropyl]-1-pyrrolidinyl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxobutyl]-*N*-methyl-L-valinamide



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SUMMARY

Although curable, more than one-third of patients with Hodgkin's lymphoma and anaplastic large cell lymphoma (ALCL) will relapse after frontline therapy. Novel therapies are needed to improve the curability and survival of relapsed and refractory patients while minimizing toxicity. Brentuximab vedotin (SGN-35) is an antibody–drug conjugate consisting of a chimeric anti-CD30 antibody chemically conjugated to the antitubulin auristatin derivative monomethylauristatin E. Results from recently completed phase I studies demonstrated significant activity and tolerability of brentuximab vedotin in patients with relapsed and refractory Hodgkin's lymphoma and ALCL. Phase II trials were recently completed in these two diseases, and combination studies have already started in patients with newly diagnosed Hodgkin's lymphoma. This paper will review the current status of brentuximab vedotin for the treatment of patients with CD30-positive lymphomas.

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PREPARATION

The brentuximab vedoxin conjugate was prepared by alkylation of the previously reduced monoclonal antibody (mAb) brentuximab (cAC10) with maleimidocaproyl-Val-Cit-*p*-aminobenzyloxycarbonyl-monomethylauristatin E in cold acetonitrile to give the nonaggregated conjugate with about eight drugs attached per antibody. The components of the conjugate were prepared separately. Monomethylauristatin E (MMAE) is totally synthetic and was prepared by known conventional methods. The protease-cleavable

dipeptide linker maleimidocaproyl-Val-Cit was attached to the *N*-terminal of MMAE through the *p*-aminobenzylcarbamate spacer by stirring maleimidocaproyl-Val-Cit-*p*-aminobenzyl alcohol *p*-nitrophenylcarbonate, MMAE and *N*-hydroxybenzotriazole in DMF. The mAb is reduced by treatment with dithiothreitol in PBS containing 50 mM borate (pH 8) at 37 °C. After gel filtration, thiol determination results in approximately eight SH groups per mAb (1).

BACKGROUND

Both Hodgkin's lymphoma and anaplastic large cell lymphoma (ALCL) are rare cancers, with an estimated 8,510 new cases of Hodgkin's lymphoma reported in the U.S. in 2009 and ALCL accounting for fewer than 5% of all cases of non-Hodgkin's lymphoma (NHL) (2). Both can affect a relatively young subset of the population (3). Although ALCL and Hodgkin's lymphoma are highly curable, many patients may not respond to their initial therapy, and in the case of Hodgkin's lymphoma, many patients who are cured may still die prematurely due to late toxic complications of their curative therapy (4). Moreover, although a fraction of patients with relapsed and refractory Hodgkin's lymphoma can be cured with second-line salvage therapy and autologous stem cell transplantation (ASCT), those whose disease relapsed after ASCT have a median survival of less than 3 years (5). Similarly, approximately 50% of patients with systemic ALCL develop recurrent disease. The impact of early mortality in this young patient population is significant, and clearly patients with relapsed Hodgkin's lymphoma and ALCL represent a patient population with an unmet medical need. Despite this, the development of new drugs for patients with Hodgkin's lymphoma and ALCL has been challenging. In fact, no new drugs have been approved for Hodgkin's lymphoma patients in over 30 years.

CD30 is a member of the tumor necrosis factor (TNF) receptor superfamily densely expressed as an integral membrane glycoprotein on Hodgkin's Reed–Sternberg (HRS) and ALCL cells. CD30 is rarely expressed by nonmalignant cells, but can be expressed on a small fraction of activated B and T lymphocytes and natural killer (NK) cells. Because its expression is highly restricted to tumor cells, CD30 is an excellent target for an immunotherapeutic approach with mAbs (6). CD30 is shed in a soluble form detected in the sera of patients, which could attenuate the therapeutic efficacy of antibodies (7-9). Activation of CD30 by CD30 ligand (CD30L) binding or crosslinking by immobilized antibodies induces trimerization of the receptor and recruitment of signaling proteins, and leads to transduction of numerous biological signals, such as cell proliferation or apoptosis (10). Antibodies against CD30 have been directly shown to induce cell growth arrest and death in a variety of CD30-expressing cell lines, in addition to their ability to kill by antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) or antibody/antigen-dependent cellular phagocytosis (ADCP).

SGN-30 (cAC10) is a first-generation chimeric naked anti-CD30 antibody constructed from the variable regions of the anti-CD30 murine monoclonal AC10 and the human γ1 heavy chain and κ light chain constant regions. Preclinical studies demonstrated antitumor activity in both in vitro and in vivo models in Hodgkin's lymphoma and CD30-expressing ALCL (11). However, SGN-30 produced no significant clinical activity in patients with relapsed Hodgkin's lymphoma and ALCL (12, 13). Similarly, a clinical trial combining SGN-30 with gemcitabine-based chemotherapy was also disappointing, as it produced unexpected pulmonary toxicity (14).

Despite these setbacks, preclinical experiments suggested that SGN-30 may have a therapeutic role in other aggressive $CD30^+$ malignancies, such as adult T-cell leukemia, although this has not been tested in a clinical setting (15). The disappointing results for unconjugated antibodies in Hodgkin's lymphoma and ALCL might be due to their neutralization in vivo by circulating soluble CD30, or due to their poor antigen-binding and/or effector cell-activating properties. However, as described below, the fact that many patients with relapsed Hodgkin's lymphoma responded to brentuximab vedotin (SGN-35) therapy suggests that soluble CD30 may not be an important factor in determining the clinical outcome of anti-CD30 antibody therapy. Thus, it is likely that the first-generation anti-CD30 antibodies, including SGN-30 and iratumumab (MDX-060), are suboptimal for activating effector cells. To bypass this limitation, a second-generation anti-CD30 antibody, XmAb-2513, was generated by humanizing the chimeric anti-CD30 antibody cAC10 by optimizing human string content. XmAb-2513 has higher affinity for CD30 compared to the parent antibody, and exhibits an approximately 20-fold increase in affinity for the FcγIIIA receptor (16). This resulted in significant improvement in antitumor activity in vitro. A phase I clinical trial with XmAb-2513 was recently initiated to examine its clinical activity in patients with relapsed $CD30^+$ malignancies.

Another strategy to improve on the clinical activity of CD30-targeted mAbs is to conjugate the antibody with toxic molecules. Brentuximab vedotin (SGN-35) is an antibody–drug conjugate (ADC) consisting of the chimeric antibody SGN-30 chemically conjugated to a synthetic analogue (monomethylauristatin E [MMAE]) of the naturally occurring antitubulin agent dolastatin 10 (1, 17). Brentuximab vedotin binds to CD30 on the cell surface and is internalized. ADC is trafficked to lysosomes and MMAE is released from the conjugate through proteolytic degradation of the drug linker (18). MMAE then binds to tubulin and disrupts the microtubule network, leading to G_2/M phase cell cycle arrest and apoptosis (Fig. 1) (19).

PRECLINICAL PHARMACOLOGY

Preclinical studies of brentuximab vedotin demonstrated antitumor activity in both in vitro and in vivo models (17, 20, 21). Potent and selective in vitro activity was demonstrated in Hodgkin's lymphoma and ALCL cell lines, with IC_{50} values ranging from 4 to 35 ng/mL for $CD30^+$ cell lines, compared with IC_{50} values of > 1000 ng/mL for $CD30^-$ cells. In an ALCL tumor xenograft model, a significant response was observed when mice were treated with brentuximab vedoxin when compared to cAC10 anti-CD30 antibody alone or unconjugated cAC10 antibody with the same amount of MMAE (Fig. 2) (1).

CLINICAL STUDIES

Brentuximab vedotin has been evaluated in phase I and II clinical trials in patients with relapsed Hodgkin's lymphoma, ALCL and angioimmunoblastic T-cell lymphoma (Table I). In the first phase I trial, brentuximab vedotin was administered by i.v. infusion every 3

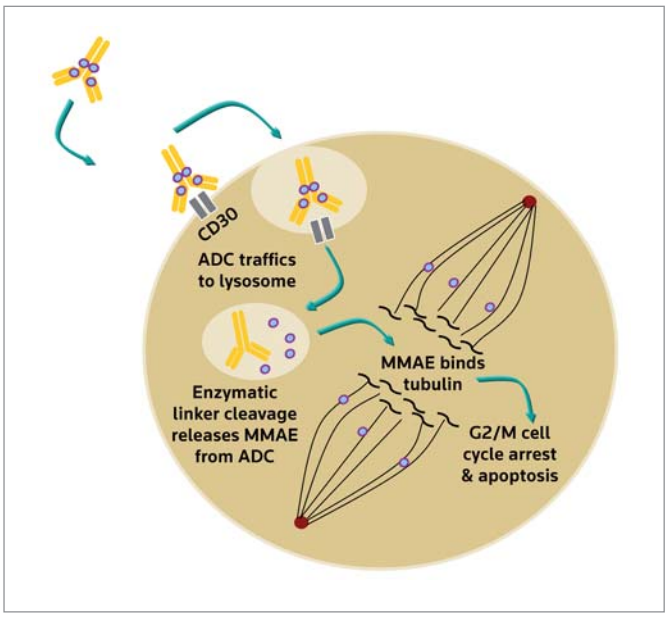

Figure 1. Brentuximab vedotin mechanism of action. ADC, antibody–drug conjugate; MMAE, monomethylauristatin E. Reprinted with permission from Seattle Genetics.

weeks. Forty-five patients with relapsed or refractory CD30$^+$ lymphoma, including Hodgkin's lymphoma and ALCL, were enrolled (22). Dose levels ranged from 0.1 to 3.6 mg/kg given over 2 h every 3 weeks. Twenty-eight evaluable patients were treated at or above the 1.2 mg/kg dose level and the objective response rate was 46% (n = 13) and the complete remission rate was 25% (n = 7). Both patients with systemic ALCL (one each in the 1.2 and 2.7 mg/kg cohorts) had a complete remission. Dose-limiting toxicities were experienced at the 2.7 mg/kg dose level, including unrelated grade 3 acute renal failure, grade 3 hyperglycemia and unrelated grade 3 prostatitis and febrile neutropenia. The maximum tolerated dose (MTD) was 1.8 mg/kg every 3 weeks. The most commonly reported adverse events include fatigue, pyrexia, nausea, diarrhea, dose-related neutropenia and peripheral neuropathy.

Based on half-life estimates of 4-6 days for ADC and 3-4 days for MMAE observed with the every-3-week dosing schedule, a weekly dosing schedule was later investigated in a phase I trial, producing similar response rates as the every-3-week schedule (23). Thirty-seven patients with Hodgkin's lymphoma, ALCL and peripheral T-cell lymphoma were treated with dose levels ranging from 0.4 to 1.4 mg/kg given i.v. over 30 min or 2 h. The objective response rate was 46% (n = 16), with 29% (n = 10) of patients achieving complete remission. The MTD was exceeded at 1.4 mg/kg given weekly. Dose-limiting toxicities included diarrhea, vomiting and hyperglycemia. The most common adverse events were peripheral neuropathy, nausea, fatigue, diarrhea, dizziness and neutropenia, most being grade 1 and 2 in severity.

Interestingly, retreatment with brentuximab vedotin can also induce clinical responses in patients who had relapsed after previously receiving brentuximab vedotin (24). In a recent report of an ongoing study, six of seven patients with relapsed Hodgkin's lymphoma and ALCL responded to retreatment with brentuximab vedotin. All treatment-related toxicities in the retreatment study were grade 1 and 2, with three patients experiencing upper respiratory infection and two peripheral sensory neuropathy.

CONCLUSIONS

Brentuximab vedotin at a dose of 1.8 mg/kg given every 3 weeks is currently being examined in a pivotal clinical trial seeking approval by the FDA for relapsed Hodgkin's lymphoma (28). If successful, this may dramatically change the options for this young patient population of relapsed and refractory patients with Hodgkin's lymphoma

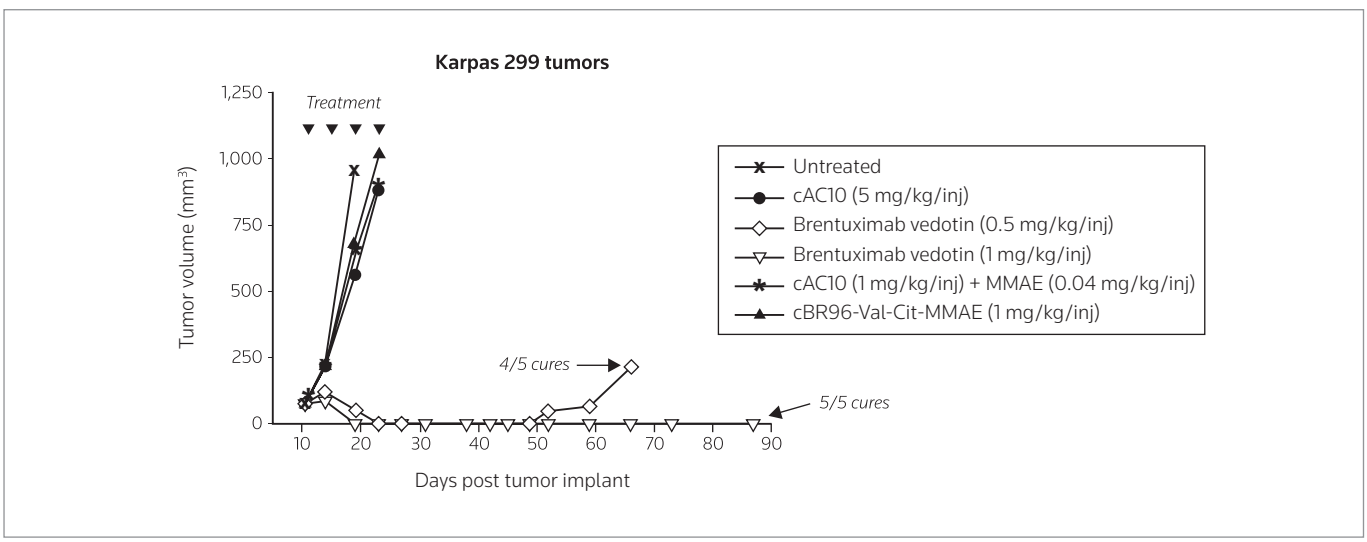

Figure 2. In vivo therapeutic efficacy of monomethylauristatin E conjugates. Reprinted by permission from Macmillan Publishers Ltd: Nature Biotechnology, Doronina, S.O., Toki, B.E., Torgov, M.X., Mendelsohn, B.A., Cerveny, C.G., Chace, D.F., DeBlanc, R.L., Gearing, R.P., Bovee, T.D., Siegall, C.B., Francisco, J.A., Wahl, A.F., Meyer, D.L., Senter, P.D. *Development of potent monoclonal antibody auristatin conjugates for cancer therapy*, 2003, 21(7): 778-784, © 2003.

Table I. *CD30-targeted studies.*

Author	Antibody	Number of patients	Overall response rate
Bartlett et al. (11)	SGN-30	N = 24 (21 HL, 3 NHL)	4% (n = 1)
Ansell et al. (25)	Iratumumab	N = 21 (16 HL, 3 ALCL, 2 TCL)	29% (n = 6)
Thertulien et al. (26)	MDX-1401	N = 12	0%
Younes et al. (27)	XmAb-2513	N = 13 (13 HL)	0%
Younes et al. (22)	Brentuximab vedotin (every 3 weeks)	N = 35 (32 HL, 2 ALCL, 1 AITCL)	46% (n = 13)
Fanale et al. (23)	Brentuximab vedotin (weekly)	N = 37 (31 HL, 5 ALCL, 1 PTCL)	46% (n = 16)
Bartlett et al. (24)	Brentuximab vedotin (retreatment)	N = 7 (6 HL, 1 ALCL)	75% (n = 6)

HL, Hodgkin's lymphoma; NHL, non-Hodgkin's lymphoma, ALCL, anaplastic large cell lymphoma; TCL, T-cell lymphoma; AITCL, angioimmunoblastic T-cell lymphoma; PTCL, peripheral T-cell lymphoma.

and ALCL. Combination strategies are currently being evaluated. A phase I trial is evaluating brentuximab vedotin in combination with ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) in newly diagnosed Hodgkin's lymphoma (29). If proven to be safe, a follow-up randomized study will be required to compare ABVD with brentuximab vedotin plus ABVD to determine the impact of this novel combination on patients' survival. Brentuximab vedotin is also currently being evaluated in clinical trials examining its role in preventing relapsed disease following autologous stem cell transplantation in patients with relapsed Hodgkin's lymphoma (30).

SOURCES

Seattle Genetics, Inc. (US); codeveloped with Millenium Pharmaceuticals, Inc. (US).

DISCLOSURES

The authors state no conflicts of interest.

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