Antibody-Drug Conjugates
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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
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,
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.

**Table 2** Monoclonal antibodies directed at malignant cell surface receptors.

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

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

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
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>
<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>Ibritumomab 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>
</tbody>
</table>
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.