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ANTIBODY-DRUG CONJUGATES

FUNDAMENTALS, DRUG DEVELOPMENT, AND CLINICAL OUTCOMES TO TARGET CANCER

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Preface

We are honored and privileged to have been part of assembling and editing *Antibody–Drug Conjugates: Fundamentals, Drug Development, and Clinical Outcomes to Target Cancer*. This is a critical field of drug discovery, development, and commercialization focused on improving a patient's quality of life by specifically targeting the disease with a highly effective therapy, while simultaneously sparing normal tissue. We worked closely with distinguished, knowledgeable, and well-known industry, academic, and government researchers, drug developers, and clinicians to present a comprehensive story with concrete examples of novel therapies across various indications in oncology. We intentionally have overlap in various chapters to ensure full coverage of essential topics, which allows for a variety of opinions and strategies to be thoroughly explored.

As the reader may be aware, in order to effectively treat cancer and improve the quality of life for patients, therapeutic oncology molecules must kill all cancer cells without adversely affecting normal cells. Combinations of cytotoxic chemotherapeutic drugs have been the traditional means to this end, but often have off-target dose-limiting toxicities in normal cells and tissues that prevent sufficient exposure to kill all tumor cells. While the advent of engineered targeted monoclonal antibodies (mAbs) significantly improved the clinical outcomes for patients with several types of cancer, optimal efficacy requires they be given in combination with cytotoxic chemotherapy. Antibody–drug conjugates (ADCs) have the advantage of specifically targeting cancer cells to deliver cytotoxic drugs. This combination has created widespread enthusiasm in the oncology drug development community as well as in patient advocacy networks and can be largely explained by the properties of these molecules in their exquisite binding specificity and their substantially decreased toxicity profile. Several approaches are being evaluated including linkage of mAbs to highly cytotoxic drugs and targeted delivery of cytotoxic drug payloads in liposomes. This book will provide academic oncologists, drug researchers, and clinical developers and practitioners with a depth of knowledge regarding the following topics: (i) ADC fundamentals, (ii) molecules, structures, and compounds

included in this class, (iii) chemistry manufacturing and controls associated with ADC development, (iv) nonclinical approaches in developing various ADCs, (v) clinical outcomes and successful regulatory approval strategies associated with the use of ADCs, and (vi) case studies/examples (included throughout) from oncology drug discovery. Readers will be educated about ADCs so that they can affect important improvements in this novel developing field. They will have practical, proven solutions that they can apply to improve their ADC drug discovery success.

We feel this book will be a valuable reference to significantly augment the scope of currently available published information on ADCs. Considering how expansive this field is and the potential benefit to researchers, clinicians, and ultimately our patients, we felt a more comprehensive book covering the newest cutting-edge information was essential to the field of oncology drug development.

Cambridge, MA and Los Angeles, CA,
30 June 2016

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Historical Perspective: What Makes Antibody–Drug Conjugates Revolutionary?

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Introduction

Developing drugs that are able to target disease and spare healthy tissue has been a long-time goal of both oncologic and non-oncologic drug development. Since the late nineteenth century, it has been recognized that effective treatment of disease by therapeutic agents is improved when therapeutics demonstrate selectiveness for foreign bodies (bacteria) or diseased cells and spare healthy cells. The development of novel and highly selective antibody–drug conjugates (ADCs) has moved us closer to this goal in cancer therapy (Figure 1). Agents such as trastuzumab emtansine (T-DM1) and brentuximab vedotin have shown promising results, particularly in patients with advanced disease who have progressed on other treatments. Combining cancer-specific antibody targets with potent cytotoxic therapies makes these agents revolutionary in their efforts to deliver potent treatments while minimizing adverse effects, coming closer to the "magic bullet" concept of Ehrlich and other early twentieth-century pharmacologists [1].

Early Work in Monoclonal Antibody Development: Ehrlich's Magic Bullets

Ehrlich and colleagues hypothesized that there may be antigens specific to tumors and bacteria that could be targeted with drugs for the treatment of cancer and infectious disease. Throughout the 1960s and 1970s, there was much work to develop specific antibodies that could be easily generated in large quantity and used for therapeutics. In a 1975 letter to the journal *Nature*,

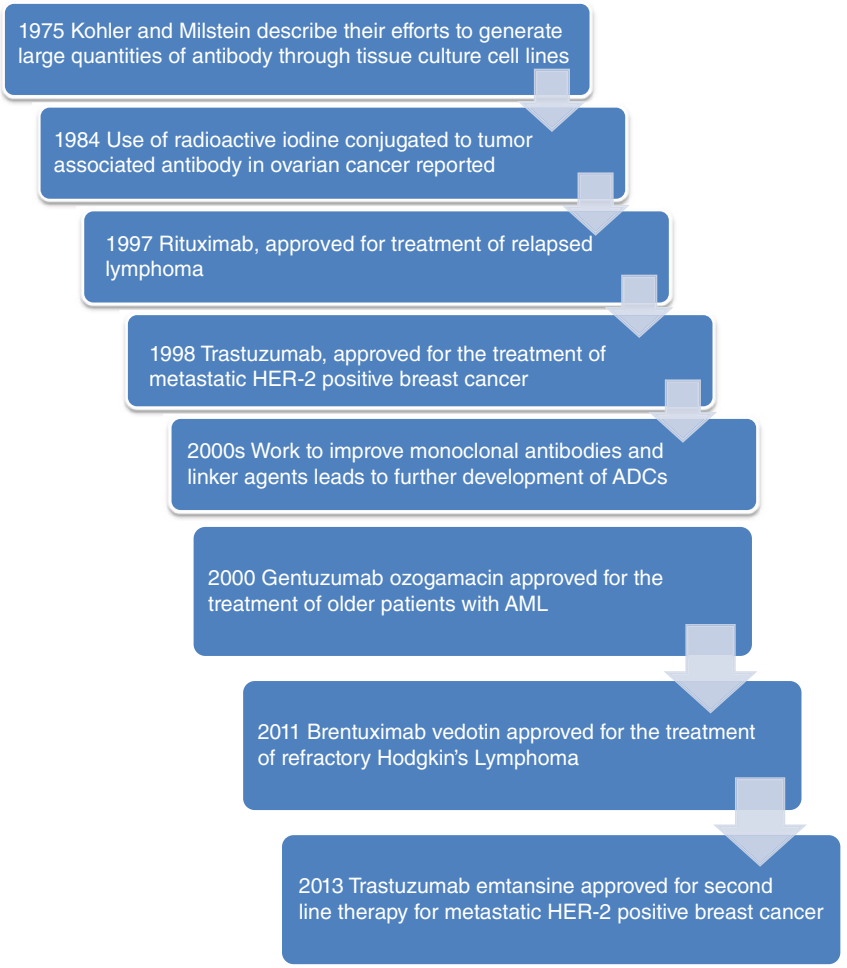

Figure 1 Timeline of events in development of ADCs.

Georges Kohler and César Milstein described the development of a mechanism to generate large quantities of antibodies with a defined specificity by fusing myeloma cells that reproduce easily in cell culture with mouse spleen cells that are antibody-producing cells [2]. By combining these two types of cells, a continuous supply of specific antibody was produced in quantities sufficient for use as therapeutic agents. As with the production of other human proteins, the use of microbial agents for antibody production further advanced the field, as these methods were able to generate antibody and antibody fragments in the quantities needed for drug development [3–5].

Subsequent work demonstrated that monoclonal antibodies could be used to identify and characterize the multiple different types of surface receptors found on cells [6, 7]. These receptors could then be used as targets for cancer therapeutics with better tumor specificity and potentially less toxicity.

Use of Monoclonal Antibodies to Identify and Treat Cancer

Early on, the potential for monoclonal antibodies in the detection and treatment of cancer was recognized as promising [8, 9]. The use of antibodies to improve tumor localization was of great interest in the 1970s and 1980s and was a first step in transitioning the use of these antibodies from tumor identification to tumor treatment [10]. Radioactive iodine was conjugated to a tumor-associated monoclonal antibody to effectively deliver cytotoxic doses of radiation to tumor sites in women with metastatic ovarian cancer with lower doses of radiation to surrounding tissues and the remainder of the body [11].

During the 1980s and 1990s, the development of monoclonal antibodies for therapeutic treatment of cancers delivered promising results. In 1997, rituximab, an anti-CD20 monoclonal antibody that targets malignant B cells, was initially approved for use in relapsed follicular lymphoma [12]. Trials demonstrated that in low-grade lymphomas, this agent had a response rate of 48%. Importantly, this therapy was relatively well tolerated with only 12% grade 3 and 3% grade 4 toxicity [13]. Subsequent trials established the role of rituximab in aggressive B-cell lymphomas as it significantly improved survival when added to standard chemotherapy [14–16].

Following the initial approval of rituximab, trastuzumab was approved in 1998 for the treatment of human epidermal growth factor receptor-2 (HER2) overexpressing metastatic breast cancer (MBC). Based on significant survival benefits in phase III clinical trials, this agent was approved in combination with paclitaxel for the first-line treatment of HER2 overexpressing MBC and as a single agent for those who had progressed on one or more previous chemotherapy regimens [17]. Similar to rituximab, trastuzumab was well tolerated with few side effects. The main safety signal reported was cardiomyopathy that was primarily seen when used in combination with anthracycline-containing regimens [18, 19]. Subsequently, a number of other agents were approved for use in solid tumor malignancies including those that target vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR). Table 1 is a comprehensive listing of monoclonal antibody that have been approved along with their approval dates and indications.

Although these agents have provided therapeutic benefits, there have been multiple efforts to enhance the efficacy of monoclonal antibodies. This has been done in a variety of ways including the development of monoclonal antibodies

Table 1 Monoclonal antibodies directed at malignant cell surface receptors.

Drug name	Target	Year approved	Initial indication
Rituximab	CD20	1997	Follicular lymphoma
Trastuzumab	HER2	1998	Metastatic HER2 overexpressing breast cancer
Alemtuzumab [20]	CD52	2001	CLL refractory to fludarabine
Cetuximab [21]	EGFR	2004	Metastatic colorectal cancer
Bevacizumab	VEGF-A	2004	Metastatic colorectal cancer
Panitumumab [22]	EGFR	2004	Metastatic colorectal cancer that is KRAS wild type and has progressed on a regimen containing a fluoropyrimidine and oxaliplatin or irinotecan
Ofatumumab [23]	CD20	2009	Refractory CLL
Obinutuzumab	CD20	2014	Combined with chlorambucil for the treatment of previously untreated patients with CLL
Ramucirumab	VEGF-2	2014	Patients with metastatic gastric or GE junction cancer that progressed on fluoropyrimidine- or platinum-containing regimen

Abbreviations: CLL, chronic lymphocytic leukemia; GE, gastroesophageal.

that target immune cells [24, 25], the development of bispecific monoclonal antibodies that target multiple cell surface receptors and link malignant cells with host immune cells [26], and the development of monoclonal antibodies through the conjugation of radioisotopes for the targeted delivery of cytotoxic radiation [27, 28]. Examples of these agents are found in Table 2.

Linking Monoclonal Antibodies with Cytotoxic Agents

The linkage of monoclonal antibodies to potent cytotoxic drugs is a further step toward enhancing the efficacy of these agents in cancer treatment. Although specific cell surface receptors on malignant cells may not be directly involved in tumor proliferation, receptors that are identified as unique to tumor cells can allow for targeted delivery of cytotoxic agents. An effective ADC consists of three primary components: a monoclonal antibody that recognizes a cell surface receptor that is expressed primarily on malignant cells, a linking agent, and a potent cytotoxic agent that is known as the "payload" [29].

Much work has been devoted to improving the linking molecule between the monoclonal antibody and the cytotoxic agent as this is a crucial component of

Table 2 Additional monoclonal antibodies approved for use.

Type of modification	Drug name	Target	Year approved
Immune cell surface receptors targeted to enhance immune response	Ipilimumab	CTLA-4	2011
	Nivolumab	PD-1	2014
	Pembrolizumab	PD-1	2015
Bispecific monoclonal antibody to link immune cell and malignant cell	Blinatumomab	CD3 and CD19	2014
Conjugate with radioisotope	Ibritumomab tiuxetan	CD20; linked to yttrium-90 for treatment	2002
	Iodine tositumomab	CD20	2003; as of February 2014, this drug has been discontinued by manufacturer and is no longer available

drug stability and potency. Effective linkers are able to maintain the cytotoxic agent on the monoclonal antibody such that it is trafficked to the targeted cancer cell and then transported into the cell where the link is then cleaved within the lysosome. This linkage allows potent cytotoxic whose dosing is limited by its toxicity to be delivered directly to malignant cells and improves the therapeutic index of these agents. Improvements in the identification and development of monoclonal antibodies to specific tumor cell targets, along with the type of cytotoxic agent and the linker used to conjugate the agents, have been critical in the development and improvement of ADC agents for use in oncology [30].

Antibody–Drug Conjugates in the Clinic

The first ADC approved for use in oncology was gemtuzumab ozogamicin (GO), a CD33 monoclonal antibody linked to a calicheamicin, a potent cytotoxic derived from bacteria. This agent was given accelerated approval based on phase II data and was approved from 2000 to 2010 for use in patients aged 60 and older with acute myeloid leukemia who were otherwise unable to be treated with standard induction chemotherapy. Food and Drug Administration (FDA) approval was withdrawn in 2010 as results from the SWOG S0106 study evaluating the use of GO combined with standard induction chemotherapy in patients younger than 60 years demonstrated no improvement in efficacy and

no difference in overall survival (OS), with a 5-year OS rate in the arm containing GO being 46–50% in the standard therapy arm [31]. This lack of survival benefit combined with toxicities observed post-approval including hepatotoxicity with severe veno-occlusive disease, infusion reactions including anaphylaxis, and pulmonary toxicity leading to Pfizer's voluntary withdrawal of the product in 2010. However, there are additional data demonstrating the benefit of this agent in acute promyelocytic leukemia and in those patients without adverse cytogenetic features [32]. Although this agent is no longer approved for routine clinical use, there may be a role for this drug in the treatment of specific subtypes and in specific populations of patients with acute myeloid leukemia [33].

Brentuximab vedotin, an ADC that links anti-CD30 activity with the antimitotic agent monomethyl auristatin E (MMAE), was the second agent approved in this class of drugs and was initially approved in 2011 for the use in refractory Hodgkin's disease (HD) and in anaplastic large-cell lymphoma (ALCL) [34, 35]. While early work on monoclonal antibodies targeting CD30 had demonstrated little therapeutic efficacy, the linkage of this antibody to the potent cytotoxic agent MMAE [36, 37] resulted in potent drug delivery to the target and enhanced treatment effect. Trials of this agent in patients who had relapsed after autologous stem cell transplant (ASCT) demonstrated an overall response rate of 75% with a complete remission in 34% of patients [38]. Subsequent trials have demonstrated the efficacy of this agent as consolidation therapy after ASCTs in patients with Hodgkin's disease who are at high risk of relapse [39]. This agent has shown significant efficacy in those patients with high-risk Hodgkin's disease as well as those with ALCLs where initial trials of naked monoclonal antibodies to CD30 demonstrated little to no efficacy [40].

Shortly after the approval of brentuximab vedotin, trastuzumab emtansine was approved in February 2013 for the treatment of HER2-positive MBC that had progressed on trastuzumab-based therapy [41]. This agent used the already effective monoclonal antibody to HER2, trastuzumab, and linked the antibody to the potent cytotoxic DM1, a maytansinoid, which is a microtubule depolymerizing agent [42]. OS with this agent in patients who had progressed on prior therapy with trastuzumab and taxane was improved by 5.8 months when compared to capecitabine and lapatinib. This agent is a significant advance for patients who have MBC that has progressed on standard anti-HER2 regimens and is well tolerated without significant alopecia or neuropathy.

Table 3 demonstrates the clinical trials and settings where each of these agents has been or is currently being evaluated. As of 1 June 2015, over 200 clinical trials evaluating ADCs across a variety of hematological and solid tumor malignancies were listed on clinical trials.gov. For both brentuximab vedotin and trastuzumab emtansine, successful use of these therapies in patients with recurrent or refractory disease has prompted evaluation of the use of these agents earlier in disease course. Data from these pivotal trials will help us to better understand the role of these agents at various stages of the treatment trajectory.

Table 3 Clinical trials evaluating brentuximab vedotin and trastuzumab emtansine.

	Trial	Study design	Results
Brentuximab vedotin	Phase II evaluation of brentuximab vedotin in patients with relapsed or refractory Hodgkin's disease after ASCT	Single-arm study evaluating safety and efficacy	CR 34% ORR 75% Median PFS 5.6 months
	Phase II evaluation of brentuximab vedotin in patients with relapsed or refractory anaplastic large-cell lymphoma	Single-arm study evaluating safety and efficacy	CR 57% ORR 86% Median PFS 13.3 months
	Phase III evaluation of brentuximab vedotin with doxorubicin, dacarbazine, and vinblastine vs. doxorubicin, bleomycin, dacarbazine, and vinblastine as frontline treatment for advanced Hodgkin's disease	RCT evaluating upfront brentuximab in place of bleomycin in the standard regimen for Hodgkin's disease	Study ongoing
	ECHELON-2 Phase III evaluation of brentuximab vedotin with doxorubicin, cyclophosphamide, and prednisone vs. doxorubicin, cyclophosphamide, vincristine, and prednisone for CD30+ peripheral T-cell lymphoma	RCT evaluating upfront brentuximab in place of vincristine for mature T-cell lymphoma	Study ongoing
	AETHERA: Phase III evaluation of brentuximab vedotin in patients at risk for relapse or progression after ASCT	RCT comparing brentuximab vs. best supportive care in patients at high risk for relapse after ASCT (primary refractory Hodgkin's lymphoma, relapsed Hodgkin's lymphoma with initial remission duration of ≤12 months, or extranodal involvement)	Median PFS 42.9 months vs. 24.1 months No difference in OS, however, 85% of those in the placebo arm went on to receive brentuximab vedotin as a subsequent line of therapy at progression
	Phase III trial of brentuximab vedotin vs. physician's choice in patients with CD30 positive cutaneous T-cell lymphoma	Randomization to brentuximab vedotin vs. physician's choice (methotrexate or bexarotene)	Study ongoing

(*Continued*)

Table 3 (Continued)

	Trial	Study design	Results
Trastuzumab emtansine	ATEMPT: Phase II trial of T-DM1 vs. trastuzumab and paclitaxel for adjuvant therapy for stage I breast cancer	Randomization to T-DM1 or trastuzumab and paclitaxel	Study ongoing
	KATHERINE: Phase III trial of T-DM1 vs. trastuzumab for those who do not achieve a pathologic complete response with neoadjuvant chemotherapy for HER2 overexpressed breast cancer	Those who have residual disease after preoperative chemotherapy with trastuzumab and taxane are randomized to receive T-DM1 or trastuzumab to complete 1 year of adjuvant HER2 targeted therapy	Study ongoing
	MARIANNE: Phase III trial of T-DM1 and pertuzumab vs. T-DM1 vs. trastuzumab and taxane	Randomization of initial treatment for HER2 overexpressed metastatic disease to one of three treatment arms: T-DM1 and pertuzumab, T-DM1, or trastuzumab and taxane	Study ongoing PFS: HT 13.7 months T-DM1 14.1 months T-DM1 + P 15.2 months
	EMILIA: Phase III trial of T-DM1 vs. lapatinib and capecitabine in patients who had progressed on trastuzumab and taxane	Randomized, open-label trial to T-DM1 vs. lapatinib and capecitabine	Median OS: T-DM1 30.9 months Lapatinib + capecitabine 25.1 months
	TH3RESA: Phase III clinical trial of T-DM1 vs. physician's choice in pretreated HER2 positive advanced breast cancer [43]	Randomized, open-label trial to T-DM1 vs. PC in patients who had received at least two lines of HER2 targeted therapy including trastuzumab and lapatinib	PFS: T-DM1 6.2 months PC 3.3 months OS: T-DM1 22.7 months PC 15.8 months

Abbreviations: ASCT, autologous stem cell transplant; CR, complete response; ORR, overall response rate; OS, overall survival; PC, physician's choice; PFS, progression free survival.

Why ADCs Are Revolutionary?

The primary goal of drug development is the creation of therapeutic agents that are effective at treating disease while minimizing the effects of the treatment on healthy tissue. This goal is closer to being reached in oncology with the successful development of ADCs that can deliver potent cytotoxic therapy to targeted malignant cells. Clinical validation of this concept has been demonstrated with two recently approved agents in cancer: brentuximab vedotin and trastuzumab emtansine. In addition, there is an exciting pipeline of multiple ADCs that are in various stages of clinical development, including agents for triple-negative breast cancer [44], platinum-resistant ovarian cancer [45], glioblastoma [46], as well as additional solid tumor and hematological malignancies. These agents move us closer to the realization of the goal of "magic bullets" that Ehrlich and colleagues conceptualized in the early twentieth century and offer exciting potential as agents that improve treatment efficacy while reducing toxicity, leading to improvements in both survival and quality of life in patients with cancer.

References

1 Strebhardt K, Ullrich A. Paul Ehrlich's magic bullet concept: 100 years of progress. *Nature Reviews Cancer* 2008;**8**:473–80.
2 Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975;**256**:495–7.
3 Harrison JS, Keshavarz-Moore E. Production of Antibody Fragments in Escherichia coli. *Annals of the New York Academy of Sciences* 1996;**782**:143–58.
4 Humphreys DP. Production of antibodies and antibody fragments in Escherichia coli and a comparison of their functions, uses and modification. *Current Opinion in Drug Discovery and Development* 2003;**6**:188–96.
5 Itakura K, Hirose T, Crea R, *et al*. Expression in Escherichia coli of a chemically synthesized gene for the hormone somatostatin. *Science (New York, NY)* 1977;**198**:1056–63.
6 Williams AF, Galfre G, Milstein C. Analysis of cell surfaces by xenogeneic myeloma-hybrid antibodies: differentiation antigens of rat lymphocytes. *Cell* 1977;**12**:663–73.
7 Milstein C, Lennox E. The use of monoclonal antibody techniques in the study of development cell surfaces. *Current Topics in Developmental Biology* 1980;**14**:1–32.
8 Ritz J, Schlossman SF. Utilization of monoclonal antibodies in the treatment of leukemia and lymphoma. *Blood* 1982;**59**:1–11.
9 Macek C Monoclonal antibodies: key to a revolution in clinical medicine. *JAMA* 1982;**247**:2463–70.

10 Goldenberg DM, Kim EE, DeLand FH, Bennett S, Primus FJ. Radioimmunodetection of cancer with radioactive antibodies to carcinoembryonic antigen. *Cancer Research* 1980;**40**:2984–92.

11 Antibody-guided irradiation of malignant lesions: three cases illustrating a new method of treatment. A report from the Hammersmith Oncology Group and the Imperial Cancer Research Fund. *Lancet* 1984;**1**:1441–3.

12 US Food and Drug Administration. 1997. (Accessed May 16, 2015, at http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/ucm107740.pdf.)

13 McLaughlin P, Grillo-Lopez AJ, Link BK, *et al*. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology* 1998;**16**:2825–33.

14 Habermann TM, Weller EA, Morrison VA, *et al*. Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology* 2006;**24**:3121–7.

15 Coiffier B, Thieblemont C, Van Den Neste E, *et al*. Long-term outcome of patients in the LNH-98.5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: a study by the Groupe d'Etudes des Lymphomes de l'Adulte. *Blood* 2010;**116**:2040.

16 Coiffier B, Lepage E, Briere J, *et al*. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *The New England Journal of Medicine* 2002;**346**:235–42.

17 FDA Approval Letter for Trastuzumab. 1998. (Accessed May 17, 2015, at http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/ucm091360.pdf.)

18 Baselga J, Tripathy D, Mendelsohn J, *et al*. Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology* 1996;**14**:737–44.

19 Cobleigh MA, Vogel CL, Tripathy D, *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. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology* 1999;**17**:2639–48.

20 FDA Approval Letter for Alemtuzumab. 2001. (Accessed June 21, 2016, at http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/ucm088605.pdf.)

21 US Food and Drug Administration *FDA Approves Erbitux for Colorectal Cancer*. US Food and Drug Administration; 2004.
22 Giusti RM, Shastri KA, Cohen MH, Keegan P, Pazdur R. FDA drug approval summary: panitumumab (Vectibix). *Oncologist* 2007;**12**:577–83.
23 US Food and Drug Administration *FDA Approves New Treatment for Chronic Lymphocytic Leukemia*. FDA; 2009.
24 Fong L, Small EJ. Anti-cytotoxic T-lymphocyte antigen-4 antibody: the first in an emerging class of immunomodulatory antibodies for cancer treatment. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology* 2008;**26**:5275–83.
25 Ascierto PA, Kalos M, Schaer DA, Callahan MK, Wolchok JD. Biomarkers for immunostimulatory monoclonal antibodies in combination strategies for melanoma and other tumor types. *Clinical Cancer Research* 2013;**19**:1009–20.
26 Topp MS, Kufer P, Gokbuget N, *et al*. Targeted therapy with the T-cell-engaging antibody blinatumomab of chemotherapy-refractory minimal residual disease in B-lineage acute lymphoblastic leukemia patients results in high response rate and prolonged leukemia-free survival. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology* 2011;**29**:2493–8.
27 Witzig TE, Gordon LI, Cabanillas F, *et al*. Randomized controlled trial of yttrium-90-labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology* 2002;**20**:2453–63.
28 Davies AJ, Rohatiner AZ, Howell S, *et al*. Tositumomab and iodine I 131 tositumomab for recurrent indolent and transformed B-cell non-Hodgkin's lymphoma. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology* 2004;**22**:1469–79.
29 Panowksi S, Bhakta S, Raab H, Polakis P, Junutula JR. Site-specific antibody drug conjugates for cancer therapy. *MAbs* 2014;**6**:34–45.
30 Nolting, B Linker technologies for antibody–drug conjugates. In: Ducry L, ed. *Methods in Molecular Biology*, 1064–3745; 1045. New York: Humana Press; 2013.
31 Petersdorf SH, Kopecky KJ, Slovak M, *et al*. A phase 3 study of gemtuzumab ozogamicin during induction and postconsolidation therapy in younger patients with acute myeloid leukemia. *Blood* 2013;**121**:4854–60.
32 Hills RK, Castaigne S, Appelbaum FR, *et al*. Addition of gemtuzumab ozogamicin to induction chemotherapy in adult patients with acute myeloid leukaemia: a meta-analysis of individual patient data from randomised controlled trials. *Lancet Oncology* 2014;**15**:986–96.
33 O'Hear C, Rubnitz JE. Recent research and future prospects for gemtuzumab ozogamicin: could it make a comeback? *Expert Review of Hematology* 2014;**7**:427–9.

34 US Food and Drug Administration. *FDA Approves Adcetris to Treat Two Types of Lymphoma*. FDA; 2011.

35 Pro B, Advani R, Brice P, *et al*. Brentuximab vedotin (SGN-35) in patients with relapsed or refractory systemic anaplastic large-cell lymphoma: results of a phase II study. *Journal of Clinical Oncology* 2012;**30**:2190–6.

36 Foyil KV, Bartlett NL. Anti-CD30 Antibodies for Hodgkin lymphoma. *Current Hematologic Malignancy Reports* 2010;**5**:140–7.

37 Forero-Torres A, Leonard JP, Younes A, *et al*. A Phase II study of SGN-30 (anti-CD30 mAb) in Hodgkin lymphoma or systemic anaplastic large cell lymphoma. *British Journal of Haematology* 2009;**146**:171–9.

38 Younes A, Gopal AK, Smith SE, *et al*. Results of a pivotal phase II study of brentuximab vedotin for patients with relapsed or refractory Hodgkin's lymphoma. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology* 2012;**30**:2183–9.

39 Moskowitz CH, Nademanee A, Masszi T, *et al*. Brentuximab vedotin as consolidation therapy after autologous stem-cell transplantation in patients with Hodgkin's lymphoma at risk of relapse or progression (AETHERA): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2015; **385**:1853.

40 Younes A, Bartlett NL, Leonard JP, *et al*. Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. *The New England Journal of Medicine* 2010;**363**:1812–21.

41 Verma S, Miles D, Gianni L, *et al*. Trastuzumab emtansine for HER2-positive advanced breast cancer. *The New England Journal of Medicine* 2012;**367**:1783–91.

42 Lewis Phillips GD, Li G, Dugger DL, *et al*. Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate. *Cancer Research* 2008;**68**:9280–90.

43 Krop IE, Kim S-B, González-Martín A, *et al*. Trastuzumab emtansine versus treatment of physician's choice for pretreated HER2-positive advanced breast cancer (TH3RESA): a randomised, open-label, phase 3 trial. *Lancet Oncology*;**15**:689–99.

44 Yardley DA, Weaver R, Melisko ME, *et al*. EMERGE: a randomized Phase II study of the antibody-drug conjugate glembatumumab vedotin in advanced glycoprotein NMB-expressing breast cancer. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology* 2015;**33**:1609–19.

45 Pipleline. (Accessed May 18, 2015, at http://www.gene.com/medical-professionals/pipeline.)

46 Hamblett KJ, Kozlosky CJ, Siu S, *et al*. AMG 595, an anti-EGFRvIII antibody drug conjugate, induces potent anti-tumor activity against EGFRvIII expressing glioblastoma. *Molecular Cancer Therapeutics* 2015.

Part I

What is an Antibody–Drug Conjugate

1

Typical Antibody–Drug Conjugates

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1.1 Introduction

1.1.1 A Simple Concept

Ever since cancer patients were first treated with cytotoxic agents with the goal of eradicating the tumor tissue, oncologists have looked to widen the therapeutic window for these agents. The goal of combination chemotherapy, pioneered by Emil "Tom" Frei and others [1], was to increase antitumor efficacy of cytotoxic drug therapy, without substantially increasing overall toxicity to the patient, by using agents with nonoverlapping dose-limiting toxicities. However, such modalities have proven only partially effective at the maximum achievable doses, limited by the severe side effects of the cytotoxic agents used. Attaching cytotoxic effector molecules to an antibody to form an antibody–drug conjugate (ADC) provides a mechanism for the selective delivery of the cytotoxic payload to cancer cells via the specific binding of the antibody moiety to cancer-selective cell surface molecules. This simple concept was thought to be a particularly attractive solution to the challenge of finding a way to increase the therapeutic window of the cytotoxic agent (Figure 1.1). Furthermore, conjugation of a small molecular weight cytotoxic agent to a large hydrophilic antibody protein is expected to restrict penetration of the cytotoxic compound across cellular membranes of antigen-negative normal cells, providing an additional mechanism by which the therapeutic index of the small molecule cytotoxin is widened, beyond that of targeted delivery. Thus, from the perspective of a medicinal chemist, an ADC is a prodrug that can only be activated within tumor cells and is excluded from normal cells by virtue of conjugation to a protein. In addition, giving the *in vivo* distribution properties of an antibody to the small molecular weight cytotoxic agent has the potential to reduce its systemic toxicity.

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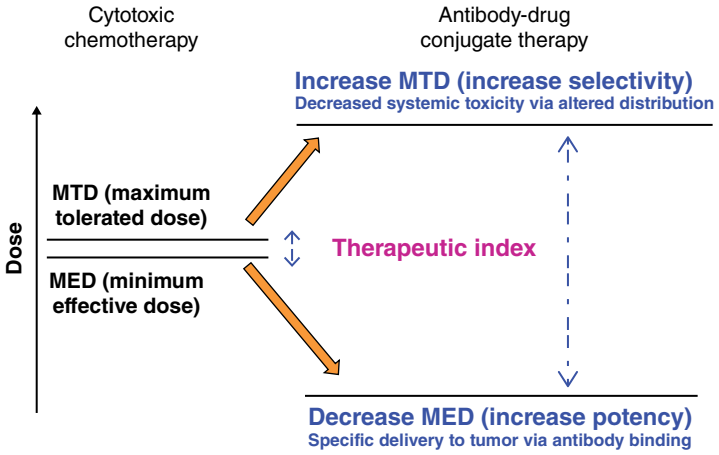


Figure 1.1 Increasing the therapeutic index of cytotoxic drugs by conjugation to antibodies.

1.1.2 Turning Antibodies into Potent Anticancer Compounds

There is another way to look at the simple concept of an ADC. Ever since the advent of monoclonal antibody technology [2], a focus of cancer research has been to develop antibodies for anticancer therapy. Indeed, four monoclonal antibodies, rituximab, trastuzumab, cetuximab, and bevacizumab, are among the most commercially successful anticancer drugs [3]. However, many more antibodies to a variety of target antigens have been tested, both in preclinical studies and in clinical trials, and have proven to have insufficient anticancer activity to be developed as therapeutic agents. In general, the immunologic mechanisms for killing malignant cells induced upon binding of antibodies to cell surface antigens present in cancers appear to be insufficient to affect significant reduction in tumor cell burden in most instances. Thus, providing an additional killing mechanism to such anticancer antibodies via conjugation to cytotoxic agents was thought to be a solution to their lack of potency. From the perspective of an immunologist, enhancing antibody activity by creating ADCs was one approach to be able to fully exploit the full potential of their exquisite specificity toward tumor cells [4–6].

1.1.3 What is a Typical ADC and How Does it Act?

A typical ADC consists of several molecules of a potent cytotoxic agent (generally in the range of two to six molecules per antibody molecule on average), which are linked covalently to side chains of particular amino acid residues of a monoclonal antibody (Figure 1.2). The chosen linker chemistry should be

- **ADC components:**
  An integrated system
  - Targeting antibody
  - Cytotoxic agent
  - Linker

- **ADC mechanism:**
  - Binds to target on surface of cancer cell
  - Internalized into the cell
  - Cytotoxic agent is released inside the cell
  - Cytotoxic agent affects its target (eg., DNA; tubulin)
  - Cell death

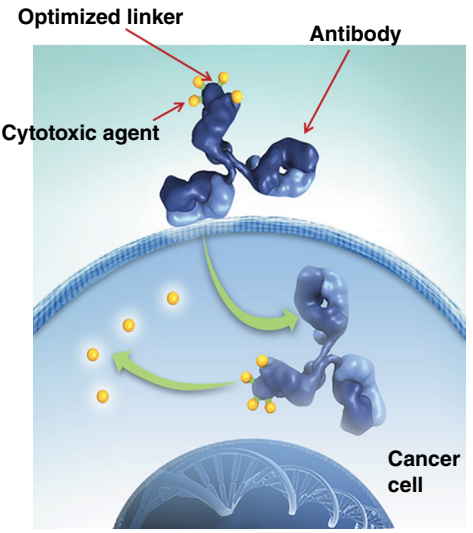


Figure 1.2 The components of an ADC and its mechanism of action. (*See color plate section for the color representation of this figure.*)

sufficiently stable during *in vivo* circulation in the bloodstream so that the payload stays linked to the antibody during the time it takes for the antibody to distribute into tissues, yet must allow release of an active cytotoxic compound once the ADC is taken up by cancer cells within tumor tissue. Once at the tumor, the antibody component of the ADC binds specifically to its target antigen on cancer cells; in the case of a typical ADC, the cytotoxic payload is liberated after internalization of the antibody–antigen complex and routing to the relevant intracellular compartment for release of an active cytotoxic compound from the ADC (Figure 1.2).

1.1.4 Simple Concept, but Not So Simple to Execute

The earliest notion in the field of ADC research was that conjugation to specific monoclonal antibodies was a way to widen the therapeutic window of existing chemotherapeutic drugs, such as the *vinca* alkaloids [7], and doxorubicin [8], following on from the early attempts to provide specificity to cytotoxic drugs by conjugation to serum immunoglobulins [9]. However, despite the early optimism generated by some of the preclinical results [8], the results of clinical trials of such conjugates were disappointing [10–12]. During the 1980s, increased knowledge of the biodistribution properties of monoclonal antibodies based on clinical dosimetry measurements with radiolabeled antibodies pointed to one explanation for such disappointing results.

It was found that the amount of antibody that could be localized to a solid tumor 24 h after administration, a time corresponding approximately to the peak delivered concentration, was only about 0.01% of the injected dose of antibody per gram of tumor tissue for a range of different antibodies, to a variety of targets in patients with a variety of tumor types [13]. Thus, it was reasoned that the lack of clinical benefit from ADCs made with conventional chemotherapeutic drugs was that not enough of these agents could be localized at the tumor via antibody-mediated delivery to have an antitumor effect. The use of these only moderately cytotoxic compounds as payloads for ADCs was at least one of the barriers to the successful execution of the ADC concept. The idea that conventional chemotherapeutic drugs were not potent enough to serve as payloads for ADCs has guided much of the subsequent research in the field [4–6].

1.2 The Building Blocks of a Typical ADC

All three parts of an ADC, the antibody, the cytotoxic payload, and the linker chemistry that joins them together, are important in designing an ideal ADC. The design goal is to add the potent tumor cell-killing mechanism afforded by the payload, while retaining all the favorable properties of the antibody in terms of *in vivo* pharmacokinetics and biodistribution, together with any intrinsic biologic activity and immunologic properties. It is beyond the scope of this chapter to discuss the properties of the cell surface target molecule, but suffice to say that selecting the right target, and matching the design of the ADC to the properties of the target, is vital to the creation of an effective therapeutic agent.

1.2.1 The Antibody

The first monoclonal antibodies used in ADCs and also in immunotoxins – antibodies conjugated to potent protein toxins such as derivatives of ricin, or diphtheria toxin [14] – were murine antibodies. However, apart from other limitations, such conjugates proved to be immunogenic in humans [10]. The advent of chimerization and a variety of humanization techniques (CDR grafting, resurfacing) for rendering murine antibodies less immunogenic or nonimmunogenic in humans [15], and the methods for cloning of human immunoglobulin genes into a variety of organisms, such as transgenic animals, bacteriophage, or yeast, for the generation of fully human antibodies [16–18], have largely addressed this problem (Figure 1.3), as has been generally borne out by the recent clinical experience with ADCs [19]. Of the 51 ADCs currently in clinical trials, at least two utilize chimeric antibodies, including the approved ADC, brentuximab vedotin, while for the other ADCs, antibody usage is, where known, fairly evenly split between humanized antibodies and fully human antibodies. Several of the humanizations were done by the method of variable domain resurfacing [15], for

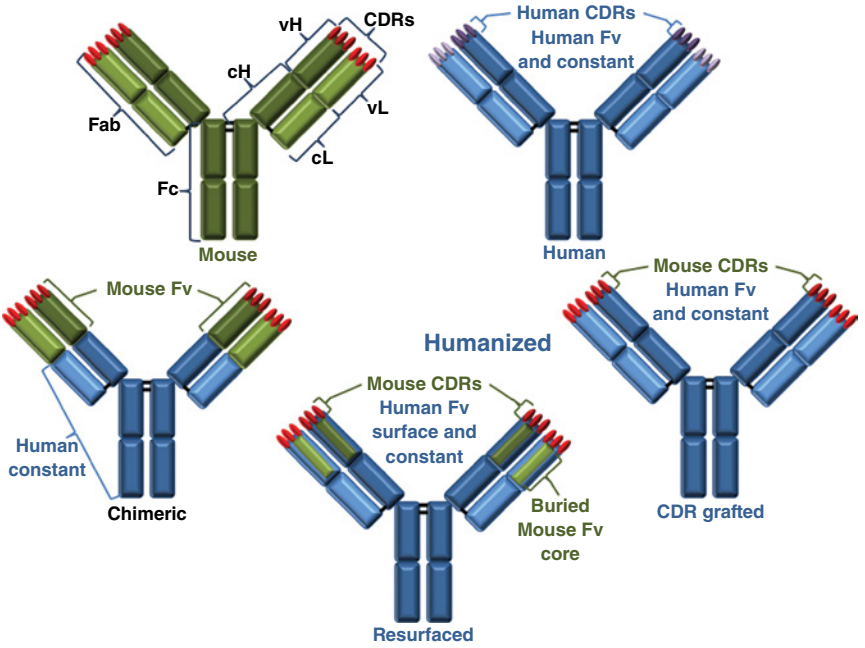


Figure 1.3 Schematic representations of a mouse (green) and a fully human (blue) monoclonal antibody, together with a chimeric antibody, and those humanized by complementarity-determining region (CDR) grafting and by variable domain resurfacing methodologies (mixed green and blue). The antibody sub-domains are indicated on the mouse antibody, including the Fab fragment, the Fc fragment, the heavy-chain (vH) and light-chain (vL) variable regions, the heavy-chain (cH) and light-chain (cL) constant regions, and the CDRs. The light chains are represented in a lighter shade of color than the heavy chains. CDRs derived from murine antibodies are in red, while CDRs generated on human IgG backbone sequences are in purple. (*See color plate section for the color representation of this figure.*)

example, the anti-CanAg antibody, cantuzumab, utilized in the first maytansinoid ADC (cantuzumab mertansine) to enter into clinical trials [20]. Recently, however, the World Health Organization decided to alter criteria for providing generic names to antibodies, resulting in the confusing situation of many humanized antibodies being given names bearing the suffix of a chimeric antibody ("-ximab"), for example, the anti-CD19 antibody, coltuximab [15, 21], and the antifolate receptor alpha (anti-FRα) antibody, mirvetuximab [22], both of which were humanized by the resurfacing method [15, 23].

1.2.1.1 Antibody Isotype in ADCs

Most of the antibodies utilized in ADCs evaluated in clinical trials to date, including those (about 20) now discontinued, have been of the human IgG_1 isotype (60 of 67 ADCs, with an additional four not disclosed, upon this author's

review of source information). In general, the Fc regions of these IgG_1 antibodies are unmodified with respect to Fc receptor binding properties so that all could be capable of inducing immune effector cell killing or complement-mediated cytotoxicity (Figure 1.4). However, at least one ADC was designed with an IgG_1 antibody having enhanced FcγR (FcγRIIIa) binding for enhanced antibody-dependent cellular cytotoxicity (ADCC) activity by virtue of being produced in an afucosylated form [24]. Thus far, no ADC with a human IgG_1 isotype in clinical development has employed an antibody with amino acid mutations known to abrogate FcγR binding, despite some speculation that such modifications may reduce certain toxicities observed in clinical trials with some ADCs [25]. Indeed, where abrogation of FcγR binding was part of the stated design goal of the resulting ADC, the IgG_4 format has been the preferred option to date, known to be used in three ADCs currently in clinical trials (gemtuzumab ozogamicin, inotuzumab ozogamicin, and indatuximab ravtansine). At least three ADCs have employed a human IgG_2 antibody, all of which were fully human antibodies generated in transgenic mice engineered to express human immunoglobulin genes in place of the corresponding mouse genes [17].

1.2.1.2 Functional Activity of the Antibody Moiety in ADCs

Antibodies for ADCs may be developed to targets where the antibody may have functional activity beyond intrinsic immunological functions of ADCC, ADCP, or CDC. The primary exemplar of this would be the approved ADC, ado-trastuzumab emtansine, wherein the antibody component, trastuzumab, inhibits HER2-driven cell growth in HER2-positive (overexpressing) breast cancer [26]. In this case, arming the antibody with a payload provides an additional mechanism for cancer cell killing over and above its intrinsic biologic and immunologic activities (Figure 1.4). In another example, antibody selection for an ADC that targets CD37 (IMGN529) was based on screening for those antibodies that could directly induce apoptotic cell death in CD37-positive tumor B cell lines. The antitumor activity of the antibody was then further augmented by arming it with a payload to create the ADC compound that was taken into clinical development [27]. For targets that have no signaling function, one would not anticipate finding antibodies that can induce any biologic function upon binding to the target, saving perhaps for immunologic effector functions triggered by antibody binding to the cell surface. In general, antibodies whose only function upon binding to tumor cells is to induce ADCC and/or ADCP often exhibit very little antitumor activity in clinical trials, sparking efforts to enhance effector functions [28]. Most ADCs in development are to such targets, where arming the antibodies with a payload to exploit their specific binding to cells is one way to provide them with a direct cell-killing function. For these targets, the antibodies should be selected for the property of efficient payload delivery, as in the example of an ADC designed to target FRα, IMGN853, recently named mirvetuximab soravtansine [22].

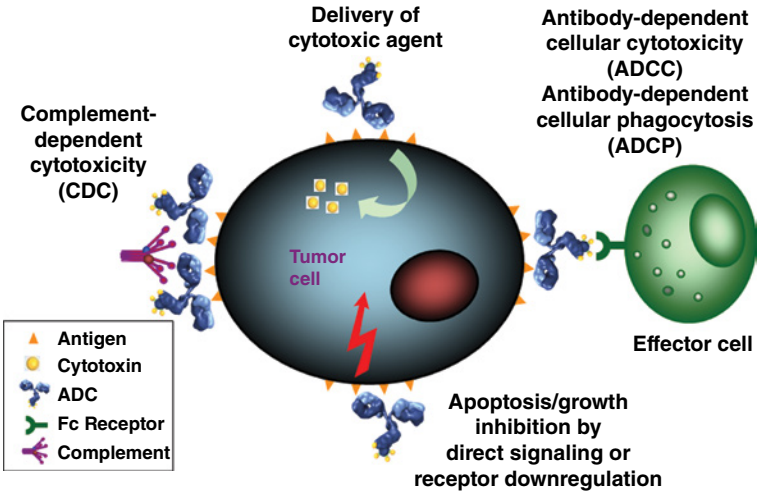


Figure 1.4 Potential cell-killing mechanisms for an ADC. Illustration of the mechanisms by which an ADC can effect cell death. For some targets and some antibodies, only the payload delivery mechanism of cell killing is operative. For other targets and antibodies, one or more of the biologic or immunologic mechanisms may also contribute to the overall activity of an ADC. (*See color plate section for the color representation of this figure.*)

Apart from specificity for their target, antibodies should bind with sufficient affinity for good retention at the tumor *in vivo*. Typically, the apparent binding affinities of the antibody component of most ADCs currently in clinical evaluation are in the range of about 0.1 to 1.0 nM. However, there is little published data regarding what the optimal binding affinity should be for an ADC. Some studies with antibodies suggest that very high affinity may compromise delivery of antibodies throughout solid tumors [29], although such findings may depend on target biology and tumor type. Since typical ADCs are designed to require intracellular release of an active payload, the antibody should be internalized upon binding to its target [30–33].

1.2.2 The Payload

For an ADC to exhibit potent antitumor activity, the cytotoxic agent that serves as the payload must be active at killing cells at the intracellular concentrations achievable within tumor cells by antibody-mediated distribution into tumor tissue followed by target-mediated uptake into tumor cells. As the constraints on payload delivery via antibody-mediated distribution and cellular uptake became better understood [13], it was reasoned that the cytotoxic compounds suitable for ADC approaches should have potency in the

picomolar range [4–6]. The structures of several highly potent cytotoxic compounds that are currently being used as payloads for ADCs are shown in Figure 1.5. All but calicheamicin, of those shown in Figure 1.5, were (or, in the case of SJG-136, are still being) evaluated in clinical trials, and all proved too toxic, with limited antitumor activity at the achievable maximum tolerated doses [5].

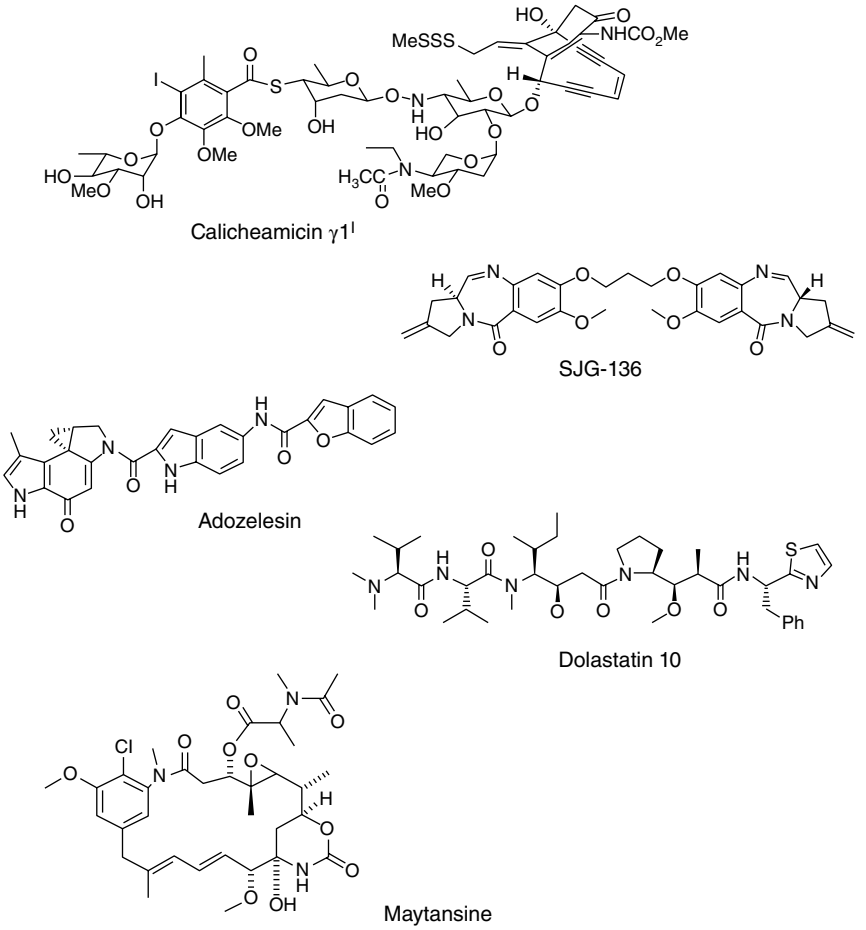


Figure 1.5 Structures of highly cytotoxic compounds developed as payloads for ADCs. Calicheamcin antibiotics cause DNA double-stranded breaks via a radical mechanism, SJG-136, a pyrrolobenzodiazepine dimer, alkylates and cross-links DNA, and the duocarmycin, adozelesin, alkylates DNA [5]. Dolastatin 10 and maytansine are potent tubulin-interacting compounds that disrupt microtubule dynamics [5, 34].

1.2.2.1 DNA-Targeting Payloads

The first ADC to receive marketing approval by FDA, gemtuzumab ozogamicin [35], used calicheamicin as the payload, a potent DNA-targeting agent that causes double-stranded breaks in the DNA resulting in cell death [5]. However, in 2010, it was withdrawn from the US market by the sponsor, 10 years after its initial approval for treating acute myeloid leukemia (AML), following an unsuccessful confirmatory phase III trial [36] and unacceptable safety profile. Subsequently, results from other trials utilizing dose fractionation have suggested patient benefit and have revived interest in this compound [37], and also in CD33 as an ADC target for AML [38]. Calicheamicin is known to be used as the payload in at least two other ADCs in current clinical testing, inotuzumab ozogamicin that targets CD22 on malignant B cells and that is in a phase III trial for treating acute B-cell leukemia [39], and an ADC that targets EphA4, a marker expressed on the cell surface of tumor stem cells in certain solid tumors [40], that is being evaluated in a phase I trial.

Another potent class of DNA-targeting agent are derivatives of the anticancer agent, SJG-136 (Figure 1.5), a pyrrolobenzodiazepine (PBD) dimer [41] that cross-links DNA, which are being assessed as payloads for three ADCs in ongoing clinical trials (e.g., see references [38] and [42]). Others include the camptothecin analog SN38 that is the payload for two ADCs [43], and a duocarmycin, a member of a family of DNA-alkylating antibiotics which includes adozelesin (Figure 1.5), that is, the payload of an ADC targeting HER2 [44]. Recently, a potent DNA-alkylating indolinobenzodiazepine dimer has been developed as a payload for ADCs, the first of which, IMGN779, entered into clinical testing in early 2016 [45].

1.2.2.2 Payloads Targeting Tubulin

Although these DNA-acting cytotoxins have the desired attribute of extraordinary high potency to be effective as an ADC payload, such compounds do have drawbacks. In general, DNA-interacting compounds are hydrophobic and may lack sufficient solubility in aqueous conditions for facile conjugation to antibodies, and some (e.g., duocarmycins) may not be stable in aqueous environments, thus requiring the use of prodrug approaches to protect the DNA-alkylating function [44]. These factors may explain why, even though the first ADC to receive approval utilized calicheamicin as the payload [35], only 11 of the 51 ADCs in clinical development at the time of writing utilize DNA-targeting compounds as payloads. Currently, the most important classes of ADC payload are potent tubulin-acting agents, which are used in 37 of the 51 ADCs in development (the payloads for three of the 51 ADCs have not yet been publicly disclosed). There are two main classes of these potent tubulin-acting agents in widespread use in ADCs undergoing clinical testing. Where the payload structures are disclosed ($n = 37$), 60% use auristatins, analogs of dolastatin 10, while 35% utilize derivatives of maytansine (Figure 1.5).

The binding of auristatins or maytansinoids to tubulin interferes with microtubule dynamicity, causing cells to arrest in the G2/M phase of the cell cycle, which ultimately results in apoptotic cell death [31, 34, 46]. Since these agents act as antimitotic agents because of their effect at disrupting the mitotic spindle, they have a natural selectivity for rapidly dividing cells. In the context of an ADC, this attribute of a payload may bring an additional level of selectivity beyond that provided by the specific binding of the antibody moiety. Target antigens are rarely completely tumor specific, their selectivity being based on differential expression on tumor versus normal cells rather than the complete absence of expression on normal cells. In any case, in most circumstances, most of the administered antibody is eventually removed from circulation for catabolism via cells of the reticuloendothelial system with only a small portion of the injected material passing through and being retained in tumor tissue [13]. Thus, the lack of cytotoxicity of these potent microtubule-acting compounds toward nondividing, or only slowly dividing, normal cells may contribute to the tolerability of ADCs made using them as payloads.

1.2.3 Linker Chemistries

An optimal linker should be sufficiently stable in circulation in the bloodstream to take advantage of the pharmacokinetic properties of the antibody moiety (the long half-life), yet should allow efficient release of an active cytotoxic compound within the tumor cell. Linkers used in typical ADCs can be characterized as either cleavable or noncleavable. The only mechanism of release of an active metabolite from an ADC utilizing noncleavable linker chemistry is by the complete proteolysis of the antibody moiety down to its constituent amino acids, which requires that following antigen-mediated internalization of the ADC, it is trafficked to lysosomes for proteolytic degradation. The active cytotoxic metabolite is thus appended with an amino acid residue, a lysine or a cysteine residue in a typical ADC – the site of attachment of the payload to the antibody via the linker. The necessity for sufficient lysosomal trafficking of the ADCs designed with noncleavable linkers means that lysosomal trafficking becomes a key selection criterion for the antibody and its target for ADCs of this design [33].

Cleavable linkers are those whose structure includes a mechanism of cleavage of chemical bonds between the amino acid attachment site on the antibody and the payload, thus freeing the active cytotoxic metabolite from any residual amino acid residue derived from the antibody attachment site. The cleavage mechanisms used in typical ADCs with cleavable linkers include the hydrolysis of acid-labile bonds in acidic intracellular compartments, proteolytic cleavage of amide bonds by intracellular proteases, and reductive cleavage of disulfide bonds by the reducing environment inside cells (see Section 1.3). It is possible that these mechanisms can operate in the pre-endosomal and endosomal compartments

of cells without a strict requirement for lysosomal trafficking, although in the case of proteolytic cleavage, one must design peptide linkers susceptible to the proteases present in such nonlysosomal compartments. When the chemical structure of the linker–payload results in the release of an unmodified payload, such linkers may be referred to as "traceless linkers." In other cases, the final active cytotoxic metabolite released intracellularly from the ADC is a derivative of the "parent" cytotoxic compound, which now includes structures and/or functional groups introduced as part of the linker chemistry. Indeed, varying the linker–payload chemistry to alter the properties of the final active metabolite is part of the design space of developing an effective, well-tolerated ADC [26, 30, 32, 47]. For example, increasing the hydrophobicity of the cytotoxic metabolite may increase the rate of transfer across cellular membranes for more efficient exit of the released payload moiety from lysosomes to enable access to its target within the cell. Alternatively, increasing its hydrophilic nature, for example, via charged groups, may decrease the rate of transmembrane transfer and thereby increase cellular retention [47, 48].

Linkers can be "stand-alone" bifunctional reagents that have one functional group designed to react with a functional group on an antibody, typically the amino group of a lysine residue or the sulfhydryl group of a cysteine residue (Figure 1.6), and a second functional group capable of reacting with an appropriate complementary functional group of the cytotoxic payload. This approach is the one taken in making ADCs using the maytansinoid platform, as exemplified by ado-trastuzumab emtansine [5, 26, 49]. Alternatively, the linker chemistry can be built into the payload as a single chemical entity, which then contains a single functional group for reaction with the antibody protein, again usually targeting either lysine amino groups or sulfhydryl groups of lysine or cysteine residues, respectively (Figure 1.6). This approach is exemplified by ADCs such as brentuximab vedotin using the auristatin platform [4, 5, 31].

1.3 Building an ADC Molecule

1.3.1 Conjugation of Payloads to Antibodies at Lysine Residues

The surface-accessible amino groups of lysine residues in an antibody make good attachment sites for a linker–payload since a sizable fraction of them can be modified without disturbing the integrity of the protein structure, thus preserving the native function and favorable pharmacokinetic properties of the antibody [5]. Most linkers/linker–payloads designed for attachment to lysine amino groups utilize *N*-hydroxysuccinimide esters, which react readily and preferentially with primary amines to form stable amide

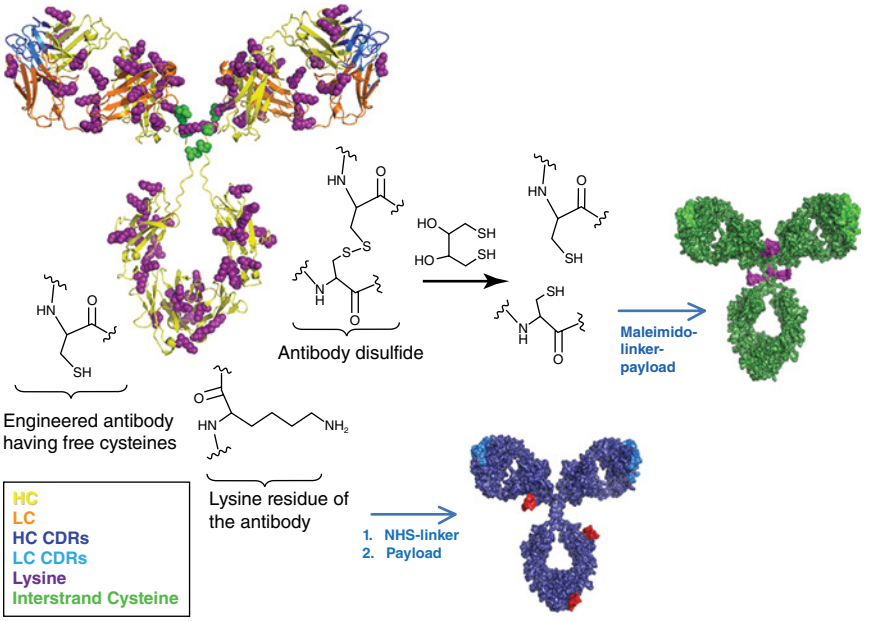


Figure 1.6 Functional groups of antibodies typically used in conjugation reactions. The ribbon diagram shows the structure of an IgG1, with the backbone color coded according to the inset. Lysine residues (purple) and those cysteine residues involved in interchain disulfide bonds (green) are shown with space-filling atomic spheres. *N*-hydroxysuccinimide ester cross-linkers (NHS-linker) are typically used for a two-step conjugation of maytansinoids (red space-filling) to lysine residues [5, 47], for example, in the preparation of ado-trastuzumab emtansine [5, 26, 49, 50]. Maleimido-linker–auristatin compounds (magenta space-filling) are typically used to conjugate auristatin derivatives to antibodies at free sulfhydryl groups formed by partial reduction of interchain cysteine–cysteine disulfide bonds [4], for example, in the preparation of brentuximab vedotin [31, 51]. Similar conjugation chemistry can conjugate payloads to sulfhydryl groups of cysteine residues introduced into antibody structures by protein engineering [38, 52, 53]. (*See color plate section for the color representation of this figure.*)

bonds between the linker and the side-chain amino group of the lysine. Lysine attachment sites are used in the approved ADC, ado-trastuzumab emtansine (Figure 1.7), and in the other maytansinoid ADCs in clinical development, as well as in calicheamicin-containing ADCs, such as gemtuzumab ozogamicin and inotuzumab ozogamicin [35, 39, 40]. The examples of typical ADC structures conjugated through lysine residues, shown in Figure 1.7, include ADCs with an acid-labile hydrazine linker (the calicheamicin conjugates), an uncleavable linker (ado-trastuzumab emtansine), and a hindered disulfide linker cleavable by the reduction of the disulfide bond (mirvetuximab soravtansine).

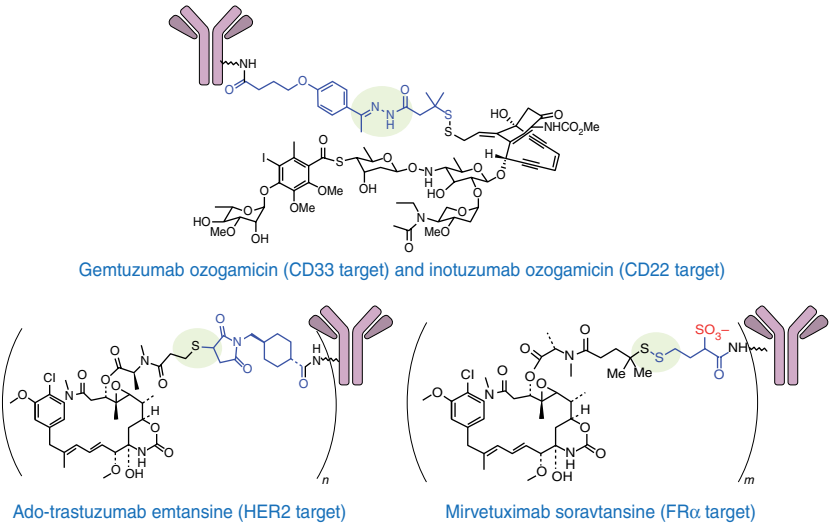


Figure 1.7 Examples of typical ADCs conjugated at lysine residues. Gemtuzumab ozogamicin and inotuzumab ozogamicin are conjugates of a calicheamicin payload where the linker includes an acid-labile hydrazone moiety (shaded gray), and also contains a hindered disulfide bond cleavable by reduction (average DAR of these ADCs are in the range of 2 to 4 – only one linker-payload structure drawn for simplicity). The two maytansinoid ADCs show examples of conjugates with either a non-cleavable link created by reaction of the sulfhydryl group of the maytansinoid DM1 with the maleimido group of the linker (thioether bond so formed is shaded gray), as in ado-trastuzumab emtansine, or with a hindered disulfide-containing link (disulfide shaded gray) that is cleavable by reduction, as in mirvetuximab soravtansine (values for **n** and **m** are between 3 and 4 maytansinoids per antibody). The linker for mirvetuximab soravtansine also bears a hydrophilic charged sulfonate group.

A typical human(ized) IgG_1 antibody contains between 80 and 90 unique lysine residues within its amino acid sequence [50, 54]. The conditions of the modification reaction between the antibody and the linker/linker–payload (e.g., reagent concentrations, reaction pH) must be carefully controlled to limit the average level of payload addition to a typical range of about three to four conjugated sites per antibody molecule. For example, the average maytansinoid-to-antibody molar ratio (also characterized as "drug"-to-antibody ratio, or DAR) for ado-trastuzumab emtansine is about 3.5 [26, 49, 50]. The ratio was selected for the defined ADC product based on (i) minimizing the amount of nonconjugated antibody and (ii) avoiding species in the mixture with very high DAR, which may be problematic in manufacturing and formulation due to higher hydrophobicity and lower solubility [26, 50]. Furthermore, higher DAR species may have altered pharmacokinetic properties, the increased hydrophobicity resulting in more rapid clearance [21]. The relative abundance of ADC species with different numbers of payloads attached per antibody molecule can

be estimated by mass spectrometry [50, 54–56]. In the case of maytansinoid ADCs, for an average DAR of about 3.5 for which three representative mass analyses are shown in Figure 1.8 (three different linker–maytansinoid species), about 70–80% of the antibody molecules have between two and five maytansinoids per antibody and > 90% of the antibody molecules have individual DAR values in the range of 1 to 6 [55]. At this average level of payload addition (DAR ~3.5), only about 3% of the antibody was nonconjugated antibody and only a similarly low proportion of antibody molecules had DAR values ≥7 [55, 56]. The distribution pattern of species with different DAR found experimentally is quite predictable for a given average DAR and can be described by statistical models, either by Poisson distribution [50] or by the binomial distribution [55]. One implication of these observations is that measurement and control of the

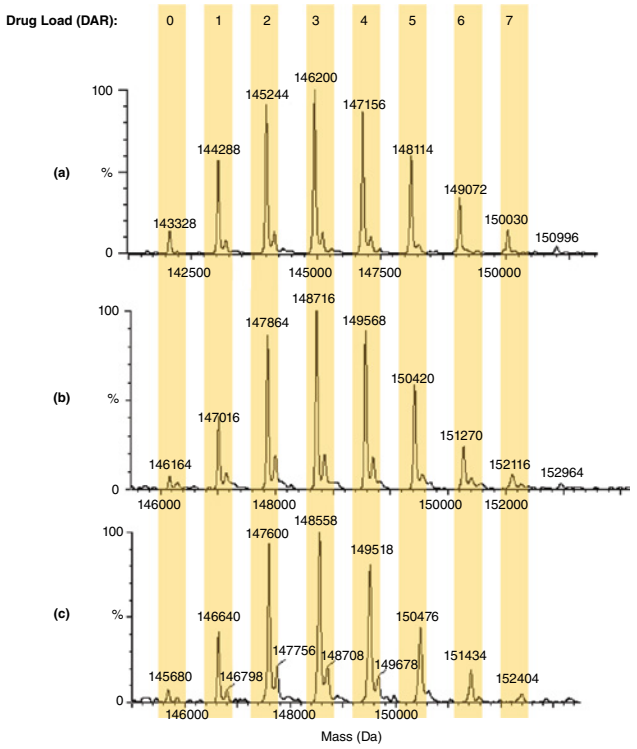


Figure 1.8 Deconvoluted mass spectra of three deglycosylated ADCs. Shown are examples of three different antibodies conjugated to three different linker–maytansinoid moieties with an average DAR value of 3.5 for each conjugate (determined spectrophotometrically). (a) mAb1-SMCC-DM1; (b) mAb2-SPP-DM1; (c) mAb3-sulfo-SPDB-DM4. Source: Adapted with permission from Goldmacher, V.S., *et al*., *Molecular Pharmaceutics*, *12*, 1738–1744, copyright 2015, American Chemical Society [55].

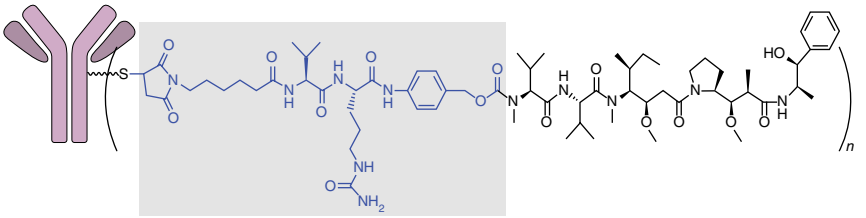
DAR value itself during conjugation reactions could be sufficient to control the levels of nonmodified antibody in the defined ADC preparation [50].

Mass spectroscopy is also a useful tool to analyze chromatographic peptide maps in order to determine the actual conjugation sites in the antibody moiety of the ADC. Depending on the sensitivity of the technique, from 40 to 70 individual lysine residues (more than half of the total possible) are partially modified in the example of maytansinoid technology [50, 54, 56]. The range of levels of modification of individual lysine residues in ado-trastuzumab emtansine, for example, is from about 25% to <1%, with a median value of about 4% [50]. Such peptide mapping techniques provide a fingerprint that can be used to compare different lots of an ADC to ensure process consistency and robustness during scale-up to commercial manufacturing.

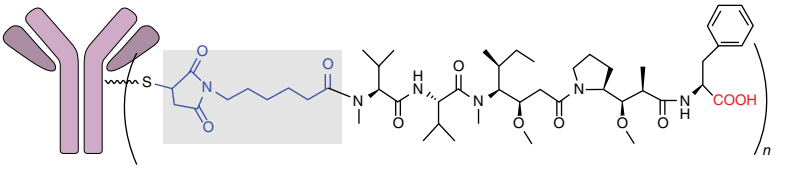
1.3.2 Conjugation of Payloads to Antibodies at Cysteine Residues

Linker–payload constructs designed for attachment to sulfhydryl groups of cysteine residues of the antibody have made use of the rapid reaction between maleimido groups and sulfhydryl groups to form thioether bonds [31, 42, 51]. The auristatin (MMAE) used as the payload of the approved ADC, brentuximab vedotin, and the auristatins used in the other ADCs in current clinical development, which use this payload class (MMAE and MMAF), are synthesized as maleimide-bearing linker–payload compounds for reaction with protein sulfhydryl groups. ADCs made with the DNA-crosslinking payload, PBD, can also be conjugated by this approach [42]. Figure 1.9 shows the structure of brentuximab vedotin and also shows a structure for an MMAF conjugate. The valine-citrulline-*para*-aminobenzyl-containing linker of brentuximab vedotin is an example of a protease-cleavable linker; the amide bond between the dipeptide and the *para*-aminobenzyl moiety is cleaved by the lysosomal protease, cathepsin B [5, 51]. It is also an example of a "traceless linker," since cleavage of the amide bond is followed by self-immolation of the *para*-aminobenzyl moiety with loss of carbon dioxide to yield MMAE as the final metabolite [5, 51]. Other payloads exploit a similar mechanism of release [42]. The MMAF conjugate has an uncleavable link so that the final metabolite released inside the cell contains the linker plus a cysteine reside [5].

Antibodies generally do not contain free, solvent-accessible sulfhydryl groups, but rather they contain cysteine residues whose sulfhydryl groups are oxidized to form disulfide bonds between pairs of cysteine residues (see Figure 1.6). An IgG_1 contains 16 disulfide bonds, four interchain disulfide bonds, two between the two heavy chains and one between each light chain and a heavy chain, and 12 intrachain disulfides. The four interchain bonds can be readily reduced and maintained as pairs of free sulfhydryl groups under nonoxidative conditions, and these can serve as sites of reaction with maleimido-linker–payload compounds [4, 5, 51].



Brentuximab vedotin (CD30 target)



Antibody-maleimido-caproyl-MMAF

Figure 1.9 Examples of typical ADCs conjugated at cysteine residues. Brentuximab vedotin, approved for the treatment of Hodgkin lymphoma and acute large cell lymphoma, is an example of an ADC containing a valine-citrulline-*para*-aminobenzyl-MMAE linker-payload moiety which includes a maleimido-caproyl-dipeptide- para-aminobenzyl linkage (shown shaded gray) that is cleavable by the cellular protease, cathepsin B. Also shown is a structure for a mAb-maleimido-caproyl-MMAF conjugate where the charged auristatin, MMAF, is linked to cysteine residues via a non-cleavable link (shown shaded gray). Values for **n** are typically about 4 in each case.

The level of interchain disulfide bond reduction is carefully controlled so that in a typical auristatin conjugate, the DAR is limited to an average of about four [51]. As mentioned earlier for lysine conjugation, the ratio of about four auristatins per antibody molecule was selected to minimize the amount of non-conjugated antibody, and also to minimize the amount of ADC species with DAR values of eight, obtained by complete reduction of the intrachain disulfide bonds of an IgG_1 antibody followed by complete reaction of all sulfhydryl groups thus produced with the maleimido-linker–payload. Such "fully-loaded" auristatin conjugates show less antitumor activity than ADCs with lower DAR, likely due to the finding that they are rapidly cleared from circulation in blood *in vivo* [57]. In brentuximab vedotin, the size distribution analysis, done by hydrophobic interaction chromatography [56, 57], shows that antibody species bearing predominantly two, four, and six molecules of auristatin per antibody are present in the defined product mixture, with small amounts (~8% each) of nonconjugated antibody and species with a DAR of eight [51, 56, 57].

The loss of interchain disulfide bonds that accompanies conjugation of maleimido-linker–payloads to an antibody may come at a cost of some degree of stability *in vivo*, which may vary from antibody to antibody, and may become

particularly apparent when such modification is pushed to completion (all four disulfides of an IgG_1 reduced to yield an ADC bearing eight linker–payload moieties) [57]. Auristatin conjugates with DAR values of eight have a very short half-life *in vivo*, likely accounting for poor efficacy, and perhaps increased toxicity [52, 57]. One approach to address this issue is to express antibody genes bearing mutations, which result in the replacement of a solvent-accessible amino acid with a cysteine residue whose unpaired sulfhydryl group can serve as a payload attachment site [38, 52]. This approach also allows an ADC to be made with a DAR value of about two, with most conjugation occurring at a single site on each half-antibody (see Figure 1.6). Such site-specific conjugation minimizes the generation of any ADC species with DAR values ≥3, which may be an important consideration when conjugating very hydrophobic payloads (e.g., see reference [38]). However, selecting the correct site in the antibody molecule up front is not trivial [58], and furthermore, having 100% of the modification at a single site could potentially increase the possibility of an adverse immune response to the ADC. Several ADCs made using site-specific cysteine conjugation have recently entered clinical trials (structures are disclosed for four such "site-specific" ADCs at time of writing). There are a variety of other site-specific conjugation approaches that are being evaluated in the research laboratory [53, 59, 60]; however, a detailed description of these approaches is beyond the scope of this chapter and is about "typical" ADCs at this time.

1.4 Attributes of a Typical ADC

1.4.1 Structural Attributes of a Typical ADC

A typical ADC with a DAR value in the range of 3.5 to 4.0 has a molecular mass that is about 2% greater than that of the corresponding "naked" antibody moiety. Ideally, the biochemical parameters of an ADC should be broadly similar to those of its nonmodified antibody moiety, behaving mostly as intact monomeric molecules upon size-exclusion chromatography, with very little aggregate present [56, 61]. However, addition of payloads does alter surface hydrophobicity properties, with effects on solubility and the propensity to aggregate. The magnitude of such effects depends on DAR and relative hydrophobicity of the payload and must be carefully studied to ensure appropriate formulation designed to minimize any aggregation [56, 61]. In many cases, ADCs can be formulated in a similar manner to the parent "naked" antibody and can be provided to the clinical pharmacy as vials of either a liquid (e.g., coltuximab ravtansine) or a lyophilized (e.g., ado-trastuzumab emtansine) drug product [62, 63]. It is important to have an accurate assay to test for the presence of any nonconjugated ("free") payload, including any small molecule linker–payload derivatives, which are typically at levels that are less than a few percent of the total conjugated

payload [56]. As mentioned earlier, size distribution analysis by mass spectroscopy or by hydrophobic interaction chromatography are important tools for characterizing the ADC product, together with other biochemical and biophysical techniques such as imaged capillary isoelectric focusing for charge-based separation of species, and peptide mapping by liquid chromatography coupled to mass spectroscopy [54, 56, 61]. Many of these biochemical and biophysical tools are the same tools that are already widely used to characterize "naked" antibody products, which are themselves heterogeneous molecules, for example, with respect to glycosylation patterns, deamidation of glutamine or asparagine, level of C-terminal lysine, and so on [54, 56, 61].

1.4.2 Functional Characteristics of a Typical ADC

1.4.2.1 *In Vitro* Properties

Ideally, conjugation of 3.0–4.0 payloads per antibody should have little or no effect on the binding specificity and apparent binding affinity of the antibody component of the ADC to its cell surface target [64, 65]. ADCs should also exhibit specificity and selectivity in their cytotoxicity upon evaluation of their *in vitro* potency. For example, a maytansinoid ADC targeting FRα made with the uncleavable SMCC-DM1 linker–payload format shows specific killing of cells expressing human FRα on their cell surface relative to an isotype-matched nonbinding control conjugate (Figure 1.10). Also shown in Figure 1.10 is the kill curve for a maytansinoid ADC targeting FRα made with a cleavable sulfo-SPDB-DM4 linker–payload format (IMGN853) that shows selectivity for antigen-mediated killing as demonstrated by the blockade of cytotoxic effects by the addition of a large excess of nonmodified "naked" antibody (Figure 1.10) [22]. Thus, for maytansinoid ADCs targeting FRα, *in vitro* cytotoxicity assays do not distinguish between a conjugate made with a cleavable linker (disulfide, in this case) and a noncleavable linker, a common observation in such *in vitro* assays [30, 32, 47]. However, differences in potency may be observed *in vivo* depending on the biology of the target [21, 22, 30, 32, 49].

It is worth noting that the magnitude of the difference between antigen-mediated killing and that of a nonbinding control ADC (about 100-fold as shown in Figure 1.10) is largely a function of antibody binding affinity to its target in these *in vitro* assays. An antibody with an apparent K_D only in the 5–10 nM range will show only a small degree of specificity *in vitro*. However, it may be that the apparent binding affinity of an antibody is only one of the factors important for *in vivo* potency of an antibody/ADC, and high affinity may actually decrease overall uptake and retention by a tumor mass *in vivo* under some circumstances [29, 66, 67].

1.4.2.2 *In Vivo* Efficacy

Once an ADC has met the biochemical quality attributes (monomeric molecule, with only low levels of aggregates and of free payload species), and exhibits

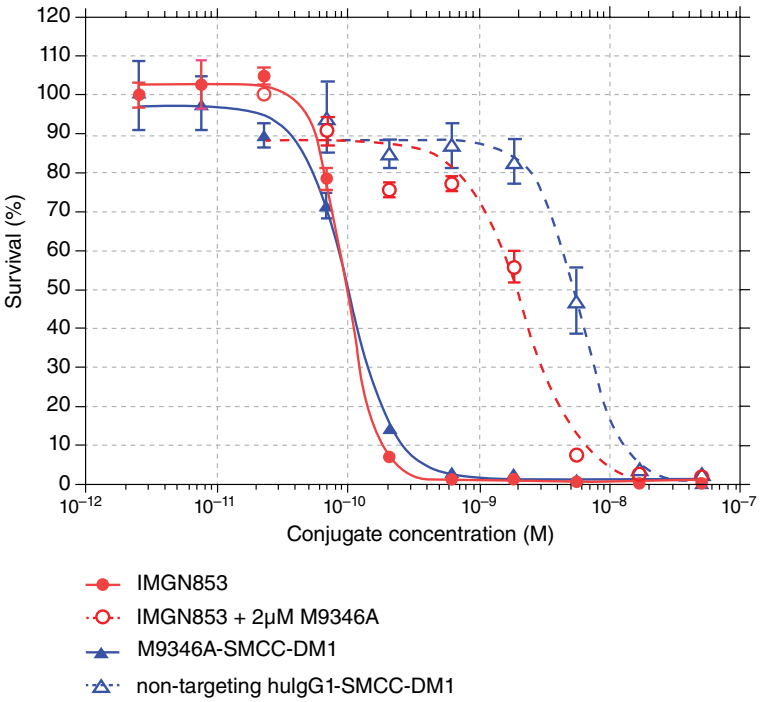


Figure 1.10 *In vitro* cytotoxicity was measured after a 5-day exposure of FRα-positive KB cells to (i) a maytansinoid ADC targeting FRα made with a cleavable sulfo-SPDB-DM4 linker-payload format (IMGN853), with (dotted line, open circles) or without (solid line, closed circles) a blocking concentration (2 μM) of the "parent" non-conjugated anti-FRα antibody (M9346A), and (ii) M9346A-SMCC-DM1, a maytansinoid ADC targeting FRα made with a non-cleavable SMCC-DM1 linker-payload format (solid line, closed triangles), compared with the cytotoxicity curve of a non-targeting huIgG1-SMCC-DM1 (dotted line, open triangles). Source: Adapted from Ab, O., *et al*., *Molecular Cancer Therapeutics*, *14*(*7*); 1605–1613, copyright 2015, American Association for Cancer Research [22].

appropriate *in vitro* activity, its antitumor activity should be evaluated in several *in vivo* models representing human tumors that are relevant indications for the particular ADC. These preclinical models can be xenograft models derived from human tumor cell lines or from patient-derived tumor xenograft models (PDx models). Clearly, it is a prerequisite that whatever *in vivo* models are to be used for the determination of antitumor activity, their levels of target antigen expression should be assessed. Immunohistochemical methods that are calibrated to have a dynamic range covering a relevant range of antigen expression levels appropriate for the cell-killing capability of the ADC are typically used for this purpose and should as closely as possible match the test method to be used on clinical samples for cancer patients.