Picking the Optimal Target for Antibody-Drug Conjugates
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OVERVIEW

Antibody-drug conjugates (ADCs) combine the cytotoxic potential of chemotherapeutic drugs with the specificity of monoclonal antibodies (mAbs). After many years of unfulfilled promise, the field of ADCs is experiencing resurgence as more is learned about each of the components of an ADC and how these components need to be combined to produce a successful therapeutic agent. Choosing an appropriate target for ADCs is a critical parameter that affects the efficacy, therapeutic window, and toxicity profile of ADCs. This review will focus on the concepts underlying the choice of the target, review specific current ADCs and their targets, and look to the future of ADCs.

Cancer therapeutics has long struggled with the need to target and destroy malignant cells while minimizing undesired collateral toxicity to normal tissue. Numerous highly cytotoxic drugs have been identified that are of limited clinical utility because they are taken up by, and cytotoxic toward, both normal and malignant cells. Monoclonal antibody (mAb) therapy is based on the concept that target cell specificity can limit toxicity. mAb successes include rituximab, trastuzumab, bevacizumab, and cetuximab. On the other hand, experience with these and other less successful mAbs has demonstrated that binding of a mAb to target antigen on a malignant cell does not always result in destruction of the malignant cell.

ADCs seek to overcome the limitations of both nonspecific cytotoxic drugs and specific but ineffective mAb therapy by combining the cytotoxic potential of the drug with the specificity of the mAb. ADCs are only successful if each component is effective. The mAb needs to bind preferentially to malignant cells at a relatively high density, and internalize in a manner that allows for release of the drug from the linker in the appropriate intracellular compartment. The linker needs to attach the drug to the mAb in a manner that is stable in the circulation, but releases the drug once it is internalized. The cytotoxic drug needs to be nontoxic when linked to the mAb in the circulation, but capable of killing tumor cells once it is internalized and released.

Brentuximab vedotin and ado-trastuzumab emtansine are the only U.S. Food and Drug Administration (FDA)-approved ADCs as of February 2013. However, the field is rapidly expanding with over 20 ADCs currently in development and in clinical trials (Tables 1 and 2), many with encouraging preliminary results. Many of the most promising ADCs are targeted toward hematologic malignancies, likely because of the availability of cell type-specific targets, sensitivity of such malignancies to cytotoxic drugs, and accessibility of the majority of malignant cells to intravenously administered therapy. However, the current pipeline also includes ADCs against solid tumors.

TUMOR TARGETS

Most ADCs recognize tumor cell surface antigens. There are complex factors we are only now starting to understand that effect the attractiveness of a given molecule as a target for ADCs. These include the size, location, and type of vesicle containing the ADC once it is internalized, the dynamic and interconnected nature of malignant cells and the tumor microenvironment, the direct antitumor effects of the mAb itself, the function of the target antigen, and the downstream cellular changes induced by successful binding of the ADC to the target. The process is dynamic. The amount of antigen expressed may change in response to treatment and the rate of internalization of an ADC may be influenced other factors on the tumor cell or in the tumor microenvironment.

EXAMPLES

The following examples of ADCs highlight some aspects of target antigen characteristics as well as some of the complexities involved in picking a suitable target for an ADC.

CD30

Targeting an antigen that is specific, highly expressed, and able to internalize. CD30 is a member of the tumor necrosis factor (TNF) family. It is, in many ways, an ideal antigen for ADCs. CD30 is not detectable on healthy tissues outside the...
immune system or on resting lymphocytes and monocytes. It was originally identified as a cell surface marker of Reed-Sternberg and Hodgkin cells and serves as a primary diagnostic marker for Hodgkin lymphoma. It is also highly expressed on anaplastic large-cell lymphoma (ALCL) and a subset of other lymphomas including diffuse large B-cell lymphoma. The malignant hematopoietic cells that express CD30 are sensitive to a variety of cytotoxic drugs. Prior efforts to treat Hodgkin Disease with unmodified anti-CD30 mAb demonstrated anti-CD30 mAb could be administered safely, and effectively target the malignant cells. Unfortunately, the clinical antitumor response to unmodified anti-CD30 mAb was limited. Anti-CD30 mAbs were found to internalize effectively after binding, a desired characteristic for an ADC target.

Brentuximab vedotin (SGN-35) is an ADC that consists of an anti-CD30 monoclonal antibody linked to three to five molecules of monomethyl auristatin E (MMAE), an antimitotic agent, via a valine citrulline peptide linker. It gained accelerated approval from the FDA in August of 2011 for treatment of relapsed or refractory systemic ALCL and relapsed or refractory Hodgkin’s Lymphoma.

HER2 Enhancing efficacy of an approved mAb. HER2 is amplified and over-expressed in a minority of breast cancers and is also over-expressed in select cases of other cancers including gastric, ovarian, endometrial, and uterine papillary serous carcinoma. HER2-targeted mAb trastuzumab has been used therapeutically for the treatment of breast cancer for over 14 years. However, there is considerable room for improvement in HER2-targeted therapy. Primary and acquired resistance are both seen with trastuzumab, as is toxicity to standard regimens. These drawbacks served as an impetus for developing an ADC against HER2. Support for developing an ADC against a HER2 target was strengthened with the understanding that trastuzumab binding triggers HER2 internalization and degradation. Trastuzumab emtansine (T-DM1), is an ADC that consists trastuzumab, joined via a stable linker to a derivative of maytansine, a highly potent cytotoxic chemotherapy. Trials of T-DM1 as a single agent and in combination with other chemotherapies have shown clinical activity and a favorable safety profile in patients with HER2-positive metastatic breast cancer. Citing an improvement in progression-free survival and median overall survival over patients treated with lapatinib plus capecitabine, the FDA approved ado-trastuzumab emtansine for patients with HER2-positive metastatic breast cancer in February of 2013.

CD33 Clinical promise despite limited specificity and lower expression. Gemtuzumab ozogamicin is an anti-CD33 IgG4 mAb linked to a semisynthetic derivative of calicheamicin that is a potent DNA-binding antibiotic that causes double stand

### Table 1. ADCs in development for Hematologic Malignancies

<table>
<thead>
<tr>
<th>Target</th>
<th>Cancer Type</th>
<th>ADC Name</th>
<th>Current Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD33</td>
<td>Relapsed AML</td>
<td>Gemtuzumab ozogamicin (Mylotarg)</td>
<td>FDA approved based on accelerated approval. Subsequently approval withdrawn.</td>
</tr>
<tr>
<td>CD22</td>
<td>ALL, NHL, B cell lymphoma</td>
<td>CMC544</td>
<td>Phase I/II/III</td>
</tr>
<tr>
<td>CD19</td>
<td>NHL, ALL, diffuse Large B Cell lymphoma</td>
<td>SAR3419</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>CD74</td>
<td>Multiple myeloma</td>
<td>hLL1-DOX</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>CD138</td>
<td>Multiple myeloma</td>
<td>BT-062</td>
<td>Phase I/IIa</td>
</tr>
<tr>
<td>CD56</td>
<td>Multiple myeloma, Small cell lung cancer, ovarian cancer</td>
<td>IMGN901</td>
<td>Phase I</td>
</tr>
<tr>
<td>CD70</td>
<td>NHL, renal cell cancer</td>
<td>SGN-75</td>
<td>Phase Ib</td>
</tr>
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</table>

Source: Clinicaltrials.gov as of February 15, 2013.
breaks. It was the first ADC approved by the FDA. Its target, CD33, is a 67-kD transmembrane cell-surface glycoprotein of the sialo adhesion family. As with anti-CD30 mAbs, unconjugated anti-CD33 mAb had been administered safely to patients, but with limited clinical efficacy. The expression of CD33 is less tumor specific than some other targets of ADCs. It is expressed by mature and immature myeloid cells and erythroid, megakaryocyte, and multipotent progenitor cells.\(^{13,14}\) CD33 mAb and ADC therefore target both acute myeloid leukemia (AML) cells and benign CD33 bearing cells. Although 90% to 95% of patients with AML are CD33 positive, the actual level of CD33 expression is relatively low at a mean of 10,000 CD33 molecules per AML cell.\(^{15}\) This level of expression may also limit efficacy.

The FDA approval for gemtuzumab ozogamicin was for the treatment of patients with CD33-positive AML in first relapse who are 60 years or older and who are not considered candidates for cytotoxic chemotherapy.\(^ {16}\) This was an accelerated approval based on phase II clinical trials that included substantial proportions of older patients with relapsed AML. Despite this early promise, gemtuzumab ozogamicin was withdrawn from the U.S. market when postmarketing confirmatory phase III analysis indicated limited efficacy in the presence of enhanced toxicity.\(^ {17}\) New data from clinical trials for older patients with AML suggest that a modification of the dose may permit patients to benefit without unacceptable toxicity.\(^ {18}\)

**CD19**

**Going back to ADC roots.** In the 1980s, Ellen Vitetta and other investigators began exploring immunotoxins composed of anti-CD19 mAb linked to ricin and other protein toxins.\(^ {19,20,21}\) These agents were highly potent, but the immunogenicity and nonspecific toxicity of the protein toxins posed major problems that limited further development.\(^ {22,23}\)

CD19 and other coreceptors found on B cells, including CD21, remain appealing targets for ADCs. Their expression is limited to B cells, they are expressed in many cases at high levels, and internalize in a manner that is attractive for ADC development.\(^ {24,25}\) In an interesting illustration of the underlying biologic complexity in choosing a target, the levels of CD21 can effect the rate of internalization of anti-CD19 antibodies and anti-CD19-MCC-DM1 causes greater cytotoxicity in the faster anti-CD19-internalizing cell lines, implying that the rate of lysosomal delivery and subsequent drug release is important.\(^ {26}\) Anti-CD19 ADCs are currently in development and have passed phase I clinical trials.\(^ {27}\)

### TARGETING THE VASCULATURE: A NOVEL APPROACH TO ADCs

Tumor growth beyond 1 mm to 2 mm in diameter requires tumor angiogenesis.\(^ {28}\) This process involves degradation of the extracellular matrix, proliferation and migration of endothelial cells, and tube formation. These new blood vessels offer another potential set of targets for ADCs with the goal of inducing selective destruction of the blood vessels within established tumors leading to ischemic tumor cell death.

An optimal ADC target in the tumor neovasculature has some parallels with ADC tumor cell targets. Such a target would be highly expressed in new blood vessels within the tumor relative to blood vessels in benign tissues. The target would also need to allow the ADC to be internalized and release its cytotoxic drug into the endothelial cells of the vessel.

When compared to tumor cell surface antigens, neovascular targets for ADCs have certain conceptual advantages.

### TABLE 2. ADCs in Development for Solid Tumors

<table>
<thead>
<tr>
<th>Target</th>
<th>Cancer Type</th>
<th>ADC Name</th>
<th>Current Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2</td>
<td>Breast cancer</td>
<td>Trastuzumab emtansine (anti HER2-SMCC-DM1)</td>
<td>FDA approved February, 2013 for metastatic breast cancer</td>
</tr>
<tr>
<td>GPNMB</td>
<td>Breast cancer, melanoma</td>
<td>CDX-O11</td>
<td>Phase I/II completed</td>
</tr>
<tr>
<td>PSMA</td>
<td>Metastatic prostate cancer</td>
<td>PSMA-ADC</td>
<td>Phase II</td>
</tr>
<tr>
<td>Ley</td>
<td>Non-small cell lung carcinoma</td>
<td>SGN-15 (cBR96-doxorubicin immunotoxin)</td>
<td>Phase II completed 2003</td>
</tr>
<tr>
<td>CA6</td>
<td>Solid malignant neoplasms</td>
<td>SAR566658</td>
<td>Phase I</td>
</tr>
<tr>
<td>CanAng</td>
<td>Solid tumors</td>
<td>IMGN242</td>
<td>Phase I</td>
</tr>
<tr>
<td>Av integrin</td>
<td>Solid tumors</td>
<td>IMGN388, IMGN368</td>
<td>Phase I</td>
</tr>
<tr>
<td>SLC44A4</td>
<td>Pancreatic Ca, Gastric Ca</td>
<td>AGS-5 ADC</td>
<td>Phase I</td>
</tr>
<tr>
<td>CEACAM5</td>
<td>Metastatic colorectal cancer</td>
<td>IMMU-130</td>
<td>Phase I</td>
</tr>
<tr>
<td>Nectin-4</td>
<td>Solid tumors</td>
<td>AGS-22M6E</td>
<td>Phase I</td>
</tr>
<tr>
<td>AGS-16</td>
<td>Kidney, RCC</td>
<td>AGS-16MBF</td>
<td>Phase I</td>
</tr>
<tr>
<td>Anti-Cripto</td>
<td>Solid tumors</td>
<td>BiiB015</td>
<td>Phase I</td>
</tr>
<tr>
<td>Carbonic Anhydrase 9</td>
<td>Solid tumors</td>
<td>BAY79-4620</td>
<td>Phase I</td>
</tr>
<tr>
<td>Mesothelin</td>
<td>Solid tumors</td>
<td>BAY94-9343</td>
<td>Phase I</td>
</tr>
<tr>
<td>TENB2</td>
<td>Prostate cancer</td>
<td>Anti-TENB2 ADC</td>
<td>Preclinical</td>
</tr>
<tr>
<td>5T4</td>
<td>Non-small cell lung cancer</td>
<td>AtmMMAF (Anti-5T4-maleimidocaproyl linker-MMAF)</td>
<td>Preclinical</td>
</tr>
</tbody>
</table>

Given that all carcinomas are dependent on angiogenesis, targets within the tumor neovasculature for antibody drug conjugates could potentially have much broader indications for use in oncology. Endothelial cells are less likely to undergo somatic mutations that lead to drug resistance. They constitute the most proximal cell layer encountered by intravenously administered drug, thus vascular-targeting ADCs are more likely to achieve higher on-target exposure levels. Potential neovasculature targets for ADCs include vascular endothelial growth factor (VEGF) and its receptors, certain integrins (αvβ3, α5β1, α6β4, α1β3, α5β5, and αvβ5) and endoglin. VEGF is particularly noteworthy as a potential target for ADCs given the success of bevacizumab, an anti-VEGF-A mAb that has been approved for a number of cancers.

CONCLUSION

ADCs offer a means to deliver highly cytotoxic small molecule drugs to a tumor by linking them to a mAb that binds to a specific tumor antigen. The concept of combining the potency of a highly toxic agent with the specificity of mAbs has been explored for over 25 years, and is now generating increased enthusiasm with over 20 ADCs in development. The choice of the target antigen is an important parameter in determining the efficacy, therapeutic window, and toxicity profile of the ADC. Antigens being explored as targets are located on the tumor cell surface, in the neovasculature and in the extracellular stroma. Optimal tumor targets are highly expressed in the tumor, have low expression in normal tissues, and internalize effectively. The search for better target antigens, particularly for solid tumors, is ongoing, as are efforts to develop better linkers and drugs for ADCs. Given the speed of progress in each of these areas, it is likely new and efficacious ADCs will continue to be developed and evaluated. Future success will come from both the development of new agents, and the exploration of combination treatments involving ADCs and other antineoplastic agents.

Disclosures of Potential Conflicts of Interest

The author(s) indicated no potential conflicts of interest.

References


