Antibody-Drug Conjugates
Antibody-Drug Conjugates: Fundamentals, Drug Development, and Clinical Outcomes to Target Cancer

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Contents

List of Contributors xvii
Preface xxi
Historical Perspective: What Makes Antibody–Drug Conjugates Revolutionary? xxiii

Part I What is an Antibody–Drug Conjugate 1

1 Typical Antibody–Drug Conjugates 3
John M. Lambert
1.1 Introduction 3
1.1.1 A Simple Concept 3
1.1.2 Turning Antibodies into Potent Anticancer Compounds 4
1.1.3 What is a Typical ADC and How Does it Act? 4
1.1.4 Simple Concept, but Not So Simple to Execute 5
1.2 The Building Blocks of a Typical ADC 6
1.2.1 The Antibody 6
1.2.1.1 Antibody Isotype in ADCs 7
1.2.1.2 Functional Activity of the Antibody Moiety in ADCs 8
1.2.2 The Payload 9
1.2.2.1 DNA-Targeting Payloads 11
1.2.2.2 Payloads Targeting Tubulin 11
1.2.3 Linker Chemistries 12
1.3 Building an ADC Molecule 13
1.3.1 Conjugation of Payloads to Antibodies at Lysine Residues 13
1.3.2 Conjugation of Payloads to Antibodies at Cysteine Residues 17
1.4 Attributes of a Typical ADC 19
1.4.1 Structural Attributes of a Typical ADC 19
1.4.2 Functional Characteristics of a Typical ADC 20
1.4.2.1  In Vitro Properties  20
1.4.2.2  In Vivo Efficacy  20
1.4.2.3  Pharmacokinetics of ADCs  23
1.5  Summary  24

Acknowledgment  24
Abbreviations  25
References  25

Part II  Engineering, Manufacturing, and Optimizing Antibody–Drug Conjugates  33

2  Selecting Optimal Antibody–Drug Conjugate Targets Using Indication-Dependent or Indication-Independent Approaches  35

Jay Harper and Robert Hollingsworth
2.1  Characteristics of an Optimal ADC Target  35
2.2  Indication-Dependent ADC Target Selection  40
2.3  Indication-Independent ADC Target Selection  48
2.4  Concluding Remarks and Future Directions  50

Acknowledgments  52

References  52

3  Antibody–Drug Conjugates: An Overview of the CMC and Characterization Process  59

Philip L. Ross and Janet Wolfe
3.1  Introduction  59
3.2  ADC Manufacturing Process  60
3.2.1  Conjugation  62
3.2.2  Conjugation – Next-Generation Chemistry  64
3.2.2.1  Conjugation – Novel Payloads  64
3.2.2.2  Conjugation – Linker Design  65
3.2.3  mAb Engineering  66
3.2.4  Purification  68
3.2.5  Formulation  68
3.3  Characterization  70
3.3.1  Quality and Stability Testing  70
3.3.2  Biochemical and Microbiological Testing  74
3.3.3  Extended Characterization  74
3.4  Comparability  76
3.5  Concluding Remarks  76

Abbreviations  77
References  78
4 Linker and Conjugation Technology; and Improvements 85
Riley Ennis and Sourav Sinha

4.1 Overview 85
4.2 Noncleavable 86
4.3 Cleavable Linkers and Self-Immolative Groups 86
4.4 Differences in Therapeutic Window of Cleavable and Noncleavable Linkers 88
4.5 Improving Therapeutic Window with Next-Generation Linker Technologies 89
4.6 Site-Specific Conjugation, Homogeneous Drug Species, and Therapeutic Window 91
4.7 Influence of Linkers on Pharmacokinetics and ADME 93
4.8 PEG Linkers to Optimize Clearance, Solubility, and Potency 93
4.9 Linkers to Optimize for Drug Resistance 94
4.10 Improving Solid Tumor Penetration with Linkers 96
4.11 Analytical Methods for Characterizing Linker Pharmacodynamics 96
4.12 Conclusion 98
References 99

5 Formulation and Stability 105
Kouhei Tsumoto, Anthony Young, and Satoshi Ohtake

5.1 Introduction 105
5.2 Stability Considerations for ADCs 106
5.2.1 Physical Stability 106
5.2.2 Chemical Stability 111
5.3 Formulation Approaches 115
5.4 Logistical Considerations 123
5.5 Summary and Close 125
References 126

6 QC Assay Development 131
Xiao Hong, Chen and Mate Tolnay

6.1 Introduction 131
6.2 Drug-to-Antibody Ratio 132
6.3 Drug Loading Distribution 133
6.3.1 Lysine-Linked ADCs 134
6.3.2 Cysteine-Linked ADCs 134
6.4 Positional Isomers 136
6.5 ADC Concentration 136
6.6 Drug-Related Substances 137
6.7 Antigen Binding Assays and Potential Impact of Drug Conjugation 137
6.8 Cell-Based Cytotoxicity Assays 139
6.9 Assays to Monitor Fc-Dependent Effector Functions to Characterize Additional Possible Mechanisms of Action 140
6.10 Immunogenicity Assays to Monitor the Immune Response to ADC 142
6.11 Conclusions 144
6.12 Key Guidance Documents 145
Acknowledgments 145
References 145

7 Occupational Health and Safety Aspects of ADCs and Their Toxic Payloads 151
Robert Sussman and John Farris
7.1 Introduction 151
7.2 Background on ADCs 152
7.2.1 Payloads 153
7.2.2 Linker Technologies 154
7.2.3 Antibodies 156
7.2.4 Partial Conjugates 156
7.3 Occupational Hazard Assessment of ADCs and Their Components 157
7.4 Occupational Implications and Uncertainties 159
7.4.1 Routes of Occupational Exposure 159
7.4.2 Binding Efficiency (Payload to Antibody) 159
7.4.3 Unintended Targets 160
7.4.4 Free Payload in Conjugation Formulation 160
7.4.5 Local Effects in the Lung 160
7.5 General Guidance for Material Handling 160
7.5.1 Handling of Powders 162
7.5.2 Handling of Solutions 162
7.6 Facility Features and Engineering Controls 163
7.6.1 HVAC and Air Pressure Relationships 164
7.6.2 Air Changes and Airflow 164
7.6.3 Recirculation and Filtration of Room Air 164
7.6.4 Changing Areas 164
7.6.5 Designated Areas 165
7.7 Specific Operational Guidance 165
7.7.1 Payload Synthesis 165
7.7.2 Conjugation 166
7.7.3 Lyophilization 166
7.7.4 Cleaning 167
7.8 Personal Protective Equipment 167
7.8.1 Chemical Protective Clothing 167
7.8.1.1 Protective Clothing 167
7.8.1.2 Gloves 167
7.8.1.3 Eye and Face Protection 168
7.8.2 Respiratory Protection 168
7.9 Training 168
7.9.1 Potent Compound Awareness Training 169
7.9.2 Standard Operating Procedures for Synthesizing and Handling ADCs 169
7.10 Industrial Hygiene Monitoring 169
7.10.1 Air Monitoring 170
7.10.2 Surface Monitoring 170
7.11 Medical Surveillance Program 171
7.12 Summary and Future Direction 172

References 172

Part III Nonclinical Approaches 177

8 Bioanalytical Strategies Enabling Successful ADC Translation 179
Xiaogang Han, Steven Hansel, and Lindsay King
8.1 Introduction 179
8.2 ADC LC/MS Bioanalytical Strategies 182
8.2.1 Nonregulated Unconjugated Payload Bioanalysis 183
8.2.2 Intact Protein Bioanalysis by LC/MS: Measurement of Drug-to-Antibody Ratio 184
8.2.3 ADC Pharmacokinetic Bioanalysis by LC/MS 186
8.2.4 Calculated Conjugated Payload Determination 187
8.2.5 Conjugated Payload Quantitation of Cleavable Linker ADCs 188
8.2.6 Conjugated Payload Quantitation by Peptide-Based Analysis 189
8.3 Non-Regulated ADC Pharmacokinetic and Immunogenicity Support Using Ligand Binding Assays 190
8.3.1 ADC Ligand Binding Assays 190
8.3.2 Reagents 191
8.3.3 ADC Reference Standards 192
8.3.4 Total Antibody Assays 192
8.3.5 ADC Assays 193
8.3.6 Target Interference in ADC Measurement 194
8.3.7 ADC Immunogenicity Assays 194
8.4 Biodistribution Assessment 195
8.5 Regulated ADC Pharmacokinetics and Immunogenicity Evaluation 196
8.5.1 ADC Assays in Regulated Studies 196
8.5.2 Regulated Ligand Binding Assays 197
8.5.3 Regulated LC/MS/MS Quantitation of Unconjugated Payload 198
8.5.4 Regulated Conjugated Payload LC/MS Assays 199
8.5.5 Regulated Anti-therapeutic Assays 199
8.6 ADC Biomeasures and Biomarkers 199
8.7 Summary 200
References 201

9 Nonclinical Pharmacology and Mechanistic Modeling of Antibody–Drug Conjugates in Support of Human Clinical Trials 207
Brian J. Schmidt, Chin Pan, Heather E. Vezina, Huadong Sun, Douglas D. Leipold, and Manish Gupta
9.1 Introduction 207
9.2 Cell Line Testing 210
9.2.1 Antigen Density 211
9.2.2 Antigen and Antibody–Drug Conjugate Internalization 211
9.2.3 Payload Processing and Binding 213
9.3 Xenograft Models 214
9.3.1 Payload Bystander Effects 215
9.3.2 Biomarker Assays 216
9.4 Nonclinical Testing to Support Investigational New Drug Applications 216
9.4.1 Antibody–Drug Conjugate Efficacious Dose Range 218
9.5 Mechanistic Modeling of Antibody–Drug Conjugates 220
9.5.1 Tumor Tissue Transport Considerations 221
9.5.2 Subcellular Trafficking 225
9.5.3 Shed Antigen and Endosomal Processing 225
9.5.4 Enhanced Pharmacokinetic Modeling to Enable Antibody–Drug Conjugate Pharmacology Predictions 226
9.5.5 Mechanistic Modeling of Antibody–Drug Conjugate Pharmacology: Accounting for Uncertainties 227
9.6 Target-Mediated Toxicity of Antibody–Drug Conjugates 228
9.7 Considerations for Nonclinical Testing Beyond Antibody–Drug Conjugate Monotherapies 229
9.8 Summary 230
Acknowledgments 231
References 231
10 Pharmacokinetics of Antibody–Drug Conjugates 245
Amrita V. Kamath
10.1 Introduction 245
10.2 Pharmacokinetic Characteristics of an ADC 246
10.2.1 ADC Biodistribution 248
10.2.2 ADC Clearance 249
10.3 Unique Considerations for ADC Pharmacokinetics 250
10.3.1 Linker Stability 250
10.3.2 Site of Conjugation and Drug Load 252
10.3.3 Cytotoxic Drug 253
10.4 Tools to Characterize ADC PK/ADME 254
10.4.1 Bioanalytical Methods 254
10.4.2 In Vitro Assays 255
10.4.3 In Vivo Studies 256
10.4.4 Pharmacokinetic/Pharmacodynamic (PK/PD) Models 256
10.5 Utilization of ADC Pharmacokinetics to Optimize Design 257
10.6 Pharmacokinetics of Selected ADCs 259
10.6.1 Ado-Trastuzumab Emtansine (Kadcyla®) 259
10.6.2 Brentuximab Vedotin (Adcetris®) 261
10.7 Summary 261
References 262

11 Path to Market Approval: Regulatory Perspective of ADC Nonclinical Safety Assessments 267
M. Stacey Ricci, R. Angelo De Claro, and Natalie E. Simpson
11.1 Introduction 267
11.2 FDA Experience with ADCs 268
11.3 Regulatory Perspective of the Nonclinical Safety Assessment of ADCs 269
11.3.1 Regulatory Guidance Available for Nonclinical Studies 270
11.3.1.1 Species Selection 272
11.3.1.2 Study Duration and Dose Regimen 275
11.3.1.3 Study Test Article 276
11.3.1.4 Pharmacology Studies 278
11.3.1.5 Pharmacokinetics/Toxicokinetics 279
11.3.1.6 Genotoxicity 280
11.3.1.7 Developmental and Reproductive Toxicology 280
11.3.1.8 First-in-Human Dose Selection 280
11.4 Concluding Remarks 282
References 283
Part IV  Clinical Development and Current Status of Antibody–Drug Conjugates 285

12 Antibody–Drug Conjugates: Clinical Strategies and Applications 287
Heather E. Vezina, Lucy Lee, Brian J. Schmidt, and Manish Gupta
12.1 Antibody–Drug Conjugates in Clinical Development 287
12.2 Therapeutic Indications 291
12.3 Transitioning from Discovery to Early Clinical Development 292
12.4 Challenges and Considerations in the Design of Phase 1 Studies 293
12.5 First-in-Human Starting Dose Estimation 293
12.6 Dosing Strategy Considerations 294
12.7 Dosing Regimen Optimization 295
12.8 Phase 1 Study Design 297
12.9 Supportive Strategies for Phase 1 and Beyond 299
12.10 Clinical Pharmacology Considerations 301
12.11 Organ Impairment Assessments 301
12.12 Drug–Drug Interaction Assessments 302
12.13 Immunogenicity 303
12.14 QT/QTc Assessments 303
12.15 Pharmacometric Strategies 307
12.16 Using Physiologically Based Pharmacokinetic and Quantitative Systems Pharmacology Models with Clinical Data 308
12.17 Summary and Conclusions 311
Acknowledgments 311
References 311

13 Antibody–Drug Conjugates (ADCs) in Clinical Development 321
Joseph McLaughlin and Patricia LoRusso
13.1 Introduction and Rationale 321
13.2 Components of ADCs in Development 321
13.2.1 Antibody 321
13.2.2 Linker 327
13.2.3 Payload 328
13.3 Landscape of ADCs 329
13.3.1 History of ADCs 329
13.3.2 FDA Approved ADCs 329
13.4 Clinical Use of ADCs 330
13.5 Future of ADCs 330
13.6 ADCs in Development 330
13.6.1 Hematological Malignancies and Renal Cell Carcinoma 330
Contents

13.6.1.1 Auristatins (MMAE and MMAF)  330
13.6.1.2 Maytansinoids (DM1 and DM4)  332
13.6.1.3 Pyrrolobenzodiazepines (PBDs)  334
13.6.1.4 Calicheamicins  335
13.6.1.5 Others  335
13.6.2 Solid Malignancies  335
13.6.2.1 Auristatins (MMAE and MMAF)  335
13.6.2.2 Maytansinoids (DM1 and DM4)  338
13.6.2.3 Others  339
13.7 Future Directions  340
References  340

14 ADCs Approved for Use: Trastuzumab Emtansine (Kadcyla®, T-DM1) in Patients with Previously Treated HER2-Positive Metastatic Breast Cancer  345
Gail D. Lewis Phillips, Sanne de Haas, Sandhya Girish, and Ellie Guardino

14.1 Introduction  345
14.2 Preclinical Development of T-DM1  348
14.3 Early Clinical Studies of T-DM1  357
14.3.1 Phase I Adverse Events (AEs)  357
14.3.2 Phase I Efficacy  358
14.3.3 Dosing Schedule  359
14.3.4 Phase II Trials  359
14.4 Clinical Pharmacology and Pharmacokinetics  361
14.5 Phase III Studies of T-DM1 in Patients with HER2-Positive MBC  362
14.5.1 EMILIA Trial  363
14.5.2 TH3RESA Trial  367
14.5.3 Treatment Exposure  369
14.5.4 Biomarkers as Predictors of Efficacy  369
14.6 Future Directions  371
14.7 Summary  373
References  374

15 ADCs Approved for Use: Brentuximab Vedotin  381
Monica Mead and Sven de Vos

15.1 Introduction  381
15.2 Early Efforts to Target CD30 with Monoclonal Antibodies  383
15.3 BV: Preclinical Data  386
15.3.1 Clinical Data: Safety/Tolerability  388
15.3.2 Clinical Data: Efficacy  391
15.3.3 CD30 Expression Level and Response to BV  393
15.4 Clinical Context  394
15.5 Mechanisms of Resistance 395
15.6 Current Research 397
15.7 Discussion 400
References 401

16 Radioimmunotherapy 409
Savita V. Dandapani and Jeffrey Wong
16.1 History of Radioimmunotherapy 409
16.2 Radioisotopes 410
16.3 Chemistry of RIT 411
16.4 Radioimmunotherapy Antibody Targets in Use Today (Table 16.2) 412
16.4.1 Hematologic Malignancies 412
16.4.1.1 CD20 412
16.5. Other Hematologic Targets 415
16.5.1 Lymphomas 415
16.5.1.1 Lym-1, CD22, CD25 415
16.5.2 Leukemias 417
16.5.2.1 CD33 417
16.6 Solid Tumors 417
16.6.1 CEA (Carcinoembryonic Antigen) 418
16.6.2 Other RIT in Solid Tumors 419
16.7 Combination Therapy with RIT: Chemotherapy and/or Radiation 420
16.7.1 RIT and Chemotherapy 420
16.8 RIT and External Beam Radiation Treatment (EBRT) 421
16.9 RIT and EBRT and Chemotherapy 421
16.10 RIT Administration 422
16.11 Future of RIT 422
References 423

Part V Future Perspectives in Antibody–Drug Conjugate Development 431

17 Radiolabeled Antibody-Based Imaging in Clinical Oncology 433
Bart S. Hendriks and Daniel F. Gaddy
17.1 Introduction 433
17.2 Applications for Clinical Antibody Imaging 434
17.3 Antibodies as Imaging Agents 435
17.4 Nuclear Imaging – Gamma Camera (Planar) Scintigraphy and SPECT 439
17.4.1 Tumor Detection and Staging 440
17.4.1.1 CEA 441
17.4.1.2 PSMA 441
17.4.1.3 TAG-72 443
17.4.1.4 Pancarcinoma Antigen 443
17.4.2 Diagnostic Assessment 444
17.4.2.1 HER2 444
17.4.2.2 EGFR 445
17.4.3 Dosimetry for Radioimmunotherapy 445
17.4.4 Early Assessment of Response 447
17.5 Nuclear Imaging - PET 448
17.5.1 68Ga 448
17.5.2 64Cu 449
17.5.3 89Zr 451
17.5.4 124I 454
17.6 Commercialization Considerations 456
17.7 Summary 461
References 462

18 Next-Generation Antibody–Drug Conjugate Technologies 473
Amy Q. Han and William C. Olson
18.1 Introduction 473
18.2 Novel Cytotoxic Payloads and Linkers 474
18.2.1 Microtubule Inhibitors 474
18.2.2 Benzodiazepine Dimers 474
18.2.3 Anthracyclines 477
18.2.4 Amatoxins 478
18.2.5 Disulfide Rebridging 479
18.2.6 FleximerTM Polymeric Linkers 481
18.3 Tailoring Antibodies for Use as ADCs 482
18.3.1 Engineered Cysteines 483
18.3.2 Enzyme-Assisted Conjugation 484
18.3.2.1 Microbial Transglutaminase 484
18.3.2.2 Formylglycine-Generating Enzyme (FGE) 485
18.3.2.3 Glucosyltransferases and Other Glycan Engineering 486
18.3.3 Non-Native Amino Acids and Selenocysteine 487
18.3.4 Alternative Formats and Masked Antibodies 488
18.3.5 ADCs Beyond Oncology 489
18.4 Conclusions 491
References 491

Index 505
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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

Kennath J. Olivier Jr. and Sara A. Hurvitz
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,
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...
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
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

### Table 2  Additional monoclonal antibodies approved for use.

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