PERSPECTIVE

nature biotechnology

The discovery and development of brentuximab vedotin for use in relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma

Peter D Senter & Eric L Sievers

Progress has been made recently in developing antibody-drug conjugates (ADCs) that can selectively deliver cancer drugs to tumor cells. In principle, the idea is simple: by attaching drugs to tumor-seeking antibodies, target cells will be killed and nontarget cells will be spared. In practice, many parameters needed to be addressed to develop safe and effective ADCs, including the expression profiles of tumor versus normal tissues, the potency of the drug, the linker attaching the drug and placement of the drug on the antibody, and the pharmacokinetic and stability profiles of the resulting ADC. All these issues had been taken into account in developing brentuximab vedotin (Adcetris), an ADC that recently received accelerated approval by the US Food and Drug Administration for the treatment of relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma (ALCL). Research is under way to extend the applications of brentuximab vedotin and to advance the field by developing other ADCs with new linker and conjugation strategies.

The concept of targeting cancer cells with ADCs has roots that can be traced back more than a century, when the German immunologist Paul Ehrlich (**Fig. 1**) proposed that if toxic molecules could be selectively delivered to disease-causing cells, it would be possible to develop therapeutic modalities with specificities that were otherwise unattainable[1]. For treating cancer, this would require a targeting agent that selectively binds to tumor cell surface antigens, coupled with a cytotoxic drug that could kill the cells once delivered. Antibodies that have been selected to distinguish between tumor and non-tumor cells are ideally suited for this purpose because they are readily available, biologically compatible, minimally immunogenic and may circulate in the body for extended periods of time. The adaptation of antibodies to Ehrlich's vision is schematically represented in **Figure 2**, which shows how ADCs can elicit specific tumor cell killing either through receptor-mediated endocytosis[2–4] or extracellular drug release[5]. Target cells are killed if sufficient drug is selectively delivered, ideally sparing normal tissues from chemotherapeutic damage.

Whereas early ADC research was undertaken with available targeting reagents such as polyclonal antibodies to human tumor antigens[6], it was not until the development of monoclonal antibody (mAb) technology by Kohler and Milstein (**Fig. 1**) in 1975 (ref. 7) that tumor targeting became technologically feasible. Soon afterward, several pharmaceutical and biotech companies actively developed ADC programs for treating cancer, with an emphasis on proof-of-principle experiments using conventional anticancer drugs targeted to tumor types for which the drugs had been already approved. Unfortunately, advanced agents from this work, such as KS1/4–desacetylvinblastine hydrazide[8] (Eli Lilly; Indianapolis) in patients with metastatic adenocarcinomas, and BR96–doxorubicin (Adriamycin)[9] (Bristol-Myers Squibb; New York) in patients with metastatic breast cancer proved to be clinically unsuccessful. Shortcomings became evident, stemming from conjugate immunogenicity, low drug potency, antigen expression on normal tissues and instability of the linkers that joined the drugs to the mAbs.

Attention then turned to ADCs that contained highly potent cytotoxic drugs that were likely to be too toxic for use in an untargeted setting. The reasoning was that the limitations of intratumoral macromolecular uptake[10] could be overcome if very few drug molecules were required to kill the target cell. Higher drug potency necessitated both choosing target antigens judiciously as well as addressing the immunogenic properties of the mAb carrier to avoid high ADC and immune complex concentrations in normal tissues. With these parameters in mind, gemtuzumab ozogamicin (Mylotarg) was developed by Wyeth (now part of Pfizer, New York) and Celltech (now part of UCB, Brussels) for the treatment of acute myeloid leukemia (AML)[11]. The drug consisted of an anti-CD33 mAb conjugated with a derivative of calicheamicin, a highly potent enediyne antibiotic. Despite encouraging clinical results that led to accelerated approval of gemtuzumab ozogamicin in 2000, a subsequent phase 3 confirmatory trial raised new concerns about the product's safety and failure to demonstrate clinical benefit[12,13]. In 2010, Pfizer voluntarily withdrew the drug from the US market. Subsequent findings in three additional randomized trials comparing standard induction chemotherapy with and without gemtuzumab ozogamicin in newly diagnosed AML patients stood in contrast to the phase 3 confirmatory study and suggested clinical benefit among certain patients—those whose AML was characterized by either 'good' or 'intermediate' risk cytogenetics[14–16]. The impact that the different phase 3 trials might have on the future development of gemtuzumab ozogamicin as a drug remains for global regulatory bodies to determine.

Several lessons can be taken from the gemtuzumab ozogamicin development program. Calicheamicin is hydrophobic, and only a few drugs can be conjugated before high levels of aggregated protein are obtained. Consequently, the manufacturing process used at the time gemtuzumab

Seattle Genetics, Bothell, Washington, USA. Correspondence should be addressed to P.D.S. (psenter@seagen.com).

Published online 10 July 2012; doi:10.1038/nbt.2289

Figure 1 Some of the key players that helped advance ADC technology. Paul Ehrlich proposed the concept of 'magic bullets' in the early 1900s (top left). In 1975, César Milstein and George Köhler described how mAbs with exquisite specificities could be produced (top right). George Pettit extracted highly potent cytotoxins from natural sources (bottom right), such as the Indian Ocean sea hare (*Dolabela auricularia*, bottom left), that eventually led us to the discovery and development of the drug component of brentuximab vedotin.

ozogamicin was developed yielded 50% unconjugated mAb in the final drug product[17,18]. The linker between calicheamicin and the mAb released 50% of bound drug in 48 h[19]. Finally, the drug component was derived from a soil microorganism, which restricted the ability to design new forms to circumvent these limitations.

Here we describe how each of these issues was addressed in the design and development of brentuximab vedotin, a clinically effective ADC for the treatment of Hodgkin lymphoma and systemic ALCL.

Selecting the right drug-linker

In developing the ADC platform technology described here, we took a lesson from the Indian Ocean sea hare, *Dolabela auricularia* (**Fig. 1**), which has been known for more than 2,000 years to harbor highly toxic extractable substances to protect itself from being eaten. George Pettit (**Fig. 1**) and co-workers characterized several toxic components from the sea hare that exhibited activities against cancer cell lines and elucidated the structure and mechanism of dolastatin 10 (**Fig. 3**), one of the most potent antimitotic agents ever described[20]. Several related molecules have been produced through total synthesis[21], establishing that the drug class, now known as the auristatins, could be prepared in large quantities. Like the vinca alkaloids, the auristatins exert anticancer activity by inhibiting tubulin polymerization. A phase 2 clinical trial with dolastatin 10 in patients with advanced breast cancer confirmed the drug's high toxicity[22]. Although minimal clinical benefit was obtained at the optimal dose of 0.4 mg m^{-2}, this did not diminish our interest in the class because we established combinatorial routes to the synthesis of thousands of auristatins that allowed us to fully explore linker technologies, and the effects of hydrophilicity, stability and potency on ADC activity. This work led to monomethyl auristatin E (MMAE; **Fig. 3**), a synthetic analog of dolastatin 10 that had high potency, water solubility and stability under physiological conditions, together with a built-in functionality for stable linker attachment. These were qualities in the cytotoxic component of the ADC that we deemed to be highly important for optimal activity[23].

We considered several linker technologies, including acid-labile hydrazones such as those present on gemtuzumab ozogamicin and BR96–doxorubicin, and disulfides that had been used for maytansinoid-containing ADCs[24]. After exploring possibilities along these lines, our attention was drawn to protease-cleavable linkers because protease activity is abundant in lysosomes, where ADCs commonly traffic, and is almost absent outside cells because of modulation by secreted protease inhibitors[25]. We could readily attach MMAE to suitable peptides through the N-terminal amine via a self-immolative spacer, *p*-aminobenzyloxycarbonyl (PABC). The purpose of the spacer is to situate the cleavable peptide away from the drug to allow facile proteolysis. Upon peptide cleavage, the PABC group rapidly fragments, leading to the release of MMAE in chemically unmodified form. The dipeptide we selected from among many was valine-citrulline (Val-Cit), which is stable in the plasma but is very rapidly hydrolyzed by lysosomal enzymes, such as cathepsin B[23,25]. We used a maleimide functionality to attach the spacer to mAb cysteine residues. In the mouse, the *in vivo* half-life of the linker we used to bind the drug to the mAb was in the range of a week[26], which was a major advancement over many of the previously reported technologies for generating ADCs. The structure of the resulting mAb-Val-Cit-PABC-MMAE is shown in **Figure 3**.

Establishing appropriate conjugation technology

The number of molecules of the drug and where they reside on the mAb carrier can impact ADC pharmacokinetics, tumor exposure and stability in the circulation[27,28]. Because of this, we and others have focused considerable attention on conjugation technology, which has resulted in a variety of methods that provide different drug configurations and different degrees of product heterogeneity (**Fig. 4**). The greatest number of species is obtained when drugs are linked through mAb lysine residues, which was the case with gemtuzumab ozogamicin[17] and the breast cancer drug trastuzumab emtansine (T-DM1; Herceptin conjugated to a derivative of maytansine; Genentech, South, San Francisco, CA, USA)[29]. This is due to a small number of drugs, on average four, distributed among the large number of lysines scattered throughout the antibody structure. In contrast, less heterogeneity is obtained by either reacting all IgG interchain cysteines with the drug[23]

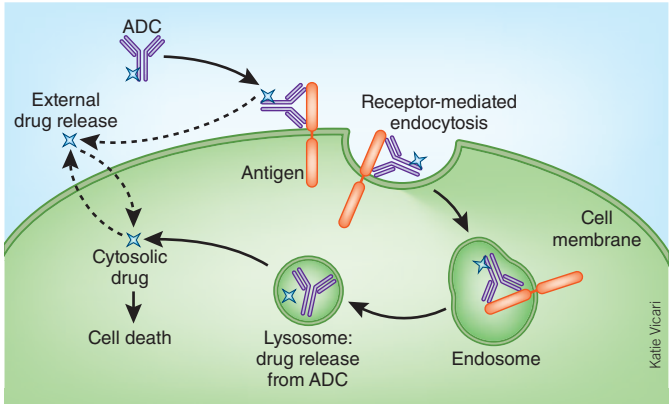

Figure 2 Mechanisms of drug delivery mediated by ADCs. Upon binding to tumor cell surface antigens, a drug conjugated to a mAb is internalized by a process known as receptor-mediated endocytosis, which can lead to drug release inside the target cell[2–4]. Alternatively, if the mAb remains bound to the antigen on the cell surface, extracellular drug release may take place, depending on the antigen that is targeted and the mode of drug attachment[5].

or using recombinant technologies that define particular cysteines for conjugation[28,30,31]. Between these extremes lies partial substitution of interchain disulfides, the method we eventually adopted.

IgGs contain reducible disulfide bonds that covalently link the heavy and light chains together. Initially, our group[23] produced highly uniform ADCs with eight drugs per antibody molecule, using all available cysteine residues generated upon mAb reduction. The resulting ADCs remained intact in the absence of the disulfides, consistent with previous reports that mAbs devoid of any interchain disulfide bonds are fully active[32]. Because the drugs were distal to the antigen-binding sites, antigen binding was not affected. However, subsequent studies demonstrated that such heavily loaded antibodies were rapidly cleared from the circulation, most likely due to differences in overall ADC hydrophobicity, and had reduced therapeutic windows compared with similarly prepared conjugates having an average of four auristatin drugs per antibody[27]. Reproducible technologies were therefore established to generate ADCs with an average of four drugs per mAb, and analytical methodologies, including liquid chromatography–mass spectrometry and hydrophobic interaction chromatography were developed to characterize the products present in the conjugation mixture[27,33]. The resulting ADCs contained primarily two, four and six molecules of drug per mAb, with four drugs per mAb being predominant. Small amounts of ADCs with 0 and 8 drugs per mAb (~8% each) were also obtained. All of the conjugated drugs resided among the heavy-heavy chain and heavy-light chain reduced disulfides. Notably, the use of partial reduction conjugation technology to generate four-loaded auristatin ADCs was applicable to nearly all IgGs tested and could be performed at scales ranging from micrograms to kilograms[34]. The yields were in the range of 95%, binding affinity was preserved and purified ADCs with minimal (<2%) aggregation levels were obtained in less than a day.

The target is critical

In choosing an appropriate antigen for the application of auristatin-based ADCs, we selected targets that were highly expressed on tumor cells, with minimal expression on normal tissues. This was motivated by the extreme potency (~10–100 pM) of four MMAE–containing ADCs[27] and information obtained from previous clinical trials with agents such as BR96-doxorubicin[9] and anti-CD44v6-DM1 (ref. 35), where target-related dose-limiting toxicities had been observed. Thus, although several target antigens have been successfully used in preclinical ADCs that contain peptide-linked auristatins[2–4], we focused on those that provided the highest levels of tumor selectivity.

CD30 is an ideal target for selective drug delivery. The antigen is highly expressed in Hodgkin lymphoma, ALCL, cutaneous T-cell lymphoma and other selected lymphoid tumors, as well as in some nonlymphoid malignancies including germ cell cancers[36]. Cross-reactivity of CD30 on normal tissues is very low, with some expression on activated, but not resting, T and B cells. CD30 is a tumor necrosis factor receptor (TNFR) superfamily member, which stimulates apoptosis[37] via TNFR-associated factor 2 degradation[38]. Because of its expression profile and its role in the regulation of cell survival, considerable interest has surrounded CD30 as a target both for anti-CD30 mAbs and for ADCs.

Hodgkin lymphoma and systemic ALCL represent substantial unmet medical needs.

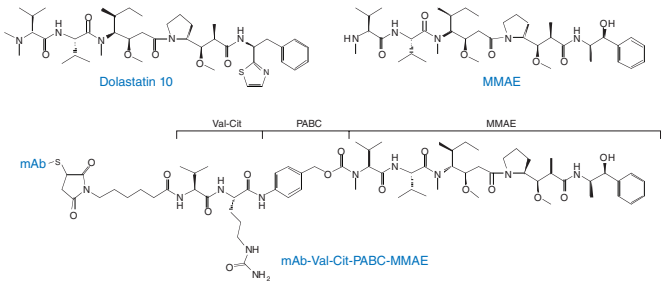

Figure 3 Structures of highly potent antimitotic drugs. Dolastatin 10 was isolated from *D. auricularia*, whereas MMAE was derived from total synthesis. MMAE is released from the mAb conjugate by proteolysis of the Val-Cit dipeptide, followed by elimination of the PABC group.

Although front-line Hodgkin lymphoma therapy with ABVD (doxorubicin (Adriamycin), bleomycin (Blenoxane), vinblastine and dacarbazine (DTIC)) and BEACOPP (bleomycin, etoposide (Vepesid), doxorubicin (Adriamycin), cyclophosphamide (Cytoxan), vincristine (Oncovin), procarbazine (Matulane) and prednisone) results in high remission rates, up to 20% of the patients are refractory and advanced-stage patients often relapse[39]. CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) therapy is used in front-line systemic ALCL treatment, but in ~40–65% of patients, the disease recurs[40]. We envisioned that targeted drug delivery through the CD30 antigen on Hodgkin lymphoma and systemic ALCL could be a novel approach to the treatment of these malignancies.

Clinical trials have been reported for unconjugated anti-CD30 mAbs. MDX-060, a fully human mAb, has been tested in a phase 1/2 trial in patients with Hodgkin lymphoma, ALCL and cutaneous T-cell lymphoma[41]. The unconjugated antibody was well tolerated and provided some evidence of clinical activity, with overall response rates (ORRs) that included complete and partial responses of 6% in Hodgkin lymphoma (4 of 63 patients) and 29% in ALCL (2 of 7 patients, both complete responses). Similar results were obtained with SGN-30, a chimeric mAb also known as cAC10 (ref. 37). Patients with Hodgkin lymphoma and ALCL were given high doses (6 or 12 mg kg^{-1}) SGN-30 weekly in a phase 2 clinical trial[42]. There were no objective responses in Hodgkin lymphoma patients, although 29% of these patients experienced stable

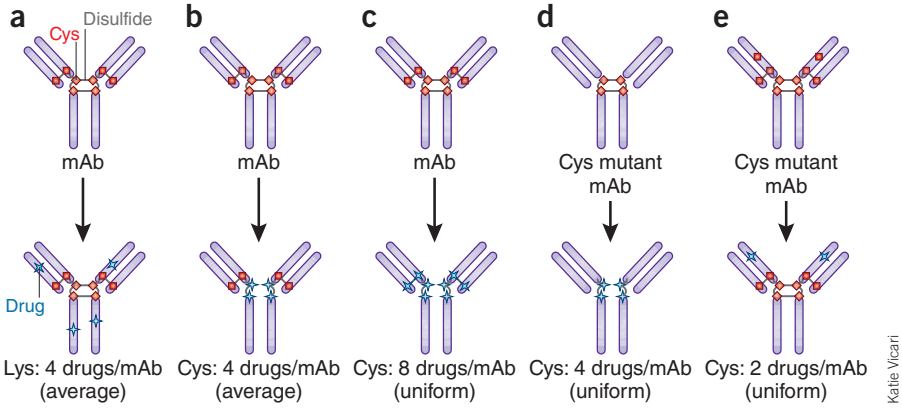

Figure 4 Drug-conjugation strategies. (**a**,**b**) Random distribution of the drugs on the mAb lysine groups (**a**)[18,24] leads to the higher levels of heterogeneity than attachment of the drugs to cysteine residues generated upon mAb reduction (**b**)[23]. (**c**–**e**) The least amount of heterogeneity is obtained by reacting all the intrachain disulfides with drug[23] (**c**) and using engineered mAbs that have defined sites for available cysteine reactivity (**d**,**e**).

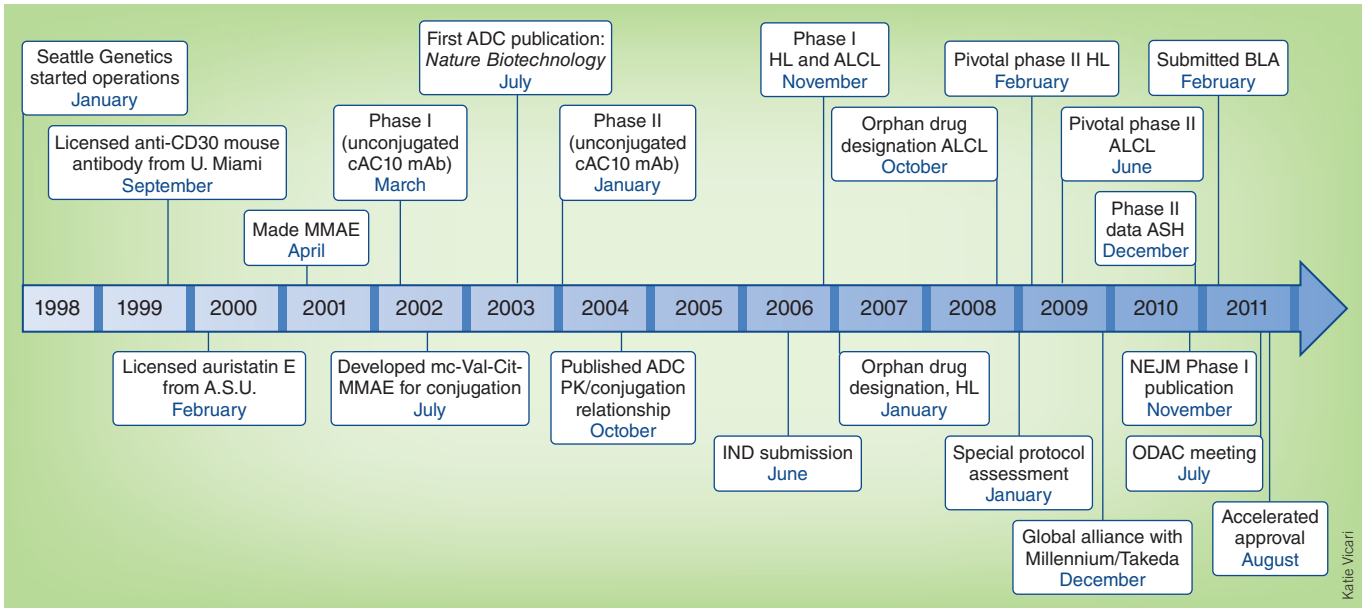

Figure 5 Some important milestones that led to accelerated approval of brentuximab vedotin. The initial work (1998–2004) surrounded the development of the mAb (cAC10), drug (MMAE) and linker (mc-Val-Cit) components, conjugation technology and preclinical activities. Clinical work with brentuximab vedotin commenced in November 2006, leading to accelerated approval on 19 August 2011. ASH, American Society of Hematology; ASU, Arizona State University; IND, investigational new drug; BLA, biologics license application; NEJM, New England Journal of Medicine; ODAC, Oncology Drugs Advisory Committee.

disease while on drug. Among ALCL patients, 5% (2 of 41 patients) achieved complete responses and 12% (5 of 41 patients) had partial responses. The response rate in a phase 1 Hodgkin lymphoma trial with an anti-CD30–ricin A chain conjugate was also quite modest[43]. Thus, although these agents were not promising enough to warrant clinical development, the data obtained, together with the antigen expression profile, supported development of an optimized and highly potent anti-CD30 ADC for the treatment of CD30-positive malignancies.

Development of brentuximab vedotin

We applied the technology described using the highly potent auristatin derivative MMAE, a protease-cleavable dipeptide linker, and a reproducible and robust method for generating active ADCs to the cAC10 mAb (chimeric IgG1), the same antibody that had been used in an unconjugated form in the Hodgkin lymphoma/ALCL phase 2 clinical trial just described. cAC10-Val-Cit-PABC-MMAE, now known as brentuximab vedotin, comprising on average four molecules of MMAE attached to cAC10 interchain cysteine residues through the protease-cleavable Val-Cit-PABC linker. The timeline for the development of this molecule from its constitutive components to accelerated approval on 19 August 2011 is illustrated in **Figure 5**.

Typical preparations of brentuximab vedotin contained less than 2% aggregated protein and bound to CD30 with unaltered affinity (3 nM) compared with the nonconjugated antibody[27,44]. The conjugate was highly potent against $CD30^+$ Hodgkin lymphoma and ALCL tumor cells *in vitro*, with half-maximal inhibitory concentration (IC_{50}) values of 3–50 pM (0.5–8 ng ml^{-1}). The effects were antigen-selective because non–CD30-expressing cells were ~1,000 times less sensitive to the effects of the ADC.

In vivo therapy studies in immunodeficient mice established that single-dose brentuximab vedotin regressed and cured established human Hodgkin lymphoma[30] and ALCL[27] tumor xenografts at doses of 1–3 mg kg^{-1}. With a maximum tolerated dose (MTD) of ~100 mg kg^{-1} in mice, the therapeutic window was pronounced[27]. Also favorable was the half-life of brentuximab vedotin, which was 14 d in mice compared with 16.7 d for the unconjugated antibody[27]. Finally, the ADC could be combined with chemotherapeutic agents used in treating Hodgkin lymphoma[45]. These studies provided the rationale for putting brentuximab vedotin into the clinic.

A phase 1 dose-escalation trial for brentuximab vedotin was initiated in 2006 in patients with $CD30^+$ malignancies[46]. Treatment was every three weeks at doses ranging from 0.1 mg kg^{-1} to 3.6 mg kg^{-1} delivered intravenously. The trial was designed to determine the MTD of the drug, with secondary objectives that included characterization of pharmacokinetics and antitumor activities. At 1.8 mg kg^{-1} every three weeks (the MTD), objective responses were obtained, including complete responses (4 of 12 patients) and partial responses (2 of 12 patients). The ORR (complete and partial responses) among all 45 patients in the trial was 38%, which included a 24% complete-response rate. During the study, low titers of antitherapeutic antibody were found in two of 40 patients tested. A second phase 1 trial of brentuximab vedotin was carried out to test its effects when administered weekly[47]. The dosing range was 0.4–1.4 mg kg^{-1}, the MTD was 1.2 mg kg^{-1} and the ORR was 59%, with 34% complete responses. The most common adverse events in both trials were peripheral sensory neuropathy, neutropenia, fatigue, nausea and diarrhea.

Encouraging data from the phase 1 trials served as a foundation for paired prospective, single-arm pivotal phase 2 trials that enrolled patients with relapsed or refractory Hodgkin lymphoma or systemic ALCL, respectively. The primary endpoint of both trials was ORR by independent radiographic review. In the Hodgkin lymphoma trial, patients were required to have had lymphoma that was progressive or recurrent after prior autologous stem cell transplant[48]. Tumor reductions were observed in 94% of the 102 enrolled Hodgkin lymphoma patients (**Fig. 6**) and the ORR was 75%. Complete responses were achieved in 34% of patients; the median duration of these responses was 20.5 months at the time of publication[48]. Similarly, among 58 patients with relapsed or refractory systemic ALCL, the ORR was 86%, and

57% of the patients experienced complete responses with a median duration of 13.2 months[40]. The most common adverse events in these paired pivotal trials were peripheral sensory neuropathy, nausea, fatigue, neutropenia and diarrhea. In August 2011, the US Food and Drug Administration (FDA; Rockville, MD, USA) granted accelerated approval for the use of brentuximab vedotin in relapsed Hodgkin lymphoma and relapsed systemic ALCL. Brentuximab vedotin is the only ADC currently approved for use, and it is the first approved drug for treating Hodgkin lymphoma in 30 years.

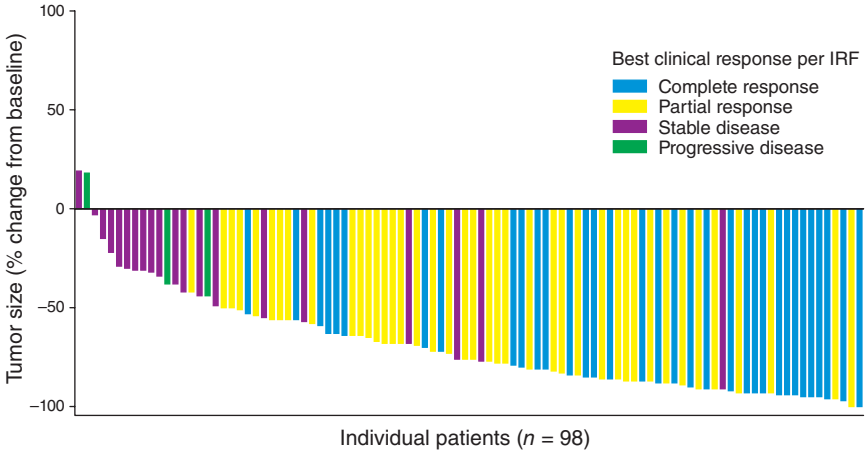

Figure 6 Maximum percentage reduction in the sum of the product of tumor diameters in individual patients ($n = 98$) per Cheson *et al.*[65]. Tumor size reductions were observed in 96 (94%) of 102 patients in the pivotal phase II Hodgkin lymphoma clinical trial[48]. Reprinted with permission from ref. 48. IRF, Independent review facility.

Prospects for the future

The power of ADC technology is evident by comparing the clinical activities of advanced conjugates with those of unconjugated antibodies that bind the same antigens. A phase 2 clinical trial with unconjugated cAC10 provided 0% ORR in Hodgkin lymphoma and 17% ORR in ALCL[42], in stark contrast to the results obtained with brentuximab vedotin: a 75% ORR for Hodgkin lymphoma[48] and 86% ORR in ALCL[40]. Consequently, several studies are ongoing with brentuximab vedotin and other auristatin-based ADCs. A randomized, placebo-controlled phase 3 trial (the AETHERA trial) is under way in Hodgkin lymphoma patients at high risk of progression after autologous stem cell transplant. Phase 2 trials are ongoing in patients with $CD30^+$ B- and T-cell lymphoma and non-lymphomatous malignancies. Phase 1 trials have also been initiated to explore the activities of brentuximab vedotin in combination with chemotherapy for the treatment of front line Hodgkin lymphoma and $CD30^+$ mature T-cell lymphomas. We and our collaborators are exploring the applicability of the ADC technology for other antigen targets in more than 15 active clinical trials. These include a phase 2 trial of CDX-011 (anti-glycoprotein nonmetastatic melanoma protein B mAb-Val-Cit-MMAE ADC) for the treatment of breast cancer and melanoma[49] and phase 1 trials in many other hematologic malignancies and solid tumors. In the next two years, we expect that a tremendous amount of new data will be available on how the auristatin technology applies to other cancer indications and other antigen targets.

New data will also be emerging for other ADCs in advanced clinical development. These include T-DM1, an ADC comprising the anti-HER2 mAb trastuzumab conjugated with an antimitotic maytansinoid anticancer drug for the treatment of HER2-positive breast cancer[29]. Trastuzumab is typically used in combination chemotherapy but has single-agent activity ranging from 15% to 26%[50–52]. T-DM1 is not only more active with ORRs of 25–64%, but offers an improved safety profile compared with traditional chemotherapy in phase 2 clinical trials[29,53–56]. The drug is currently in two randomized phase 3 clinical trials. The EMILIA trial is investigating T-DM1 activity against the lapatinib and capecitabine combination in patients who failed prior trastuzumab-based therapies for metastatic breast cancer. The MARIANNE trial is a randomized study comparing the effects of single-agent T-DM1, T-DM1 plus pertuzumab and trastuzumab drug combinations in front-line therapy for advanced metastatic breast cancer.

Another ADC of interest is inotuzumab ozogamicin, an anti-CD22–calicheamicin conjugate in a phase 3 clinical trial for the treatment of acute lymphocytic leukemia (ALL[57]). As with gemtuzumab ozogamicin, the cytotoxic component kills cells by binding to the minor groove of DNA and inducing double-strand breaks. In phase 1/2 clinical trials, the anti-CD22 mAb epratuzumab gave 18% ORR in indolent non-Hodgkin lymphoma[58]. A recently reported phase 2 clinical trial of inotuzumab ozogamicin in patients with relapsed ALL demonstrated a 57% ORR, with 18% (9 of 49 patients) having complete responses. This highly potent ADC (phase 2 dose 1.8 mg m^{-2}) has promising indications for the treatment of refractory and relapsed ALL.

Whereas much of the focus on ADCs is on how they perform clinically, the field is rapidly advancing and expanding owing to substantial technological advancements in conjugate design and composition. Beyond the auristatin, maytansinoid and calicheamicin drug components described here, new highly potent drugs for ADCs are emerging with distinct mechanisms of activity. These include duocarmycins[59] and pyrrolobenzodiazepine dimers[60], both of which alkylate DNA after binding to the minor groove, the RNA polymerase inhibitor amanitin[61] and many others.

Great strides have also been made in conjugation technology. As noted above, lysine and native mAb cysteine modification lead to multiple ADC species that differ in potency and pharmacokinetics. Site-specific introduction of cysteine residues into the antibody structure has been used to make highly uniform ADCs that not only have pronounced activities[31], but may also provide levels of stability beyond what can be achieved normally[28]. Research is under way to exploit these findings and to extend them by introducing unnatural or modified amino acids into antibody structures for the purposes of specific drug attachment[62,63]. New scaffolds for drug delivery that differ from mAbs in substantial ways are also under investigation, and studies with them should provide insights into the effects of size, clearance and distribution on therapeutic efficacy[64].

Thus, the approval of brentuximab vedotin represents not only an advancement for the treatment of relapsed Hodgkin lymphoma and systemic ALCL but also a major milestone for ADCs as a whole. This biologic drug evolved over many years, as insights had been made into the structure-activity relationship of the auristatins, new linker technologies and conjugation protocols were developed, and the merits of targeting the CD30 antigen became apparent. The role of ADCs in chemotherapy of cancer is likely to grow in the near future, as existing

molecules advance toward approval and technological innovations, such as those reported here, are put into practice.

ACKNOWLEDGMENTS

We are grateful for meaningful and enduring contributions by our colleagues at Seattle Genetics and various international cancer centers, our corporate partner, Millennium, the Takeda Oncology Company and to patients with life-threatening lymphoma, who collectively participated in clinical trials of brentuximab vedotin —ultimately leading to its approval by the FDA.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

Published online at http://www.nature.com/doifinder/10.1038/nbt.2289.
Reprints and permissions information is available online at http://www.nature.com/reprints/index.html.

1. Schwartz, R.S. Paul Ehrlich's magic bullets. *N. Engl. J. Med.* **350**, 1079–1080 (2004).
2. Senter, P.D. Potent antibody drug conjugates for cancer therapy. *Curr. Opin. Chem. Biol.* **13**, 235–244 (2009).
3. Carter, P.J. & Senter, P.D. Antibody-drug conjugates for cancer therapy. *Cancer J.* **14**, 154–169 (2008).
4. Wu, A.M. & Senter, P.D. Arming antibodies: prospects and challenges for immunoconjugates. *Nat. Biotechnol.* **23**, 1137–1146 (2005).
5. Bernardes, G.J. *et al.* A traceless vascular-targeting antibody-drug conjugate for cancer therapy. *Angew. Chem. Int. Edn Engl.* **51**, 941–944 (2012).
6. Arnon, R. & Sela, M. In vitro and in vivo efficacy of conjugates of daunomycin with anti-tumor antibodies. *Immunol. Rev.* **62**, 5–27 (1982).
7. Köhler, G. & Milstein, C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **256**, 495–497 (1975).
8. Petersen, B.H., DeHerdt, S.V., Schneck, D.W. & Bumol, T.F. The human immune response to KS1/4-desacetylvinblastine (LY256787) and KS1/4-desacetylvinblastine hydrazide (LY203728) in single and multiple dose clinical studies. *Cancer Res.* **51**, 2286–2290 (1991).
9. Tolcher, A.W. *et al.* Randomized phase II study of BR96-doxorubicin conjugate in patients with metastatic breast cancer. *J. Clin. Oncol.* **17**, 478–484 (1999).
10. Jain, R.K. Tumor physiology and antibody delivery. *Front. Radiat. Ther. Oncol.* **24**, 32–46 (1990).
11. Sievers, E.L. & Linenberger, M. Mylotarg: antibody-targeted chemotherapy comes of age. *Curr. Opin. Oncol.* **13**, 522–527 (2001).
12. Petersdorf, S. et al. Preliminary results of Southwest Oncology Group Study S0106: an international intergroup phase 3 randomized trial comparing the addition of gemtuzumab ozogamicin to standard induction therapy versus standard induction therapy followed by a second randomization to post-consolidation gemtuzumab ozogamicin versus no additional therapy for previously untreated acute myeloid leukemia. *Blood* **114**, abstract 790 (2009). Paper presented at the American Society of Hematology National Meeting, New Orleans, December 5–9, 2009.
13. Ravandi, F. Gemtuzumab ozogamicin: one size does not fit all—the case for personalized therapy. *J. Clin. Oncol.* **29**, 349–351 (2011).
14. Burnett, A.K. *et al.* Identification of patients with acute myeloblastic leukemia who benefit from the addition of gemtuzumab ozogamicin: results of the MRC AML15 trial. *J. Clin. Oncol.* **29**, 369–377 (2011).
15. Castaigne, S. *et al.* Effect of gemtuzumab ozogamicin on survival of adult patients with de novo acute myeloid leukaemia (ALFA-0701): a randomised, open-label, phase 3 study. *Lancet* **379**, 1508-1516 (2012).
16. Walter, R.B., Appelbaum, F.R., Estey, E.H. & Bernstein, I.D. Acute myeloid leukemia stem cells and CD33-targeted immunotherapy. *Blood* advance online publication, doi:10.1182/blood-2011-11-325050 (27 January 2012).
17. Bross, P.F. *et al.* Approval summary: gemtuzumab ozogamicin in relapsed acute myeloid leukemia. *Clin. Cancer Res.* **7**, 1490–1496 (2001).
18. Hollander, I., Kunz, A. & Hamann, P.R. Selection of reaction additives used in the preparation of monomeric antibody-calicheamicin conjugates. *Bioconjug. Chem.* **19**, 358–361 (2008).
19. Boghaert, E.R. *et al.* Determination of pharmacokinetic values of calicheamicin-antibody conjugates in mice by plasmon resonance analysis of small (5 microl) blood samples. *Cancer Chemother. Pharmacol.* **61**, 1027–1035 (2008).
20. Pettit, G.R. *et al.* The isolation and structure of a remarkable marine animal antineoplastic constituent: dolastatin 10. *J. Am. Chem. Soc.* **109**, 6883–6885 (1987).
21. Pettit, G.R. *et al.* Antineoplastic agents 365. Dolastatin 10 SAR probes. *Anticancer Drug Des.* **13**, 243–277 (1998).
22. Perez, E.A. *et al.* Phase II trial of dolastatin-10 in patients with advanced breast cancer. *Invest. New Drugs* **23**, 257–261 (2005).
23. Doronina, S.O. *et al.* Development of potent monoclonal antibody auristatin conjugates for cancer therapy. *Nat. Biotechnol.* **21**, 778–784 (2003).
24. Chari, R.V. Targeted cancer therapy: conferring specificity to cytotoxic drugs. *Acc. Chem. Res.* **41**, 98–107 (2008).
25. Dubowchik, G.M., Mosure, K., Knipe, J.O. & Firestone, R.A. Cathepsin B-sensitive dipeptide prodrugs. 2. Models of anticancer drugs paclitaxel (Taxol), mitomycin C and doxorubicin. *Bioorg. Med. Chem. Lett.* **8**, 3347–3352 (1998).
26. Alley, S.C. *et al.* Contribution of linker stability to the activities of anticancer immunoconjugates. *Bioconjug. Chem.* **19**, 759–765 (2008).
27. Hamblett, K.J. *et al.* Effects of drug loading on the antitumor activity of a monoclonal antibody drug conjugate. *Clin. Cancer Res.* **10**, 7063–7070 (2004).
28. Shen, B.Q. *et al.* Conjugation site modulates the *in vivo* stability and therapeutic activity of antibody-drug conjugates. *Nat. Biotechnol.* **30**, 184–189 (2012).
29. LoRusso, P.M., Weiss, D., Guardino, E., Girish, S. & Sliwkowski, M.X. Trastuzumab emtansine: a unique antibody-drug conjugate in development for human epidermal growth factor receptor 2-positive cancer. *Clin. Cancer Res.* **17**, 6437–6447 (2011).
30. McDonagh, C.F. *et al.* Engineered antibody-drug conjugates with defined sites and stoichiometries of drug attachment. *Protein Eng. Des. Sel.* **19**, 299–307 (2006).
31. Junutula, J.R. *et al.* Site-specific conjugation of a cytotoxic drug to an antibody improves the therapeutic index. *Nat. Biotechnol.* **26**, 925–932 (2008).
32. Andersen, D.C. & Reilly, D.E. Production technologies for monoclonal antibodies and their fragments. *Curr. Opin. Biotechnol.* **15**, 456–462 (2004).
33. Sun, M.M. *et al.* Reduction-alkylation strategies for the modification of specific monoclonal antibody disulfides. *Bioconjug. Chem.* **16**, 1282–1290 (2005).
34. Lyon, R.P., Meyer, D.L., Setter, J.R. & Senter, P.D. Conjugation of anticancer drugs through endogenous monoclonal antibody cysteine residues. *Methods Enzymol.* **502**, 123–138 (2012).
35. Tijink, B.M. *et al.* A phase I dose escalation study with anti-CD44v6 bivatuzumab mertansine in patients with incurable squamous cell carcinoma of the head and neck or esophagus. *Clin. Cancer Res.* **12**, 6064–6072 (2006).
36. Deutsch, Y.E., Tadmor, T., Podack, E.R. & Rosenblatt, J.D. CD30: an important new target in hematologic malignancies. *Leuk. Lymphoma* **52**, 1641–1654 (2011).
37. Wahl, A.F. *et al.* The anti-CD30 monoclonal antibody SGN-30 promotes growth arrest and DNA fragmentation in vitro and affects antitumor activity in models of Hodgkin's disease. *Cancer Res.* **62**, 3736–3742 (2002).
38. Duckett, C.S. & Thompson, C.B. CD30-dependent degradation of TRAF2: implications for negative regulation of TRAF signaling and the control of cell survival. *Genes Dev.* **11**, 2810–2821 (1997).
39. Connors, J.M. State-of-the-art therapeutics: Hodgkin's lymphoma. *J. Clin. Oncol.* **23**, 6400–6408 (2005).
40. Pro, B. *et al.* Brentuximab vedotin (SGN-35) in patients with relapsed or refractory systemic anaplastic large cell lymphoma: results of a phase 2 study. *J. Clin. Oncol.* advance online publication doi:10.1200/JCO.2011.38.0402 (21 May 2012).
41. Ansell, S.M. *et al.* Phase I/II study of an anti-CD30 monoclonal antibody (MDX-060) in Hodgkin's lymphoma and anaplastic large-cell lymphoma. *J. Clin. Oncol.* **25**, 2764–2769 (2007).
42. Forero-Torres, A. *et al.* A phase II study of SGN-30 (anti-CD30 mAb) in Hodgkin lymphoma or systemic anaplastic large cell lymphoma. *Br. J. Haematol.* **146**, 171–179 (2009).
43. Schnell, R. *et al.* A Phase I study with an anti-CD30 ricin A-chain immunotoxin (Ki-4.dgA) in patients with refractory CD30+ Hodgkin's and non-Hodgkin's lymphoma. *Clin. Cancer Res.* **8**, 1779–1786 (2002).
44. Kim, K.M. *et al.* Anti-CD30 diabody-drug conjugates with potent antitumor activity. *Mol. Cancer Ther.* **7**, 2486–2497 (2008).
45. Oflazoglu, E., Kissler, K.M., Sievers, E.L., Grewal, I.S. & Gerber, H.P. Combination of the anti-CD30-auristatin-E antibody-drug conjugate (SGN-35) with chemotherapy improves antitumour activity in Hodgkin lymphoma. *Br. J. Haematol.* **142**, 69–73 (2008).
46. Younes, A. *et al.* Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. *N. Engl. J. Med.* **363**, 1812–1821 (2010).
47. Fanale, M.A. *et al.* A phase I weekly dosing study of brentuximab vedotin in patients with relapsed/refractory CD30-positive hematologic malignancies. *Clin. Cancer Res.* **18**, 248–255 (2012).
48. Younes, A. *et al.* Results of a pivotal phase II study of brentuximab vedotin for patients with relapsed or refractory Hodgkin's lymphoma. *J. Clin. Oncol.* advance online publication, doi:10.1200/JCO.2011.38.0410 (29 May 2012).
49. Keir, C.H. & Vahdat, L.T. The use of an antibody drug conjugate, glembatumumab vedotin (CDX-011), for the treatment of breast cancer. *Expert Opin. Biol. Ther.* **12**, 259–263 (2012).
50. Baselga, J. *et al.* Phase II study of efficacy, safety, and pharmacokinetics of trastuzumab monotherapy administered on a 3-weekly schedule. *J. Clin. Oncol.* **23**, 2162–2171 (2005).
51. Cobleigh, M.A. *et al.* Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J. Clin. Oncol.* **17**, 2639–2648 (1999).
52. Vogel, C.L. *et al.* Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J. Clin. Oncol.* **20**, 719–726 (2002).
53. Burris, H.A. III *et al.* Phase II study of the antibody drug conjugate trastuzumab-DM1 for the treatment of human epidermal growth factor receptor 2 (HER2)-positive breast cancer after prior HER2-directed therapy. *J. Clin. Oncol.* **29**, 398–405 (2011).
54. Hurvitz, S.A., Hu, Y., O'Brien, N. & Finn, R.S. Current approaches and future directions in the treatment of HER2-positive breast cancer. *Cancer Treat. Rev.* advance online publication, doi:10.1016/j.ctrv.2012.04.008 (4 June 2012).
55. Krop, I.E. *et al.* Phase I study of trastuzumab-DM1, an HER2 antibody-drug conjugate, given every 3 weeks to patients with HER2-positive metastatic breast cancer. *J. Clin. Oncol.* **28**, 2698–2704 (2010).
56. Krop, I.E. *et al.* A phase II study of trastuzumab emtansine in patients with

human epidermal growth factor receptor 2-positive metastatic breast cancer who were previously treated with trastuzumab, lapatinib, an anthracycline, a taxane, and capecitabine. *J. Clin. Oncol.* advance online publication, doi: 10.1200/JCO.2011.40.5902 (29 May 2012).
57. Kantarjian, H. *et al.* Inotuzumab ozogamicin, an anti-CD22-calecheamicin conjugate, for refractory and relapsed acute lymphocytic leukaemia: a phase 2 study. *Lancet Oncol.* **13**, 403–411 (2012).
58. Leonard, J.P. *et al.* Phase I/II trial of epratuzumab (humanized anti-CD22 antibody) in indolent non-Hodgkin's lymphoma. *J. Clin. Oncol.* **21**, 3051–3059 (2003).
59. Beck, A., Senter, P. & Chari, R. World Antibody Drug Conjugate Summit Europe: February 21-23 2011, Franfurt, Germany *MAbs* 3, 331–337 paper presented at the World Antibody Drug Conjugate Summit Europe, Frankfurt, Germany, February 21–23, 2011.
60. Hartley, J.A. *et al.* SG2285, a novel C2-aryl-substituted pyrrolobenzodiazepine dimer prodrug that cross-links DNA and exerts highly potent antitumor activity. *Cancer Res.* **70**, 6849–6858 (2010).
61. Moldenhauer, G. *et al.* Therapeutic potential of amanitin-conjugated anti-epithelial cell adhesion molecule monoclonal antibody against pancreatic carcinoma. *J. Natl. Cancer Inst.* **104**, 622–634 (2012).
62. Liu, C.C. & Schultz, P.G. Adding new chemistries to the genetic code. *Annu. Rev. Biochem.* **79**, 413–444 (2010).
63. Wu, P. *et al.* Site-specific chemical modification of recombinant proteins produced in mammalian cells by using the genetically encoded aldehyde tag. *Proc. Natl. Acad. Sci. USA* **106**, 3000–3005 (2009).
64. Friedman, M. & Stahl, S. Engineered affinity proteins for tumour-targeting applications. *Biotechnol. Appl. Biochem.* **53**, 1–29 (2009).
65. Cheson, B.D. *et al.* Revised response criteria for malignant lymphoma. *J. Clin. Oncol.* **25**, 579–586 (2007).