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CANCER CELL TARGETING: WHY ARE SO FEW ANTIBODY-DRUG CONJUGATES FDA APPROVED?

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CANCER CELL TARGETING: WHY ARE SO FEW ANTIBODY-DRUG CONJUGATES FDA APPROVED?

An Interactive Qualifying Project Report

Submitted to the Faculty of

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In partial fulfillment of the requirements for the

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This report represents the work of WPI undergraduate students submitted to the faculty as evidence of completion of a degree requirement. WPI routinely publishes these reports on its website without editorial or peer review. For more information about the projects program at WPI, please see http://www.wpi.edu/academics/ugradstudies/project-learning.html
ABSTRACT

Antibody-drug conjugates (ADCs) are a type of targeted therapy for killing cancer cells. ADCs combine the power of antibodies to recognize and bind specific proteins on tumor cells with the power of newly developed highly potent cytotoxic drugs for killing cells. Although the targeting idea appears simple, to date only two ADC drugs have received FDA approval. The goal of this IQP project was to evaluate ADC technology by assessing its technical and regulatory problems to help determine the obstacles for gaining FDA approval. Our team performed a review of the current research literature, and conducted interviews with academic, industry, and legal experts. We conclude that ADCs have great potential, and in some cases have been shown to cause complete cancer remissions. The few ADCs that have been approved target well-chosen antigens with excellent properties, and had years of research prior to approval. All ADCs cause adverse side-effects, but they are usually manageable, and are out-weighed by the need for new drugs for treating relapsed cancer that does not respond to other therapies. We recommend that ADC research be continued, especially in the areas of combination therapies, non-internalizing ADCs that target the tumor vasculature, immunotoxins and radioimmune therapies to complement ADCs, and site-specific conjugations to control the number and locations of drug attachments to the antibodies.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title Page</td>
<td>01</td>
</tr>
<tr>
<td>Abstract</td>
<td>02</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>03</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>04</td>
</tr>
<tr>
<td>Authorship</td>
<td>06</td>
</tr>
<tr>
<td>Project Goals</td>
<td>07</td>
</tr>
<tr>
<td>Executive Summary</td>
<td>08</td>
</tr>
<tr>
<td>Literature Review, Antibody-Drug Conjugates (ADCs)</td>
<td>23</td>
</tr>
<tr>
<td>Section-1: Introduction to Antibodies and ADCs</td>
<td>23</td>
</tr>
<tr>
<td>Section-2: ADC Pre-Clinical Testing</td>
<td>36</td>
</tr>
<tr>
<td>Section-3: ADC Clinical Trials and Safety</td>
<td>52</td>
</tr>
<tr>
<td>Section-4: ADC Legalities</td>
<td>75</td>
</tr>
<tr>
<td>Section-5: ADC Recent Advances and Directions</td>
<td>86</td>
</tr>
<tr>
<td>Methods</td>
<td>105</td>
</tr>
<tr>
<td>Results/Findings</td>
<td>106</td>
</tr>
<tr>
<td>Conclusions and Recommendations</td>
<td>118</td>
</tr>
<tr>
<td>Appendix</td>
<td>121</td>
</tr>
<tr>
<td>Example Questions</td>
<td>121</td>
</tr>
<tr>
<td>Interview Preamble</td>
<td>122</td>
</tr>
</tbody>
</table>
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### AUTHORSHIP

<table>
<thead>
<tr>
<th>Author</th>
<th>Topics Covered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kimberley Kirchner</td>
<td>Introduction to Antibodies and Antibody-Drug Conjugates</td>
</tr>
<tr>
<td>William Mosby</td>
<td>ADC Pre-Clinical Testing</td>
</tr>
<tr>
<td>Alex Gallant</td>
<td>ADC Clinical Trials and Safety</td>
</tr>
<tr>
<td>Austin LaBastie</td>
<td>ADC Legalities</td>
</tr>
<tr>
<td>Alex Kolodziejczak and Sean St. Pierre</td>
<td>ADC Recent Advances and Future Directions</td>
</tr>
</tbody>
</table>
PROJECT GOALS

The overall goal of this IQP project was to document and evaluate the technology of antibody-drug conjugates (ADCs) for targeting and killing cancer cells, to determine why only two of the approximately 40 ADCs currently in human testing have received FDA approval, and to map the steps for moving forward with other ADC drugs.

The specific objectives were to:

1. Develop a comprehensive assessment of the scientific experiments that led to the development of antibody-drug conjugates, and discuss the technique’s potential applications.
2. Characterize what key scientific and IVF stakeholders believe are the strengths and weaknesses of this technology, and their legal hurdles.
3. Evaluate the obtained evidence, and prioritize the remaining problems.
4. Recommend potential solutions to the remaining problems.
Antibody-drug conjugates (ADCs) are a type of targeted drug therapy for specifically killing cancer cells. ADCs combine the power of antibodies (that recognize and bind specific proteins) with the power of new highly potent drugs that kill cells in very small quantities. An ADC drug contains an antibody directed against a tumor antigen (that is hopefully lacking in normal cells), chemically conjugated to a cytotoxic drug via a linker. Although the ADC idea seems simple and powerful, ADCs have not achieved their full potential. While over 30 different ADCs are under development, only three have received FDA approval, and one of those was withdrawn from the market when it was shown to increase patient death.

Some ADCs have undergone strong pre-clinical testing, including using human tumor cell lines grown in vitro, and using mouse xenograft models (mice growing human tumors in vivo). But even when an ADC drug shows strong pre-clinical data, this does not guarantee a strong performance in clinical trials. In the ADC clinical trials performed to date, all ADCs have adverse side-effets, but they appear to be mostly manageable, and the drugs appear to be relatively safe compared to the symptoms of the advanced cancer. However, ADCs show varying levels of effectiveness. If a drug has been shown to improve patient survival by only a few months, does this warrant FDA approval? What types of side-effects are seen, and can they be minimized? Do the needs of cancer patients with very poor prognosis (ADCs are given to a patient only if he no longer responds to other drugs) outweigh the adverse side-effects caused by the ADCs? What are the key problems remaining for FDA approval of more ADCs?

The overall goal of this IQP project was to document and evaluate the technology of antibody-drug conjugates, to assess their technical and legal problems, and to make recommendations for moving forward. The specific objectives were to: 1) develop a comprehensive assessment of the published scientific experiments related to ADCs (including pre-clinical experiments that led to the development of ADCs, the clinical trial data including safety, the legal hurdles for approving new ADC drugs, and the technique’s potential future applications), 2) characterize what key scientific stakeholders believe are the strengths and weaknesses of this technology, and their legal concerns, 3) evaluate all of the obtained evidence, and prioritize the remaining problems, and 4) recommend potential solutions to remaining problems.

To accomplish objective-1, we performed a review of the current literature, including reputable academic journal articles, relevant books, scholarly websites, and other pertinent materials. To accomplish objective-2, we conducted interviews with various researchers in academia and industry who had developed or tested ADCs in animals or humans. We also interviewed legal experts to clarify remaining problems for gaining FDA approval for ADC drugs. After performing the Literature Review and interviews, the team synthesized the collected information to ascertain the strength of the evidence for and against ADC drugs, and created recommendations for moving forward.
Cancer and the Need for New Drugs

The National Cancer Institute defines cancer as a term for a group of diseases in which abnormal cells divide without control and which can invade nearby tissues (NCI, 2017). At the cellular level, cancer has several hallmarks, including: genomic instability, deregulated cell signaling causing cell division and growth, sustained cell proliferation, resistance to cell death, and evasion from the patient’s immune system (Hanahan and Weinberg, 2011). As a result of these changes, cancer is difficult to treat. A cancer statistic review performed by the National Cancer Institute shows that survival rates range widely, from about 8% to 18% for difficult to treat cancers (like pancreatic, lung, and liver cancers), to greater than 65% for cancers of the colon, breast, kidney, and prostate (that grow slowly and are easier to treat if detected early) (Howlader et al., 2017). The poor survival rates for some cancers are observed even when using the best anti-cancer therapies, such as chemotherapies to block dividing cells (e.g., taxanes, anthracyclines, platin), small-molecule inhibitors (to block specific cellular signal transduction pathways), or bio-therapeutics (e.g., therapeutic antibodies against cell surface receptors). These therapies are not fully effective, and all of them have adverse side-effects, so scientists are constantly trying to develop new therapies that are more effective with fewer side-effects.

Introduction to Antibodies

Antibodies are molecules secreted by a B-cell, a type of white blood cell. Antibody molecules migrate through the blood and bind to a foreign protein (or parts thereof, antigens) to help inactivate it. Each antibody is highly specific for the protein it was designed to detect (Murphy and Weaver, 2016). The general structure of an antibody is the shape of a ‘Y’. Each upper arm of the ‘Y’ (which bind the antigen) is referred to as the N-terminus (amino-terminus). The N-termini are variable between different antibodies and dictate their binding properties. The lower base of the ‘Y’ is referred to as the C-terminus (carboxy-terminus) and is a constant region (Murphy and Weaver, 2016). The variable region is unique to each antibody, and the human immune system can produce over $5 \times 10^{13}$ different types of antibodies (Murphy and Weaver, 2016). There are five main classes of antibodies (IgA, IgD, IgE, IgG, and IgM), and the constant region remains the same within each class. The constant regions allow the antibody to interact with effector molecules and phagocytic cells that internalize the antibody-antigen complex. Once an antibody binds its antigen, based on its type of constant region, it signals for a specific effector function that leads to destruction of the pathogen.

Introduction to Antibody-Drug Conjugates

Based on the high specificity of interaction between an antibody and its antigen, scientists have engineered antibodies to bind to cancer cells. With a few exceptions, in most cases simply attaching an antibody by itself to a cancer cell antigen does not necessarily kill the cell (Thomas et al., 2016). So, scientists have designed a new type of anti-cancer drug, antibody-drug conjugates (ADCs). These consist of an antibody targeting a specific tumor antigen (usually on the cell surface) linked to a highly potent cytotoxic poison (the cargo). Most ADCs are designed to be “internalizing”: once the ADC binds to its target antigen, the cell engulfs the ADC and releases the toxin inside the cell, thus killing it (Pastan et al., 2006). To make a good ADC, the
antibody should strongly bind the antigen, the antigen should be highly expressed on the tumor cell (not normal cells), the linker should be stable in the patient’s circulation to avoid releasing the toxin too soon, and the cargo drug should be highly potent since only a few molecules will enter the cell.

In the ADC production process, monoclonal antibodies against a particular surface tumor antigen are usually produced in cell culture, and are then bound to a toxin using a linker (Pastan et al., 2006; Chiu and Gilliland, 2016). It is essential that the target antigen is as specific as possible for the cancer, so that the ADC will mostly bind and kill the tumor cells not normal cells (Thomas et al., 2016). In some cases this may not be possible, such as with leukemia where the target antigen may also be present in a low amount on normal cells. Some ADCs have been modified to remove the domains that bind normal cells (Pastan et al., 2006). Currently, all ADCs are linked to their drugs covalently through one of four chemical methods: cysteine disulfide bond conjugation, glycol-conjugation, protein tags, or amino acid incorporation (Pastan et al., 2006; Agarwal and Bertozzi, 2015; Thomas et al., 2016). The linker can be placed at various locations on the antibody, including the variable region, hinge region, constant region, or all three simultaneously (Agarwal and Bertozzi, 2015). Usually two to four cargo molecules are attached to each antibody such that the antibody retains all of its antigen-binding properties and is not cleared from the body too quickly (Hughes, 2010). The linker functions to keep the drug bound to the antibody on its journey to the target, and becomes degraded once the ADC enters the cell (Thomas et al., 2016). Linkers have been designed to be sensitive to proteases, pH, or glutathione, and are cleavable or non-cleavable (Jain et al., 2015). Cleavable linkers utilize enzymes inside the target cell to release the drug from the antibody (Jain et al., 2015). This ensures that the linker is stable in the bloodstream, and breaks down only when being digested by a target cell. Non-cleavable linkers do not release the drug once they enter the cell; when the antibody and linker are digested, the drug is released (Jain et al., 2015).

ADCs are typically administered to the patient intravenously. They bind to the target cell and are engulfed by an endocytic vesicle (Pastan et al., 2006; Bouchard et al., 2014). Once the intracellular vesicle fuses with a lysosome, the environment turns acidic and enzymes are released that digest the linker releasing the cargo into the cytoplasm where it works to kill the cell. Drugs commonly used in ADCs bind DNA (leading to its cleavage or alkylation), block tubulin (inhibiting cell division), or inhibit RNA polymerases (blocking RNA synthesis) (Thomas et al., 2016).

Example ADCs

Two ADCs are currently approved by the FDA: Trastuzumab emtansine (Kadcyla®) and Brentuximab vedotin (Adcetris®) (Thomas et al., 2016). A third ADC, Gemtuzumab ozogamicin (Mylotarg®), was initially approved but later withdrawn (Richwine, 2010). Kadcyla® was developed by Genentech, and is manufactured by Lonza. It is used to treat HER2-positive metastatic breast cancer in patients who are resistant to other treatments (Niculescu-Duvaz, 2010; LoRusso et al., 2011; Lopus, 2011; Verma et al., 2012; About Kadcyla, 2017). Kadcyla is composed of an antibody (Trastuzumab or Herceptin) against the HER2 receptor on the surface of some breast cancer cells conjugated to the drug DM1 (a derivative of maytansine) using a non-cleavable thioether linker (Barok et al., 2014; Jain et al., 2015). Of the various ADC drugs, Kadcyla is unique in that its antibody component by itself is sometimes
capable of blocking Her2-positive breast cancer. The binding of “naked antibody” Herceptin to Her2 receptor on the cell surface prevents receptor dimerization, inhibiting activation of the MAPK, PI3-kinase, AKT kinase pathways that otherwise stimulate cell division (Verma et al., 2012). Once ingested by the cell and released into the cytoplasm, DM1 inhibits microtubule assembly causing apoptosis (Barok et al., 2014; Jain et al., 2015; About Kadcyla, 2017).

Adcetris® is marketed by Seattle Genetics Inc. in North America, and by Takeda Oncology in the rest of the world. It is used to treat CD30-positive lymphoproliferative disorders, including Hodgkin lymphoma (HL) and anaplastic large cell lymphoma (ALCL) (Van de Donk and Dhimolea, 2012; Brentuximab vedotin, 2016). Protein CD30 often occurs on the surface of these tumor types, but rarely on normal cells (Küppers and Hansmann, 2005). Adcetris contains an antibody (Brentuximab or cAC10) targeting CD30 on HL and ALCL tumors, conjugated to 3-5 molecules of the drug monomethyl auristatin E (MMAE) by a cathepsin-cleavable linker (Van de Donk and Dhimolea, 2012). The antibody is produced in mammalian Chinese Hamster Ovary (CHO) cells, while the small molecule components are produced by chemical synthesis. Once engulfed by the cell and cleaved from its linker, MMAE binds to tubulin, inhibits mitosis, and initiates apoptosis (Van de Donk and Dhimolea, 2012; Francisco et al., 2003; Vaklavas and Forero-Torres, 2012).

Mylotarg® is manufactured by Wyeth (now Pfizer), and was FDA approved in May of 2000 to treat acute myeloid leukemia (Gemtuzumab Ozogamicin, 2015). Mylotarg is composed of an antibody (IgG4 κ hP67.6) against CD33 found on leukemia cells (Gemtuzumab Ozogamicin, 2015; Wyeth Pharmaceuticals, 2005), conjugated to 2-3 molecules of the drug calicheamicin (Gemtuzumab Ozogamicin, 2015). Calicheamicin binds to double stranded DNA causing breaks, leading to cell death (Wyeth Pharmaceuticals, 2005).

Currently, more than 40 ADCs are in the clinical trial stages of development (Thomas et al., 2016), and the future of ADCs seems bright. But in some cases the ADCs are not very effective, and all of the ADCs have adverse side-effects. Therefore, it is necessary to continue developing improved ADCs that are more effective with fewer side-effects.

Pre-Clinical ADC Testing

Pre-clinical testing of a drug in mice and on cell lines is necessary before proceeding to human clinical trials. ADC pre-clinical testing includes the use of cancer cell lines in vitro, the use of human cancers xenografted into mice, and more rarely monkey experiments. ADC drugs have been designed against a variety of surface antigens present on the surface of different types of tumor cells.

Our review of the literature included summarizing the results of pre-clinical experiments for ADCs against: CD19 (ADC SAR-3419) (Lutz et al., 2006; Gerber et al., 2009; Al-Katib et al., 2009; Carol et al., 2013), CD22 (ADC Inotuzumab ozogamicin or CMC-544) (DiJoseph et al., 2004a; 2004b; 2006; Shor et al., 2015), CD30 (ADC Brentuximab vedotin, Adcetris®, SGN-035) (Francisco et al., 2003; Hamblett et al., 2004; Okeley et al., 2010), CD33 (ADC Gemtuzumab ozogamicin, Mylotarg®) (Hamann et al., 2002), Her2 (ADC Trastuzumab emtansine, Kadcyla®, T-DM1) (Lewis-Phillips et al., 2008; Juntila et al., 2008; Kellogg et al., 2011; Phillips et al., 2014), gpNMB (ADC Glembatumumab vedotin (CDX-011) (formerly
Overall, the pre-clinical results showed that ADCs have the potential to slow or eliminate various types of cancers, both in vitro (cancer cell lines) and in vivo (xenograft models). The ADC drugs showed various degrees of effectiveness against a large variety of cancers, from inactive to complete tumor regressions. Use of the antibody alone, or the cargo toxin alone, produced no tumor killing, providing a proof-of-principle that combining the antibody with cargo (as with ADCs) strongly elevates the anti-tumor effects of either agent alone. In binding experiments, conjugation of the antibody to the cargo did not appear to alter the cell-binding ability of the antibody. In some cases, the researchers were able to correlate the extent of anti-tumor activity with the extent of target antigen expression on the tumor. Because some tumors as they mature alter the expression of surface markers, this suggests that a best practice would be to frequently monitor the patient’s tumor for target antigen expression to determine which patients most likely will benefit from the therapy.

The pre-clinical models allowed a more thorough testing than could be done with human clinical trials. For example, for some ADCs the pre-clinical tests allowed the testing of a wide range of doses: doses as low as 1 mg/kg showed tumor reductions, and doses as high as 30 mg/kg showed no signs of toxicity. A variety of cargo loads were also tested, showing that ADCs with cargo loads of 4-8 molecules per ADC were generally more active than those with only 1-2. A variety of linkers were also tested, showing that linker optimization is important for each type of ADC. The pre-clinical models also allowed the performance of specific types of experiments that could never be performed in humans, such as knocking down target antigen in tumor cell lines using small interfering RNAs to show decreasing surface antigen expression prevents destruction of the cell, and over-expressing the target antigen on normal cells to make them sensitive to the ADC. This shows the importance of the target antigen to the cell destruction. Other pre-clinical studies showed that delivering the ADC drug long-term post-cancer remission prevented disease re-occurrence (so long as the patient did not form antibodies against the ADC drug), so ADC use long-term might be tested in clinical trials to help ensure a patient’s cancer does not return. Most of the pre-clinical studies showed relatively minor adverse-effects of the ADCs; but the most serious side-effect observed in mice was neutropenia, so this might be expected to be a problem in the clinical trials, and should be monitored closely.

**ADC Clinical Trials and Safety**

Over 30 different ADCs are currently being investigated in patients (Sasson and Blanc, 2013), and this has provided a wealth of safety information for this new type of cancer drug. Our review of the literature in this area focused on some of the best investigated target antigens, including those with FDA approval. We summarized the data from clinical trials using ADCs against the following antigens: **CD19** (ADC SAR-3419) (Phase-I trials: Younes et al., 2009; Coiffier et al., 2011), **CD22** (ADCs Inotuzumab ozogamicin and CAT-8015, Moxetumomab pasudotox) (Phase-I trials: Advani et al., 2010; Kreitman et al., 2012) (Phase-II trials: Kantarjian.
et al., 2012; Wagner-Johnson et al., 2015), **CD30** (ADC Brentuximab vedotin, Adcertis®) (Phase-I trials: Seattle Genetics, 2010; Younes et al., 2010; Younes et al., 2013) (Phase-II trials: Younes et al., 2012; Pro et al., 2012), **CD33** (ADC Gemtuzumab ozogamicin, Mylotarg®) (Phase-III: Castaigne et al., 2012; Petersdorf et al., 2013; Hills et al., 2014) (Phase-II: Daver et al., 2016), **Her2** (ADC Kadcyla®, Trastuzumab emtansine, T-DM1) (Phase-I: Krop et al., 2010) (Phase-II: Burris et al., 2011; Perez et al., 2014; Phillips et al., 2014) (Phase-III: Verma et al., 2012; Krop et al., 2014), **gpNMB** (ADC Glembatumumab vedotin, CDX-011, formerly CR011-vcMMAE) (Phase-I: Hamid et al., 2010; Bendell et al., 2014) (Phase-II: Yardley et al., 2015), and **Trop-2** (ADC Sacituzumab govitecan, IMMU-132) (Phase-I: Starodub et al., 2015; Faltas et al., 2016).

Overall, the review of the clinical ADC literature showed that ADC drugs have a wide range of activity, and generally produce relatively mild and treatable adverse-effects. Importantly, almost all of the clinical trials were performed on patients who had relapsed from cancer, or whose cancer did not respond to previous therapies; only one trial was performed on newly diagnosed patients. Thus, the overall patient prognosis without further treatment was very low. An example of a strong response rate was seen with a CD22-targeting ADC that showed an overall response rate of 86%, with 46% achieving complete remission (Kantarjian et al., 2012). And another example was CD30-targeting Adcertis, where 21 of 22 patients (95%) achieved complete remission (Pro et al., 2012).

Two best practices were identified in the review of the literature which may be worth following up in interviews. The first best practice pertains to measuring chromosome cytogenetic abnormalities in the patients. Cytogenetic abnormalities include chromosome inversions, insertions, translocations, etc. Some of the Mylotarg clinical trials for leukemia patients showed that the survival benefit of the drug was best in patients with no additional cytogenetic abnormalities (other than the initial abnormality causing the leukemia). While in patients with adverse cytogenetics, Mylotarg provided no detectable benefit. This suggests that patients with additional chromosomal inversions or translocations incur new changes in cellular biochemistry that increase drug resistance. So, perhaps in best practice, patients should be monitored for cytogenetic abnormalities to help determine which patients are most likely to improve with ADC treatment.

The second best practice observed in the clinical trial literature was the assay of the extent of over-expression of target antigen in the patients. In several trials, patients with confirmed high expression levels of the target antigen showed higher response rates to the ADC, and the risk of cancer progression was lower. Antigen levels were assayed by immunohistochemistry, reverse transcriptase polymerase chain reaction (RT-PCR), or *in situ* hybridization. Thus, pre-analyzing the patient’s tumor to determine the extent of target antigen expression might help predict which patients most likely will benefit from the ADC treatment. A patient whose tumor no longer expresses the target antigen will not be targeted by the ADC.

With respect to safety, from the clinical trial data it was determined that all ADC drugs show side-effects. Of three ADC drugs approved by the FDA, one has been withdrawn from the market due to patient deaths and an apparent lack of efficacy. And all three FDA-approved drugs carry “black box warning labels” on their packaging, informing physicians of the most serious side-effects. The follow-up interviews will help weigh the adverse effects of the ADCs and their management against the side-effects of standard chemotherapy treatments, and will also be
weighed against the very poor prognosis of patients with these types of relapsed cancers who have few other options. But overall, the ADC side-effects appear to be more manageable than those caused by current chemotherapy treatments, and are far less worse than the very poor prognosis of patients with relapsed cancer. Neutropenia (low neutrophil count) was almost always observed in the clinical trials, but it was not fatal. As an example of safety data, a Phase-II trial of Adcetris on 102 patients with refractory/relapsed Hodgkin’s lymphoma showed that the most common treatment-related adverse effects were peripheral neuropathy, nausea, fatigue, neutropenia, and diarrhea (Younes et al., 2012), while a second Phase-II study showed Grade 3 or 4 (serious) adverse events of neutropenia (21%), thrombocytopenia (14%), and peripheral sensory neuropathy (12%) (Pro et al., 2012).

However, it remains unclear from the literature what causes the side-effects. Are the adverse reactions caused by expression of the target antigen in normal tissues in addition to the cancer? Or are the side-effects caused by off-target effects, such as the release of the cytotoxic cargo into the surrounding cells? Hopefully, the interviews can help resolve this issue, and will also help validate our conclusion that the side-effects are relatively manageable. In addition, interviews with the researchers might help determine whether the benefits observed of slowing or eliminating cancer outweigh the negative side-effects, especially in view of the poor prognosis of a patient with recurring cancer. The researchers who design ADCs might also suggest directions for future research.

**ADC Legalities**

Three different ADCs have been approved by the U.S. Food and Drug Administration (FDA) for treating various forms of cancer. One of the ADC drugs was subsequently withdrawn. The FDA approval process for new drugs is complex, with efficacy and safety likely being the main hold-ups for new ADC approvals.

In general, the purpose of the FDA approval process is to help protect consumers from drugs that are harmful or ineffective. Drug development and approval involves several key steps, including: pre-clinical testing, Investigational New Drug (IND) Application, Phase-I testing, Phase-II testing, Phase-III testing, New Drug Application (NDA), and Post-Marketing testing (Phase-IV) (Lipsky and Sharp, 2001). During pre-clinical testing, the FDA requires researchers to perform animal tests before humans are exposed to a new drug. For ADC drugs, this includes testing in vitro against cultured cancer cell lines, testing in mice engrafted with human cancer cells (xenograft mice), and (less frequently) testing in monkeys. The main objective of pre-clinical testing is to obtain preliminary animal data on drug safety and activity. If this data looks promising, the investigators file an IND application that includes drug chemical composition and manufacturing data, animal test results (including safety), the rationale for testing a new compound in humans, strategies for protecting human volunteers, and a plan for clinical testing (Stave and Joines, 1997). If the FDA approves the IND, the process heads into the three phases of clinical testing. Phase-I focuses mostly on drug safety and pharmacology in humans at various doses (Lipsky and Sharp, 2001). Phase-II studies are designed to obtain data on drug effectiveness, optimum drug dose, best method of delivery (IV, oral, etc.), and dosing interval, all assayed while focusing on safety (Walters, 1992; Heilman, 1995). During Phase-III, researchers attempt to confirm their previous Phase-II findings using a larger group of patients. Only about 27% of IND drugs make it past Phase-III. If the Phase-III data look promising, the
investigators file a New Drug Application (NDA). The NDA contains all the preclinical and clinical information obtained during the testing phase, including drug chemical composition, manufacturing procedures, toxicity, human pharmacokinetics (half-life and bio-distribution), clinical trial results (within the U.S. and elsewhere), and proposed labeling (Lipksy and Sharp, 2001). If the drug is approved, it can be marketed. Sometimes, the FDA requests a Post-Marketing Study (Phase-IV) to provide more data if something remains unclear or if new problems are identified with further use of the drug.

Prior to the modernization acts discussed below, the overall FDA approval process took approximately 8-12 years (Heilman, 1995). But the review time has now been shortened to about 12.6 months for normal drugs, and 6 months for an accelerated review (Drugs.com, 2013). All three of the FDA-approved ADC drugs (Mylotarg, Adcetris, and Kadcyla) received FDA approval under an accelerated review process. Accelerated reviews are sometimes granted when no other alternative drugs are available to treat a particular disease, or when the clinical trial data are very strong (Drugs.com, 2013). The Prescription Drug User Fee Act of 1992 (PDUFA) was designed to help shorten the FDA review time by allowing the FDA to collect user fees from IND filers to help enhance the review process (Walters, 1992). In addition, the FDA Modernization Act of 1997 (FDAMA) extended the use of the user fees, and streamlined the drug approval process (FDAMA, 1997).

The first ADC drug approved by the FDA was Mylotarg® (Gemtuzumab ozogamicin). Based on the promising initial data observed in preliminary clinical trials, Mylotarg was FDA-approved on May 17, 2000 under an accelerated FDA-approval process (FDA, 2000) for treating patients over the age of 60 with relapsed acute myeloid leukemia (AML), or for other AML patients not considered a candidate for standard chemotherapy (Bross et al., 2001). But within a year of Mylotarg’s initial approval, more serious adverse events were observed, including adult respiratory distress syndrome (ARDS), hepatotoxicity, anaphylaxis, veno-occlusive disease (VOD), and death (Nelson, 2010). The appearance of these more serious events resulted in the FDA requiring a “black box warning label” on Mylotarg’s package (Giles et al., 2001; Wadleigh et al., 2003). In June 2010, Mylotarg was voluntarily withdrawn from the market when additional clinical trial data and post-marketing experience showed the drug increased patient death and was no better than conventional therapies (Nelson, 2010; Richwine, 2010). In September 2017, almost seven years after withdrawing it from the market, Pfizer resubmitted their application for FDA review as a Biologics License Application (BLA) (Stanton, 2017) based on favorable data obtained since 2010.

The second ADC drug approved by the FDA was Adcetris®. In North America Adcetris is marketed by Seattle Genetics Inc., and in the rest of the world by Takeda Oncology. It has undergone numerous clinical trials, generally showing high efficacy and relatively manageable side-effects. Seattle Genetics submitted an FDA application on February 28, 2011, to treat HL and ALCL patients (Fierce Biotech, 2011), and on August 19, 2011, the drug was granted an accelerated FDA approval (Genetic Engineering and Biotechnology News, 2011). However, on January 13, 2012, the FDA announced that Adcetris had been linked to two cases of progressive multifocal leukoencephalopathy (defective formation of myelin), so the FDA now requires the manufacturer to add a “black box warning” to the drug packaging warning of this risk. Adcetris has also been approved in Europe by the European Medicines Agency (EMA). In 2009, the drug initially received approval for use as an “orphan product” (a drug that is used to treat a disease that affects no more than 5 people in 10,000) (Hofland, 2011), but in view of favorable clinical
data, in 2016 it was given EMA marking authorization. The basis of the favorable review by the EMA was discussed in a 2016 European Public Assessment Report (EPAR), stating that the EMA strongly factored in the drug’s relatively manageable adverse effects, the patient’s poor expected outcomes during relapse, and the lack of other suitable drug treatments for relapsed patients (European Medicines Agency, 2016).

The third ADC drug approved by the FDA was Kadcyla® (Trastuzumab emtansine). The antibody was developed by Genentech (a subsidiary of Roche), the linker and toxin were developed by ImmunoGen (Waltham, MA), and the complete ADC is manufactured by Lonza (Pollack, 2013). Kadcyla underwent an accelerated 6-month FDA priority review, reserved for therapies for diseases with no alternative treatments, or that provide significant improvements over other marketed products (Drug.com, 2013). In 2010, Genentech initially applied for accelerated FDA review on the basis of the results of a single Phase-II study, but on August 26th, 2010, the FDA refused to review it in accelerated mode because all of the available treatment choices had not yet been tested in their patients (News-Medical.net, 2010). So, Genentech continued their studies with an amended Phase-III trial after treating the patients with other treatment options. Kadcyla was approved on February 22, 2013, based on the positive clinical trial data of women with advanced Her2-positive breast cancer who were already resistant to antibody treatment alone (Verma et al., 2012). Kadcyla comes with its own set of side-effects which must be weighed against the potential benefit of blocking the breast cancer growth, but the adverse effects were tolerated better than those seen with standard chemotherapies. In the U.S., Kadcyla packaging carries a “black box warning” for liver toxicity, heart damage, and fetal harm if given to pregnant women.

The high cost of ADC drugs is an important issue for consumers. Kadcyla is a good example. In the U.K., the National Institute for Health and Care Excellence (NICE) and the National Health Service England (NHS) determine whether a particular drug remains on the Cancer Drugs Fund (CDF) list of medicines paid by the government. The normal CDF cut-off for an end-of-life drug like Kadcyla is about £50,000 per year (The Guardian, 2015) or £30,000 per quality-adjusted life years (NICE, 2014). Roche’s initial price was high, a reported £90,000 per patient per year (Pollack, 2013), nearly double the CDF cut-off, placing the drug in jeopardy for being removed from the list. In 2014, a draft guidance document produced by NICE concluded that Kadcyla’s price was too high for the CDF list (NICE, 2014). But in 2015, Roche compromised and lowered their price to £5,900 per month. At the new price, a typical 9.6 month treatment would cost about £56,640, bringing the drug close to the CDF cut-off. So, the NICE and NHS England committees have, for now, left Kadcyla on the list (The Guardian, 2015). Hopefully, other pharmaceutical companies will follow Roche’s lead of cutting ADC drug prices to be more affordable.

Overall, the legal portion of the ADC Literature Review summarized the FDA approval process for ADC drugs, and discussed the three approved ADCs. All three of the FDA-approved ADC drugs were reviewed under an accelerated program, either due to strong and clear clinical data better than other available treatments, or because the drugs provided a treatment for a disease with no other options available at the time of the review. All three approved ADCs have side-effects requiring black box warnings on the packaging, but they mostly appear to be manageable, transient, and less prevalent than those seen with current chemotherapy treatments. A current ADC trend appears to be the testing of drug combinations, as demonstrated by
combining the ADC Adcetris with the immune checkpoint inhibitor Opdivo®, and we will obtain further information on this promising combination approach in interviews.

**ADC Recent Advances and Future Directions**

Although ADC drugs show great promise, they are not perfect. In all clinical trials performed to date, ADCs have caused adverse side-effects in the patient, and is sometimes ineffective against the cancer. The future approval of ADCs under development may likely depend on improving their clinical performances to warrant their use (Panowski et al., 2014). Thus, there is room for continued ADC improvement.

The first-generation ADC drugs (1<sup>st</sup>-ADCs) as originally developed came with a variety of problems, including lack of efficacy and adverse side-effects. Thus, much research has gone into creating second-generation ADCs (2<sup>nd</sup>-ADCs) with superior properties (Thomas et al., 2016). 1<sup>st</sup>-ADCs usually contained mouse monoclonal antibodies against the target protein. But injecting a mouse antibody into human patients often stimulated an immune response against the drug, lowering drug effectiveness. 2<sup>nd</sup>-ADCs contain mouse-human chimera antibodies or fully humanized antibodies that produce less of a response in patients. 1<sup>st</sup>-ADCs also had short half-lives in the blood, releasing their toxic payload too soon into the circulation instead of in the tumor cell. In some cases the linkers released the payload too early, before reaching the tumor cell. 2<sup>nd</sup>-ADCs use improved linker chemistries (such as the use of disulfide linkages, dipeptide linkages, and hydrazine linkages) to hold the payload tighter, releasing it only in the acidic environment of the internalization vesicle. In addition, 1<sup>st</sup>-ADCs used first-generation cytotoxic payloads (such as doxorubicin, vinblastine, or methotrexate). Those first-generation payloads had inhibitor concentration-50 values (IC<sub>50</sub>) in the micromolar range, while 2<sup>nd</sup>-ADCs use newly designed payloads (such as Calicheamicin, Maytansine derivatives like DM1, or Auristatins like MMAE) that are far more toxic, with IC<sub>50</sub>’s in the nanomolar range (same effectiveness at 1000-fold lower concentrations).

Several key steps must occur for ADCs to work properly (Loganzo et al., 2016). These steps include: movement of the ADC through the patient’s circulation without losing its toxic cargo, binding of the ADC to the target antigen on the surface of the tumor cell, lack of targeting of the ADC to normal cells, internalization of the ADC into an endocytic vesicle, binding of the endocytic vesicle to a lysosome to acidify the compartment and release enzymes into the endosome, degradation of the linker and sometimes antibody to release the toxic cargo, movement of the cargo into the cytoplasm, and targeting of the cargo to a cellular component such as DNA or tubulin to prevent cell division. Inefficient function of any one of these steps can create an inactive drug. Problems independent of the tumor itself include: premature loss of the cargo drug into the blood, and poor pharmacokinetics (ADME) (poor absorption, distribution, metabolism, excretion) (Kraynov et al., 2016). In other cases, the environment surrounding the tumor limits access to large molecules like ADCs. These environmental changes might include the increased formation of vascular barriers such as basement membranes or increased formation of extracellular matrix. In other cases, the tumors themselves become resistant to the ADC treatment by altering one or more of the steps needed by ADCs to kill the...
cell (Loganzo et al., 2016). Each of these steps presents a research opportunity to improve second-generation ADCs relative to early versions of the drugs.

ADC drug design is a subject of much research in the biotech industry. One example of a company designing new ADCs is Tarveda Therapeutics (Watertown, MA), a clinical stage biopharmaceutical company (Tarveda.com, 2017). Their main technology is Pentarins™, which are potent, selective, miniaturized ADCs designed to penetrate more deeply into solid tumors than normal ADCs. According to Richard Wooster, Tarveda’s President of Research and Development, “The result of our [Pentarin platform of miniaturization] is a drug that is about 15 times smaller [than antibodies], and are likely to penetrate deeper into the tumor” (Ledford, 2016). Another example of a biotech company investigating ADCs is Mersana Therapeutics (Cambridge, MA). This company uses a patented “Fleximer technology” to design biodegradable ADC drugs with improved drug solubility, pharmacokinetics, reduced immunogenicity, and optimized drug loading (Mersana.com, 2017). The platform attempts to increase control over when, where, and how the ADCs are released. According to Timothy Lowinger, Mersana’s Chief Scientific Officer, “This [technology] allows the company to attach 15 molecules of the drug to each polymer, rather than the usual 3-4 (Ledford, 2016). Their most developed drug is XMT-1522 that targets Her-2 in breast cancer, currently in Phase-I clinical trials (Mersana.com, 2017).

A future ADC direction is antibody-type switching. 1º-ADCs used murine antibodies which tended to produce immune responses against the ADCs, lowering their efficacy (Teicher et al., 2011). 2º-ADCs use humanized mouse-human hybrids or purely human antibodies which are less immunogenic in patients than mouse antibodies, and are 100 to 1000-fold more potent. An example of an ADC containing a mouse-human chimera antibody is Adcetris (Deng et al., 2013), while an example of an ADC with a fully human antibody is CDX-011 (Keir and Vahdat, 2012).

Another important future direction for ADC research is the identification of new target antigens. Some of the ADC side-effects occur when the targeted tumor antigen is also expressed to some extent in normal tissues, so new unique tumor antigens need to be identified. In other cases, only a portion of a patient’s tumor cells express the target antigen, or the tumor down-regulates the antigen as it matures or metastasizes. Cells not expressing target antigen are not targeted by the ADC, so it is important to keep identifying new target antigens. A recent example of a newly identified target antigen is Her-3 that is over-expressed in breast cancer cells metastasized to the brain (Kodack et al., 2017). Another example is CD32a which may be a marker for latently HIV-infected T-cells which might help eliminate this cell population (Descours et al., 2017).

Another ADC direction worth pursuing in the future is the use of dual-targeted therapies. This approach uses two ADCs targeting the same antigen but carrying different cytotoxic cargos, or alternatively uses two different ADCs targeting different antigens on the same tumor. An example of the first approach is the use of the ADC Kadcyla to target Her-2-positive breast cancer cells combined with Pertuzumab (Perjeta) that binds the Her-2 receptor preventing its dimerization (Phillips et al., 2014). An example of the second approach has not
yet been done, but in theory could be done with Kadcyla (targeting Her-2-positive breast cancer cells) combined with an ADC against Her-3 (targeting metastasized cells).

With respect to **payload improvements**, if a patient becomes resistant to a specific type of cytotoxic agent delivered by an ADC, perhaps using an ADC targeting the same antigen but carrying a different payload can overcome the resistance. This approach has already been successful in mouse models of non-Hodgkin lymphoma (NHL) that became resistant to a CD22-targeting ADC. Altering the payload from MMAE to nemorubicin (which targets DNA), while still targeting CD22, overcame the resistance even when delivering only half the payload per cell (Yu et al., 2015). For other payload alterations, switching to 20º-drugs with potent toxicities in the picomolar range might help. For example, PBD compounds bind DNA in a sequence specific manner in its minor groove have picomolar activity against tumors (Flygare et al., 2012). Another promising ADC payload is α-Amanitin (Flygare et al., 2012). This toxin is a cyclic peptide of eight amino acids, and is the most deadly of all the amatoxins found in mushrooms, such as the death cap or the destroying angel (Michelot and Labia, 1988). α-Amanitin kills by inhibiting DNA transcription (the production of RNA from a DNA template), which is required by all cells. These payloads are in the early stages of testing, and have shown significant signs of success against a wide range of tumor cells.

Another future trend is the use of **non-internalizing ADCs** that target the tumor’s extracellular matrix. For example, the formation of new blood vessels (angiogenesis) is a common feature of many solid malignancies, and new vessel formation rarely occurs in normal adults, so targeting angiogenesis markers might be excellent targets for ADC therapy (Casi et al., 2012). Since angiogenesis markers are similar for various tumors, one ADC of this type could be used to treat a variety of tumors. Work has already begun using human mAbs specific to the extra-domain B (EDB) of fibronectin, which has been identified as a marker of angiogenesis (Palumbo et al., 2011).

Important ADC advances have also been made with **linker chemistries**. Linker stability is necessary to allow the conjugate to circulate in the bloodstream for an extended period of time without prematurely releasing its cytotoxic agent. Linker stability has a major influence on ADC properties such as pharmacokinetics, overall toxicity, and efficacy (Perez et al., 2014). ADC linkers currently under development fall under two main classes: cleavable and non-cleavable. For the cleavable linkers, there are three different release mechanisms: acid-sensitive linkers, lysosomal protease-sensitive linkers, and glutathione-sensitive linkers. For non-cleavable linkers, their main advantage is a greater stability in the circulation which prevents early release of the drug potentially eliminating the bystander effect.

**Conjugation chemistry** is another future area of ADC research. The 1º-ADC production methods produced heterogeneous mixtures of molecules (Panowski et al., 2014), with drugs being added to any solvent-accessible reactive amino acid (Agarwal et al., 2015). But the structural heterogeneity produced molecules with different properties, resulting in a wide range of activities and unpredictable results in patients (Panowski et al., 2014; Agarwal et al., 2015). The newer methods of drug attachment to antibodies use site-specific conjugations that eliminate product heterogeneity and improve conjugate stability. These new methods include the use of: 1) engineered cysteine residues, 2) unnatural amino acids, or 3) enzymatic conjugation through glycol-transferases and transglutaminases.
Radioimmunoconjugates are radioactive isotopes conjugated to antibodies targeting tumor cells. Radioimmunotherapy (RIT) has been under development for over 30 years, with little progress. But with new advancements in the field of targeted antibody cancer therapy, there is new excitement for RIT. Similar to ADCs, the choice of antibody and cytotoxic agent are critical for RIT efficacy. “The path length of penetration of the radioactive emission should match the size of the targeted tumor” (with small tumors using RITs with short range emissions) (Kraeber-Bodéré et al., 2014). Clinical trials have shown some promising results for RIT drugs targeting CD-20 in non-Hodgkin B-Cell Lymphoma using Yttrium-90 as the radionuclide (Zevalin®; Spectrum Pharmaceuticals) (Kraeber-Bodéré et al., 2014). Other research is directed toward the development of radioimmunoconjugates targeting the Her-2 antigen expressed in breast cancer using Lu-177 as the radionuclide (Bhusari et al., 2017).

Immunotoxins (ITs) are very potent molecules consisting of an antibody (or antibody fragment) linked to a bacterial or plant toxin rather than a traditional chemotherapeutic drug (Hassan et al., 2015). Immunotoxins work in a manner similar to ADCs, targeting specific tumor antigens and internalization into a tumor cell, but the toxin kills the cell by inhibiting protein synthesis. Because immunotoxins carry a cargo that kills a cell by blocking translation instead of blocking cell division like ADCs, this allows immunotoxins to be used against tumors that are no longer actively dividing (Hassan et al., 2015). Immunotoxins targeting CD-22 have produced complete remissions in refractory hairy cell leukemia and acute lymphoblastic leukemia in children (Wayne et al., 2011; Kreitman et al., 2012). As with ADCs, their large size may hinder penetration into solid tumors, so research has focused on creating IT’s with reduced size for both the antibody and toxin (Pastan et al., 2006). For example, immunotoxin SS1P targets mesothelin on mesothelioma cells and contains Pseudomonas toxin PE38. SS1P initially showed low activity due to antibody responses against the toxin, but when the patients were treated with an immunosuppressive regimen to lower the T and B cell responses, SS1P showed stronger activity (Mossoba et al., 2011; Hassan et al., 2013). In patients with advanced treatment-refractory mesothelioma, treatment with pentostatin and cyclophosphamide (to lower the immune response) plus SS1P immunotoxin significantly decreased the formation of anti-SS1P antibodies, allowing more cycles of SS1P to be given, which resulted in durable and major tumor regressions in three of the ten evaluable patients (Hassan et al., 2013).

Overall, this section of the project identified several different steps that are required for an ADC drug to function. Loss of any one of these steps can result in an inactive drug. New second-generation ADCs are being designed to overcome tumor resistance, be more active, and have fewer side-effects. Areas of future ADC research include: antibody-type switching, the identification of new target antigens, dual-targeted therapies, payload improvements, non-internalizing ADCs, improvements in linkers and conjugation chemistries, and the use of radioimmunoconjugates and immunotoxins. In addition, more research should be performed to identify mechanisms for how tumors become resistant to ADCs. To facilitate this, in vitro and in vivo mouse models could be developed for ADC resistance for various types of tumors by treating them long-term with an ADC until resistance occurs, and then characterizing the mechanism of the resistance.
Executive Summary Conclusions

Based on the research performed for this IQP project, our team made several conclusions and recommendations. It was initially unclear from our review of the literature why, with few exceptions, naked antibodies (not conjugated to a cytotoxic drug) do not efficiently kill cancer cells. Our interviews with immunologists and oncologists indicated that an active population of natural killer cells (NKs) is critical for killing the cancer cell, but NKs are low in concentration in the tumor environment. In addition, relatively few antibodies actually bind to the surface of the tumor cell. So, a more efficient mode of killing is required, such as using ADCs conjugated to highly potent drugs. ADCs have the additional advantage of killing a tumor cell regardless of the function of the surface antigen, while naked antibodies need to block growth pathways.

But if ADCs are such a good idea, why are only two currently approved by the FDA? From our interviews, the consensus appears to be that the two successful ADCs (targeting CD30 and HER2) were developed early on, when ADCs were relatively novel, making their approval easier as no other drug served the same purpose. And they were among the earliest ADCs researched, providing more time for the lengthy FDA approval process. In addition, the CD30 and HER2 target antigens are among the best targets in the entire cancer field: they are abundant on the tumor cell surface, expressed by a majority of the tumor cells, highly accessible to the ADC antibody, and are capable of internalizing the ADC. Subsequent ADC targets have not been able to provide the high degrees of efficacy and survival improvements that are needed for gaining FDA approval. We conclude that the choice of target antigen is critical in determining ADC activity, and we recommend that research be continued into identifying new targets.

Our review of the literature showed that all ADC drugs tested to date have adverse side-effects. Even the two currently approved ADCs have “black box” warnings on their packages concerning their side-effects. But it was unclear from the literature what caused the side-effects. Our interviews with physicians using ADCs in clinical trials indicated that the side-effects vary with each ADC: in some cases they are caused by off-target effects, where the cytotoxic drug released from the ADC diffuses out of the target cell into a nearby cell, killing it. In other cases, the physicians stated their side-effects were caused by the surface antigen being present on normal cells, decreasing their function. Several interviewees indicated their side-effects were manageable by using other drugs. One best practice seems to be the development of a linker that leaves a positive charge on the payload drug when cleaved. This makes the drug unable to diffuse out of the target cell to cause off-target effects. We recommend that this best practice be explored further.

Our review of the literature also showed that ADCs are expensive. In this report we discussed the example of Kadcyla in the U.K. whose initial price for a typical treatment was £56,640. But based on pressure from cancer charities and government committees, the manufacturer agreed to lower the price, keeping it on the list of drugs paid by the British government. We hope that other ADC manufacturers follow suit. However, ADC research and development is also expensive. In the U.S., it remains unclear even after interviewing co-chairs of the Moonshot Blue Ribbon Panel whether money for the Cancer Moonshot pertains to ADCs, so developing new ADCs may need to rely on other sources of funding.

Our review of the literature also identified several potential new directions for ADC research. These directions include the use of combination ADC therapies (the use of two or
more ADCs targeting different antigens or carrying two different cargos), the use of non-
internalizing ADCs targeting the extracellular matrix (ECM), the use of immunotoxins, the use
of radio-immunoconjugates, and the use of site-specific conjugations. Our interviews with
scientists performing dual ADC therapies indicated that toxicities are a problem with this
approach, as different sets of side-effects are caused by different ADCs, so this should be
carefully monitored. The non-internalizing ADC approach has already had some success in
animal models by blocking newly forming tumor vasculature. If this approach can be further
developed, the ADC could, in theory, be used to treat any type of tumor that requires new
vasculature, including a majority of solid tumors. Our interviews with scientists using
immunotoxins (ITs) indicated that it is important to alter the toxins to minimize patient immune
responses against the drug which lowers their effectiveness. Using both ADCs and ITs with two
methods of killing the cell has promise, and should be tested further. With respect to site-
specific attachment, new chemistries allow researchers to control the number of cytotoxic drugs
attached to each antibody, and to control the exact site of attachment. Our interview with
scientists using this technology indicated that the number of drugs per antibody, and their
locations, strongly affect ADC activity. So, we conclude that these parameters are important,
and should be optimized for each ADC drug.

Overall, we conclude that ADCs represent an interesting method for fighting cancer that
in some cases kill tumor cells more efficiently than “naked” antibodies alone. Some ADCs have
been shown to cause complete tumor regressions in some patients. The few ADCs already
approved by the FDA likely did so because of their excellent choice of target antigen and their
long history of research prior to approval. Although all ADCs currently have adverse side-
effects, they appear to be manageable in most cases, and are far better than the patient’s poor
prognosis without treatment. We recommend that ADC research be continued, especially on the
use of combination therapies, non-internalizing ADCs targeting the tumor vasculature, the use of
immunotoxins and radioimmune therapies to complement ADCs, and site-specific conjugations
to control the number and locations of drug attachments to the antibodies.
Cancer and the Need for New Drugs

The National Cancer Institute defines cancer as a term for a group of diseases in which abnormal cells divide without control, which can invade nearby tissues (NCI, 2017). The main types of cancer include: 1) carcinomas (cancers that initiate in skin or tissues that line or cover internal organs), 2) sarcomas (cancers that initiate in bone, cartilage, fat, muscle, blood vessels, or other connective tissues), 3) leukemia (cancers that initiate in blood-forming tissue, such as the bone marrow), 4) lymphomas and myelomas (cancers that initiate in cells of the immune system), and 5) central nervous system cancers (cancers that initiate in the tissues of the brain and spinal cord) (NCI, 2017).

At the cellular level, cancer has several hallmarks; these include: genomic instability, deregulated cell signaling causing cell division and growth, sustained cell proliferation, resistance to cell death, and evasion from the patient’s immune system (Hanahan and Weinberg, 2011). As a result of these changes, cancer is difficult to treat. A cancer statistics review performed by the National Cancer Institute shows that the average 5-year cancer survival rate in the U.S. for all cancers is about 69% (Howlader et al., 2017). The survival rates range widely, from about 8% to 18% for difficult to treat cancers like pancreatic, lung, and liver cancers, to greater than 65% for cancers of the colon, breast, kidney, and prostate, that grow slowly and are easier to treat if detected early.

The wide range of cancer survival rates are seen even with the best cancer treatments. Research over the past several decades has improved cancer survival using a variety of types of drugs, including: traditional chemotherapy to block dividing cells (e.g., taxanes, anthracyclines, platins), small-molecule inhibitors of specific cellular signal transduction pathways (e.g., kinase inhibitors), or bio-therapeutics (e.g., therapeutic antibodies against cell surface receptors). But these drugs are not fully effective, and all of them have adverse side-effects, so scientists are constantly trying to develop new therapies that are more effective with fewer side-effects.

The main topic of this IQP project is a new class of anti-cancer therapy termed antibody-drug conjugates (ADCs). ADCs are a type of targeted therapy in which a specific type of antibody (that targets the drug to a specific protein on the surface of a tumor cell) is conjugated to a cytotoxic drug (that kills the tumor cell) (Pastan et al., 2006; Bouchard et al., 2014). ADCs combine two important properties: the high specificity of an antibody-antigen interaction and the ability of newly designed highly potent toxins to kill cells. ADCs represent a line of therapy often given to a patient after all other therapies have failed, and they have proven to be a great method at treating cancers that do not respond to chemotherapy or radiation alone. To introduce the reader to the topic of ADCs, a brief discussion of the immune system and antibodies is first provided.
Introduction to the Immune System and Antibodies

Pathogens are everywhere, and it is well known that there are more foreign cells in and on the human body than cells composing it. How is it that people do not become sick from all of these organisms that surround us? The answer lies within the body’s immune system which protects the body against infectious agents, pathogens, and toxins using a variety of mechanisms (Murphy and Weaver, 2016). In recent years, scientists have begun to understand the immune system in great detail, and these advances have aided the development of immunological therapies.

The body’s first line of defense is the presence of anatomic barriers such as skin, mucosa, and epithelium (Murphy and Weaver, 2016). The pathogen must pass these barriers to enter the body and cause an infection. Anatomic barriers sometimes contain a variety of antimicrobial proteins that attempt to kill the microbes (Murphy and Weaver, 2016). Once the foreign invader breaches an anatomic barrier, the body has additional proteins that signal infection and target cell death. One important class is the complement system that consists of more than thirty different plasma proteins that signal numerous effector mechanisms that lead to a systemic eradication of the pathogen (Murphy and Weaver, 2016). This response then triggers other responses of the immune system.

The immune system is divided into two main categories: innate and adaptive immunity. All the body’s immune cells are derived from hematopoietic stem cells (HSCs) (Figure-1).

![Figure-1: Differentiation of Hematopoietic Stem Cells (HSCs). HSCs produce myeloid lineages (innate immunity) (diagram left), and lymphoid lineages (adaptive immunity) (diagram right) (Bartis and Pongracz, 2011).](image-url)
HSCs differentiate to form myeloid lineages (innate immunity) and lymphoid lineages (adaptive immunity) (Bartis and Pongrácz, 2011). The differentiated cells of the innate immune system do not have specific interactions with a foreign pathogen, but instead indiscriminately kill anything they detect as “foreign”. One example of an innate immune cell is a macrophage which migrates throughout the body engulfing and digesting foreign particles and signaling additional immune responses such as fever and inflammation. Other innate immune cells bind to pathogens and kill them in other ways. Some signal for the complement system, while others release cytotoxic granules and enzymes onto the pathogen’s surface (Murphy and Weaver, 2016). The cells of the adaptive immune system (B cells and T cells) recognize and target one specific type of antigen by producing antibodies or cytotoxic T cells, respectively.

T and B cells originate by differentiation from the “Common Lymphoid Progenitor Cell” (Figure-1, upper right) which occurs in the bone marrow (Bartis and Pongrácz, 2011; Murphy and Weaver, 2016). Immature T cells migrate to the thymus where they mature. Immature B cells remain and mature in the bone marrow. As B and T cells begin to mature, they undergo random gene rearrangements to produce an array of receptors. However, once the cells are activated by binding to a foreign antigen presented by an antigen presenting cell (APC), they mature further to commit to that antigen. The APC or dendritic cell is the link between innate and adaptive immunity (Murphy and Weaver, 2016). The APC indiscriminately ingests a pathogen, breaks it down into small peptides, and presents the foreign peptide/MHC complex to a T cell. Once the T cell and APC successfully bind, the T cell proliferates and differentiates into effector T cells (helper T (T_H) cells or cytotoxic T cells) (Murphy and Weaver, 2016). The cytotoxic T cells bind to the foreign antigen on an infected cell and initiates cell death. B cell receptors also bind to antigens, engulf them, and present them to the T_H cells. The T_H cell then produces cytokines which signal the B cell to proliferate and secrete antibodies (Murphy and Weaver, 2016).

Antibodies are molecules secreted by a B cell. These molecules migrate through the blood and bind to the antigen they are specific for once they encounter it. Each antibody has a specific antigen to which it binds, and can also bind other effector molecules (Murphy and Weaver, 2016). The general structure of an antibody is the shape of a ‘Y’ (Figure-2). Each upper arm of the ‘Y’ (which bind the antigen) is referred to as the N terminus (amino-terminus). The N-termini are variable between different antibodies. The lower base of the ‘Y’ is referred to as the C-terminus (carboxy-terminus) and is a constant region (Murphy and Weaver, 2016).

![Figure-2: Diagram of a Typical Antibody Structure.](attachment:image.png) The N-terminus contain the variable regions (red) that specifically bind an antigen, and the C-terminus contains the constant regions (blue) (Murphy and Weaver, 2016).
The variable region of the antibody binds antigen, and it has a unique binding pocket for the antigen. The variable region is unique to each antibody, and the human immune system can produce over $5 \times 10^{13}$ different antibodies (Murphy and Weaver, 2016). The vast diversity of antibodies results from random DNA recombination and joining of different segments of variable domain-encoding genes in maturing B cells. The constant region remains the same within an antibody class so that it can interact with effector molecules and phagocytic cells that internalize the antibody-antigen complex. A different view of the antibody structure (Figure-3) shows it is composed of two heavy chains (green in the diagram) and two light chains (yellow in the diagram) which are linked by disulfide bonds. Each light chain is identical, so each arm of the ‘Y’ is able to bind to two identical antigens. The hinge region of the antibody (located at the junction of the ‘Y’) allows the antibody to be flexible so that it can bind to antigens at different distances from each other. This allows the antibody to have increased binding strength for the antigen, increasing its avidity. Antibodies and antigens fit into each other’s shape and bind by non-covalent forces (Murphy and Weaver, 2016).

![Figure-3: Diagram of Antibody Chains.](image)

There are five common classes of antibodies, each with their own unique constant regions: immunoglobulin A (IgA), immunoglobulin D (IgD), immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin E (IgE) (Murphy and Weaver, 2016). A single B cell undergoes class switching that joins the antigen-binding variable region to different constant regions. This class producing process always begins with IgM, followed by IgD, IgG, IgA, and finally IgE (Murphy and Weaver, 2016). Once an antibody has bound to its antigen, based on its type of constant region it signals for a specific effector function that leads to destruction of the pathogen. There are three main ways that an antibody can help destroy a pathogen (Figure-4). One method is neutralization (diagram left), where the antibody binds to and blocks the binding site of the pathogen or toxin keeping it from binding to a cell, or facilitates its uptake into a macrophage cell. Secondly, antibody-antigen complexes can become opsonized (diagram center) which facilitates its uptake into macrophage cells. Thirdly, the constant region of antibodies can induce complement system activation which signals plasma proteins to bind to and puncture the pathogen’s membrane, leading to cell lysis (diagram right). The complement system also coats the pathogen’s membrane with proteins that attract phagocytic cells (Murphy and Weaver, 2016). The various immune responses generated by each antibody class are summarized in Figure-5.
Figure 4: Three Main Methods of Pathogen Destruction by Antibodies. Antibodies can help cause destruction of pathogens in 3 main ways: neutralization (diagram left), opsonization (diagram center), and complement activation (diagram right) (Murphy and Weaver, 2016).

![Diagram of pathogen destruction by antibodies]

Figure 5: Functions of the Five Main Classes of Antibodies. The class of antibody is specified by its constant region, and this specifies how the antibody helps inactivate the pathogen (Murphy and Weaver, 2016).

<table>
<thead>
<tr>
<th>Functional activity</th>
<th>IgM</th>
<th>IgD</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
<th>IgA</th>
<th>IgE</th>
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<tbody>
<tr>
<td>Neutralization</td>
<td>+</td>
<td>−</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
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<tr>
<td>Opsonization</td>
<td>+</td>
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<td>++</td>
<td>*</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Sensitization for killing by NK cells</td>
<td>−</td>
<td>−</td>
<td>++</td>
<td>−</td>
<td>++</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Sensitization of mast cells</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+++++</td>
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<tr>
<td>Activates complement system</td>
<td>+++++</td>
<td>−</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>−</td>
<td>+</td>
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</tr>
</tbody>
</table>
Antibody-Drug Conjugates

Due to the highly specific nature of the interaction of an antibody with its antigen, scientists have engineered antibodies to bind to specific types of cancer cells. Many patients suffer from cancers that are unable to be treated by chemotherapy or radiation treatments. Unfortunately, in most cases, simply attaching an antibody alone to a cancer cell does not necessarily lead to its demise (Thomas et al., 2016). So, scientists have designed a new type of anti-cancer drug, antibody-drug conjugates (ADCs) that consist of an antibody against a specific antigen on the surface of a tumor cell, conjugated to a cytotoxic poison that can kill a malignant cell (Figure-6) (Thomas et al., 2016). Once the ADC binds to its targeted antigen, the cell will engulf the ADC and release the toxin inside the cell, thus killing it (Pastan et al., 2006). The antibody should have high affinity for the tumor antigen, the antigen should be highly expressed on the tumor cell (not normal cells), the linker should be stable in the patient’s circulation to avoid releasing the toxin too soon, and the drug should be highly potent since only a few molecules will be present in the tumor cell.

**Figure-6: Diagram of ADC General Structure.** Shown is the general structure of an antibody-drug conjugate, consisting of an antibody (diagram left), bound to a cytotoxic drug (green) using a linker (diagram center). Also shown are desirable properties of each structural component (Thomas et al., 2016).

In the ADC production process, monoclonal antibodies against a particular surface tumor antigen are produced in cell culture, and are bound to a toxin using a linker (Pastan et al., 2006; Chiu and Gilliland, 2016). It is essential that the target antigen is as specific as possible for the cancer, so that the ADC will only bind and kill the tumor cells, not normal cells (Thomas et al., 2016). In some cases this may not be possible, such as with leukemia where the target antigen may also be present in a low amount on normal cells, so adverse side effects can be observed in the clinical trials. The drug or toxin must also not bind normal cells by itself, so in some cases its cellular binding domains may be removed (Pastan et al., 2006). The antibody then becomes
linked to the toxin or drug. All ADCs are linked to their drugs covalently through one of four chemical methods: cysteine conjugation, glycol-conjugation, protein tags, or amino acid incorporation (Agarwal and Bertozzi, 2015; Thomas et al., 2016). The most common linkers have disulfide bonds or other peptide linkages (Pastan et al., 2006). The linker can be placed at various locations on the antibody, including the variable region, hinge region, constant region, or all three simultaneously (Agarwal and Bertozzi, 2015). Usually two to four drug molecules are attached to each antibody so that the antibody retains all of its antigen binding properties and is not cleared from the body too quickly (Hughes, 2010). The linker functions to keep the drug bound to the antibody on its extracellular journey to the target, and becomes degraded once the ADC enters the cell (Thomas et al., 2016). Linkers have been designed to be sensitive to proteases, pH, and glutathione, and are cleavable or non-cleavable in nature (Jain et al., 2015). Cleavable linkers utilize enzymes inside the target cell to release the drug from the antibody (Jain et al., 2015). This ensures that the linker is stable in the bloodstream, and breaks down only when being digested by a target cell. Non-cleavable linkers do not release the drug once they enter the cell; when the entire antibody and linker are digested, the payload is released (sometimes with part of the linker attached) (Jain et al., 2015).

ADCs are typically administered to the patient intravenously. They bind to the target cell and are engulfed by an endocytic vesicle (Figure-7) (Pastan et al., 2006; Bouchard et al., 2014). Once the vesicle fuses with a lysosome, the environment turns acidic and enzymes are released that digest the linker releasing the drug into the cytoplasm. There the drug works to kill the cell, for example by strongly binding tubulin to prevent its polymerization thus blocking cell division. Drugs commonly used for ADCs either bind DNA leading to its cleavage or alkylation, block tubulin, or inhibit RNA polymerase II and III blocking RNA synthesis (Thomas et al., 2016).

![Figure-7: General ADC Function.](image)

ADCs (upper left) bind to a surface marker on the target cell (green) and become endocytosed into a vesicle (upper center). The vesicle binds with a lysosome that acidifies the compartment and releases enzymes that degrade the linker or the entire ADC, releasing the drug (red) into the cytoplasm that causes cell death (Bouchard et al., 2014).
ADC Drug Examples

Currently, only two ADC drugs have been approved by the FDA: Trastuzumab emtansine and Brentuximab vedotin (Thomas et al., 2016). A third ADC, Gemtuzumab ozogamicin (Mylotarg), was initially FDA approved but was later withdrawn (Richwine, 2010).

Trastuzumab emtansine, commercially named Kadcyla, was developed by Genentech (a subsidiary of Roche), and is manufactured by Lonza. It is used to treat HER2-positive metastatic breast cancer in patients who are resistant to other treatments (Niculescu-Duvaz, 2010; LoRusso et al., 2011; Lopus, 2011; Verma et al., 2012; About Kadcyla, 2017). It is composed of an antibody (Trastuzumab or Herceptin) against the HER2 receptor conjugated with the drug DM1 (a derivative of maytansine) using a non-cleavable thioether linker (Barok et al., 2014; Jain et al., 2015). Of the ADC drugs, Kadcyla is unique in that its antibody component by itself is sometimes capable of blocking Her2-positive breast cancer. The binding of Herceptin to Her2 receptor on the cell surface prevents receptor dimerization, inhibiting activation of the MAPK, PI3-kinase, AKT kinase pathways that otherwise would lead to cell division (Verma et al., 2012).

Kadcyla’s FDA approval was based predominantly on encouraging clinical trial data of women with advanced Her2-positive breast cancer who were resistant to the antibody treatment alone (Verma et al., 2012). The ADC drug improved the median patient survival by about 5.8 months (30.9 months versus 25.1 months) compared to standard chemotherapy. It was FDA approved on February 22, 2013, specifically for women with Her2-positive metastatic breast cancer that does not respond to antibody treatment alone or to chemotherapy (Pollack, 2013).

Kadcyla is injected intravenously (IV) where it travels through the patient’s circulation to the tumor where it binds the HER2 receptor, and becomes endocytosed (Figure-8) (Barok et al., 2014). Once the vesicle (named early endosome in the diagram) fuses with a lysosome (diagram lower left), the antibody and linker are digested by lysosomal enzymes stimulated by the acidic environment, and the DM1 (unaffected by enzymes) is released into the cytoplasm where it inhibits microtubule assembly (diagram lower right), causing apoptosis (Barok et al., 2014; Jain et al., 2015; About Kadcyla, 2017).
Figure-8: Mechanism of Action of Trastuzumab emtansine (Kadcyla). The ADC binds to the HER2 receptor (upper left) and becomes endocytosed into an early endosome. When the endosome fuses with a lysosome (lower left), enzymes from the lysosome degrade the antibody and linker, releasing DM1 into the cytoplasm which inhibits microtubule formation (lower right), ultimately resulting in apoptosis (Barok et al., 2014).

The other current FDA-approved ADC drug is Brentuximab vedotin, commercially named Adcetris. Adcetris is marketed by Seattle Genetics Inc. in North America, and by Takeda Oncology in the rest of the world. It is used to treat CD30-positive lymphoproliferative disorders, including Hodgkin lymphoma (HL) and anaplastic large cell lymphoma (ALCL) (Van de Donk and Dhimolea, 2012; Brentuximab vedotin, 2016). Protein CD30 often occurs on the surface of cells of these tumor types, but rarely on normal cells (Küppers and Hansmann, 2005). Adcetris contains an antibody (Brentuximab or cAC10) (yellow in the diagram) engineered to bind receptor CD30 present on HL and ALCL tumors, conjugated to 3-5 molecules of the drug monomethyl auristatin E (MMAE) (purple in the diagram) by a cathepsin-cleavable linker (blue in the diagram) (Figure-9) (Van de Donk and Dhimolea, 2012). The antibody is produced in mammalian Chinese Hamster Ovary (CHO) cells, while the small molecule components are produced by chemical synthesis.
Figure-9: Structure of Adcetris. This antibody-drug conjugate consists of a mouse-human chimeric monoclonal antibody against CD30 (yellow) linked via a cathepsin-cleavable linker (blue) to 3-5 units of the cytotoxic drug monomethyl auristatin-E (MMAE) (purple) that strongly binds tubulin and blocks cell division (Francisco et al., 2003).

Adcetris is administered to the patient intravenously, and with a half-life of 4-6 days, travels through the circulation to the tumor where it binds the CD30 receptor and is endocytosed into an endosome. Once the endosome fuses with a lysosome, the linker is cleaved by cathepsin lysosomal enzyme, releasing (MMAE) into the cytoplasm. MMAE binds to tubulin, inhibiting mitosis and initiating apoptosis (Van de Donk and Dhimolea, 2012; Francisco et al., 2003; Vaklavas and Forero-Torres, 2012).

Adcetris has undergone numerous trials, and has shown high efficacy and manageable side-effects. For example, in a 2010 clinical trial of HL patients (Seattle Genetics Press Release, 2010), 34% of the patients showed complete remission, 40% showed partial remission, and 94% showed tumor reductions. Additionally, in a 2010 clinical trial of ALCL patients (Miller, 2010) 97% of the patients showed measurable tumor reductions. On August 19, 2011, the drug was granted an accelerated FDA approval (Genetic Engineering and Biotechnology News, 2011; Fierce Biotech, 2011).

The third ADC drug approved by the FDA (although it was later withdrawn) is Gemtuzumab ozogamicin, commercially named Mylotarg. It was manufactured by Wyeth (now Pfizer), and was FDA approved in May of 2000 to treat acute myeloid leukemia (Gemtuzumab Ozogamicin, 2015). Mylotarg is composed of an antibody (IgG4 κ hP67.6) against the CD33 antigen found on leukemic cells (Gemtuzumab Ozogamicin, 2015; Wyeth Pharmaceuticals, 2005), conjugated with two to three molecules of the drug calicheamicin (N-acetyl-γ-calicheamicin) by a linker (4-(4-acetylphenoxy)butanoic acid) (Gemtuzumab Ozogamicin, 2015). Mylotarg is intravenously injected into the patient where it migrates to the CD33 antigen on the tumor cells. Upon binding with its antigen, the ADC is endocytosed, and fuses with a lysosome. The drop in pH caused from lysosomal fusion degrades the linker, releasing the drug into the cytoplasm (Gemtuzumab Ozogamicin, 2015). Calicheamicin binds to double stranded DNA and causes breaks, leading to apoptosis (Wyeth Pharmaceuticals, 2005).
In clinical trials, Mylotarg initially showed promise, resulting in its accelerated review and FDA approval in 2000. But in later clinical trials, Mylotarg caused serious side-effects, including myelosuppression (suppression of bone marrow) in 98% of the patients (likely because CD33 is also present on normal hematopoietic cells), respiratory problems, and veno-occlusive disease (VOD), so the FDA required a “black box” warning label on the packaging (Giles et al., 2001; Wadleigh et al., 2003). A clinical study of five years showed that Mylotarg led to increased death rates (4.3% higher than placebo), and had no apparent benefits to patients, so Pfizer withdrew Mylotarg from the market in October of 2010 (Richwine, 2010).

Currently, more than 40 ADCs are in the clinical trial stages of development (Thomas et al., 2016). The future for immunological therapies including ADCs has endless possibilities. But in some cases, the ADCs are not 100% effective, and all ADCs have some adverse side-effects (discussed later), so it is necessary to continue investigations of the human immune system, and to invest in research that aims to engineer antibodies against otherwise untreated cancers. With further research, newer more effective ADCs with fewer side-effects can be engineered to improve human health.

Section-1 References


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Section-2: Pre-Clinical ADC Testing

William Mosby

The purpose of this section of the Literature Review is to discuss some of the pre-clinical testing done with various antibody-drug conjugates (ADCs). Pre-clinical testing includes the use of cancer cell lines in vitro, the use of human cancers xenografted into mice, and monkey experiments. These pre-clinical experiments laid the groundwork for subsequent human clinical trials which are discussed later in Section-3. This chapter is divided into sub-headings based on the antigen targeted by the ADC.

CD19

CD19 is a transmembrane glycoprotein belonging to the immunoglobulin Ig superfamily (Del Nagro et al., 2005). Its expression is restricted to B-cells; it is expressed from early pre-B stages through B-cell differentiation, including mature B-cells. It is then down-regulated at the plasma cell stage. Thus, CD19 acts as a broad marker of B-cells. For example, it is a broader marker of B-cells than CD20 which is expressed only in the later stages of B-cell development. CD19 is expressed in all B-cell cancers, including all types of B-cell lymphomas, B-cell chronic lymphocytic leukemia, and non-T acute lymphoblastic leukemia (ALL) (the latter cannot be targeted by CD20) (Anderson et al., 1984; Scheuermann and Racila, 1995).

CD19 has been the target of antibody-drug conjugates (ADCs) to kill B-cell tumors. For example, SAR-3419 is an ADC drug containing a humanized monoclonal antibody against CD19 linked via a cleavable linker to 3-4 molecules of the tubulin inhibitor DM-4 (Blanc et al., 2011). In pre-clinical testing using lymphoma xenograft models, SAR-3419 induced complete tumor regressions and improved mouse survival (Lutz et al., 2006). SAR-3419 has also shown in vivo efficacy in a diffuse large B-cell lymphoma (DLBCL) model (Al-Katib et al., 2009). In both of these pre-clinical models, SAR-3419 caused complete tumor remission at the highest doses, while the lower doses caused tumor growth delays. In the Lutz et al. study, 100% of the mice were tumor-free by the end of the study, while treatment with the DM4 agent alone or the antibody alone produced no significant tumor regressions. This shows that the conjugation of the two components together is required for the observed antitumor effect.

In 2009, scientists in the Department of Pre-Clinical Therapeutics at Seattle Genetics investigated the use of an anti-CD19 ADC against non-Hodgkin lymphoma (NHL) cell lines and xenograft mice (Gerber et al., 2009). The drug showed potent tumor cell killing against CD19-positive NHL cells in vitro, including cancer cells resistant to antibody treatment alone. The ADC drug also showed durable tumor regressions in vivo against NHL tumors xenografted into mice. Importantly, CD19 had previously been reported to form dimers with CD21, raising worries that over-expression of CD21 on the tumor surface might interfere with drug interaction with CD19, but this potentially negative effect was not observed in vitro or in vivo (Gerber et al., 2009).

In a more recent SAR-3419 study (Carol et al., 2013), scientists at the Children's Cancer Institute for Medical Research, University of New South Wales (Sydney) tested SAR-3419 in
mouse xenograft models of B-cell precursor acute lymphoblastic leukemia (BCP-ALL) and in models of mixed lineage leukemia ALL (MLL-ALL). SAR-3419 was administered either as a single agent or in combination with chemotherapy. Their data showed that SAR-3419 significantly delayed the progression of 4 of 4 (100%) BCP-ALL mice, and 3 of 3 (100%) of MLL-ALL mice, although the mouse numbers were relatively small. Importantly, the drug effectiveness was found to correlate with the extent of CD19 surface expression on the tumor cells, so perhaps a future best practice will be to monitor antigen expression in the patients to ensure they can benefit from the ADC drug. The ADC was also found to delay cancer progression in chemo-resistant xenografts by up to 82 days. When the drug was administered long-term post-remission, it prevented disease re-occurrence, so this provides interesting data that ADCs might be used to prevent cancer re-occurrence.

**CD22**

CD22 is a molecule belonging to the SIGLEC family of lectins (Hatta et al., 1999). It is found on the surface of mature B-cells and to a lesser extent on immature B-cells, but it is not as broadly expressed in B-cells as CD19. CD22 has also been the target of ADCs. One example of an ADC targeting CD22 is Inotuzumab ozogamicin (CMC-544). This ADC was developed by Pfizer and Union Chimique Belge (UCB) for patients with Non-Hodgkin Lymphoma (NHL). It consists of a humanized monoclonal antibody against CD22 (Inotuzumab) linked to a cytotoxic derivative of calicheamicin which damages DNA (Takeshita et al., 2009).

In 2004, scientists in the Department of Oncology Discovery at Wyeth Research (Pearl River, NY) investigated the activity of CMC-544 against CD22-positive B-cell lymphoma (BCL) lines in vitro (DiJoseph et al., 2004a; 2004b). Their data showed that CMC-544 had potent cytotoxic activity in the model. With respect to binding affinity, the ADC bound B-cells in the same nano-molar range as free antibody, indicating that conjugation of the antibody to the drug cargo did not alter cell binding. In xenograft mouse models, CMC-544 prevented the initial establishment of BCL tumors in the mice, and potently inhibited the growth of pre-established BCL tumors. The use of non-conjugated antibody or the drug cargo alone were ineffective in their assays, indicating the conjugation of drug to antibody was the basis for the blocked tumor growth (DiJoseph et al., 2004a; 2004b). The same team followed this 2004 study with another in 2006, comparing the activity of CMC-544 with that of Rituximab antibody alone (which targets CD20) (DiJoseph et al., 2006). Their data showed that the antibody alone somewhat reduced the growth of NHL cells in vitro, but the addition of CMC-544 was more effective. In xenograft experiments, CMC-544 was more effective at inhibiting the growth of established NHL grafts and increased the lifespan of the mice. 90% of the CMC-544 treated mice lived at least 125 days, while only 20% of the antibody alone group survived that long (DiJoseph et al., 2006).

In 2015, a team of scientists in the Oncology Research Unit at Bio-Conjugates Discovery and Development, Pfizer Worldwide Research and Development (Pearl River, NY) published a summary of their pre-clinical testing of Inotuzumab ozogamicin against Non-Hodgkin’s lymphoma (NHL) (Shor et al., 2015). They concluded that the drug potently induced tumor regressions in mouse xenograft models of NHL, either when used alone or when used in combination with anti-CD20 Rituximab antibody.
**CD30**

ADCs targeting CD30 are among the best characterized of all the ADCs. CD30 is a cell membrane protein belonging to the tumor necrosis factor receptor (TNFR) family (Gorzyca et al., 2003). It is expressed by activated, but not by resting T- and B-cells, and is also associated with anaplastic large cell lymphoma (ALCL) and classic Hodgkin lymphoma (HL). An example of an ADC targeting CD30 is Brentuximab vedotin (Adcetris, SGN-035), one of only two current FDA-approved ADCs (Deng et al., 2013). Adcetris consists of a mouse-human chimeric monoclonal antibody against CD30 (Brentuximab or cAC10) linked to 3-5 units of the cytotoxic drug monomethyl auristatin-E (MMAE) that binds tubulin and keeps it from polymerizing.

With respect to pre-clinical data, monoclonal antibodies (alone without cargo) targeting CD30 (such as cAC10) have shown activity *in vitro* against HL and ALCL cancers, increasing tumor cell arrest and DNA fragmentation (Wahl et al., 2002). But the antibody alone had only modest data in clinical trials, so ADCs targeting CD30 were developed. In 2003, scientists at Seattle Genetics showed that Adcetris released only 2% of its cargo into human plasma when incubated for 10 days, but quickly released it cargo once it was internalized in a cell (Francisco et al., 2003). The release of MMAE into the cell blocked mitosis at the G2/M stage and induced apoptosis. The drug was strongly potent against CD30-positive cancer cell lines *in vitro* (IC<sub>50</sub> < 10 ng/ml), and was 500-fold less active against CD30-negative cells. In xenograft models of HL and ALCL, Adcetris caused tumor reductions at doses as low as 1 mg/kg. Mice treated with 30 mg/kg showed no signs of toxicity (Francisco et al., 2003).

In addition, a lab at Seattle Genetics tested Adcetris with various cargo loads of 2, 4, and 8 MMAE molecules per antibody (named E2, E4, and E8, respectively) (Hamblett et al., 2004). Their data indicated that the activity *in vitro* was directly dependent on the extent of cargo loading: E8 had the highest load and the highest activity. *In vivo*, E4 and E8 were similarly active, but E2 required higher doses. The maximum tolerated dose of E2 was at least double that of E4 and E8. They concluded that the amount of drug loading is an extremely important ADC design parameter (Hamblett et al., 2004). Given this data, it will be important to use drugs of equal loading when trying to compare the data of various clinical trials.

Adcetris strongly induced cell death *in vitro* for CD301 macrophage cell lines, and in mouse ALCL or HL xenograft models it showed anti-tumor activity at doses as low as 1 mg/kg (Okeley et al., 2010). When combined with chemotherapy agents, such as doxorubicin, bleomycin, vinblastine, dacarbazine, or gemcitabine, Adcetris increased their efficacy (Oflazoglu et al., 2008).

**CD33**

CD33 (also called Siglec-3; sialic acid binding Ig-like lectin-3) is a transmembrane receptor expressed mostly on myeloid cells, but it is also found on some lymphoid cells (Garnache-Ottou et al., 2005). CD33 binds sialic acids, therefore it is a member of the SIGLEC family of lectins. An example of an ADC drug in this category is Gemtuzumab ozogamicin (Mylotarg). Mylotarg is a humanized anti-CD33 monoclonal antibody covalently linked to a derivative of calicheamicin. Mylotarg was the first ADC drug approved for use in the U.S. (Bross et al., 2001). It was FDA-approved in 2000, marketed by Wyeth, and manufactured by
Pfizer to treat acute myelogenous leukemia (AML). But Mylotarg was withdrawn in 2010 when its clinical trials showed it increased patient death and was no better than conventional therapies (Nelson, 2010; Richwine, 2010).

A pre-clinical testing of various Mylotarg linkers allowed the optimization of drug design (Hamann et al., 2002). The authors attached the cargo (calicheamicin) to the CD33 antibody using a bi-functional linker which is stable at physiological pH 7.4. This allows efficient release of the cargo at the acidic pH inside lysosomes. The average loading of calicheamicin on the antibody was determined to be about 2.5. The optimized Mylotarg ADC showed significantly increased activity against HL-60 leukemia cells *in vitro*.

**Her2**

Human epidermal growth factor-2 (Her2) (also known as CD340, Neu, or ERBB2) is a member of the epidermal growth factor family of cell surface receptors (Mitri et al., 2012). Over-expression of Her2 occurs in approximately 15-30% of breast cancers, and is strongly associated with increased cancer recurrence and poor patient prognosis (Tan and Yu, 2007). Her2 over-expression can also occur on cancers of the ovary, stomach, lung, and uterus (Rüschoff et al., 2012; Buza et al., 2014).

An example of an ADC targeting Her2 is *Trastuzumab emtansine (Kadcyla, T-DM1)* (Niculescu-Duvaz, 2010; LoRusso et al., 2011; Lopus, 2011). Kadcyla is one of the two ADC drugs currently approved by the FDA. It was developed by Genentech (Roche), manufactured by Lonza, and contains a humanized antibody against Her2 (Trastuzumab or Herceptin) linked via a non-reducing thioether linker to the cytotoxic drug emtansine (DM-1). DM1 strongly binds to tubulin in the cell to inhibit microtubule assembly, and does so with greater potency than either vincristine or vinblastine (Widdison et al., 2006). The parent compound of DM1 is maytansine, which is a highly cytotoxic natural product. Maytansine failed human cancer trials due to a high level of systemic toxicity, but it does well linked to a targeting antibody. Scientists at ImmunoGen, Inc. (Cambridge, Massachusetts) synthesized a series of second-generation maytansinoids (including DM1) with disulfide or thio substitute groups which are even more potent than maytansine (Widdison et al., 2006).

In pre-clinical testing at Genentech (South San Francisco, CA), Kadcyla showed favorable activity and toxicity profiles (Lewis-Phillips et al., 2008). The drug activity was tested *in vitro* against both tumor cells and normal cells. *In vivo* activity was determined in mouse breast cancer models. Toxicity was tested in rats as measured by body weight loss. Their data showed that the toxicity of Kadcyla with its stable non-reducing linker was negligible compared to the high toxicity of free DM1 or to ADCs containing reducing linkers (presumably the reducing linkers were broken down in the blood releasing their toxic cargo too soon). Potent cell-killing activity was observed against all Her-2 over-expressing tumors, but not against normal cells or tumor cells low in Her-2 (Lewis-Phillips et al., 2008). In addition, Kadcyla seems to retain the anti-tumor properties of the anti-Her-2 Herceptin antibody alone. Kadcyla was found to inhibit Her-2 signaling pathways, and to flag Her-2-positive tumor cells for destruction by antibody-dependent cellular cytotoxicity (ADCC) (Junttila et al., 2008).
More recently, scientists at ImmunoGen, Inc. (Waltham, MA) investigated the \textit{in vitro} and \textit{in vivo} activity of various types of linkers used to attach the maytansine cargo to the Her2 antibody \cite{Kellogg2011}. Their data showed that conjugates with sterically hindered disulfide linkages were resistant to reduction in plasma. In xenograft mouse models \textit{in vivo}, ADC conjugates with an intermediate level of disulfide bond stability in the linker showed the highest activity, while all the ADCs showed the same activity \textit{in vitro}. The authors concluded that the magnitude of drug activity is strongly affected by the type of linker.

Kadcyla is unique among the various ADC drugs in that its antibody component by itself (without the DM-1) can sometimes inhibit Her2-positive breast cancer cells; the binding of the antibody to the Her2 receptor prevents its dimerization, inhibiting activation of the MAPK, PI3-kinase, AKT kinase pathways that would normally lead to cell division \cite{Verma2012}. Scientists at Genentech Inc. (South San Francisco, CA) showed that Kadcyla \textit{in vitro} retains the same ability to inhibit PI3 kinase and AKT kinase pathways, and increase antibody-dependent cellular cytotoxicity (ADCC) as the parent antibody alone \cite{Junttila2011}.

In 2014, a study coordinated by scientists in the Departments of Biostatistics, Product Development Oncology, and Translational Oncology at Genentech, Inc. (South San Francisco, CA) performed pre-clinical testing on Her-2-positive cultured cells and on mouse xenograft models using an interesting dual-targeting approach \cite{Phillips2014}. The treatment combined the ADC Kadcyla with Pertuzumab (Perjeta) that prevents dimerization of the Her-2 receptor. Receptor dimerization leads to activation of growth stimulating signal transduction pathways. Their data showed that the combined treatment synergistically inhibited cancer cell proliferation \textit{in vitro}, and enhanced anti-tumor efficacy \textit{in vivo}. This combined treatment approach using two drugs that target the same receptor using different mechanisms may be a trend of the future, and is worth gaining additional information in IQP interviews.

**Glycoprotein NMB (gpNMB)**

Glycoprotein non-metastatic melanoma protein-B (gpNMB) (also called GPNMB or osteoactivin) is a transmembrane protein expressed in a variety of cell types, including melanocytes, osteoclasts, osteoblasts, and dendritic cells \cite{Weterman1995}. gpNMB is also over-expressed in aggressive melanoma, glioma, and breast cancer \cite{Tse2006,Kuan2006,Rose2007}. An example of an ADC directed against gpNMB is \textit{Glembatumumab vedotin (CDX-011) (formerly CR011-vcMMAE)}. CDX-011 consists of a fully human monoclonal antibody against gpNMB conjugated via a valine-citrulline linker to the potent microtubule inhibitor monomethyl auristatin-E (MMAE) \cite{Naumovski2010,Keir2012}.

In pre-clinical studies performed at CuraGen Corporation (Branford, CT), an antibody against gpNMB by itself did not inhibit the \textit{in vitro} growth of melanoma cells, but when the antibody was linked to MMAE, the ADC potently and specifically inhibited the growth of gpNMB-positive melanoma cells \cite{Tse2006}. This group also showed that knocking down gpNMB protein expression in tumor cells using small interfering RNAs prevented destruction of the cell, and that over-expressing gpNMB on normal cells using genetic engineering made the cells sensitive to destruction by the ADC. So, this data shows the importance of the target antigen to the cell destruction. In mouse xenograft models, the ADC showed anti-tumor effects
that were directly dependent on the dose of the ADC, including some complete remissions at doses as low as 1.25 mg/kg (Tse et al., 2006).

In 2007, the same team at CuraGen Corporation extended their pre-clinical testing of CDX-011 (then termed CR011-vcMMAE) by performing dose titrations (Pollack et al., 2007). The ADC was found to induce melanoma tumor regression in mouse xenograft models at doses ranging from 1.25 to 80 mg/kg, delivered IV every 4 days for 4 treatments. The regressions did not regrow during the 200 day observation period. The drug had a half-life of about 10.3 days in mice. Injecting the drug either as a single bolus injection, or performing intermittent dosing, showed no differences in drug activity. Injecting antibody alone or MMAE alone produced no anti-tumor effect, again showing the importance of linking the drug to the antibody (Pollack et al., 2007).

In 2010, scientists in the Department of Medicine at the Goodman Cancer Research Centre of McGill University (Montréal, Canada) showed that the gpNMB antigen expression correlates with a more invasive phenotype for breast cancer cells and reduces patient survival, and the presence of gpNMB antigen sensitized breast cancer cells in vitro to killing from the CDX-011. (Rose et al., 2010). Thus, gpNMB is a prognostic marker for poor outcome in breast cancer patients, and the CDX-011 ADC drug targeting this antigen should be developed clinically.

**Trop-2**

Trophoblast cell-surface antigen-2 (Trop-2) (also known as tumor-associated calcium signal transducer-2, or epithelial glycoprotein-1 antigen, EGP-1), is a member of a cell surface receptor family with at least two other members (Linnenbach et al., 1993). Trop-2 is over-expressed on many types of epithelial tumors, especially the more aggressive types, but it is also expressed at low levels on normal tissues (Cubas et al., 2009; Trerotola et al., 2013; Ambrogi et al., 2014). An antibody-drug conjugate targeting Trop-2 is Sacituzumab govitecan (IMMU-132) (Cardillo et al., 2011). IMMU-132 is a humanized monoclonal antibody against Trop-2 (hRS7) linked to SN-38, the active metabolite of irinotecan, an inhibitor of topoisomerase-I. Blocking topoisomerase keeps DNA from unwinding during replication, killing the cell.

IMMU-132 has undergone much pre-clinical testing, especially from a team of researchers at Immunomedics, Inc. (Morris Plains, New Jersey). This team evaluated several types of linkers for release of the SN-38 cargo from the antibody (Moon et al., 2008; Govindan et al., 2009; Cardillo et al., 2011; Govindan et al., 2013). Some linkers released the cargo within a few hours, while other linkers released only after several days. The linker selected by the team as best optimized contains a short hydroxyl residue to enhance solubility, and it had an “intermediate level” half-life which the researchers preferred. The SN-38 cargo was released both within the acidic environment of the lysosome and within the tumor micro-environment. A safety study performed in monkeys indicated that neutropenia is the dose-limiting factor. The only other side-effect observed frequently was diarrhea (Cardillo et al., 2011).
5T4

Trophoblast glycoprotein (5t4) (also known as TPBG), is an onco-fetal antigen expressed on tumor-initiating cells (TIC) (Sapra et al., 2013). TICs represent the most aggressive cells in the tumor; if they remain in the patient following conventional therapies, they can relapse, so targeting TICs directly could improve patient survivability (Sapra et al., 2013). 5T4 is also a cell surface antigen that internalizes rapidly, giving it the potential to efficiently deliver ADC’s into tumor cells. 5T4 is largely located in solid carcinomas, especially Non-Small Cell Lung Cancer (NSCLC), and is limited in normal adult tissue.

An antibody-drug conjugate targeting 5T4 is A1mcMMAF. A1mcMMAF contains a humanized anti-5T4 antibody linked to the potent tubulin inhibitor monomethylauristatin F (MMAF) by a non-cleavable maleimidocaproyl (mc) linker (Sapra et al., 2013). In 2011, a group of scientists from the Oncology Research Unit at Pfizer (Pearl River, NY) did pre-clinical tests on A1mcMMAF (Oncofetal Antigen, 2011). In a mouse model using patient-derived NSCLC xenografts, the ADC caused tumor regressions without regrowth for up to 3 months, while treatment with vehicle or toxin conjugated to control antibodies showed no activity (Oncofetal Antigen, 2011). In 2013, the same team of scientists extended the pre-clinical testing (Sapra et al., 2013). The ADC showed potent antitumor activity both in vitro and in vivo against a variety of 5T4-positive tumors. The results for the in vitro test showed that the ADC inhibited the growth of 5T4-expressing tumor cell lines (MDAMB435/5T4, MDAMB468, and MDAMB361-DYT2) in a concentration-dependent manner, but as expected was not active against 5T4-negative lymphoma cells. In the in vivo experiment, the ADC caused complete inhibition of growth or regression of 4 different kinds of 5T4-tumors (37622A1 PDX, MDAMB468, MDAMB361DYT2, H1975). The ADC induced long-term tumor regressions for up to 100 days, with each model showing responses to the drug, even at doses as low as 3 mg/kg dosed every 4 days. No deaths or significant adverse events were recorded throughout the observation period. When given to monkeys at high doses of 10 mg/kg, the ADC showed no obvious toxicities, and had a half-life of approximately 5 days (Sapra et al., 2013).

Further experiments with the ADC mentioned above were done in 2017 at the University of Manchester (UK) using tumor cells derived from patients with acute lymphoblastic leukemia (ALL) (McGinn et al., 2017). They showed that antigen 5T4 was highly expressed in tumor cells derived from patients with high levels of residual cancer following initial treatment with chemotherapy. Treatment of mice engrafted with 5T4-positive ALL leukemia cells significantly improved survival, and showed no apparent toxicity. In mice engrafted with patient-derived 5T4-positive ALL leukemia cells, treatment with dexamethasone plus the ADC significantly improved survival (p=0.0006) relative to either therapy alone.

DLL3

Delta-like 3 (DLL3) is a protein expressed on the surface of small-cell lung cancer (SCLC) and large-cell neuroendocrine carcinoma (LCNEC), but it is rarely expressed in healthy adult tissue, which makes it an ideal target for ADCs (Saunders et al., 2015). An ADC targeting DLL3 is SC16LD6.5. Which combines a humanized anti-DLL3 monoclonal antibody to a DNA damaging dimer toxin pyrrolobenzodiazepine (PBD) (Saunders et al., 2015).
In 2015, scientists at Stemcentrx Inc. (South San Francisco, California) tested SC16LD6.5 in pre-clinical trials (Saunders et al., 2015). They implanted NOD/SCID mice, a strain of inbred immunodeficient mice, with patient-derived SCLC or LCNEC tumor cells. They observed that the ADC induced durable tumor regressions across multiple models. They also used serial transplantation experiments to show a lack of tumor recurrence after the exposure. The tumor regressions directly correlated with DLL3 expression, showing the importance of the target antigen in the therapy. The observed toxicities consisted of reversible myelosuppression, mild kidney degeneration, skin thickening, and hyperpigmentation. The adverse effects appear to be due to off-target effects of the drug. There were no reported animal deaths or other significant adverse events (Saunders et al., 2015).

EpCAM

Human epithelial cell adhesion molecule (EpCAM) (also known as CD326) is a type I membrane glycoprotein expressed in tumor initiating cells (TICs), making it a potential target for ADCs (Moldenhauer et al., 2012). Anti-EpCAM antibodies showed promise in past studies, but showed no tumor regression in a phase II clinical trial. α-amanitin-glutarate-chiHEA125 (chiHEA125-Ama) is an ADC that targets EpCAM. This ADC was created by chemical cross-linking α-amanitin (a toxin that inhibits DNA transcription) with chiHEA125 (a human-mouse chimera anti-EpCAM monoclonal antibody) (Moldenhauer et al., 2012). This ADC was investigated in pre-clinical testing by a team of scientists at the German Cancer Research Center (Heidelberg, Germany) using both in vitro and in vivo methods (Moldenhauer et al., 2012). For their in vitro experiment, they used a [3H]-thymidine incorporation assay to measure the effect of the ADC on DNA replication in human pancreatic (BxPc-3 and Capan-1), colorectal (Colo205), breast (MCF-7), and bile duct (OZ) cancer cell lines. The ADC inhibited growth of all the cell lines tested, with IC50’s ranging from 0.25 nanomolar to 5.4 picomolar (extremely active). In their in vivo experiment, they injected subcutaneous human BxPc-3 pancreatic carcinoma xenograft tumors into mice and found that a single 50 µg/kg dose of the ADC inhibited tumor growth by 79%. Two doses of 100 µg/kg given a week apart led to complete tumor regression in nine out of ten mice. When treated at extremely high doses, the authors reported 30%-50% of the mice experiencing pronounced liver toxicity, but there were no reports of death or other significant adverse events at the lower doses (Moldenhauer et al., 2012).

Fibronectin

Fibronectin is a glycoprotein of the extracellular matrix that binds to membrane-spanning receptor proteins called integrins (Palumbo et al., 2011). The EDB domain of fibronectin can be used as a marker for tumor angiogenesis, as blood vessel formation is rare in healthy adults. Fibronectin molecules containing the EDB domain are synthesized during the formation and growth of a majority of tumors. An ADC that targets fibronectin-EDB could eradicate cancer by destroying the tumor’s blood vessels, starving the tumor. An example of an ADC that targets fibronectin-EDB is SIP(L19)-PS. This ADC was synthesized by combining PS (a photosensitizer that absorbs in the red spectrum), and L19 (a human monoclonal antibody against fibronectin-EDB (Palumbo et al., 2011). In 2010, a study done by scientists at the Swiss Federal Institute of Technology (Zurich, Switzerland) tested this ADC in two mouse models of cancer (F9 and A431). Their results showed the ADC when activated by radiation lead to disruption of
the tumor vasculature, and complete and long-lasting cancer eradication. They also showed that natural killer cells (NKs) are essential for the tumor eradication. There was no mention of any adverse side-effects (Palumbo et al., 2011). Their results show that shutting down the tumor vasculature can cause tumor death.

**Mesothelin**

Mesothelin is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein, and is overexpressed in several human tumors, including the majority of ovarian and pancreatic adenocarcinomas, and in 100% of epithelial mesotheliomas (Golfier et al., 2014). Expression in normal tissues is mostly restricted, being found mainly in single cell layers lining the pleura, pericardium, and peritoneum. Mesothelin may play a role in the metastatic spread of cancer cells in the ovaries. **Anetumab Ravtansine (BAY 94-9343)** is an example of an ADC that targets mesothelin. It consists of a human anti-mesothelin antibody conjugated to the maytansinoid tubulin inhibitor DM4 via a disulfide-containing linker (Golfier et al., 2014).

In a 2014 study performed by scientists at Bayer HealthCare Pharmaceuticals (Berlin/Wuppertal, Germany), they examined the binding properties of BAY 94-9343 and its effects on tumor cells in vitro and in vivo in xenograft mice (Golfier et al., 2014). In vitro, BAY 94-9343 demonstrated potent and selective cytotoxicity against mesothelin-expressing cells with an IC$_{50}$ of 0.72 nmol/L, without affecting mesothelin-negative or non-proliferating cells. In vivo, BAY 94-9343 localized specifically to mesothelin-positive tumors and inhibited tumor growth, in both subcutaneous and orthotopic xenograft models. In addition, BAY 94-9343 was able to induce a bystander effect on neighboring mesothelin-negative tumor cells (likely from DM4 released from nearby apoptotic tumor cells). There was no mention of adverse effects or death in the report (Golfier et al., 2014).

**Section-2 Conclusions**

The pre-clinical results described in this section of the Literature Review show that antibody-drug conjugates have the potential to slow or eliminate various types of cancers, both in vitro using cultured cancer cell lines and in vivo using mouse xenograft models. The ADC drugs showed various degrees of effectiveness against a large variety of cancers, including complete cancer remissions in some cases. Use of the antibody alone, or the cargo toxin alone, produced no tumor killing, providing a proof-of-principle that combining the targeting property of an antibody with the potent cell killing ability of new cytotoxic drugs strongly elevates the anti-tumor effects of either agent alone. Targeting the cytotoxic drug directly to the tumor allows it to be used at concentrations far in excess of what could be tolerated with a systemic administration of the toxin. Some of the pre-clinical studies assayed antibody binding affinity to the surface antigen, showing that the ADC drug bound in the same nano-molar range as free antibody. This shows that conjugation of the antibody to the drug cargo did not alter its cell binding ability. Other studies performed pre-clinically assayed the amount of free unconjugated toxin in the blood, and found very little. This shows that the ADC linkers held together until the drugs were internalized in the cancer cells. With respect to side-effects, most of the pre-clinical studies showed relatively minor adverse-effects; the most serious side-effect observed in mice
was neutropenia, so this might be expected to be a problem in the clinical trials, and should be monitored closely.

With respect to best practices, some of the pre-clinical studies showed that the extent of anti-tumor activity directly correlated with the extent of over-expression of target antigen on the tumor. If the tumor no longer expressed the target antigen, the ADC drug was inactive against that tumor. So, this data implies that a best practice would be to frequently monitor the patient’s tumor for the level of target antigen expression to determine which patients most likely will benefit from the therapy or whether the ADC therapy should be continued. Other pre-clinical studies showed that delivering the ADC drug long-term post-cancer remission prevented disease re-occurrence (so long as the patient did not form antibodies against the ADC drug), so ADC use long-term might be tested in clinical trials to help ensure a patient’s cancer does not return.

Overall, the pre-clinical data discussed in this section support moving forward with human clinical trials for most of the ADC drugs, while using the best practice of frequently assaying for the presence of the target antigen to determine which patients will most likely benefit from the therapy.

Section-2 References


The purpose of this section of the Literature Review is to discuss some of the data obtained in human clinical trials using various antibody-drug conjugates (ADCs). Over 27 different ADCs are currently being investigated in patients (Sasson and Blanc, 2013), and this has provided a wealth of safety information for this type of drug. Special attention is paid to the safety of those ADCs that have been FDA approved. This section is divided into subsections based on the surface antigen targeted by the ADC.

CD19

Cluster of differentiation-19 (CD19) (also known as B-lymphocyte antigen) is a protein found on the surface of B-cells, a type of white blood cell that produce antibodies (Scheuermann and Racila, 1995). Because it is a marker for B-cells, CD19 has long been used to help diagnose cancers that arise from B-cells, such as lymphoma and leukemia, and cells strongly expressing CD19 are found in these types of tumors (Del Nagro et al., 2005). An example of an antibody-drug conjugate (ADC) directed against CD19 is SAR-3419. SAR-3419 contains a humanized monoclonal antibody against CD19, linked via a cleavable linker to 3-4 molecules of the tubulin inhibitor DM-4 (Blanc et al., 2011). SAR-3419 was designed to treat B-cell malignancies of all types, including all B-cell lymphomas, B-cell chronic lymphocytic leukemia, and non-T acute lymphoblastic leukemia (ALL) (the latter cannot be targeted by CD20).

Two Phase-I clinical trials have been performed using SAR-3419 in patients with relapsed B-cell non-Hodgkin’s lymphoma (NHL). NHL is one of the most common cancers in the United States, accounting for about 4% of all cancers. In 2017, approximately 72,240 people (40,080 males and 32,160 females) will be diagnosed with NHL, and about 20,140 people will die from this cancer (American Cancer Society, 2017). In the SAR-3419 clinical trials, the patients were dosed once every 3 weeks (Younes et al., 2009), or once every week (Coiffier et al., 2011). In the 2009 trial, other than ocular problems (such as blurred vision) no other clinically significant toxicities were observed in over 10% of the patients. There were no clinically significant hematologic or gastrointestinal toxicities, so the linker held up well, and there was little evidence of free toxic DM-4 in the blood. The drug lasted from 4-6 days in the patients, independent of dose. Tumor reduction was seen in more than half of the 35 evaluable patients. No effect was observed on normal B-cells (that also express CD-19), perhaps because their count was low due to previous therapies (Younes et al., 2009). In the 2011 trial (Coiffier et al., 2011), ocular toxicity was also noted, but the incidence (2%) and severity were lower. The hematological toxicity was insignificant, and a majority of patients showed a reduction in their lymphoma, which is highly encouraging. Thus, both of these phase-I tests showed no significant toxicity with some anti-tumor activity.
CD22

CD22 is a molecule belonging to the SIGLEC family of lectins. It is found on the surface of mature B-cells, and to a lesser extent on immature B-cells (Hatta et al., 1999). CD22 is not as broadly expressed in B-cells as CD19. An example of an antibody-drug conjugate targeting CD22 is Inotuzumab ozogamicin (also called CMC-544 or INO). This ADC was developed by Pfizer and Union Chimique Belge (UCB) for patients with Non-Hodgkin Lymphoma (NHL), and consists of a humanized monoclonal antibody against CD22 (Inotuzumab) linked to a cytotoxic agent of the class calicheamicin (which damages DNA) (Takeshita et al., 2009). Binding of the drug to a tumor cell and its internalization allows hydrolysis of the acetyl butyrate linker and the release of the cytotoxic calicheamicin which kills the cell (Advani et al., 2010).

With respect to clinical trials, in 2010, scientists in the Department of Hematologic Oncology and Blood Disorders at the Cleveland Clinic (Cleveland, OH) published the results of their Phase-I trial of Inotuzumab ozogamicin for treating 79 patients with refractory B-cell Non-Hodgkin’s Lymphoma (NHL) (Advani et al., 2010). The purpose of the Phase-I was to determine maximum tolerated dose (MTD), drug safety, and preliminary efficacy. The drug was delivered by IV once every 3-4 weeks, at doses ranging from 0.4 to 2.4 mg/m². The MTD was determined to be 1.8 mg/m². Common adverse effects at the MTD were thrombocytopenia (decrease in platelets) (90% of patients), asthenia (weakness) (67%), nausea (51%), and neutropenia (decrease in neutrophils) (51%). The overall response rate was 39% for all 79 patients, which increased to 68% for patients with follicular NHL. The authors concluded that the drug demonstrated efficacy against NHL, with reversible thrombocytopenia as the main adverse event (Advani et al., 2010).

In 2012, a team of scientists at the National Cancer Institute of the National Institutes of Health (Bethesda, MD) reported the results of their Phase-I trial of CAT-8015 (Moxetumomab pasudotox) in patients with hairy cell leukemia (HCL) (Kreitman et al., 2012). CAT-8015 contains an antibody against CD22 fused to a truncated peptide of Pseudomonas endotoxin. The drug is a second-generation ADC, modified from a previous ADC (CAT-3888) at the antigen-binding site to facilitate a 14-fold increase in CD22 binding affinity. The Phase-I trial enrolled 28 HCL patients previously refractory to two prior chemotherapy treatments, and was a dose escalation to test safety and preliminary responses. The doses ranged from 5-50 µg/kg, delivered every other day for 3 doses, with up to 16 cycles repeated at 4-week intervals. At these doses, no dose-limiting toxicity was observed. Minor side-effects, seen in 25-64% of the patients, included: hypo-albuminemia (low serum albumin), aminotransferase elevations (mild liver damage), edema, headache, hypotension, nausea, and fatigue. Interestingly, 10 of 26 evaluable patients initially made neutralizing antibodies against the drug (which could have diminished drug effectiveness), but after the first cycle of treatment, this antibody response diminished. The overall response rate was 86%, with 46% of the patients achieving complete remission (Kreitman et al., 2012). Based on these impressive Phase-I results, the U.S. National Cancer Institute has initiated a Phase-III trial (not yet published) of CAT-8015 (Hirschler, 2013). AstraZeneca indicated the Phase III clinical trial would test Moxetumomab pasudotox in patients with hairy cell leukemia who have not responded to (or relapsed from) previous therapies.

Another clinical trial performed in 2012 was in the Department of Leukemia at the MD Anderson Cancer Center (Houston, TX) (Kantarjian et al., 2012). This was a Phase-II trial (identifier NCT-01134575) of 49 patients with refractory or relapsed acute lymphocytic
leukemia (ALL) treated with Inotuzumab ozogamicin. The dose was 1.8 mg/m2 IV every 3-4 weeks. CD22 was expressed in more than 50% of the blasts in all patients. The overall response rate was 57%. The most frequent adverse effects were: fever (41%), hypotension (26%), and grade 1-2 liver problems (24%). Two patients died within 4 weeks of starting treatment, but it was not clear whether the deaths resulted from the treatment or the cancer (Kantarjian et al., 2012).

In 2015, a large team of scientists at ten different medical centers (based at Washington University School of Medicine, St. Louis, MO) published the results of their Phase-II trial of 63 patients with high-risk relapsed/refractory diffuse large B-cell lymphoma (DLBCL). The patients were treated with Inotuzumab ozogamicin (INO) plus rituximab antibody (R-INO), followed by high dose chemotherapy and an autologous stem cell transplant (SCT) (Wagner-Johnson et al., 2015). Of the 18 patients that underwent the SCT plus the ADC treatment, 61.1% showed 2-year progression-free survival. For all patients, the 1-year and 2-year progression-free survival rates were 28.9% and 25.3%, respectively. Common grade 3 or 4 side-effects during the R-INO portion of the treatment included: thrombocytopenia, lymphopenia, and neutropenia.

Thus, with respect to ADC drugs targeting CD22, two have undergone clinical trials, including two Phase-II trials for NHL, where the initial data appeared good. In 2013, Pfizer discontinued a Phase-III trial for its CD22-targeting ADC when it appeared to not extend patient lifespan (Pfizer, 2013), but ten other CD22 clinical trials are still ongoing, including a Phase-III for acute lymphoblastic leukemia (ALL) (Trial Identifier: NCT01564784) (Clinical Trials.gov).

**CD30**

CD30 is a cell membrane protein belonging to the tumor necrosis factor (TNF) receptor family. CD30 is expressed on activated, but not resting, T- and B-cells, and is associated with anaplastic large cell lymphoma (ALCL) and classic Hodgkin lymphoma (HL) (Gorzyca et al., 2003). Drugs targeting CD30 are one of the best investigated of all the ADCs. A well characterized example in this class is Brentuximab vedotin (Adcetris®, SGN-035), one of only two currently FDA-approved ADCs (Deng et al., 2013). Adcetris consists of a mouse-human chimeric monoclonal antibody against CD30 (Brentuximab or cAC10) linked to 3-5 units of the drug monomethyl auristatin-E (MMAE) that binds tubulin and keeps it from polymerizing.

With respect to clinical trials, in 2010 scientists at Seattle Genetics, Inc. released their data from the first clinical trial of Adcetris for 102 patients with refractory/relapsed HL. The data was presented at the 52nd American Society of Hematology meeting in Orlando (Seattle Genetics, 2010). Their results showed that 75% of the patients achieved an objective response, and 34% achieved complete remission.

Also in 2010, another team of scientists in the Department of Lymphoma and Myeloma at the University of Texas MD Anderson Cancer Center (Houston, TX) published the results of their Phase-I multi-center testing of Brentuximab vedotin on 45 patients with refractory/relapsed HL and ALCL (Younes et al., 2010). They tested doses ranging from 0.1 to 3.6 mg/kg body weight delivered every 3 weeks. The data showed that the maximum tolerated dose was 1.8 mg/kg, and at this dose 50% of the patients showed a response. Tumor regressions were observed in 36 of 42 measurable patients, with 11 complete remissions. The most common side-
effects were fatigue, pyrexia, diarrhea, nausea, neutropenia and peripheral neuropathy (Younes et al., 2010).

In 2012, the same MD Anderson team mentioned above extended their clinical analysis of Brentuximab vedotin with a Phase-II study of 102 patients with refractory/relapsed HL who had received an autologous (self) stem cell transplant (Younes et al., 2012). The patients were treated IV with 1.8 mg/kg Adcetris for 3 weeks. Their data showed an overall response rate of 75%, with complete remission in 34% of the patients. After a median observation time of 1.5 years, 31 patients (30%) were alive and free of documented disease. The most common treatment-related adverse effects were peripheral neuropathy, nausea, fatigue, neutropenia, and diarrhea (Younes et al., 2012).

Also in 2012, a different team of scientists at the Fox Chase Cancer Center (Philadelphia, PA) published the results of their Phase-II multicenter trial of Brentuximab vedotin in 58 patients with relapsed/refractory anaplastic large-cell lymphoma (ALCL) (Pro et al., 2012). The patients received a dose of 1.8 mg/kg IV every 3 weeks on an outpatient basis. 50 of the 58 patients (86%) showed responses: 33 (57%) showed complete remission, and 17 (29%) showed partial remission. Grade 3 or 4 adverse events included neutropenia (21%), thrombocytopenia (14%), and peripheral sensory neuropathy (12%) (Pro et al., 2012).

The MD Anderson team mentioned previously also investigated the use of Brentuximab vedotin on newly diagnosed patients with HL, using either the ADC alone or ADC combined with standard treatments (Younes et al., 2013). The team performed a Phase-I dose-escalation study on 51 patients, testing from 0.6 to 1.2 mg/kg drug IV every 2 weeks (study identifier NCT01060904). The maximum tolerated dose (MTD) when combined with standard treatment was not exceeded at the highest dose tested here. 21 of 22 patients (95%) achieved complete remission! Adverse events were generally grade 1 or 2, but occurred in 41% of all patients. However, the authors stated that an “unacceptable” number of patients showed pulmonary toxic effects (44%) when the ADC was combined with a chemotherapy containing bleomycin, so the authors recommended omitting bleomycin from the combination (Younes et al., 2013).

Thus, for ADCs against CD30, several Phase-I and II studies have been performed. The data included several complete remissions in patients with HL and ALCL, which the Adcetris drug manufacturer termed “unprecedented responses with manageable toxicity” with very high overall response rates (Vaklavas and Forero-Torres, 2012). Adcetris remains one of only two ADC drugs currently FDA-approved.

CD33

CD33 (also called Siglec-3; sialic acid binding Ig-like lectin 3) is a transmembrane receptor expressed on myeloid cells, but it is also found on some lymphoid cells (Garnache-Ottou et al., 2005). CD33 binds sialic acids, therefore it is a member of the SIGLEC family of lectins. An example of an ADC drug that targets CD33 is Gemtuzumab ozogamicin (GO; Mylotarg), containing an antibody against CD33 linked to the cytotoxic drug calicheamicin (DNA damaging) (Tsimberidou et al., 2006). Mylotarg was the first ADC drug approved for use in the U.S. (Bross et al., 2001), and it has the highest number of Phase-III trials for any ADC drug. Mylotarg was FDA-approved in 2000, marketed by Wyeth, and manufactured by Pfizer.
for treating patients with acute myelogenous leukemia (AML). However, in 2010, Mylotarg was withdrawn from the market when clinical trials showed it increased patient death and was no better than conventional therapies (Nelson, 2010; Richwine, 2010).

Since Mylotarg’s withdrawal in 2010, a few additional clinical trials have been performed where the drug shows significant anti-cancer activity. In 2012, a large team of scientists at the Hôpital Mignot, Université Versailles-Saint Quentin (Le Chesnay, France) reported their results of a Phase-III randomized study of Mylotarg on 280 patients with newly acquired acute myeloid leukemia (AML) (Castaigne et al., 2012). The study was performed at 26 different hematology centers in France. The authors stated that the data from previous Phase-III trials on Mylotarg were contradictory, so they decided to compare two groups: Mylotarg at a dose of 3 mg/m$^2$ plus a chemotherapy treatment versus a group with chemotherapy alone (control) to determine whether the combination was too toxic. Comparing the control versus the ADC/chemo combination, their data showed an increase in patient event-free survival (EFS) from 17.1% to 40.8% (p=0.0003). The relapse-free survival (RFS) increased from 22.7% to 50.3% (p=0.0003). With respect to adverse effects, persistent thrombocytopenia increased from 3% to 16% (p<0.0001), however this did not increase the death rate. The authors concluded that the use of lower doses of GO allowed a successful simultaneous addition of chemotherapy treatment to significantly improve patient outcomes, and that further Phase-III trials with GO are warranted (Castaigne et al., 2012).

In 2013, scientists at the Fred Hutchinson Cancer Research Center (Seattle, WA) reported the results of their Phase-III study of GO at 6 mg/m$^2$ combined with “induction therapy” (daunorubicin and cytarabine) compared to induction therapy alone, in 637 patients with acute myeloid leukemia (AML) (Petersdorf et al., 2013). The patient’s complete remission rate was about the same between the two groups: 69% for GO/induction compared to 70% for induction alone (p = 0.59). And the 5-year relapse-free survival rate was also about the same, 43% GO/induction versus 42% for induction alone (p=0.40). The authors concluded that the addition of GO to induction therapy provided no additional benefit (Petersdorf et al., 2013).

In 2014, scientists in the School of Medicine at Cardiff University (Cardiff, UK) performed a Phase-III meta-analysis of published PubMed reports and data obtained from individual trial lists on 3325 patients with acute myeloid leukemia (AML) (Hills et al., 2014). They compared two groups: Mylotarg + induction therapy versus a control of induction therapy alone. Their data showed that the addition of Mylotarg to the induction therapy did not improve the rate of complete remissions (p=0.3), but Mylotarg did lower the risk of cancer relapse (p=0.0001), and it improved overall patient survival at the 5-year mark (p=0.01). At 6 years, the survival benefit of GO was especially pronounced (p=0.0006) in patients with “favorable cytogenetics”. This group showed no additional chromosomal translocations or inversions. In patients having adverse cytogenetics, Mylotarg provided no detectable benefit (p=0.9). With respect to adverse effects, doses of Mylotarg at 3 mg/m$^2$ were associated with fewer early deaths than the higher dose of 6 mg/m$^2$ (both doses showed equal efficacy). The authors concluded that Mylotarg provides significant survival benefits to patients that have no adverse cytogenetic alterations, and that the drug should be re-assessed for approval (Hills et al., 2014). This study points out an excellent method for helping determine which patients may best benefit from Mylotarg treatments, those with no observable cytogenetic abnormalities, and perhaps assaying for cytogenetic abnormalities should be performed as a best practice.
In 2016, scientists in the Department of Leukemia at the University of Texas MD Anderson Cancer Center (Houston, TX) published a Phase-II study of Mylotarg at a dose of 3 mg/m² combined with decitabine (a DNA synthesis inhibitor) (Daver et al., 2016). The study was performed on 110 patients with relapsed acute myeloid leukemia (AML) or with high-risk myelodysplastic syndrome. The authors speculated that the decitabine might open up the chromatin structure in the leukemia cells making them more sensitive to damage from the calicheamicin component of the Mylotarg which binds to the DNA molecule to induce double-stranded breaks. Complete remission (CR) was seen in 3 of 5 patients (60%) in their group-2, which included patients with refractory disease that lasted longer than one year. The most frequent side-effects observed were nausea, mucositis, and hemorrhage (Daver et al., 2016).

Her2

Human epidermal growth factor receptor-2 (Her2) (also known as CD340, Neu, or Erbb2) is a member of the epidermal growth factor family of cell surface receptors (Mitri et al., 2012). Over-expression of Her2 occurs in approximately 15-30% of breast cancers, and is strongly associated with increased cancer recurrence and poor prognosis (Tan et al., 2007). Her2 over-expression can also occur on cancers of the ovary, stomach, lung, and uterus (Rüschoff et al., 2012; Buza et al., 2014).

An example of an antibody-drug conjugate against Her2 is Kadcyla® (Trastuzumab emtansine; T-DM1). Kadcyla was developed by Genentech/Roche, and is one of two current FDA-approved ADC drugs (the other is Adcetris against CD30). Kadcyla contains a humanized antibody against Her2 (Trastuzumab or Herceptin) linked to cytotoxic drug emtansine (DM-1) that binds microtubules preventing their polymerization (Niculescu-Duvaz, 2010; LoRusso et al., 2011; Lopus, 2011). Kadcyla is unique among ADCs in that its antibody component by itself (without the DM-1) can sometimes inhibit Her2-positive breast cancer cells; the binding of the antibody to the Her2 receptor prevents its dimerization, inhibiting activation of the MAPK, PI3-kinase, AKT kinase pathways that otherwise leads to cell division (Verma et al., 2012).

The first Kadcyla clinical trial was performed in 2010 by scientists at the Dana-Farber Cancer Institute (Boston, MA) (Krop et al., 2010). They reported the findings of their Phase-I study of Kadcyla for treating breast cancer patients who relapsed after receiving antibody therapy alone. They performed a dose escalation from 0.3 to 4.8 mg/kg once every 3 weeks, and then monitored safety, pharmacokinetics, and preliminary efficacy. Their data showed that thrombocytopenia was dose-limiting at the highest dose tested (4.8 mg/kg), so the authors concluded that the maximum tolerated dose (MTD) was slightly lower at 3.6 mg/kg. The drug half-life was 3.5 days. The most common drug-related adverse events were thrombocytopenia, elevated transaminases, fatigue, nausea, and anemia. No serious cardiac events that would have required drug lowering were observed. The confirmed response rate at the maximum tolerated dose was 44%. The authors concluded that Kadcyla caused mild and reversible toxicity, and it appeared to be active in the relapsed patients (Krop et al., 2010).

The same team of scientists (led by HA Burris who was senior author on the previous study, but was now associated with the Sarah Cannon Research Institute in Nashville, TN) published the results of their Phase-II study of Kadcyla for 112 breast cancer patients who had
relapsed after receiving antibody therapy alone (Burris et al., 2011). The team stayed with the previously identified maximum tolerated dose of 3.6 mg/kg once every 3 weeks. The overall response rate was 25.9%, with a median progression-free survival of 4.6 months. The drug appeared to be well tolerated; the most frequent side-effects were only at grade-1 or -2 (mild). Observed grade-3 (serious) problems included hypokalemia (lowered serum potassium levels) (8.9%), thrombocytopenia (8.0%), and fatigue (4.5%), although these were observed only in a small minority of patients. The authors concluded that the treatment was generally well tolerated (Burris et al., 2011). Importantly, the response rates were higher in patients with confirmed Her-2-positive tumors (assayed by immuno-histochemistry or by reverse transcriptase polymerase chain reaction), so this best practice of verifying the presence of the surface antigen target in a patient’s tumor should be continued.

In 2012, a Phase-III trial coordinated by scientists at the Sunnybrook Odette Cancer Center (Toronto) investigated the use of Kadcyla on 991 patients with refractory breast cancer (trial number NCT00829166) (Verma et al., 2012). The patients were resistant to antibody therapy alone plus a taxane chemotherapy, and were randomly assigned to two groups: Kadcyla versus a control group that received a chemotherapy combination of lapatinib and capecitabine. Comparing the control group to the Kadcyla group, the data showed that progression-free patient survival increased from 6.4 months to 9.6 months (p<0.001), overall survival increased from 25.1 months to 30.9 months (p<0.001), and the patient response rate increased from 30.8% to 43.6% (p<0.001). Grade-3 (serious) adverse events decreased from 57% to 41%. Thrombocytopenia and liver damage were higher with Kadcyla, while diarrhea, nausea, vomiting, and erythrodysthesia were higher with the chemotherapy. The authors concluded that Kadcyla significantly improved progression-free and overall patient survival, with less toxicity than chemotherapy.

On the basis of the previously mentioned clinical trials, the FDA approved Kadcyla on February 22, 2013, for women with Her2-positive metastatic breast cancer that had not previously responded to antibody treatment alone or to chemotherapy (Pollack, 2013).

Even after FDA approval, the clinical trials for Kadcyla continued. In 2014, the team of scientists mentioned previously at the Dana-Farber Cancer Institute (Boston) published their Phase-III data of 602 patients in 22 countries of Kadcyla against refractory Her2-positive advanced breast cancer (Krop et al., 2014). They compared two patient groups: Kadcyla and a physician’s choice chemotherapy. Their data showed that Kadcyla increased progression-free survival from 3.3 to 6.2 months (p<0.0001), slightly improved overall survival (p=0.0034), and decreased level-3 adverse events (from 43% to 32%). The Kadcyla group showed higher incidence of thrombocytopenia (5% versus 2%), but had lower incidence of neutropenia and diarrhea. The authors concluded that due to the strong safety and efficacy profile of Kadcyla, it should now be considered the new standard for treating patients with refractory Her2-positive breast cancer (Krop et al., 2014).

Also in 2014, another team of researchers based at the Mayo Clinic in Jacksonville, FL, published their Phase-II study using Kadcyla in patients with previously untreated Her2-positive breast cancer (clinical trial identifier NCT00679341) (Perez et al., 2014). They compared two groups: Kadcyla (T-DM1, N=67) versus trastuzumab antibody and docetaxel (HT) (N=70). Importantly, this team assayed the level of Her2 expression in the tumors using immuno-histochemistry, in situ hybridization, and reverse transcriptase polymerase chain reaction (RT-
PCR). They found that the risk of disease progression was lower with T-DM1 treatment than with HT (hazard ratio 0.59), and the disease risk lowered further in patients whose tumors showed levels of Her2 greater than the mean (hazard ratio 0.39) (Perez et al., 2014). This data shows that when using ADCs to treat tumors, pre-analyzing the tumors to determine the extent of antigen expression might help predict which patients are most likely to benefit from the ADC treatment.

Another Her-2 clinical trial performed in 2014 was coordinated by scientists in the Departments of Biostatistics, Product Development Oncology, and Translational Oncology at Genentech, Inc. (South San Francisco, CA) (Phillips et al., 2014). This Phase-I/II trial was performed on 3 patients (Phase-I), and 9 patients (Phase-II), with Her-2-positive breast cancer using a dual-targeting approach that used the ADC Kadcyla combined with Pertuzumab (Perjeta) to prevent dimerization (activation) of the Her-2 receptor. Dimerization of the receptor leads to activation of signal transduction pathways that induce cell growth. The patients had been pre-treated with antibody alone and chemotherapy, and the cancer had metastasized. They were treated with the maximum tolerated ADC dose of 3.6 mg/kg every 3 weeks plus a standard dose of Perjeta. Their data showed that the combined treatment caused only mild grade-1 and -2 adverse events which were treatable, so the authors concluded that the combination should be tested further in larger trials (Phillips et al., 2014). This combination approach, at least for the Her-2 situation, appears to be an interesting future application of ADCs, and is worth gaining additional information in IQP interviews.

**Glycoprotein NMB (gpNMB)**

Glycoprotein non-metastatic melanoma protein-B (gpNMB) (also called GPNMB, osteoactivin, dendritic cell-heparin integrin ligand, or hematopoietic growth factor inducible neurolin-1) is a transmembrane glycoprotein expressed in a variety of cell types, including melanocytes, osteoclasts, osteoblasts, and dendritic cells (Weterman et al., 1995). gpNMB is also over-expressed in aggressive melanoma, glioma, and breast cancer (Tse et al., 2006; Kuan et al., 2006; Rose et al., 2007). In one study, gpNMB was detected in 71% of breast tumors (Burris et al., 2009), but others state it is more likely found in 40-60% of breast cancers (Bendell et al., 2014). An example of an antibody-drug conjugate directed against gpNMB is Glembatumumab vedotin (CDX-011) (formerly CR011-vcMMAE). CDX-011 consists of a fully human IgG2 monoclonal antibody against gpNMB conjugated via a valine-citrulline linker to the potent microtubule inhibitor monomethyl auristatin-E (MMAE) (Naumovski and Junutula, 2010; Keir and Vahdat, 2012).

Relatively few clinical trials have been done with ADCs against gpNMB. In 2010, scientists published a Phase-I data of melanoma patients treated with CDX-011 (Hamid et al., 2010). This study identified a dose of 1.88 mg/kg once every 3 weeks as the maximum tolerated dose (MTD), and recommended a Phase II study be performed in melanoma patients.

In 2014, scientists at the Sarah Cannon Research Institute (Nashville, TN) published their Phase-I/II study of Glembatumumab vedotin in 42 patients with locally advanced or metastatic breast cancer (Bendell et al., 2014). Initially, the maximum tolerated dose (MTD) was determined to be 1.34 mg/kg (limited by the patient’s worsening neuropathy), but the MTD was increased to 1.88 mg/kg (their formal Phase-II dose) after eliminating patients with baseline
neuropathy. Of 19 patients tested for the presence of gpNMB-positive tumors, 16 (84%) were positive for the target antigen. At the Phase-II dose, median progression-free survival was 9.1 weeks for all patients, and 18.0 weeks for patients with gpNMB-positive tumors, indicating the presence of the target antigen increased patient survival without tumor growth. The authors concluded that CDX-011 has an acceptable safety profile, and the preliminary evidence of anti-tumor activity is promising and requires future confirmation (Bendell et al., 2014).

In 2015, this same team published their Phase-II “EMERGE” trial for Glembatumumab vedotin in 124 patients with advanced refractory breast cancer (Yardley et al., 2015). The investigators compared the ADC versus an investigator choice (IC) chemotherapy. The data showed that the overall response rates were approximately equal for the two groups, but when analyzing a subset of patients that over-expressed gpNMB to ≥ 25% of control cells, the response rate increased from 9% to 30%. The ADC drug showed less hematologic toxicity than the chemotherapy, but produced more rashes, pruritus (itching), neuropathy, and alopecia. The authors concluded that the ADC was well tolerated, and although their primary end point was not met for all enrolled patients, the drug activity was pronounced in patients that strongly over-expressed gpNMB (Yardley et al., 2015). So, this data shows that patients having tumors that strongly over-express the target antigen will best benefit from the ADC treatment.

Trop-2

Trophoblast cell-surface antigen-2 (Trop-2) (also known as tumor-associated calcium signal transducer-2, or epithelial glycoprotein-1 antigen), is a carcinoma-associated cell surface glycoprotein receptor that is a member of a family with at least two other members (Linnenbach et al., 1993). Trop-2 is over-expressed on many types of epithelial tumors, especially the more aggressive types, but it is also expressed on some normal tissues (Cubas et al., 2009; Trerotola et al., 2013; Ambrogi et al., 2014). An antibody-drug conjugate targeting Trop-2 is Sacituzumab govitecan (IMMU-132) (Cardillo et al., 2011). IMMU-132 consists of a humanized monoclonal antibody against Trop-2 (hRS7) linked to SN-38, the active metabolite of irinotecan, an inhibitor of topoisomerase-I. Blocking topoisomerase keeps DNA from unwinding during replication, killing the cell.

The first-in-human Phase-I clinical trial of IMMU-132 was published in 2015 by a team of scientists at the Indiana University Health Goshen Center for Cancer Care (Goshen, Indiana) (clinical trial NCT01631552) (Starodub et al., 2015). The authors performed a dose-escalation experiment on 25 patients with various types of solid tumors to determine the maximum tolerated dose (MTD). The doses ranged from 8-18 mg/kg. The MTD was determined to be 12 mg/kg for one cycle of treatment, but that dose could not be continued for additional cycles due to the formation of neutropenia. After extended treatments at a lower dose of 10 mg/kg, no level-4 (serious) adverse events were observed, and grade-3 toxicities were fatigue, neutropenia, diarrhea, and leukopenia. With respect to preliminary anti-cancer activity data, 16 of the 25 patients (64%) maintained stable disease, which was the best response observed (there were no complete remissions), but the authors did not pre-select their patients on the basis of Trop-2 expression. The authors concluded that IMMU-132 had acceptable toxicity and encouraging activity (Starodub et al., 2015).
In 2016, scientists in the Division of Hematology and Medical Oncology, Weill-Cornell Medical College (New York) published their Phase-I data on Sacituzumab govitecan in patients with metastatic platinum-resistant urothelial carcinoma (PRUC) (clinical trial NCT01631552) (Faltas et al., 2016). PRUC patients currently have no FDA-approved therapies. Of the six patients tested, three (50%) showed progression-free survival. The drug was well tolerated, so the authors have initiated a new Phase-II study.

In addition to the published literature, several other clinical trials are ongoing for various ADC drugs; some these are summarized in the table below. Some scientists have estimated that over 27 ADCs are undergoing clinical evaluation (Sasson and Blanc, 2013).

Table of Antibody-Drug Conjugates in Clinical Trials as of June, 2016.
Data obtained from: Lancet Oncology, 17: e254-e262.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Antigen Target</th>
<th>Cytotoxic Payload</th>
<th>Cancer Target</th>
<th>Clinical Trial Phase</th>
<th>Clinical Trials.gov Identifier</th>
</tr>
</thead>
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<tr>
<td>Sacituzumab govitecan (IMMU-132)</td>
<td>TROP2</td>
<td>SN-38</td>
<td>Triple-negative breast cancer</td>
<td>3</td>
<td>NCT02574455 NCT02161679</td>
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<tr>
<td>Inotuzumab ozogamicin (CMC-544)</td>
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<td>Calicheamicin</td>
<td>Acute lymphoblastic leukaemia</td>
<td>3</td>
<td>NCT01564784</td>
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<tr>
<td>Anetumab ravtansine (BAY 94-9343)</td>
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<td>DM4</td>
<td>Mesothelioma</td>
<td>2</td>
<td>NCT02610140</td>
</tr>
<tr>
<td>Gemtuzumab ozogamicin</td>
<td>CD33</td>
<td>Calicheamicin</td>
<td>Acute myeloid leukaemia, acute promyelocytic leukemia</td>
<td>2</td>
<td>NCT01409161 NCT01869803</td>
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<tr>
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<td>MMAF</td>
<td>Glioblastoma</td>
<td>2</td>
<td>NCT02573324 NCT02343406</td>
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<tr>
<td>Glembatumumab vedotin (CDX-011)</td>
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<td>MMAE</td>
<td>Osteosarcoma, melanoma, triple-negative breast cancer</td>
<td>2</td>
<td>NCT02487979 NCT02302339</td>
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<tr>
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<td>Mirvetuximab soravtansine (IMGN-853)</td>
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<td>DM4</td>
<td>Folate receptor α-positive epithelial ovarian cancer</td>
<td>2</td>
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</table>
### Safety of Gemtuzumab Ozogamicin (Mylotarg)

The first ADC drug approved by the FDA was Mylotarg (Gemtuzumab ozogamicin, GO), a humanized antibody against CD33 (frequently over-expressed on the surface of acute myeloid leukemia cells), linked to a cytotoxic drug of the calicheamicin class (Tsimberidou et al., 2006). Mylotarg was FDA-approved on May 17, 2000 (FDA, 2000) under an accelerated FDA-approval process for two types of cancer patients: those over the age of 60 with relapsed acute myeloid leukemia (AML), and for AML patients not considered a candidate for standard chemotherapy (Bross et al., 2001). However, in 2010, Mylotarg was withdrawn from the market when clinical trials showed it increased patient death and appeared to be no better than conventional therapies (Nelson, 2010; Richwine, 2010). The drug did not appear to extend survival for patients with previously untreated AML, and the fatality rate was 5.7% for Mylotarg patients compared to 1.4% for patients without the drug (Richwine, 2010).

With respect to safety, during Mylotarg’s initial clinical trials, the most frequent side-effects were relatively mild, including: shivering, fever, nausea, and vomiting. But within a year
of Mylotarg’s approval in 2000, more serious adverse events were observed, including: adult respiratory distress syndrome (ARDS), hepatotoxicity, anaphylaxis, veno-occlusive disease (VOD), and death (Nelson, 2010). These serious effects resulted in the FDA requiring a “black box warning label” on the package (Giles et al., 2001; Wadleigh et al., 2003), and eventually the drug was withdrawn in 2010. One key variable shown to be very important when observing VOD was whether the leukemia patient had undergone a bone marrow transplant within a short period of time after Mylotarg administration, in this situation 3 of 14 (21%) died of VOD (Wadleigh et al., 2003), compared to 14 of 119 patients (12%) who had not undergone a bone marrow transplant (Giles et al., 2001).

Following Mylotarg’s withdrawal in 2010, four clinical trials were performed that may indicate the drug might be safer than originally thought. A 2012 Phase-III trial of 280 patients with newly acquired AML performed at 26 different hematology centers in France showed that Mylotarg increased the incidence of persistent thrombocytopenia from 3% to 16% (p<0.0001), however this did not increase patient death rate (Castaigne et al., 2012). A 2013 Phase-III trial performed at the Fred Hutchinson Cancer Research Center (Seattle, WA) compared Mylotarg combined with “induction therapy” (daunorubicin and cytarabine) to induction therapy alone, in 637 patients with (AML) (Petersdorf et al., 2013). The drug was shown to be as safe as the induction therapy, but provided no additional anti-cancer benefit. A Phase-III trial in 2014 at Cardiff University (Cardiff, UK) performed a meta-analysis of PubMed reports supplemented with data obtained from individual trial lists of 3,325 AML patients (Hills et al., 2014). The study compared Mylotarg combined with induction therapy versus a control of induction therapy alone. Their data showed that doses of Mylotarg at 3 mg/m² produced fewer early deaths than 6 mg/m² yet was equally effective. So, using the minimum effective dose appears to be an effective strategy. A Phase-II study done in 2016 at the University of Texas MD Anderson Cancer Center (Houston, TX) of Mylotarg at a dose of 3 mg/m² combined with decitabine (a DNA synthesis inhibitor) on 110 patients with either relapsed AML or high-risk myelodysplastic syndrome, showed that the most frequently observed side-effects were nausea, mucositis, and hemorrhage, each of which were treatable (Daver et al., 2016).

Based on the overall safety and efficacy observed in the post-2010 clinical trials, in September of 2017, Pfizer re-submitted their application for FDA review as a Biologics License Application (BLA) (Stanton, 2017). Pfizer expects an FDA decision on this re-submission by September, 2017.

Safety of Brentuximab Vedotin (Adcetris, SGN-035)

The second ADC drug approved by the FDA is Adcetris, a mouse-human chimeric monoclonal antibody against CD30 (Brentuximab or cAC10) linked to three to five units of the cytotoxic drug monomethyl auristatin-E (MMAE) (Francisco et al., 2003). Adcetris is designed to treat patients with Hodgkin Lymphoma (HL) or patients with systemic anaplastic large-cell lymphoma (ALCL) that over-express CD30 on their tumor cells (Küppers and Hansmann, 2005). Adcetris is manufactured by Seattle Genetics, who received accelerated FDA-approval on August 19, 2011 (Genetic Engineering and Biotechnology News, 2011).

With respect to safety, Adcetris has been studied alone and in combination with other chemotherapy agents. The clinical trials were discussed in detail in Section-3 of the Literature
Review, but the safety information from them can be summarized. A 2010 Phase-I clinical trial based at the University of Texas MD Anderson Cancer Center (Houston, TX) was performed on 45 patients with refractory/relapsed HL and ALCL; the most common side-effects observed were fatigue, pyrexia (fever), diarrhea, nausea, neutropenia (decreased neutrophils) and peripheral neuropathy (irreversible tingling numbness and hyper-sensitivity to cold) (Younes et al., 2010). The same team performed a Phase-II trial in 2012 on 102 patients with refractory/relapsed HL who had received an autologous (self) stem cell transplant; the most common adverse effects were peripheral neuropathy, nausea, fatigue, neutropenia, and diarrhea (Younes et al., 2012). Another Phase-II trial was performed in 2012 at the Fox Chase Cancer Center (Philadelphia, PA) in 58 patients with relapsed/refractory anaplastic large-cell lymphoma (ALCL) (Pro et al., 2012). The adverse events observed in this trial included neutropenia (decreased neutrophils) (21%), thrombocytopenia (decreased platelets) (14%), and peripheral sensory neuropathy (12%) (all at grade-3 or -4). In 2013, the MD Anderson team mentioned earlier also performed a Phase-I trial on 51 newly diagnosed patients with HL, using either Adcetris alone or in combination with standard treatments (Younes et al., 2013). The adverse events observed most frequently were grade-1 or -2 (mild), but occurred in 41% of all patients. The authors stated that an “unacceptable” number of patients showed pulmonary toxic effects (44%) when the ADC was combined with a chemotherapy containing bleomycin, so the authors recommended omitting bleomycin from the combination (Younes et al., 2013), and this safety recommendation is followed to this date.

Thus, for Adcetris, several phase-I and II studies have been performed, and they show that the most common adverse reactions are neutropenia (decreased neutrophils), thrombocytopenia (decreased platelets), and peripheral sensory neuropathy. An independent review of Adcetris drug safety by scientists at the Comprehensive Cancer Center at the University of Alabama at Birmingham (Vaklavaas and Forero-Torres, 2012) concluded that the drug had an overall manageable toxicity profile, but that peripheral neuropathy remained an important consideration, so this may limit long-term administration of the drug. On January 13, 2012, the FDA announced that Adcetris had been linked to two cases of progressive multifocal leukoencephalopathy (defective formation of myelin), so the FDA now requires the manufacturer to add a “black box warning” to the drug packaging warning of this risk.

Safety of Trastuzumab Emtansine (Kadcyla, T-DM1)

The third ADC drug approved by the FDA is Kadcyla (Trastuzumab emtansine, T-DM1). The antibody portion of the drug was developed by Genentech (a subsidiary of Roche), and the linker and toxin were developed by ImmunoGen (Waltham, MA) (Pollack, 2013). Kadcyla contains a humanized mouse antibody (Trastuzumab or Herceptin) against surface protein Her2 linked to the cytotoxic drug emtansine (DM-1) that binds microtubules preventing their polymerization (Niculescu-Duvaz, 2010; Lopus, 2011; LoRusso et al., 2011). Kadcyla’s accelerated FDA-approval occurred on February 22, 2013, to treat women with Her2-positive metastatic breast cancer that does not respond to antibody treatment alone or to chemotherapy (National Cancer Institute, 2013; Pollack, 2013; Drugs.com, 2013).

With respect to safety, much information has been gained on Kadcyla from clinical trials. The first Kadcyla clinical trial was performed in 2010 by scientists at the Dana-Farber Cancer Institute (Boston, MA) (Krop et al., 2010). This team reported the findings of their Phase-I
dose-escalation study of breast cancer patients who relapsed after receiving antibody therapy alone. Their data showed that thrombocytopenia became dose-limiting at the highest dose tested (4.8 mg/kg), and concluded that the maximum tolerated dose (MTD) was slightly lower at 3.6 mg/kg. The most common drug-related side-effects were thrombocytopenia, elevated transaminases, fatigue, nausea, and anemia, which the authors concluded were mild and reversible. In 2011, the same team did a Phase-II study of 112 breast cancer patients who had relapsed after receiving antibody therapy alone (Burris et al., 2011). They stayed with the previously identified maximum tolerated dose of 3.6 mg/kg once every 3 weeks. The drug appeared to be well tolerated; the most frequent side-effects were mild (grade-1 or -2) and treatable. Less frequent side-effects were grade-3 (serious) problems such as hypokalemia (lowered serum potassium levels) (8.9%), thrombocytopenia (8.0%), and fatigue (4.5%), although these were in a small minority of patients. The authors concluded that the treatment was generally well tolerated. In 2012, scientists at the Sunnybrook Odette Cancer Center (Toronto) performed a Phase-III trial on 991 patients with refractory breast cancer (Verma et al., 2012), comparing Kadcyla to chemotherapy. Kadcyla decreased the grade-3 (serious) adverse events from 57% to 41%. Thrombocytopenia and liver damage were higher with Kadcyla, while diarrhea, nausea, vomiting, and erythrodysthesia were higher with the chemotherapy. The authors concluded that Kadcyla significantly improved progression-free and overall patient survival, with less toxicity than chemotherapy. In 2014, the same team mentioned above at Dana-Farber Cancer Institute (Boston) published their Phase-III data of 602 patients in 22 countries for patients with refractory Her2-positive advanced breast cancer (Krop et al., 2014). They compared two patient groups: Kadcyla versus a physician’s choice chemotherapy. Their data showed that Kadcyla decreased the level-3 adverse events from 43% to 32%, increased thrombocytopenia (5% versus 2%), but lowered neutropenia, and diarrhea. Also in 2014, another team of researchers at the Mayo Clinic (Jacksonville, FL) published their Phase-II study on patients with previously untreated Her2-positive breast cancer, comparing patients treated with Kadcyla (N=67) to patients receiving antibody plus docetaxel (N=70) (Perez et al., 2014). They found that the side-effects were mostly manageable, and that the risk of cancer progression was low when the patients had high levels of Her-2 target antigen.

Thus, Kadcyla comes with its own set of side-effects which must be weighed against the potential benefit of blocking the breast cancer growth. During clinical trials, the drug manufacturer claims that the most common adverse effects seen were fatigue, nausea, muscle pain, thrombocytopenia (low platelet counts), headache, and constipation (Kadcyla Prescribing Information, 2013), however others showed that more severe adverse-effects can include liver damage, liver failure, nodular regenerative hyperplasia, heart damage, lung disease, and peripheral neuropathy (Amiri-Kordestani et al., 2014). Most of the scientists seem to agree that the adverse effects were better tolerated than those caused by standard chemotherapies. In the U.S., Kadcyla packaging carries a black box warning for liver toxicity, heart damage, and fetal harm if given to pregnant women.

**Section-3 Conclusions**

This section of the Literature Review summarized some of the clinical trial data for various ADC drugs. The focus was predominately on drugs having the largest numbers of clinical studies, but a few other ADCs were also discussed. As expected, the two current FDA-approved drugs (Adcetris and Kadcyla) showed the strongest data in clinical testing, showing
relatively mild and treatable adverse-effects, and strong activity. Neutropenia (low neutrophil count) was almost universally observed among all the clinical trials as an adverse event, but it was not fatal. Almost all of the clinical trials were performed on patients who had relapsed from cancer, or whose cancer did not respond to previous therapies; only one trial was performed on newly diagnosed patients. Thus, the overall patient prognosis was expected to be very low. Doubling patient survival rate, for example from an expected few months (controls) to almost a year (ADC), was deemed significant. A few patients showed complete remission, although most studies at best showed stable tumor progression.

Two best practices were identified in this review of the literature which may be worth following up in interviews. The first pertains to measuring chromosome cytogenetic abnormalities in the patients. Cytogenetic abnormalities include chromosome inversions, insertions, translocations, etc. Some of the Mylotarg clinical trials for leukemia patients showed that the survival benefit of the drug was most pronounced in patients with no additional cytogenetic abnormalities (other than the initial abnormality causing the leukemia). While in patients with adverse cytogenetics, Mylotarg provided no detectable benefit. This may imply that patients with additional chromosomal inversions or translocations incur new changes in cellular biochemistry that increase drug resistance. So, perhaps in best practice, patients should be monitored for cytogenetic abnormalities to help determine which patients are most likely to improve with ADC treatment.

The second best practice observed in the clinical trial literature was the determination of the extent of over-expression of target antigen in the patients. In several trials, patients with confirmed high expression levels of the target antigen showed higher response rates to the ADC, and the risk of cancer progression was lower. Antigen levels were assayed by immuno-histochemistry, reverse transcriptase polymerase chain reaction (RT-PCR), or in situ hybridization). Thus, pre-analyzing the patient’s tumor to determine the extent of antigen expression might help predict which patients are most likely to benefit from the ADC treatment. A patient whose tumor no longer expresses the target antigen obviously will not be targeted by the ADC drug. This practice of assaying target expression levels should be continued. If a patient no longer expresses the target antigen, another drug should be chosen.

With respect to safety, from the clinical trial data it can be seen that the side-effects caused by ADC drugs are not trivial. Of three ADC drugs approved by the FDA, one has been withdrawn from the market due to patient deaths and an apparent lack of efficacy. And all three FDA-approved drugs carry “black box warning labels” on their packaging, informing physicians of the most serious side-effects. The follow-up interviews will help weigh the adverse effects of the ADCs against the side-effects of standard chemotherapy treatments currently used to treat the cancer, and will also be weighed against the very poor prognosis of patients with these types of relapsed cancers who have few other options. But overall, the ADC side-effects appear to be more manageable than those caused by current chemotherapy treatments, and are far better than the very poor prognosis of their relapsed cancer.

However, it remains unclear from the literature what causes the ADC side-effects. Are the adverse reactions caused by expression of the target antigen in normal tissues in addition to the cancer? Or are the side-effects caused by non-target effects, such as the release of the cytotoxic cargo into the surrounding cells? In the next phase of this IQP, information obtained from interviews may help resolve this issue, and will also help validate our conclusion that the
side-effects are relatively manageable. In addition, interviews with the researchers might help determine whether the benefits observed of slowing or eliminating cancer outweigh the negative side-effects, especially in view of the poor prognosis of a patient with recurring cancer. The researchers who design ADCs might also suggest directions for future research.

Section-3 References


https://www.accessdata.fda.gov/drugsatfda_docs/nda/2000/21174_Mylotorg.cfm


Kadcyla Prescribing Information (2013)  


Currently, three different antibody-drug conjugates (ADCs) have been approved by the U.S. Food and Drug Administration (FDA) for treating various forms of cancer. One of the ADC drugs was subsequently withdrawn from the market. This section of the Literature Review focuses on the approval process for ADC drugs, and attempts to identify remaining hold-up steps for other ADC drugs.

**Summary of the FDA Drug Approval Process**

The purpose of FDA approval is to help protect consumers from drugs that harm the consumer or are ineffective. Drug development and approval is a complex process involving several steps (Lipsky and Sharp, 2001); these steps are summarized in Table-I:

**Table-I: Summary of the FDA Approval Process**
Adapted from: http://www.phrma.org/charts/approval.html

<table>
<thead>
<tr>
<th>Phase</th>
<th>Preclinical</th>
<th>Phase-I</th>
<th>Phase-II</th>
<th>Phase-III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects:</td>
<td>Lab and animal studies</td>
<td>20-100 healthy volunteers</td>
<td>100-300 patient volunteers</td>
<td>1,000-3,000 patient volunteers</td>
</tr>
<tr>
<td>Purpose:</td>
<td>Assess safety and preliminary activity</td>
<td>Determine safety and dosage</td>
<td>Evaluate effectiveness and side-effects</td>
<td>Verify effectiveness and monitor long-term side-effects</td>
</tr>
<tr>
<td>Time Course: Prior to the FDA Modernization Act of 1997</td>
<td>Year-1 and -2</td>
<td>Year-3</td>
<td>Year-4 and -5</td>
<td>Year-6, -7, -8</td>
</tr>
<tr>
<td>% Drugs Passed:</td>
<td>100%</td>
<td>70% of INDs</td>
<td>33% of INDs</td>
<td>27% of INDs</td>
</tr>
</tbody>
</table>

The first phase of the FDA approval process is **pre-clinical testing**. The FDA requires researchers to perform animal tests *before* humans are exposed to a new drug. Prior to the modernization act (discussed below), this stage typically lasted 1-2 years, and, for purposes of our ADC discussion, includes testing *in vitro* against cultured cancer cell lines, testing in mice engrafted with human cancer cells (xenograft mice), and (less frequently) testing in monkeys. The main objectives of this stage of the process are to obtain animal data on drug safety and to
obtain preliminary data on drug activity. The tests must show that the drug is not toxic at the doses most likely to be effective.

If the pre-clinical data look promising, the drug manufacturer files an **Investigational New Drug (IND) Application** with the FDA. This application includes drug chemical composition and manufacturing data, animal test results (including safety), the rationale for testing a new compound in humans, strategies for protecting human volunteers, and a plan for clinical testing (Stave and Joines, 1997).

If the FDA approves the IND, the process heads into **Phase-I**. This stage focuses on the safety and pharmacology of the drug in humans (Lipsky and Sharp, 2001). Typically, various doses of a drug are given to a small group of 20-100 healthy volunteers who are then closely supervised for adverse effects. In cases of severe or life-threatening illnesses, volunteers with the disease may be used in place of healthy volunteers. The Phase-I study begins with a small dose, which is then gradually increased until serious adverse effects are seen. On average, about 70% of Phase-I drugs are found to be safe enough to enter Phase-II.

**Phase-II** studies are designed to obtain data on drug effectiveness. To avoid unnecessarily exposing a human volunteer to a potentially harmful drug, Phase-II experiments use the least number of patient volunteers to gain sufficient statistical power to determine efficacy. On average, Phase-II trials use 100-300 patients suffering from the disease the drug is designed to treat. Variables tested in this phase can include optimum drug dose, best method of delivery (IV, oral, etc.), and dosing interval, all assayed while focusing on safety (Walters, 1992; Heilman, 1995). Only 33% of IND drugs make it past this stage of the process, either because they turn out to be ineffective, or they have serious safety problems that were not apparent in Phase-I.

During **Phase-III**, researchers attempt to confirm their previous Phase-II findings using a larger group of patients. This stage can last from 2 to 10 years, and can involve thousands of patients at multiple sites. Phase-III focuses more precisely on defining optimum dosage, while continuing to focus on safety. In fact, throughout the entire clinical process, safety comes first, so the FDA and investigators are constantly in communication with each other (Lipksy and Sharp, 2001). However, when analyzing large patient populations, often the earlier encouraging Phase-I and II data fail to replicate, and only 27% of IND drugs make it past Phase-III.

If the Phase-III data look promising, the drug manufacturer files a **New Drug Application (NDA)** with the FDA. An NDA contains all the preclinical and clinical information obtained during the testing phase, including drug chemical composition, manufacturing procedures, toxicity, human pharmacokinetics (half-life and bio-distribution), clinical trial results (within the U.S. and elsewhere), and proposed labeling (Lipksy and Sharp, 2001). The FDA either approves the NDA, rejects the NDA, or requests additional data before making a decision. If the drug is not approved, the applicant is given the reasons why, including information that could be provided by the manufacturer to allow acceptance. Sometimes the FDA makes a tentative approval recommendation, requesting that a minor deficiency or labeling issue be corrected before formal approval.

Following acceptance (approval) of the NDA, the drug can be marketed. Sometimes, the FDA requests a **Post-Marketing Study (Phase-IV)** to provide more data if something remains
unclear or if new problems are identified upon further use of the drug. A Phase-IV might also be requested to study the drug in a specific population of individuals, such as high-risk individuals, pediatric patients, or elderly patients, or to follow the long-term use of the drug (Leonard, 1994). As a drug gains wider usage, a critical element of the approval is the “Medwatch requirement” stating that physicians must report any observed adverse complications at quarterly intervals for the first 3 years after a drug’s approval.

The overall FDA approval process prior to the enactment of the FDA Modernization Act of 1997 (see below) took about 8-12 years (Heilman, 1995), but the review time has now been shortened to about 12.6 months for normal drugs, and 6 months for an accelerated review (see below) (Drugs.com, 2013). A 1997 overview of the pharmaceutical industry performed by scientists at Glaxo Wellcome Inc. (Research Triangle Park, North Carolina) estimated the cost of developing a new drug to average $359 million (Stave and Joines, 1997).

### Expediting the Drug Approval Process

All three of the current FDA-approved ADC drugs (Mylotarg, Adcetris, and Kadcyla) (discussed below) received FDA approval under an accelerated review process. Accelerated reviews are sometimes granted when no other alternative drugs are available to treat a particular disease, or when the clinical trial data are very strong (Drugs.com, 2013). The Prescription Drug User Fee Act of 1992 (PDUFA) was designed to help shorten the FDA review time by allowing the FDA to collect user fees from IND filers to help enhance the review process (Walters, 1992). The act requires the FDA to review a “standard drug application” (if the drug is similar to other drugs already on the market) within 12 months, and requires review of a “priority drug application” (if the drug provides important new advances for treating a disease not available with other drugs) within 6 months. As with other reviews, the FDA often interacts with the drug manufacturer to obtain more information.

In addition, the Food and Drug Administration Modernization Act of 1997 (FDAMA) extended the use of the user fees, and streamlined the drug approval process (FDAMA, 1997). The act increases patient access to experimental drugs, and allows drug manufacturers to provide peer reviewed papers on diseases not included in the original IND application to expand the drug uses to other diseases. Proponents of the accelerated review process argue it works fairly well; prior to the act, the average review time was 8-10 years, and in 1999 after passage of the act, the average review time lowered to 12.6 months. As part of an accelerated review, the FDA requires the Phase-IV post-market study to confirm the accelerated data.

Critics of the updated FDA approval process argue that allowing drugs to be approved based on a single clinical trial, expanding the use of special accelerated reviews, and allowing the use of alternative data end points, have allowed dangerous drugs to be approved (Lurie and Sasich, 1999). But others argue there is no hard evidence of increased drug risks following the changes (Friedman et al., 1999).
Gemtuzumab Ozogamicin (Mylotarg)

The first ADC drug approved by the FDA was Mylotarg® (Gemtuzumab ozogamicin). Mylotarg is a humanized antibody against protein CD33 (frequently over-expressed on the surface of acute myeloid leukemia cells), linked to a cytotoxic drug of the calicheamicin class. Pfizer acquired Mylotarg when it bought Wyeth in October 2009 (Richwine, 2010).

Based on the promising initial data observed in preliminary clinical trials, Mylotarg was FDA-approved on May 17, 2000 under an accelerated FDA-approval process (FDA, 2000). It was approved for treating patients over the age of 60 with relapsed acute myeloid leukemia (AML), or for other AML patients not considered a candidate for standard chemotherapy (Bross et al., 2001). The approved dose was 9 mg/m² intravenous over 4 hours, repeated in 2 weeks.

As with all anti-cancer drugs, ADCs have side-effects. These adverse events are weighed against the potential benefit of the drug. The most frequent side-effects initially observed with Mylotarg were relatively mild, including shivering, fever, nausea, and vomiting. But within a year of Mylotarg’s initial approval, more serious adverse events were observed, including adult respiratory distress syndrome (ARDS), hepatotoxicity, anaphylaxis, veno-occlusive disease (VOD), and death (Nelson, 2010). The appearance of these more serious events resulted in the FDA requiring a “black box warning label” on Mylotarg’s package (Giles et al., 2001; Wadleigh et al., 2003).

In June 2010, Mylotarg was voluntarily withdrawn from the market when additional clinical trial data and post-marketing experience showed the drug increased patient death and was no better than conventional therapies (Nelson, 2010; Richwine, 2010). Mylotarg was the first drug withdrawn after being approved under the FDA’s accelerated review process (Richwine, 2010). The drug did not appear to extend survival for patients with previously untreated AML, and the fatality rate was 5.7% for Mylotarg patients compared to 1.4% for patients without the drug (Richwine, 2010).

In September 2017, almost 7 years after withdrawing it from the market, Pfizer resubmitted their application for FDA review as a Biologics License Application (BLA) (Stanton, 2017). The resubmission is based on favorable data obtained from a recent randomized Phase-III study (ALFA-0701) in 280 adult patients with newly diagnosed AML, and a meta-analysis of over 3,000 patients from some of the earlier trials. Pfizer expects an FDA decision on this re-submission by September, 2017.

Brentuximab Vedotin (Adcetris, SGN-035)

The second ADC drug approved by the FDA is Adcetris®. In North America Adcetris is marketed by Seattle Genetics Inc., and in the rest of the world by Takeda Oncology. It consists of a mouse-human chimeric monoclonal antibody against CD30 (Brentuximab or cAC10) linked via a cathepsin-cleavable linker to three to five units of the cytotoxic drug monomethyl auristatin-E (MMAE) (Francisco et al., 2003). MMAE very strongly binds tubulin and prevents it from polymerizing, leading to cell cycle arrest. The antibody portion of the ADC is produced by mammalian Chinese Hamster Ovary (CHO) cells, while the linker and MMAE are produced by
Adcetris is administered intravenous (IV), and its half-life is about 4-6 days as it is slowly cleared by the liver. Adcetris is designed to treat patients with Hodgkin Lymphoma (HL) and patients with systemic anaplastic large-cell lymphoma (ALCL). Protein CD30 often occurs on the surface of cells of these tumor types, but only rarely on normal cells (Küppers and Hansmann, 2005).

The clinical trials for Adcetris were discussed earlier in the Literature Review, but here suffice it to say that the drug has undergone numerous trials, showing high efficacy and relatively manageable side-effects. For example, in a 2010 clinical trial of HL patients (Seattle Genetics Press Release, 2010), 34% of the patients showed complete remission, 40% showed partial remission, and 94% showed tumor reductions. With respect to safety, Adcetris has been studied both alone and in combination with other chemotherapy agents. In two phase-II trials, the most common adverse reactions were chemotherapy-induced peripheral neuropathy (irreversible tingling numbness and hyper-sensitivity to cold), neutropenia (drop in neutrophil count), fatigue, nausea, anemia, upper respiratory tract infection, diarrhea, fever, rash, thrombocytopenia (decreased platelet count), cough, and vomiting (Vaklavas and Forero-Torres, 2012).

Seattle Genetics submitted an FDA application on February 28, 2011, to treat HL and ALCL patients (Fierce Biotech, 2011), and on August 19, 2011, the drug was granted an accelerated FDA approval (Genetic Engineering and Biotechnology News, 2011). However, on January 13, 2012, the FDA announced that Adcetris had been linked to two cases of progressive multifocal leukoencephalopathy (defective formation of myelin), so the FDA now requires the manufacturer to add a “black box warning” to the drug packaging warning of this risk.

Recently, Seattle Genetics and Bristol-Myers Squib partnered to test a drug combination therapy of Bristol-Myers’ Nivolumab (Opdivo®) (a drug that blocks checkpoint inhibitor PD-1 to stimulate the immune system) and Seattle Genetics’ ADC Adcetris® in patients with relapsed or refractory classical Hodgkin lymphoma (HL). The initial results of a Phase-I/II trial were reported at the 58th American Society of Hematology (ASH) Annual Meeting in San Diego, December 3-6, 2016 (ADC Review, 2017). The drug combination showed a 62% complete response rate with an acceptable safety profile. According to Alex Herrera, M.D., lead trial investigator and assistant professor at the City of Hope Medical Center (Duarte, CA), “The [combination approach] is a promising investigational approach as it combines a targeted therapy with a therapy designed to activate the immune system, and the combination may have synergistic activity. The preliminary results are compelling and support further exploration of this novel regimen, free of traditional chemotherapy” (ADC Review, 2017). On June 2, 2017, the two companies provided an update on their collaboration at the 53rd annual meeting of the American Society of Clinical Oncology, stating they plan to initiate a Phase-III trial of their drug combination beginning mid-2017 (ADC Review, 2017).

Adcetris has also been approved in Europe. In Europe, drugs are approved by the European Medicines Agency (EMA). In 2009, the drug initially received approval for use as an “orphan product” (a drug that is used to treat a disease that affects no more than 5 people in 10,000) (Hofland, 2011). But with favorable clinical data, in 2016 it was given EMA marking authorization. The basis of the favorable review by the EMA was discussed in a 2016 European Public Assessment Report (EPAR) for Adcetris (European Medicines Agency, 2016). The report stated they found Adcetris beneficial for HL and ALCL patients whose cancer had come back or
had not responded to previous therapy, but they also noted a relatively limited amount of clinical data and a lack of true controls (untreated patients) (although this is normal in cancer trials). The EMA strongly factored in the drug’s relatively manageable adverse effects, the patient’s poor expected outcomes during relapse, and the lack of other suitable drug treatments for relapsed patients. So, the EMA concluded that the benefits are greater than the risks, and Adcetris was given marketing authorization, although they await additional data on the long-term effects of the drug. The drug will be reviewed annually by the EMA in view of any new information (European Medicines Agency, 2016).

**Trastuzumab Emtansine (Kadcyla, T-DM1)**

The third ADC drug approved by the FDA is Kadcyla® (Trastuzumab emtansine). The antibody was developed by Genentech (a subsidiary of Roche), the linker and toxin were developed by ImmunoGen (Waltham, MA), and the complete ADC is manufactured by Lonza (Pollack, 2013). Kadcyla is ImmunoGen’s first FDA-approved product, although they have been working on ADC drugs for over three decades. Kadcyla is a humanized mouse antibody against surface protein Her2 (Trastuzumab or Herceptin) linked to cytotoxic drug emtansine (DM-1) that binds microtubules preventing their polymerization (Niculescu-Duvaz, 2010; Lopus, 2011; LoRusso et al., 2011).

Kadcyla underwent an accelerated 6-month FDA priority review, reserved for therapies for diseases with no alternative treatments, or that provide significant improvements over other marketed products (Drug.com, 2013). In 2010, Genentech initially applied for accelerated FDA review on the basis of the results of a single Phase-II study, but on August 26th, 2010, the FDA refused to review it in accelerated mode because all available treatment choices had not yet been tested in their patients (News-Medical.net, 2010). So, Genentech continued their studies with an amended Phase-III trial after treating the patients with other treatment options. Kadcyla was approved on February 22, 2013, based on the positive clinical trial data of women with advanced Her2-positive breast cancer who were already resistant to antibody treatment alone (Verma et al., 2012). In those studies, the data showed that Kadcyla improved the median patient survival by 5.8 months (30.9 months versus 25.1 months) compared to standard chemotherapy (National Cancer Institute, 2013; Pollack, 2013; Drugs.com, 2013).

Kadcyla comes with its own set of side-effects which must be weighed against the potential benefit of blocking the breast cancer growth. During clinical trials, the most common adverse effects seen were fatigue, nausea, muscle pain, thrombocytopenia (low platelet counts), headache, and constipation (Kadcyla Prescribing Information, 2013). Less common, but more severe, adverse effects included liver damage, liver failure, nodular regenerative hyperplasia, heart damage, lung disease, and peripheral neuropathy (Amiri-Kordestani et al., 2014). However, the adverse effects were tolerated better than those with standard chemotherapies. In the U.S., Kadcyla packaging carries a black box warning for liver toxicity, heart damage, and fetal harm if given to pregnant women.

The high cost of ADC drugs is an important issue for consumers, and is perhaps worth pursuing in IQP interviews. In the U.K., the National Institute for Health and Care Excellence (NICE) and the National Health Service England (NHS) determine whether a particular drug remains on the Cancer Drugs Fund (CDF) list of medicines paid by the government. The normal
CDF cut-off for an end-of-life drug like Kadcyla is about £50,000 per year (The Guardian, 2015). This is equal to about £30,000 per quality-adjusted life years (NICE, 2014). Roche’s initial price was high, a reported £90,000 per patient per year (Pollack, 2013), nearly double the CDF cut-off, placing the drug in jeopardy for being removed from the list. In 2014, a draft guidance document produced by NICE concluded that Kadcyla’s price was too high for the CDF list (NICE, 2014). But in 2015, Roche compromised and lowered their price to £5,900 per month; for a typical 9.6 month treatment duration, this new price would be about £56,640, bringing the drug close to the CDF cut-off. So, the NICE and NHS England committees for now have left Kadcyla on the list (The Guardian, 2015). Hopefully, other pharmaceutical companies will follow Roche’s lead of cutting drug prices to be more affordable.

**Other ADC Drugs**

Not all ADC drugs are being actively developed by biotech companies. In some cases, the clinical trial data that initially looked promising did not repeat in larger trials, so the company stops developing the drug. For example, in 2015 Sanofi began trimming its oncology division, abandoning the ADC drug SAR-3419 (Garde, 2015). SAR-3419 targets CD19 on the surface of diffuse large B-cell lymphomas and other blood cancers. In 2014, the SAR-3419 Phase-II data looked promising, but after a series of setbacks, Sanofi decided to cut about 100 researchers from their oncology division, merging the remaining researchers within their global R&D divisions (Garde, 2015).

Another example of an ADC drug with mixed clinical trial data is the CD22-targeting Inotuzumab ozogamicin, used to treat non-Hodgkin’s lymphoma (NHL). On May 20, 2013, Pfizer announced they were discontinuing their Phase-III study of this drug because it did not meet the main objective of improving overall patient survival (Pfizer Press Release, 2013). The company stated that there are over 70 different types of lymphoma, and they are trying to determine which type of lymphoma is best treated by Inotuzumab. They did not mention whether they monitored their phase-III patients for over-expression of the target CD22 prior to treatment, although doing so appears to be a best practice.

**Section-4 Conclusions**

This part of the Literature Review summarized the FDA approval process for ADC drugs, and discussed the three ADCs with FDA approval. All three of the FDA-approved ADC drugs were reviewed under an accelerated program, either due to strong and clear clinical data better than other available treatments, or because the drugs provided a treatment for a disease with no other options available at the time of the review. Based on the information found in this section of the Literature Review, we believe that the three current FDA-approved ADC drugs have clearly documented serious side-effects, but they mostly appear to be manageable, transient, and less prevalent than those seen with current chemotherapy treatments. We also believe that the patient’s extremely poor prognosis during relapse of the cancer should strongly factor into decision, and far outweighs the relatively minor adverse effects. We predict that drug efficacy likely is the most important variable for FDA approval for ADCs, as a lack of it resulted in the withdrawal of Mylotarg. We plan to pursue this point of improving ADC efficacy in IQP interviews. We also showed that a current ADC trend appears to be the testing of drug
combinations, as demonstrated by combining the ADC Adcetris with the immune checkpoint inhibitor Opdivo®, and will get further information on this promising combination approach in interviews.

Section-4 References


Fierce Biotech (2011) Seattle Genetics Submits BLA to FDA for Brentuximab Vedotin in Relapsed or Refractory Hodgkin Lymphoma and Systemic ALCL. Feb 28, 2011 12:05pm.


Section-5: ADC Recent Advances and Future Directions
Sean St. Pierre and Alex Kolodziejczak

As discussed in the previous sections of the Literature Review, ADC drugs are an exciting advance in cancer therapeutics. ADCs link a potent cytotoxic cancer-killing drug (the cargo) to an antibody that targets the cargo to a specific antigen which is over-expressed on the surface of the tumor. But ADC drugs are not perfect. In all clinical trials performed to date, ADCs have caused side-effects in the patient, and in some cases the ADC is ineffective against the cancer. In addition, some scientists have indicated that the future approval of numerous ADCs under development might depend on improving their clinical performances to produce stronger effects that warrants each drug’s use (Panowski et al., 2014). Thus, there is room for continued improvement of ADC drugs. The purpose of this section of the Literature Review is to summarize some of the problems seen with first generation ADC drugs, discuss some design advances found in the new second-generation ADCs, and discuss future directions for further ADC improvements.

Comparison of First and Second Generation ADC Drugs

First generation ADC drugs (1º-ADCs) as originally developed came with a variety of problems, including lack of efficacy and adverse side-effects, so much research has gone into creating second-generation ADCs (2º-ADCs) with superior properties (Thomas et al., 2016). Table-I below compares some of the properties of 1º-ADCs and 2º-ADCs. 1º-ADCs usually contained mouse monoclonal antibodies against the target protein. But injecting a mouse antibody into human patients in some cases stimulated an immune response against the drug, lowering drug effectiveness. 2º-ADCs contain either mouse-human chimera antibodies or humanized mouse antibodies that produce less of a response in patients. Using fully human antibodies, produced by mammalian cell culture, should produce even less of an immune response in the patient. 1º-ADCs also have short half-lives in the blood, releasing their toxic payload too soon into the circulation instead of in the tumor cell. In some cases the linkers used released the payload too early, before reaching the tumor cell. 2º-ADCs use improved linker chemistries (such as the use of disulfide linkages, dipeptide linkages, and hydrazine linkages) to hold the payload tighter, releasing it only in the acidic environment of the internalization vesicle. In addition, 1º-ADCs use first generation cytotoxic payloads (such as doxorubicin, vinblastine, or methotrexate) with inhibitor concentration-50 values (IC$_{50}$) in the micromolar range, while 2º-ADCs use newly designed payloads (such as Calicheamicin, Maytansine derivatives like DM1, or Auristatins like MMAE) that are far more toxic, with IC$_{50}$’s in the nanomolar range (they have the same effectiveness at 1000-fold less concentrations).
Table-I: Comparison of First and Second Generation ADCs
Adapted from Thomas et al., 2016.

<table>
<thead>
<tr>
<th>Property</th>
<th>First Generation ADCs</th>
<th>Second Generation ADCs</th>
<th>Future ADCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody:</td>
<td>Mouse Antibody</td>
<td>Mouse/Human Chimera or Humanized Mouse Antibodies</td>
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<tr>
<td>Immunogenicity:</td>
<td>Highly Immunogenic</td>
<td>Decreased Immunogenicity</td>
<td>Non-Immunogenic</td>
</tr>
<tr>
<td>Linker:</td>
<td>Drug release prematurely into the blood, low drug release inside cancer cell.</td>
<td>Less drug release into blood and stronger release inside cell: Disulfide Linkages, Dipeptide Linkages, Hydrazone Linkages</td>
<td>Structures that are more specifically lysed in the lysosomal environment</td>
</tr>
<tr>
<td>Cytotoxic Cargo:</td>
<td>Micromolar IC₅₀: Doxorubicin, Vinblastine, Methotrexate</td>
<td>Nanomolar IC₅₀: Calicheamicin, Maytansines: DM1 Auristatins: MMAE</td>
<td>Cargos with different mechanism of actions to kill the tumor cell in a variety of ways.</td>
</tr>
</tbody>
</table>

**ADC Problems**

Several key steps must occur for ADCs to work properly (Figure-1) (Loganzo et al., 2016). These steps include: movement of the ADC through the patient’s circulation without losing its toxic cargo, binding of the ADC to the target antigen on the surface of the tumor cell, lack of targeting of the ADC to normal cells, internalization of the ADC into an endocytic vesicle, binding of the endocytic vesicle to a lysosome to acidify the compartment and release enzymes into the endosome, degradation of the linker and sometimes antibody to release the toxic cargo, movement of the cargo into the cytoplasm, and targeting of the cargo to a cellular component such as DNA or tubulin to prevent cell division. Inefficient function of any one of these steps can create an inactive drug. Thus, each of these steps presents a research opportunity to improve second-generation ADCs relative to early versions of the drugs.
Problems independent of the tumor itself include: premature loss of the cargo drug into the blood, and poor pharmacokinetics (ADME) (poor absorption, distribution, metabolism, excretion) (Kraynov et al., 2016). In other cases, the environment surrounding the tumor limits access to large molecules like ADCs. These environmental changes might include the increased formation of vascular barriers such as basement membranes or increased formation of extracellular matrix. In other cases, the tumors themselves become resistant to the ADC treatment by altering one or more of the steps needed by ADCs to kill the cell (Loganzi et al., 2016). Each of these key steps is discussed below, along with a potential solution.

1. Lowered Surface Antigen Expression: The ADC therapeutic molecule must successfully bind a tumor-specific antigen on the surface of the tumor cell. As a tumor matures in the patient, if it lowers expression of the surface antigen, it lowers binding of the drug to the cell (Loganzi et al., 2015a). For example, DNA changes that occur as a tumor evolves in a patient could result in down-regulation of a surface protein. This has already been shown to be the case for ADC drugs. For example, down-regulation of Her2 surface protein was shown to occur in cell models of ADC-resistant tumors (Loganzi et al., 2015b). And for the CD30-targeting ADC Brentuximab vedotin (BV), long-term treatment of CD30-positive cancer cells with an escalating dose of BV for
several months resulted in a 655-fold increased resistance to BV, accompanied by a 38% reduction of CD30-positive cells and a 79% reduction of CD30 fluorescence on the cell surface (Chen et al., 2015). Potential Solution: Create ADCs that target different tumor-specific antigens on the same cancer cell, or discover new tumor antigens that satisfy this requirement.

2. Increased ADC Re-cycling to the Cell Surface without Drug Activation: The ADC drug must be internalized by receptor-mediated endocytosis (RME) into an endosome that eventually fuses with a lysosome. The fusion process acidifies the compartment and releases degradative enzymes that digest the linker and/or antibody, releasing the drug into the cytoplasm. If the drug is not released into the cytoplasm, it cannot kill the tumor cell. Or if the drug is internalized but quickly returned to the cell surface, it will not become activated. Potential Solution: Use a drug to temporarily block non-RME uptake.

3. Use of an Alternative Vesicle for Entrance: If the ADC is not internalized by a vesicle that fuses with a lysosome, as mentioned in step-2 the drug does not become activated. RME vesicles are usually coated by clathrin (clathrin-coated vesicles), but clathrin-independent uptake can occur in caveolae which do not fuse with lysosomes. As an example, in an in vitro model of Her-2-positive cancer induced to be resistant to ADC drug T-DM1, proteomic sequencing showed alterations in several proteins involved in vesicle transport and lysosome/endosome biogenesis (Loganzo et al., 2015c). In addition, when gastric carcinoma cells were made resistant to T-DM1 by cyclical exposure to the ADC long-term over several months, the cells increased their resistance to the drug by more than 100-fold and upregulated several proteins including Caveolin-1 associated with the alternative caveolae vesicles (Sung et al., 2016). Potential Solution: Use a drug to temporarily block caveolae uptake.

4. Altered Cell Death or Cell Survival Pathways: Some drugs kill the cell by activating cell death pathways (apoptosis) or by decreasing the cell’s survival pathways. If the tumor cell alters these pathways, the drug’s effectiveness can be lowered. Potential Solution: Engineer an ADC with a different drug that uses a different mechanism to kill the cell.

5. Improper ADC Catabolism: Any impairment of ADC antibody or linker degradation inside the RME vesicle will result in inactive drug. In general, ADC drugs containing non-cleavable linkers require nearly complete catabolism of the antibody in the vesicle to release drug, while enzymatically-cleavable linkers merely need to be cleaved once to release drug (Doronina et al., 2006). For example, in a T-DM1-resistant in vitro cancer model, using a T-DM1 drug prepared with a non-cleavable linker was less effective than using a drug made with a protease-cleavable linker (Loganzo et al., 2015a). Potential Solution: Alter the type of linker present in the ADC to increase its activity.

6. Improper Release of the ADC from the Vesicle: The cytotoxic cargo must bind its cellular target (usually tubulin or DNA) to kill the cell. This means that the drug must be released from the vesicle into the cytoplasm. Loss of a lysosomal transporter protein
could reduce this necessary release step. For example, Hamblett and colleagues identified SLC46A3 as a protein required for the transport of maytansine cargo from the lysosome to the cytoplasm (Hamblett et al., 2015). When this protein was mutated in the tumor, the drug lost its effectiveness. **Potential Solution:** Use a drug to stimulate lysosomal protein transport, or deliver the required SLC46A3 transport protein by gene therapy.

### 7. Increase in Drug Efflux Transporter Activity

Drug transporters such as MDR1 and MRP1 transport drugs from the cytoplasm to the cell exterior which inactivates the drug. Increasing drug transporter activity lowers the drug concentration inside the cell, lowering its effectiveness. With respect to ADCs, chronic treatment of a mouse xenograft model of non-Hodgkin Lymphoma (NHL) using a CD22-targeting ADC increased the expression of MDR1 that exports toxins outside a cell (Yu et al., 2015). **Potential Solution:** Change the ADC drug to a different cargo that is not exported by the upregulated transporter, or use a drug to lower transporter activity.

### 8. Alterations of Cargo Drug Target

The ADC cargo requires a specific cellular target to kill the cell, usually DNA or tubulin. But some tumor cells can become resistant to a specific anti-cancer drug by altering the target (Gillet and Gottesman, 2010; Holohan et al., 2013). In the case of ADCs, a drug might initially target tubulin to kill actively-dividing cells, but upon maturation the tumor acquires tubulin mutations and no longer binds the drug (Kavallaris, 2010). **Potential Solution:** Use a second ADC with a different cargo that binds a different cellular target.

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**Tarveda Therapeutics**

ADC drug design is a subject of much research in the biotech industry. This includes the design of improved second-generation ADCs. One example is Tarveda Therapeutics (Watertown, MA). Tarveda is a clinical stage biopharmaceutical company developing second-generation ADC drugs (Tarveda.com, 2017). Their main technology is **Pentarins™**, which are potent, selective, miniaturized ADCs designed to penetrate more deeply into solid tumors than normal ADCs. Data using first-generation ADCs against solid tumors indicated they were generally less effective than the ADCs against leukemia or breast tumors. According to Richard Wooster, Tarveda’s President of Research and Development, “The result of our [Pentarin platform of miniaturization] is a drug that is about 15 times smaller [than antibodies], and are likely to penetrate deeper into the tumor” (Ledford, 2016).

Tarveda is developing a pipeline of several different Pentarins to treat a wide range of cancers (Figure-2). The company’s lead Pentarin drug is **PEN-221**. This drug is designed to target the somatostatin receptor over-expressed in neuroendocrine tumors and in small-cell lung cancers. The drug is currently in Phase-I/II testing. Tarveda’s next lead candidate is **PEN-866**, a Pentarin designed to target Heat Shock Protein-90 (hsp-90) located within a variety of solid tumors, including colorectal cancer, small-cell lung cancer, sarcomas, and pancreatic cancers. In
earlier pre-clinical testing, PEN-866 induced complete tumor regressions in mouse xenograft models of small-cell lung cancer, pancreatic cancer, ovarian cancer, and sarcoma (Tarveda.com, 2017). PEN-866 is currently in IND studies and is scheduled to enter clinical trials in 2017. Other Pentarins are also being developed by the company that carry a variety of extremely potent payloads (discovery stage), and Pentarins that target a variety of other tumor-specific proteins (discovery stage).

<table>
<thead>
<tr>
<th>Programs</th>
<th>Discovery</th>
<th>IND Enabling Studies</th>
<th>Phase 1/2a Clinical Development</th>
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<tbody>
<tr>
<td>PEN-221 (Somatostatin)</td>
<td></td>
<td></td>
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<tr>
<td>Neuroneuroendocrine &amp; Small Cell Lung Cancers</td>
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<tr>
<td>PEN-866 (HSP90)</td>
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<tr>
<td>Small Cell Lung &amp; Pancreatic Cancers and Sarcomas</td>
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<td>HSP90 Pentarins</td>
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<td>With Potent Payloads</td>
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<td>Novel Pentarins</td>
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<tr>
<td>To Other Cell Surface Targets</td>
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Figure-2: Pipeline of “ADC” Pentarin Drugs Under Development at Tarveda Therapeutics. Pentarin drugs are similar to ADCs except they are miniaturized. Shown are the four classes of “ADC” Pentarin drugs being developed at Tarveda, including: PEN-221 (directed against somatostatin-presenting cancers including neuroendocrine tumors and small cell lung cancers) (Phase-I/II); PEN-866 (directed against hsp-90 on small cell lung cancer and pancreatic cancer) (IND studies), other HSP-90 Pentarins with a variety of potent cargos (discovery stage), and other Pentarin drugs against a variety of surface proteins (discovery phase) (Tarveda.com, 2017).

According to the company’s website, the Pentarin platform allows drugs to be designed for optimum efficacy while maintaining their miniature size to penetrate deeper into the tumors. Altering the synthetic components of the Pentarin allows more precise control of the pharmacokinetics, bio-distribution, tumor accumulation, tumor penetration, and cancer cell uptake of Pentarins, increasing cancer cell death. One of their patented procedures uses nanoparticles enclosing the Pentarins to penetrate the perivascular region of the tumor using the leaky tumor vasculature, then they release the Pentarin to bind the tumor cells.

Mersana Therapeutics

Another biotech company investigating second-generation ADC drugs is Mersana Therapeutics (Cambridge, MA). This company uses a patented Fleximer technology to design biodegradable ADC drugs with improved drug solubility, pharmacokinetics, reduced immunogenicity, and optimized drug loading (Mersana.com, 2017). The Fleximer platform allows an ADC to be custom designed with specific properties to overcome current ADC limitations. The platform attempts to increase control over when, where, and how the ADCs are
released. If the drug can deliver higher quantities of payload directly to a tumor, it could more effectively treat the tumor and reduce unwanted side-effects. The ADC can be customized to vary the type and quantity of payload, or to alter the target antigen. According to Timothy Lowinger, Chief Scientific Officer, “This [technology] allows the company to attach 15 molecules of the drug to each polymer, rather than the usual 3-4 (Ledford, 2016). Their most developed drug is XMT-1522 that targets Her-2 in some types of breast cancers. This drug is currently in Phase-I clinical trials (Mersana.com, 2017).

Switching Antibodies

As stated above, the first-generation ADCs used murine antibodies directed against human antigen targets. These early ADCs were evaluated in human clinical trials, but had limited success due to the ADC immunogenicity, a lack of potency, or insufficient selectivity for tumor versus normal tissue (Teicher et al., 2011). Fully human antibodies can now be produced in mice. Immuno-deficient mice (lacking their own immune system) are injected with human bone marrow cells containing hematopoietic stem cells (HSCs). The human HSCs colonize the bone marrow of the mouse, and then differentiate into a functional immune system that includes the production of human antibodies from B-cells. B-cells producing human antibodies can be isolated from these mice and fused with myeloma cells to make a cell line that is easy to grow in culture. Procedures also exist for producing mouse-human chimera antibodies. Both of these types of antibodies are less antigenic in patients than mouse antibodies and are 100 to 1000-fold more potent than first-generation ADCs. An example of an ADC containing a mouse-human chimera antibody is Brentuximab vedotin (Adcetris), one of only two currently FDA-approved ADCs. It consists of a mouse-human chimeric monoclonal antibody directed against CD30 (Deng et al., 2013). An example of an ADC containing a fully human antibody is Glembatumumab vedotin (CDX-011). It consists of a fully human monoclonal antibody against gpNMB conjugated to the potent microtubule inhibitor monomethyl auristatin-E (MMAE) (Naumovski and Junutula, 2010; Keir and Vahdat, 2012).

Identifying New Target Antigens

A very important future direction for ADC research is the identification of new target antigens. As mentioned previously, some of the off-target effects of ADC drugs occur when the targeted tumor antigen is also expressed to some extent in normal tissues. An example of this problem was the initial use of ADCs against KS1 (for non-small cell lung cancer) and against BR96 (for breast cancer). Both of those antigens are also found in normal tissues, and their presence resulted in ADC toxic side-effects (Elias et al., 1990; Tolcher et al., 1999). In other cases, only a portion of a patient’s tumor expresses the target antigen, so those tumor cells are not eliminated by the ADC drug. In other cases, a tumor might initially express an antigen, but then down-regulate its expression as the tumor matures or metastasizes.
A recent example of the identification of a new target antigen **Her-3**. A team of scientists based primarily in the Department of Radiation Oncology at Massachusetts General Hospital (MGH) and Harvard Medical School (HMS) (Boston, MA) noticed in mouse models of breast cancer that Her-2-positive breast cancer cells (with an over-activated PI3K-pathway), were blocked by inhibition of PI3K, while breast cancer cells that had metastasized to the brain no longer responded to the PI3K inhibitors (even though the inhibitors penetrated those cells) (Kodack et al., 2017). Upon further analysis, the authors determined that Her-3 had become over-expressed in the metastasized brain cancer cells. Blocking Her-3 function produced significant tumor growth delay and improved mouse survival (Kodack et al., 2017). Thus, Her-3 might be an effective antigen for targeting Her-2-resistant tumor cells as they start to metastasize.

Another recent example of the identification of a new target antigen is **CD32a**. A team of scientists from the Institut de Génétique Humaine, Laboratoire de Virologie Moléculaire, Université de Montpellier (Montpellier, France) performed a gene expression profile on CD4-positive human T-cells infected *in vitro* with HIV-1 (Descours et al., 2017). HIV initially establishes a productive infection where the infected cells produce and release active virus. But as the infection progresses further, the retrovirus establishes a quiescent or latent infection. Latently infected cells are not killed by the virus, nor are they eliminated by anti-retroviral drugs, so the presence of these latently infected cells is one of the major remaining hurdles for developing an HIV cure. The Université de Montpellier team identified 103 genes specifically upregulated during latent HIV infection, including 16 transmembrane proteins. Of the 16 transmembrane proteins, CD32a was upregulated the strongest, and it was not present in normal cells or in lytically infected cells. Although only 0.012% of the CD4-positive cells expressed CD34a, these cells represent a major source of HIV during rebounds if the antiretroviral drugs are withdrawn (Descours et al., 2017). So, targeting CD34a with an ADC drug might help eliminate the HIV reservoir from the body.

**Dual-Targeting Approaches**

Another ADC procedure that might be worth pursuing in the future is the use of dual-targeting therapies. This approach uses two ADCs against the same receptor but that work by two different mechanisms, or alternatively it uses two different ADCs that target different receptors on the same tumor. An example of the first approach is the use of the ADC Kadcyla to target **Her-2**-positive breast cancer cells combined with Pertuzumab (Perjeta) that binds **Her-2** preventing its dimerization (Phillips et al., 2014). Preventing Her-2 dimerization blocks the activation of signal transduction pathways that lead to cell growth. The combined dual-ADC treatment inhibited cancer cell proliferation *in vitro*, enhanced anti-tumor activity in mouse xenograft models, and showed only mild grade-1 and -2 adverse effects in Phase-I/II clinical trials with preliminary evidence of efficacy (Phillips et al., 2014).

An example of the second dual-ADC approach that target different receptors has not yet been done, but in theory could be done with Kadcyla (targeting **Her-2** breast cancer cells)
combined with an ADC against **Her-3** (targeting metastasized cells) (not developed yet). The study mentioned previously in the Department of Radiation Oncology at Massachusetts General Hospital (MGH) and Harvard Medical School (HMS) (Boston, MA) showed that Her-2 breast cancer cells increase their expression of Her-3 when they metastasize to the brain (**Kodack et al., 2017**). So, perhaps using two different ADCs might prevent the metastasis. This second approach might also work in cancer patients where the tumor cells do not all express the target antigen, such as breast cancer patients where only a portion of the cells are Her-2 positive, or patients with large-cell lymphoma where only a portion are CD30-positive. But this dual-ADC approach would necessitate identifying new tumor antigens for various cancers to serve as alternative ADC targets.

**Altering the Payload to Overcome Resistance**

If a cancer patient becomes resistant to a cytotoxic agent delivered by one type of ADC drug, perhaps using an ADC that targets the same antigen but carries a different payload might help overcome the resistance. This altered payload approach has already been successful in the case in mouse models of non-Hodgkin lymphoma (NHL) that had become resistant to a CD22-targeting ADC. Altering the payload from MMAE to nemorubicin (which targets DNA), while still targeting CD22, overcame the resistance even when delivering only half the payload per cell (**Yu et al., 2015**).

**Payload Advancements**

The first-generation ADCs included cytotoxic payloads such as doxorubicin, vinblastine, or methotrexate. These drugs inhibit cell growth with inhibitor concentration-50 values (IC$_{50}$) in the micromolar range. Second-generation ADCs use newly designed payloads such as Calicheamicin, Maytansine derivatives (DM1), or Auristatins (MMAE). These new drugs target either DNA or tubulin, and are far more toxic than previous drugs with IC$_{50}$’s in the nanomolar or picomolar range (same effectiveness at 1000-fold to 1,000,000-fold less concentrations). These payloads have proven very effective against a wide range of human tumor cells.

Research continues to identify new types of payloads. One new type of payload is the Pyrrolobenzodiazepine class (PBDs). PBDs are a class of naturally occurring anti-tumor drugs that bind DNA in a sequence specific manner in its minor groove (**Flygare et al., 2012**). This type of drug kills the cell by crosslinking opposing strands of the target cell’s DNA. PBDs have been shown to have picomolar activity against tumor cell lines. Another promising ADC payload is the RNA polymerase II inhibitor $\alpha$-Amanitin (**Flygare et al., 2012**). This toxin is a cyclic peptide of eight amino acids, and is the most deadly of all the amatoxins found in mushrooms, such as the death cap or the destroying angel (**Michelot and Labia, 1988**). $\alpha$-Amanitin kills by inhibiting DNA transcription (the production of RNA from a DNA template), which is required by all cells. These payloads are in the early stages of testing, and have shown significant signs of success against a wide range of tumor cells.
Vascular Targeting ADCs

So far, most of the ADC research has focused on targeting tumor-associated antigens expressed on the surface of cancer cells (Casi et al., 2012). This type of ADC utilizes antibodies linked with a toxic payload which becomes internalized in the cell through receptor-mediated endocytosis. As discussed earlier in this section, the success of this type of ADC requires several steps to occur, including the movement through barriers associated with large solid tumors, binding to the cell surface, internalization into an endosome, fusion with a lysosome, and release of the drug into the cytoplasm. But if any of these steps malfunctions, the cytotoxic payload will never reach the intended target.

An alternative approach has been developed that targets markers expressed in the newly forming tumor neo-vasculature. This strategy uses non-internalizing antibodies (Casi et al., 2012). Since the formation of new blood vessels (angiogenesis) is a common feature of all malignancies, and new vessel formation rarely occurs in normal adults, these markers might be excellent targets for ADC therapy (Casi et al., 2012). An advantage of neo-vascular targeting is that one ADC drug could be used to treat a variety of tumors, as most tumors need new vasculature. Research on angiogenesis makers focuses on the sub-endothelial extracellular matrix (ECM). ECM markers are typically more stable than surface tumor markers, and this would allow the antibodies to have a residence time of days to weeks, which would increase the time the toxic drug could act. Work has already begun using human mAbs specific to the extra-domain B (EDB) of fibronectin, which has been identified as a marker of angiogenesis (Palumbo et al., 2011). A team from the Swiss Federal Institute of Technology (Zurich, Switzerland) designed ADC SIP(L19)-PS, which combines PS (a photo-sensitizer that absorbs in the red spectrum), and L19 (a human monoclonal antibody against fibronectin-EDB). They tested their ADC in two mouse models of cancer (F9 and A431), and showed that activating the ADC by radiation causes disruption of the tumor vasculature, and produces complete and long-lasting cancer eradication (Palumbo et al., 2011).

Linker Optimization

An important ADC component that was slightly overlooked in first generation ADC drugs was linker chemistry. Linker stability is necessary to allow the conjugate to circulate in the bloodstream for an extended period of time without prematurely releasing its cytotoxic agent. Linker stability has a major influence on ADC properties such as pharmacokinetics, overall toxicity, and therapeutic index (Perez et al., 2014).

ADC linkers currently under development fall under two main classes: cleavable linkers and non-cleavable linkers. There are three different release mechanisms for cleavable linkers: acid-sensitive linkers, lysosomal protease-sensitive linkers, and glutathione-sensitive linkers. Acid-sensitive linkers contain an acid labile group, such as a hydrazone, that easily undergoes hydrolysis in the low pH of the lysosomal compartment, releasing the drug into the tumor. This
type of linker was employed in first-generation ADCs such as the first FDA approved ADC, Mylotarg (Casi et al., 2012). Mylotarg’s clinical trials showed adverse effects due to linker instability, and it was withdrawn from the market. Research is currently being done to try to improve linkers that use this acid-labile drug release mechanism. Lysosomal protease-sensitive linkers initiate proteolysis after fusion with a lysosome. Lysosomal proteases, such as Cathepsin B, are used to recognize and cleave a specific dipeptide bond, releasing the drug from the conjugate. This method was used successfully in the FDA-approved Adcetris, which is currently still on the market. The third type of cleavable linker, glutathione-sensitive linkers, “exploit the high level of intracellular reduced glutathione to release free drug inside the cell” (Panowski et al., 2013). Disulfide bonds within the linker are stable in the bloodstream, but can be reduced by intracellular glutathione and release the free drug (Perez et al., 2014).

The main advantage of non-cleavable linkers is their greater stability in the circulation than cleavable linkers. Their heightened stability prevents early release the drug potentially eliminating the bystander effect. This seems like a safer option, but the chemistry becomes a little more complicated since the linker is never detached. This process relies on complete degradation of the antibody once the ADC is internalized, and the payload must do its job while being chemically modified since its chemical structure is altered by the linker extension.

Linker chemistry has become a major focus of ADC research and is forcing the evolution of the ADC field. New theories are currently being tested in preclinical trials, as well as optimization of proven technologies discussed previously. Other tumor-associated proteases, such as legumain, have been identified that release the ADC payload in non-lysosomal compartments, giving promise to finding more efficient linker release mechanisms (Perez et al., 2014). Since the linker is mainly responsible for the intracellular release of the toxic drug, linker chemistry must be precisely engineered to control toxicity. Linker selection is not only dependent on the mechanism used for delivering the drug, but is also dictated by the choice of target antigen and the desired payload.

**Site-Specific Conjugation**

Conjugation chemistry is an area of ADC research that has recently started seeing advancements. The first-generation ADC production methods produced heterogeneous mixtures of molecules (Panowski et al., 2014). The traditional conjugation of drugs to antibodies occurs at solvent-accessible reactive amino acids. Those methods used non-specific acylation of lysine residues, and alkylation of cysteine thiols (Agarwal et al., 2015). As an example of drug heterogeneity, the attachment of a drug to lysine residues results in 0-8 conjugated drug molecules per antibody, on both the heavy and light chains, at 40 different locations (20 lysine residues per chain). This results in the production of more than one million different types of ADC molecules per reaction (Wu et al., 2005; Wang et al., 2005). Each type of molecule has distinct properties, resulting in a wide range of activities that produced somewhat unpredictable results in patients (Panowski et al., 2014). A diverse product such as this will contain a variety of conjugates that all have their own set of properties that affect pharmacokinetics, toxicity, aggregation, antigen affinity, and drug release (Agarwal et al., 2015). Cysteine conjugation
produces a slightly less diverse drug mixture than lysine-based conjugation due to a more limited number of reactive sites, but it still produces a heterogeneous mixture.

The newer methods of drug attachment to antibodies use site-specific conjugations that eliminate product heterogeneity and improve conjugate stability. These new methods include: 1) engineered cysteine residues, 2) unnatural amino acids, or 3) enzymatic conjugation through glycol-transferases and transglutaminases. In the first approach, extra cysteine residues are engineered into the antibody as sites for drug attachment, allowing the antibody to keep its normal disulfide bonds intact, maintaining its structure. Cysteine residues can be engineered in a way that will make specific sites much more reactive than others, yielding a site-specific product. Using a cysteine residue engineering approach, a team of scientists at Genentech prepared homogeneous ADCs conjugated to IgGs, which they dubbed THIOMABs (Agarwal et al., 2015). They used a reduction/reoxidation approach in which they reduced engineered cysteine residues and inter-chain disulfide bonds simultaneously, and then used an oxidizing agent to reform only the inter-chain disulfide bonds, leaving open a specific reactive cysteine thiol that could be used for attaching the drug. This new method generated site-specific ADCs with 2.0 drug molecules per antibody (Casi et al., 2012). Genentech showed that the site-specific ADCs had a greatly improved therapeutic index.

The second new site-specific strategy uses unnatural amino acids inserted at a desired location in the antibody. The amino acids used to date include selenocysteine and acetylphenylalanine. Seleno-cysteine is similar to cysteine, but contains selenium instead of sulfur. Its selenolate group is more reactive than its thiolate counterpart, so the drugs tend to add onto that site first (Panowski et al., 2014). The unnatural amino acid acetylphenylalanine contains a keto group that can be specifically conjugated to a drug through an oxime ligation (Panowski et al., 2014).

The third new site-specific strategy uses enzymatic conjugations with glycotransferases or transglutaminases. Glycotransferase can attach a chemically active sugar residue to a glycosylation site on an antibody, and then the drug is conjugated to the chemical handle on the sugar residue. Alternatively, transglutaminase can be used to form a bond between an amine group on the linker or drug and an engineered glutamine residue on the antibody. Both platforms are being investigated for the production of ADCs.

**Radioimmunotherapy**

Radioimmunoconjugates are radioactive isotopes conjugated to antibodies targeting tumor cells. Radioimmunotherapy (RIT) has been under development for over 30 years with little progress, but with new advancements in the field of targeted antibody cancer therapy, there is new excitement for RIT. Similar to ADCs, the choice of antibody and cytotoxic agent are critical for RIT efficacy. “The path length of penetration of the radioactive emission should match the size of the targeted tumor” (Kraeber-Bodéré et al., 2014). Clinical trials have shown some promising results for RIT drugs targeting antigen CD-20 in non-Hodgkin B-Cell Lymphoma using Yttrium-90 as the radionuclide (drug Zevalin®; Spectrum Pharmaceuticals, Henderson, NV). Zevalin is approved for use in the U.S. Europe, Asia, and Africa (Kraeber-Bodéré et al., 2014).
Current research is directed toward development of radioimmunoconjugates targeting the Her-2 antigen expressed in breast cancer. The chosen cytotoxic radionuclide for this research is Lu-177 due to its favorable characteristics (Bhusari et al., 2017). In addition, isotopes with shorter tissue penetration, such as alpha emitters Bismuth-213 or Astatine-211, are being investigated for use with microscopic tumors. Other developments in the areas of targeted antibody design, such as site specific drug (isotope) conjugation, will eventually be applied to RIT therapies.

Immunotoxin Therapy for Cancer Treatment

Immunotoxins are very potent molecules consisting of an antibody (or antibody fragment) linked to a bacterial or plant toxin rather than a traditional chemotherapeutic drug (Hassan et al., 2015). Immunotoxins work in a manner similar to ADCs, targeting specific tumor antigens and internalization into a tumor cell. But different than ADCs, the toxin kills the cell by inhibiting protein synthesis, not by binding tubulin to block cell division or binding DNA to cause its fragmentation. Because immunotoxins carry a cargo that kills a cell by blocking translation instead of blocking cell division like ADCs, this allows immunotoxins to be used against tumors that are no longer actively dividing (Hassan et al., 2015).

Immunotoxins targeting CD-22 have produced complete remissions in refractory hairy cell leukemia and acute lymphoblastic leukemia in children (Wayne et al., 2011; Kreitman et al., 2012). But, similar to ADCs, immunotoxins have had less success with solid tumors (Hassan et al., 2015). Large molecules, such as ADCs and immunotoxins, have trouble penetrating inside solid tumors. So, some scientists have engineered smaller molecules with better penetration. For example, a team of scientists at the Center for Cancer Research, National Cancer Institute (Bethesda, MD) used protein engineering to produce a small immunotoxin consisting of the Fv variable (antigen-binding) fragment of an antibody against a cancer protein fused to a truncated form of Pseudomonas exotoxin-A (PE) termed PE38 (Pastan et al., 2006). Natural PE has three domains: Domain I binds the cell surface of most cells, Domain II enables the toxin to be cut by furin, separating the Fv from the toxin, and Domain III catalyzes the inactivation of translational elongation factor-2, inhibiting protein synthesis. The authors removed Domain-I to prevent the toxin from attaching to the surface of normal cells, and replaced it with the Fv domain of antibodies against various cancer proteins, including interleukin-6 and TGF-alpha (Kreitman et al., 1992), and against erbB2 (Batra et al., 1992).

The same team of scientists from the National Cancer Institute discussed above has also investigated the use of immunotoxins against mesothelioma by targeting the mesothelin protein. Mesothelin is highly expressed in many human malignancies including mesothelioma, and cancers of the pancreas, lung, stomach, ovary and bile duct (Ordóñez, 2003). The team created an immunotoxin, named SS1P, consisting of the Fv fragment that binds mesothelin conjugated to their truncated Pseudomonas toxin PE38. In their initial clinical trials, SS1P had limited activity because 90% of the patients developed antibodies against the immunotoxin, lowering its
effectiveness. So the team testing an immunosuppressive regimen to lower the T and B cell responses to allow longer term use of the drug (Mossoba et al., 2011; Hassan et al., 2013). Using mice with a functional immune system, the team showed that pretreatment with a drug combination of pentostatin (that kills T cells) and cyclophosphamide (that kills B cells) eliminated anti-SS1P antibody formation (Mossoba et al., 2011). And in patients with advanced treatment-refractory mesothelioma, treatment with pentostatin, cyclophosphamide, and SS1P together significantly decreased the formation of anti-SS1P antibodies allowing more cycles of SS1P to be given, which resulted in durable and major tumor regressions in three of the ten evaluable patients (Hassan et al., 2013).

In addition, the same team identified residues in the PE Pseudomonas toxin that were being recognized by T and B cells as foreign and removed them to create a less immunogenic toxin. The team determined that most of the toxin Domain II could be deleted, so long as they retained 11 residues required for furin processing. Not only was the altered toxin less immunogenic, it was also more active in killing cancer cells, 8-fold less toxic to mice, and it greatly diminished capillary leak syndrome in rats (Weldon et al., 2013). With respect to B-cell binding, the team identified several residues in Domain-III that were being recognized by B-cells; they mutated seven hydrophilic residues to hydrophobic alanine residues to reduce the binding (Liu et al., 2012). The new toxin was too small, 43-kDa, which would rapidly be removed from the circulation by the kidneys, so the team collaborated with Roche Diagnostics to produce a larger molecule which they fused to an antibody against mesothelin to produce immunotoxin RG7787 with a size of about 72-kDa. RG7787 is currently in phase I testing against mesothelin-positive malignancies (Hassan et al., 2015).

Section-5 Conclusions

This section of the Literature Review identified several different steps that are required for an ADC drug to function. Loss of any one of these steps can result in an inactive drug. Second-generation ADCs are being designed to overcome tumor resistance, be more active, and have fewer side-effects. One area that needs much more research is the identification of more tumor-specific antigens not found in normal tissues. This will allow ADCs to better target the tumor instead of normal tissues, and will also allow the use of more than one ADC to target a tumor if resistance against one antigen arises. In addition, more research should be performed to identify mechanisms for how tumors become resistant to ADCs. To facilitate this, in vitro and in vivo mouse models could be developed for ADC resistance for various types of tumors by treating them long-term with an ADC drug until resistance occurs, and then characterizing the mechanism of the resistance. In the future, to increase the effectiveness, the ADC could be combined with an immunotoxin or radioimmune drug targeted to the same tumor cells but which kills the cell by a different mechanism. This strategy would be particularly important if some of the tumor cells have become resistant to the ADC.
Section-5 References


Kreitman RJ, Siegall CB, Chaudhary VK, FitzGerald DJ, Pastan I (1992) Properties of Chimeric


METHODS

To accomplish objective-1, we performed an extensive review of the current research literature, including reputable academic journal articles, relevant books, scholarly websites, newspaper articles, and other pertinent materials.

To accomplish objective-2, we conducted interviews with various academic or medical researchers who had developed or used antibody-drug conjugates on animals or humans, or who are FDA legal experts, to determine their range of opinions on the strengths and weaknesses of this technology, and to identify remaining problems for gaining FDA approval for new ADC drugs.

Who: The interviewees included academic and industry experts on antibody-drug conjugates, and legal experts on the FDA approval process for new drugs. The technical experts will help answer questions identified from our Literature Review search, and will help prioritize the problems. The legal experts will help discern the remaining legal hurdles for gaining approval to use more ADCs in the U.S. Some of the interviewees will initially be identified by referral from the project advisor, Dave Adams. Other interviewees will be identified from the published literature as authors on key scientific papers, or by referral from the initial interviews.

Where and When: Whenever possible, interviews were conducted in person, but the majority were performed by email, phone, or Skype.

How: We developed our interview questions based on our background research. A preliminary set of questions is shown in the Appendix. Based on our background search of each interviewee, we designed a pertinent initial question. Any subsequent questions were based on the interviewee’s response to the initial question. The Appendix shows the range of topics covered in our interviews.

With respect to the method of the interview, after establishing contact with an interviewee, we informed the interviewee about the purpose of our project, and asked for permission to quote them (see interview preamble in the Appendix). If the need arose for confidentiality, we protected the interviewee by either not quoting them directly, or by giving them the right to review any quotations used in the final published report, explaining that the interview is voluntary, and explaining that they may stop the interview at any time or refuse to answer any question. To further increase the number of interviewees, at the end of an interview, we sometimes asked the interviewee to recommend other potential stakeholders we might interview.

With respect to the total number of interviews performed for our project, we discontinued our interviews once we had obtained sufficient information to represent all sides of the ADC problem, or when the unclear points had been clarified.

To accomplish objectives-3 and 4, the IQP team synthesized all of the collected information in our literature research, interviews, and follow-up interviews to ascertain the strength of the evidence for and against ADC drugs, and created recommendations for further research.
RESULTS / FINDINGS

Our review of the ADC literature identified several problems that needed clarification in subsequent interviews. The problems we chose to focus on in interviews were: 1) Why, in general, don’t antibodies alone (naked antibodies without payload) kill tumor cells? 2) Why are only two ADC drugs approved by the FDA? 3) What causes the ADC side-effects? 4) What are some of the problems associated with the high cost of ADC drugs? 5) Are ADC drugs included for funding in President Obama’s Cancer Moonshot program? 6) What are some future directions for ADC research? We performed interviews with scientists who helped develop ADC drugs, doctors who performed human ADC clinical trials, and legal experts who participated in the approval of ADC drugs.

Antibodies Alone versus ADCs

Our review of the literature remained unclear about why, with few exceptions, treating cancer patients with “naked” antibodies alone (not conjugated to a payload) typically do not kill cancer cells. After all, shouldn’t the body manufacture antibodies against the body’s forming tumor and kill it? To increase our understanding of this issue, we interviewed Dr. Dario Neri, a Professor in the Department of Chemistry and Applied Biosciences at the Swiss Federal Institute of Technology (Zürich). Dr. Neri was the corresponding author on a 2012 article published in the Journal of Controlled Release, July 20; 161(2): 422-428, “Antibody-Drug Conjugates: Basic Concepts, Examples and Future Perspectives”. Dr. Neri responded to our question about “naked” antibodies by saying:

In principle, anti-cancer “naked” IgG’s would be the perfect drug, as they would be “silent” (i.e., not toxic) until they (i) bind to a surface antigen on the tumor cell; (ii) recruit NK cells through the interaction of the Fc portion of the antibody molecule with Fc-gammaRIII receptors on NK cells; and (iii) cause the killing of tumor cells by targeted degranulation of the recruited NK cells. Unfortunately, this strategy does not work effectively against the majority of cancers, for a number of reasons: 1) Antibodies typically show suboptimal accumulation on tumor cells, because of a slow and inefficient extravasation and diffusion into tumor masses, and 2) There is an insufficient number of NK cells within the tumor mass [to perform the killing].

Thus, Dr. Neri’s explanation about why “naked” antibodies are generally less effective than ADCs is that antibodies show poor accumulation on the tumor surface, and the killing without a conjugated payload is dependent on natural killer (NK) cells whose concentrations are low in the tumor environment. Presumably by linking a highly potent cytotoxic payload to the antibody for ADCs, the attachment of only one antibody to the cell surface in theory can kill the cell.

On the same topic, we also interviewed Dr. Anna M. Wu, PhD, a Professor at the Crump Institute for Molecular Imaging in the David Geffen School of Medicine, UCLA (Los Angeles). Dr. Wu was a corresponding author on a 2005 article in Nature Biotechnology, 23: 1137-1146, “Arming Antibodies: Prospects and Challenges for Immunoconjugates”. When asked the “naked antibody” question, Dr. Wu indicated that some of the same ways that tumors avoid the body’s
immune response in the first place which allows tumor growth, such as “downregulating MHC to avoid antigen detection, engaging immune checkpoint inhibitors, and secreting immuno-suppressive cytokines”, may explain why antibody formation against surface antigens is low. Although this information did not directly address our question about naked antibodies versus ADCs, because both of those therapies use delivered antibodies, not antibodies induced in vivo, she made a very important point about why antibody formation against surface tumor antigens is generally low in vivo.

On this same topic, we also interviewed Dr. Beverly A. Teicher, PhD, Chief of the Molecular Pharmacology Branch at the National Cancer Institute (Bethesda). Dr. Teicher was corresponding author on a 2011 article in the journal of Clinical Cancer Research, Oct 15; 17(20): 6389-6397, “Antibody Conjugate Therapeutics: Challenges and Potential”. Dr. Teicher’s response to our question about naked antibodies provided examples of several antibodies that, by themselves, sometimes work against cancer, even when not conjugated to a cytotoxic payload. Her examples of sometimes effective naked antibodies included: trastuzumab [Herceptin, binds HER2], cetuximab [binds the epidermal growth factor receptor], rituximab [binds CD20], bevacizumab [binds VEGF-a], alemtuzumab [binds CD52], and panitumumab [binds the epidermal growth factor receptor]. She stated that “all of these ‘naked’ antibodies target cell surface proteins, and block/inhibit pathways that are critical for cell survival and/or proliferation”, and went on to say that the advantage of ADCs is they “open the possibilities to targeting abundant cell surface proteins that are not directly involved in critical cell survival pathways. So, the advantage of ADCs over “naked” antibodies is they can kill a cell by physically tethering to it, without regard to the function of the tumor antigen itself.

Thus, with respect to the naked antibody question, we conclude that to kill a tumor cell, a naked antibody bound to a tumor cell needs an active NK cell population, and these cells are low in concentration in the environment surrounding tumors. In addition, relatively few antibodies bind to the surface of the tumor cell. ADCs, being conjugated to highly potent drugs, need relatively few (or one) antibodies bound to the tumor cell to kill it, and do not need NK cells. Moreover, to kill a cell, naked antibodies need to block growth pathways, while ADC target antigens can have any function.

**Why Are Only Two ADC Drugs Approved?**

Currently, only two ADC drugs have been approved by the FDA. A third ADC was originally approved, but was subsequently withdrawn. With approximately 40 ADC drugs currently under development, and the ADC antitumor mechanism apparently straightforward, we were puzzled as to why so few ADCs have been approved. To shed light on this, we interviewed Dr. John M. Lambert, of ImmunoGen, Inc. (Waltham, MA). Dr. Lambert was one of two corresponding authors on a 2014 paper in the Journal of Medicinal Chemistry, Aug 28; 57(16): 6949-6964, “Ado-trastuzumab Emtansine (T-DM1): An Antibody-Drug Conjugate (ADC) for HER2-Positive Breast Cancer”. When asked his opinion about why only two ADC drugs are currently approved, he replied:

You ask a fundamental question for the field!! My short answer is: the biggest hurdle in my opinion is to identify the right target antigen for optimal activity of an ADC. CD30 (on Hodgkin Lymphoma) and HER2 (on HER2-positive breast cancer) are “good”
targets: they have high surface density, uniform expression on 100% of the tumor cells, they internalize sufficiently well to import the cytotoxic payload, and those antigens are located on tumors that seem readily accessible to antibodies. After these ADC successes, the field made ADCs to all sorts of targets on the surface of many different types of cancer cells – but in the end, many of the targets do not bring into the cell enough payload to kill the majority of the tumor in an in vivo setting. To get FDA approval, one needs to have good anti-tumor activity at well tolerated doses. Many of the targets chosen for the current ADC technologies turned out to have insufficient anti-tumor activity at the maximum tolerated doses.

The field now has lots of “tools”, such as different payloads, linkers, etc. The trick is finding the right target on a type of tumor that is susceptible to the payload class, and where the target allows delivery of sufficient payload to kill most all the tumor cells.

Another point is it has proven really hard to extrapolate the parameters of anti-tumor activity, and overall toxicity, from preclinical animal models to humans. ADCs combine characteristics of large antibody molecules with characteristics of small molecule cytotoxics which, I think, makes it hard to judge the probability for clinical success from preclinical information.

Perhaps, with ImmunoGen’s maytansinoid [payload] technology…the first patient was dosed with a maytansinoid-ADC in December 1999, we can now begin to understand this a little better. There is now clinical information for several different ADCs to compare with the corresponding preclinical data. We are also optimistic about mirvetuximab soravtansine [an ADC against folate-alpha-positive cancers], now in a phase 3 clinical trial.

On this same topic, we also interviewed Dr. Ian E. Krop of the Dana-Farber Cancer Institute, Harvard University School of Medicine (Boston, MA). Dr. Krop was the corresponding author on a 2014 paper in *Lancet Oncology*, 2014 Jun; 15(7): 689-699, “Trastuzumab Emtansine versus Treatment of Physician’s Choice for Pretreated HER2-Positive Advanced Breast Cancer (TH3RESA): A Randomised, Open-Label, Phase 3 Trial”. When asked his opinion about why only two ADC drugs had been approved, he responded: “Good question. There will be others approved soon, I think. The delays happened mainly because they needed to develop better payloads (toxins) and identify good targets besides HER2 and CD30”. So, Dr. Krop agrees with Dr. Lambert that the early approved ADC drugs had excellent surface targets.

We also interviewed Dr. Thomas H. Pillow, a scientist in the Department of Research & Early Development, Genentech, Inc. (South San Francisco, CA). Dr. Pillow was a corresponding author on a recent article in *Chemical Science*, 2017 Jan 1; 8(1): 366-370, “Decoupling Stability and Release in Disulfide Bonds With Antibody-Small Molecule Conjugates”. When asked his opinion about why so few ADCs have received approval, he stated:

“Good question. There are a couple of things to consider with regard to only two ADCs being FDA approved. One is the long time it takes to achieve approval. Research on linking HER2 to drugs started in the late 90s, and took until 2013…more than 10 years. Now consider that most of the ADC research has been really done after 2010, you can imagine that the impact of this research will take some time to bring new approved drugs
to the market. The other thing to think about is that HER2 and CD30 are some of the best
targets for ADCs (due to many reasons, among them being copy number, access to
antigen, etc.). Other solid tumor targets are much more challenging, and have been
limited in general to the toxicity caused by the agents. A narrow therapeutic index has
been a constant challenge [most ADCs show a narrow window of effective
concentrations before becoming toxic], and research is trying to understand and expand
on it. I think we’re making progress but time will tell”.

The next interview was with Dr. Alfred Zippelius of the Department of Biomedicine,
University Hospital and University of Basel, Switzerland. Dr. Zippelius was a corresponding
author on a 2015 paper in Science Translational Medicine, 7(315): 315ra188, “Trastuzumab
Emtansine (T-DM1) Renders HER2+ Breast Cancer Highly Susceptible to CTLA-4/PD-1
Blockade”, which involved pre-clinical animal testing. When asked his opinion about why more
ADC drugs have not been approved, he stated it was “probably due to a lack of [good] tumor
antigens, and the ADC technology which still needs to be improved, especially regarding
instability.

On this same topic we also interviewed Dr. Daniel J. DeAngelo, MD, PhD, in the
Department of Medical Oncology, Dana-Farber Cancer Institute (Boston, MA). Dr. DeAngelo
was corresponding author on a 2003 paper in Blood, 102(5): 1578-1582, “Prior Gemtuzumab
Ozogamicin Exposure Significantly Increases The Risk of Veno-Occlusive Disease in Patients
Who Undergo Myeloablative Allogeneic Stem Cell Transplantation”. Gemtuzumab ozogamicin
is an ADC consisting of a monoclonal antibody against CD33 conjugated to a cytotoxic drug
from the class of calicheamicin that damages DNA. It was designed to be used against CD33-
positive acute myelogenous leukemia (AML). When asked about why so few ADCs have FDA
approval, he stated “there is a barrier to approval….the need to show an overall survival benefit
in the absence of toxicities, which is not trivial”.

The next interview was with Dr. Stephen M. Ansell, MD, PhD, in the Division of
Hematology, Mayo Clinic (Rochester, MN). Dr. Ansell was sole author on a summary article in
why so few ADC drugs have been approved, and what is the biggest hurdle for gaining approval,
he stated that “the target antigen to which the ADC is directed determines the efficacy, and the
results with different targets vary”.

The last interview in this section was with Dr. Alan K. Burnett, a Professor in the
School of Medicine, Cardiff University (Cardiff, UK). Dr. Burnett was corresponding author on
Ozogamicin to Induction Chemotherapy in Adult Patients With Acute Myeloid Leukaemia: A
Meta-Analysis of Individual Patient Data From Randomised Controlled Trials”. When asked
about why so few ADCs are approved, and the remaining hurdles, he stated:

“In the case of gemtuzumab [in the U.S.], it was approved by the FDA in a single arm
study nearly 20 years ago, mostly because it was novel. [It was later withdrawn]. In
Europe, it also got a favourable opinion around then, but the company could not
guarantee the manufacturing, so the CHMP [Committee for Medicinal Products for
Human Use] looked at it again, and the members had changed, and they voted it down. It
was approved in Japan...and still is. We import it for all our UK trial patients. The general question of the paucity of immunoconjugates in AML may reflect that there is no clear leukaemia-specific antigen to target. So all [the targeted antigens] are also present on normal cells. Anti-CD123 may work, but the Seattle Genetics drug recently failed on toxicity grounds and those studies are now closed. ADCs are also expensive to manufacture”.

Thus, for the question as to why only two ADC drugs have been approved, the consensus appears to be that the two successful ADCs (targeting CD30 and HER2) were developed early on (when ADCs were relatively novel, making approval easier, and providing more time for the approval), and the CD30 and HER2 target antigens are among the best in the field: they are abundant on the tumor cell surface, expressed by a majority of the tumor cells, highly accessible to the ADC antibody, and can internalize the ACD drug. Subsequent ADC targets have not been able to provide the high degrees of efficacy and survival improvements that are needed for gaining FDA approval. Other interviewees (discussed below) indicated they felt that more ADC drug approvals will occur very soon.

**Causes of ADC Side-Effects**

Our review of the literature showed that all ADC drugs showed side-effects in clinical trials, but it remained unclear what caused the side-effects. Were they caused by the presence of the target antigen on normal cells, so the ADC drug kills them too? Or were they caused by off-target effects of the toxic payload, with the toxic payload diffusing out of the target cell to kill normal cells? To shed light on this problem, we interviewed Dr. David M. Goldenberg, a scientist at Immunomedics, Inc. (Morris Plains, NJ). Dr. Goldenberg was corresponding author on a 2015 paper in Clinical Cancer Research, 21(17): 3870-3878, “First-in-Human Trial of a Novel Anti-Trop-2 Antibody-SN-38 Conjugate, Sacituzumab Govitecan, for the Treatment of Diverse Metastatic Solid Tumors”. Their study observed neutropenia as a side-effect of the ADC treatment. When asked his opinion about what caused the neutropenia, Dr. Goldenberg responded: “[The neutropenia is caused by the] SN-38 [payload] drug. It can also be induced by [administering] the pro-drug Irinotecan by itself [the chemical precursor for active SN-38]”. Thus, Dr. Goldenberg thinks the neutropenia he observed is not caused by normal healthy neutrophils expressing the Trop-2 target antigen, but is an off-target effect caused by the toxic payload lowering neutrophil numbers after its release from the cell. The neutropenia is also observed when treating patients only with the payload drug unbound to antibody.

The next interview on this topic was with Dr. Sven Golfier of Bayer HealthCare Pharmaceuticals (Berlin, Germany). Dr. Golfier was corresponding author on a 2014 paper in Molecular Cancer Therapeutics, 13(6): 1537-1548, “Anetumab Radvantsine: A Novel Mesothelin-Targeting Antibody–Drug Conjugate Cures Tumors with Heterogeneous Target Expression Favored by Bystander Effect”. The authors developed a new ADC (Anetumab Radvantsine, BAY 94-9343), consisting of a human antibody against mesothelin conjugated to the maytansinoid tubulin inhibitor DM4. Mesothelin is frequently over-expressed in mesothelioma, ovarian, pancreatic, and lung adenocarcinomas, with limited expression in normal cells. A test of the ADC in patient-derived xenograft mouse models showed efficient drug internalization and complete tumor eradication in most of their mouse models, but also showed a by-stander effect. When asked to clarify whether the by-stander effect might be caused by toxic DM4 diffusing
from the mesothelin-positive target cell after release from the ADC to damage surrounding mesothelin-negative cells, he stated:

“I am happy to learn that our publication raises your interest, and I am glad to respond to your question. It is correct, that neighboring mesothelin-negative cells are killed by DM4, which has been previously taken up as an intact ADC by a mesothelin-positive cell and is then degraded. In fact, SPDB-DM4 degradation results in a cell permeable metabolite with a bystander effect. In contrast, the proteolytic cleavage of e.g. Kadcyla (Trastuzumab-SMCC-DM1) leaves a Lysine attached at DM1. Therefore this metabolite is not cell permeable and has no bystander effect. We [also] believe that killing of target-negative cells in close proximity to target-positive cells is desirable….assuming that it is also a malignant proliferating cell. In fact, we believe that [our ADC] Anetumab Ravtansine is characterized by a double specificity: First targeting mesothelin-positive cells by the antibody, and second killing proliferating cells by the DM4 toxophore. We treated mesothelin-positive primary mesothelial cells with Anetumab Ravtansine, and it did not kill those cells under conditions which kill proliferating cancer cells”.

We next interviewed Dr. Linda T. Vahdat, MD, of the Weill Cornell Medical College (New York, NY). Dr. Vahdat was corresponding author on a 2015 paper in the Journal of Clinical Oncology, 33: 1609-1619, “EMERGE: A Randomized Phase II Study of the Antibody-Drug Conjugate Glembatumumab Vedotin in Advanced Glycoprotein NMB–Expressing Breast Cancer”. The authors described the results of their phase-II randomized trial of the ADC Glembatumumab vedotin in patients with refractory breast cancer. When asked her opinion about ADC side-effects, she stated they “vary by which drug is conjugated to the antibody, but I would not say they are uniformly better when the drug is conjugated to an antibody versus not”. With respect to whether the side-effects are caused by antibody targeting normal cells or bystander effects, she stated “It is probably all of the above”. When asked whether the side-effects can be avoided in patients, she stated “Sometimes”. Are target-negative tumor cells killed by the ADC? “I am not sure. There is something called “innocent bystander” effect, and that may have a more global effect when using these targeted agents”.

We next interviewed Dr. Anjali Advani, MD, of the Cleveland Clinic, Department of Hematologic Oncology and Blood Disorders, Taussig Cancer Center (Cleveland, OH). Dr. Advani was corresponding author on a 2010 paper in the Journal of Clinical Oncology, 28(12): 2085-2093, “Safety, Pharmacokinetics, and Preliminary Clinical Activity of Inotuzumab Ozogamicin, a Novel Immunoconjugate for the Treatment of B-Cell Non-Hodgkin’s Lymphoma: Results of a Phase I Study”. In this paper, the authors reported their Phase-I data on maximum tolerated dose, safety, and pharmacokinetics (distribution in the body) of the ADC Inotuzumab ozogamicin (CMC-544) (a humanized antibody against CD22 conjugated to cytotoxic agent calicheamicin). The drug was tested in patients with relapsed or refractory CD22 B-cell non-Hodgkin’s lymphoma (NHL). When asked about side-effects seen with Inotuzumab, he replied they are “manageable” and “vary based on the actual ADC construct and what the target is”. He agreed with our assessment that in this case the side-effects were caused by targeting of Inotuzumab to normal cells, but thinks Inotuzumab “will likely get FDA approved soon for treating acute lymphocytic leukemia (ALL); the Phase 3 ALL trial was just completed and published in NEJM this year”.

111
Thus, with respect to causes of ADC side-effects, we conclude that the side-effects observed vary with each ADC, and sometimes are caused by off-target effects (Dr. Goldenberg’s Trop-2 example), and in other cases are caused both by off-target effects and by normal cells expressing the target antigen. At least, in some cases, the side-effects appear to be manageable. One important point for reducing potential off-target effects is to conjugate the cytotoxic drug to the antibody using a linker that produces a charged residue when cleaved; the charge makes the drug unable to diffuse from the target cell to the surrounding cells. Although, in some cases, it might be desirable to target dividing cells surrounding the target cell.

**ADC Funding Issues**

The development and testing of ADC drugs is expensive, and so is the cost of ADC drugs to cancer patients. The problem of the high cost of ADC drugs to patients is best exemplified by the example of Kadcyla in the United Kingdom. **Delyth Morgan** is the Chief Executive of “Breast Cancer Now”, the largest breast cancer charity in the United Kingdom. Ms. Morgan was quoted in an article in *The Guardian* on November 4, 2015, “Breast Cancer Drug Kadcyla to Remain on NHS After Manufacturer Lowers Price”. Kadcyla, the ADC targeting HER2 in breast cancer, is manufactured by Roche, and was scheduled to be dropped from England’s Cancer Drugs Fund (CDF), a list of drugs the British government pays for. England’s National Institute for Health and Care Excellence (NICE) had determined that Kadcyla was no longer cost-effective (it was too expensive). According to the article, cost appeared to be the only reason Kadcyla was going off the approved list, because “NICE recognized that Kadcyla works well, and can significantly extend women’s lives without severe side-effects”. Kadcyla is given to women only if the drug Herceptin (the Her2 antibody alone) fails to work. The original price for Kadcyla, according to the article, was £5,900 (pounds) per month, which over an average treatment time of 9.6 months would total about £56,640. An earlier article from NICE dated 8-8-14 stated the cost was somewhat higher at £90,000. Breast Cancer Now organized a petition to Roche to drop the price of Kadcyla. The petition was signed by more than 42,000 people. The focus of the 2015 article was that Roche finally agreed to lower the cost of Kadcyla down to £50,000, the threshold for approved drugs used for end-of-life treatments, to allow it to remain on the list of drugs paid by the government. The Chief Executive of Breast Cancer Now, **Delyth Morgan**, was quoted in the article, saying: “We’re pleased that our voices have been heard. It’s encouraging to learn that Roche and NHS England [who will actually do the financing] have been able to come to a deal, but patients relying on other delisted drugs, such as the breast cancer drug Avastin [kicked off the list] for future treatments will no doubt be devastated. There’s a bigger problem with our drug access and pricing system that will not go away”. When we contacted Ms. Morgan, she agreed with our summary of the problem, and gave us permission to quote her.

**Are ADC Drugs Included in the Cancer Moonshot Program?**

The “Cancer Moonshot” was former President Obama’s charge to then Vice President Joseph Biden to achieve the ambitious goal of making a decade’s worth of cancer research progress in five years, and to bring the most promising science and clinical developments to all cancer patients in the near term. The “Cancer Moonshot Blue Ribbon Panel” published a report in 2016 describing their recommendations for accelerating cancer research in the U.S. The Blue
Ribbon Panel and its seven working groups were given the very important charge of assessing where we are fighting cancer today, and to imagine what could be done if the entire cancer community was galvanized by the support, coordination, and infusion of funding that the Cancer Moonshot promised. One of the recommendations of the panel was to organize a “Cancer Immunotherapy Translational Science Network” that would “develop and implement a national strategy to discover and evaluate novel immune-based approaches to treat and prevent both adult and pediatric cancers.” The network would facilitate tumor procurement and comprehensive profiling, including cancers from diverse populations (including racial and ethnic minorities and other underserved patient populations), and develop and implement a national strategy to discover new immune targets and evaluate novel immune-based approaches. It was not clear from our reading of the report whether the “immune-based approaches” slated for development included ADCs. Most of the detail cited in that section of the report referred to T-cell therapies against cancer, not ADCs.

To attempt to clarify whether the Cancer Moonshot initiative includes ADC drugs, we interviewed Dr. Elizabeth Jaffee, MD, Professor and Deputy Director for Translational Research at the Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine. Dr. Jaffee is an international leader in the development of immune-based therapies for pancreatic and breast cancers, and was one of three Co-Chairs of the Cancer Moonshot Blue Ribbon Panel. When asked her opinion about whether the moonshot program and its funding would include developing new ADCs, Dr. Jaffee replied “yes, it would”. However, this point was not agreed to by another co-chair of the same panel, Dr. Dinah S. Singer, PhD, Deputy Director (Acting) of the National Cancer Institute, and Director, Division of Cancer Biology, National Institutes of Health (Bethesda, MD). When asked the same question, Dr. Singer replied “As you’ve noted, the focus of the recommendation is on approaches to T-cell activation and relief of immunosuppression. While ADC approaches are not explicitly excluded, it is unlikely that they would be considered immunotherapies”.

So, at this point it remains unclear whether the substantial Cancer Moonshot funds will include developing new ADC drugs. So, developing new ADCs may need to rely on other sources of funding.

**Combination ADC Therapies**

One of the future directions for ADC research identified in our search of the literature was the potential use of dual ADC therapies….the use of two different ADC drugs targeting two different antigens on the tumor cells. To determine whether experts in the ADC field consider this a serious idea, we interviewed a leading ADC scientist (who wishes to remain anonymous) in the Department of Medicine, Massachusetts General Hospital Cancer Center, Harvard Medical School (Boston, MA). This scientist was a corresponding author on a 2017 paper in *Science Translational Medicine*, whose lab has identified HER3 as a marker for breast cancer cells that have metastasized to the brain. When asked whether his lab has considered doing a combination therapy of an ADC directed against HER2 (a common marker for breast cancer) plus an ADC directed against HER3, he replied: “That is a very good question. The antibodies that we use block HER3 signaling, and are not yet ADCs. As you suggest, it could be interesting to explore HER3 ADCs for this indication, but I think one would have to perform careful experimentation to make sure that the combo of HER2 and HER3 ADCs are not too toxic, and they maintain the
same level of activity as we observed in the mouse studies with the naked antibodies”. So, in theory this scientist agrees that a combination ADC approach might be useful, but cautions against toxicity.

On this same topic of dual therapy, we also interviewed another ADC scientist (anonymous), of the Edwin L. Steele Laboratory, Department of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School (Boston, MA). When asked the same question about dual ADC therapies, he replied:

Thank you for your interest in our work! Based on our findings, dual inhibition of HER2 and HER3 is necessary to achieve efficacious signaling inhibition. In the case of ADCs, their efficacy is mostly driven by the delivery of their cytotoxic component, and monotherapy may be sufficient to control brain metastatic disease. We recently showed for example that the ADC T-DM1 (ado-trastuzumab emtansine) alone can control brain metastases from HER2-positive breast cancer in preclinical models, a result which is supported by further preclinical and clinical findings. We do not have data on the combination of anti-HER2 and anti-HER3 ADCs. While such a combination could be effective, the toxicity associated with it should be critically considered.

On the same topic, we also interviewed Dr. Scott J. Dylla, PhD, Vice President of R&D, Chief Scientific Officer, AbbVie Stemcentrx LLC, 450 East Jamie Court, South San Francisco, CA 94080. Dr. Dylla was corresponding author on a 2015 paper in *Science Translational Medicine*, 7(302): 302ra136, “A DLL3-Targeted Antibody-Drug Conjugate Eradicates High-Grade Pulmonary Neuroendocrine Tumor-Initiating Cells In Vivo”. Their article describes the development of ADC “SC16LD6.5”, comprised of a humanized antibody against DLL3 conjugated to a DNA-damaging toxin pyrrolobenzodiazepine (PBD). They tested their drug in mouse xenograft models containing patient-derived human tumor cells (patient-derived xenografts, PDXs) with high grade pulmonary endocrine tumors. Their results showed evidence of “durable tumor regression”. When asked whether they have identified other potential target antigens on this type of tumor or tested a combination therapy, he stated they “have identified several potential targets, some which have largely non-overlapping expression with DLL3”. But he provided no indication they had tested a combination therapy.

We also interviewed Dr. Vaskar Saha, a scientist in Paediatric Oncology, Division of Molecular & Clinical Cancer Sciences, University of Manchester (UK). Dr. Saha was corresponding author on a 2017 article in *Haematologica*, Jun; 102(6): 1075-1084, “Targeting the 5T4 Oncofetal Glycoprotein With an Antibody Drug Conjugate (A1mcmmaf) Improves Survival in Patient-Derived Xenograft Models of Acute Lymphoblastic Leukemia”. Dr. Saha has done much pre-clinical testing of ADC drugs. When asked whether he had tested a dual therapy with one ADC targeting antigen 5T4 and another ADC targeting a leukemic marker (such as CD22), he replied that he had “not tested that yet”. We also interviewed the other co-corresponding author on the same paper, Dr. Peter L. Stern of the same university. When asked the same question about he had tested dual therapies, he replied “the short answer is no, but it [dual ADC approach] might be a useful approach”.

So, with respect to combination ADC therapies, the scientists we interviewed indicated in general it might be a good idea, but cautioned against potential toxicities of using two different ADCs, each causing their own set of side-effects. So, toxicity would have to be monitored
carefully. In an example case, using two different antibodies (against HER2 and HER3) was shown to be required for blocking growth-inducing signal transduction pathways, so in this case the dual ADC approach might strongly help block the growth-inducing signal transduction and also kill the tumor cells.

Non-Internalizing ADCs

Most ADC drugs developed to date target antigens on the surface of tumors. In these cases, the ADC must be internalized by receptor-mediated endocytosis (RME) to kill the cell. But this approach does not always work well for large solid tumors with physical and kinetic barriers, and does not work well for many types of tumor antigens (thus the problem of getting more ADCs approved by the FDA). Our review of the literature identified non-internalizing ADCs as a potential future direction for research. Non-internalizing ADCs target antigens in the extracellular matrix (ECM) outside cells, or on endothelial cells lining tumor capillaries, and are not necessarily internalized by a cell. A particularly interesting application of non-internalizing ADCs is to target special angiogenesis markers in the sub-endothelial ECM (surrounding new capillaries) to block the formation of new blood vessels during tumor expansion. Since most solid tumors require an expanding blood supply, these ADCs would have the advantage of being active against a variety of tumors.

To obtain more information on the importance of this topic, we interviewed Dr. Dario Neri of the Department of Chemistry and Applied Biosciences, Swiss Federal Institute of Technology (Zürich). Dr. Neri was corresponding author on a 2012 review article in the Journal of Controlled Release, 161: 422-428, “Antibody-Drug Conjugates: Basic Concepts, Examples, and Future Perspectives”. Targeting the ADC to endothelial cells that line tumor capillaries has potential, but the cytotoxic drug might be released directly into the circulatory system and damage healthy cells. The authors suggested that perhaps a better strategy is to target angiogenesis markers in the sub-endothelial extracellular matrix (ECM). These targets are more stable than antigens on the surface of tumor cells, so the ADC drug lasts longer in that location. The authors also provided an example of antibody L19 that targets “extra domain-B” (EDB) of fibronectin, a marker of angiogenesis. EDB is not present in normal tissue, but becomes inserted into fibronectin during angiogenesis, and is highly abundant in many aggressive tumors. Dr. Neri’s lab is developing antibodies against EDB-fibronectin. When Dr. Neri was asked about his team’s progress on developing ADC’s targeting ECM proteins, he indicated that his lab has just published some work on an ADC containing an antibody (F16) targeting tenascin-C, a glycoprotein present in the ECM: Gébleux R, Stringhini M, Casanova R, Soltermann A, Neri D, 2017, “Non-Internalizing Antibody-Drug Conjugates Display Potent Anti-Cancer Activity Upon Proteolytic Release of Monomethyl Auristatin E in the Subendothelial Extracellular Matrix”, International Journal of Cancer, 2017 Apr 1: 140(7): 1670-1679. Because the ADC is not expected to become internalized in a cell, which normally would cleave the linker in the vesicle), they equipped the ADC with a “self-immolative spacer”. At a dose of 7 mg/kg, the ADC cured tumor-bearing mice containing human tumors U87, A431, and MDA-MB-231. The authors stated that they were surprised that their valine-citrulline linker was cleaved in the extracellular environment, as it is normally cleaved inside an intracellular vesicle. So, more research is needed to determine the mechanism of linker cleavage. If this class of ADC could be developed, it might provide a treatment for a variety of tumors that share the need for increased vasculature.
**Immunotoxins**

Immunotoxins (ITs) are a type of cancer therapy consisting of an antibody targeting a tumor antigen conjugated to a cytotoxic bacterial *toxin* (like diphtheria toxin) that kills the tumor cell. ITs are different than ADCs, the latter are conjugated to cytotoxic drugs instead of bacterial toxins. Initially, the usefulness of IT’s was limited by the strong antigenicity of the bacterial toxin payload; the patients mounted an immune response against the drugs, lowering IT effectiveness. To determine whether scientists have overcome this initial limitation, we interviewed **Dr. Ira Pastan**, MD, NIH Distinguished Investigator, Co-Chief, Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health (Bethesda, MD). Dr. Pastan was corresponding author on a 2015 review article in *Clinical Cancer Research*, 22(5): 1055-1058, “New Life for Immunotoxin Cancer Therapy”. Dr. Pastan’s lab is currently developing alternative strategies for IT’s, including: 1) delivering the IT drug combined with chemotherapy agents (pentostatin and cyclophosphamide) to temporarily lower the patient’s immune response so they don’t mount an immune response against the drug, and 2) using recombinant DNA technology to make recombinant toxins that are less immunogenic. When asked whether ITs are more effective than ADCs, he replied “You are looking at it the wrong way: It is not either/or. Cancer is complicated, and most drugs do not work or do not work very well, so different approaches are needed. Each has advantages and disadvantages”. So, Dr. Pastan believes that there is a need to develop both ITs and ADCs to fight cancer, to not just focus on single ADC treatments, but multi-mechanism approaches.

**Site-Specific Drug Conjugation**

Our review of the literature also showed that the activity of ADCs might be improved by controlling the number of drugs attached to each antibody, and controlling the site of attachment. First-generation methods attached the drugs to random positions on the antibody, but newer approaches attach the drugs to specific locations. To help determine how important controlling drug attachment sites to antibodies are to the ADC field, we interviewed **Dr. Carlos Garcia-Echeverria** of the Lead Generation to Compound Realization Group at Sanofi (Vitry-sur-Seine, France). Dr. Garcia-Echeverria was corresponding author on a 2014 review article in *Bioorganic and Medicinal Chemistry Letters*, 24: 5357-5363, “Antibody-Drug Conjugates: A New Wave of Cancer Drugs”. When asked about the importance of site-specific conjugations, he replied:

“We know that the drug-to-antibody ratios (DAR’s) significantly impact the anti-tumor activity and tolerability of ADCs. This is a parameter that needs to be optimized on a case-by-case basis for each ADC. Similarly, it is fair to assume that not all of the molecules in an ADC mixture at a given average DAR value may have the same activity (e.g. processing after internalization may be affected by the positioning of the modified lysine), but macroscopically we are measuring the activity of the total mixture. I do not recall articles describing how the positioning of a modified cysteine (in the case of a thio-mAb ADC) affects the biological activity, although I briefly described this approach in the article, and this type of information would be needed to properly address your question”.
Thus, Dr. Garcia-Echeverria agreed with our assessment that the position of attachment of the cytotoxic drug to the antibody can affect ADC activity. So, this variable needs to be tested and optimized for each ADC drug by creating ADCs with drugs attached to known locations on the antibody. He also stated that even if we achieved a homogeneous ADC product (where the drug is attached to a known location on the antibody), there would still be a heterogeneous activity…with some molecules being taken up by the cell and processed, while other molecules are not. So, using site-specific conjugation would improve ADC activity, but other variables also remain important to overall activity.
CONCLUSIONS and RECOMMENDATIONS

Based on the research performed for this project, our team has made several conclusions and recommendations. It was initially unclear from our review of the literature why, with few exceptions, naked antibodies (not conjugated to a cytotoxic drug) do not efficiently kill cancer cells. Once an antibody correctly targets a cancer cell and binds to it, natural killer cells (NKs) roaming the body should bind to the Fc portion of the antibody and facilitate the killing. Our interviews with immunologists and oncologists indicated that indeed, an active NK population is critical for killing the cancer cell, but NKs are low in concentration in the tumor environment. In addition, relatively few antibodies actually bind to the surface of the tumor cell. ADCs, conjugated to highly potent drugs, need relatively few antibodies bound to the tumor cell to kill it, and do not need NK cells. The cytotoxic payload by itself can kill the cell once internalized. So, ADCs in general are more efficient at killing tumor cells than “naked” antibodies. ADCs are used to treat patients only after they do not respond to “naked” antibody treatments. ADCs also have the advantage of killing a tumor cell regardless of the function of the surface antigen, while naked antibodies need to block cell growth pathways. So, ADCs can target a wider range of antigens.

But if ADCs are such a good idea, why are only two currently approved? From our interviews, the consensus appears to be that the two successful ADCs (targeting CD30 and HER2) were developed early on, when ADCs were relatively novel. At that time, few other drugs had been developed to treat relapsed cancer, so there was less competition. And the early development allowed more data to be included in the FDA application. In addition, the CD30 and HER2 target antigens are among the best in the entire cancer field: they are abundant on the tumor cell surface, expressed by a majority of the tumor cells, highly accessible to the ADC antibody, and are capable of internalizing the ADC. Subsequent ADC targets have not been able to provide the high degrees of efficacy and survival improvements that are needed for gaining FDA approval. Other interviewees indicated they felt more ADC drug approvals will occur very soon, as more data is obtained. The interviews underscore the importance of target antigen selection for ADC performance and approval, and we recommend research be continued to identify new target antigens on various types of tumors.

Our review of the literature showed that all ADC drugs tested to date have adverse side-effects. Even the two currently approved ADCs have “black box” warnings on their packages concerning their side-effects. But it was unclear from the literature what caused the side-effects. Our interviews with physicians using ADCs in clinical trials indicated that the side-effects vary with each ADC: in some cases they are caused by off-target effects, where the cytotoxic drug released from the ADC diffuses out of the target cell into a nearby cell, thus killing it. In other cases, the physicians stated the side-effects were caused by the surface antigen being present on normal cells, so the ADC targets normal cells, diminishing their functions. Importantly, regardless of the cause, several interviewees indicated their side-effects were manageable by using other drugs. One important finding from the interviews is to use a newly designed linker that leaves a positive charge on the drug when it is cleaved. This makes the cargo unable to diffuse out of the original target cell, and we recommend using this new type of linker if off-target effects are a problem. One interviewee stated that he actually liked the off-target effects,
as they allowed the ADC to kill nearby dividing cells (presumably cancer cells) surrounding the target cell, even if they don’t express the target antigen.

Our review of the literature showed that ADCs are expensive drugs. For example, in the U.K., the original price for Kadcyla was £5,900 (pounds) per month, which over an average treatment time of 9.6 months totals about £56,640. Kadcyla was in the process of being dropped from the list of drugs paid by the British government, but the charity “Breast Cancer Now” organized a petition to Roche to drop the price. The petition was signed by more than 42,000 people, and eventually Roche agreed to lower the cost of the drug down to £50,000, the threshold for drugs used for end-of-life treatments, to allow it to remain on the list. We hope that other ADC manufacturers follow suit. Unfortunately, ADC research is expensive. In the U.S., former President Obama initiated the “Cancer Moonshot” program, a charge to former Vice President Joseph Biden to achieve the ambitious goal of making a decade’s worth of cancer research progress in five years, and to bring the most promising science and clinical developments to all cancer patients in the near term. This program would provide significant funds for cancer research. How the money was to be spent was summarized in a report of the “Cancer Moonshot Blue Ribbon Panel”, but following our reading of the report, it remained unclear whether the moonshot would include ADC research; frequently mentioned were T-cell therapies. We interviewed two of the co-chairs of the panel, one agreed the funds would include ADC research, and the other disagreed. So, developing new ADCs may need to rely on other sources of funding.

Our review of the literature also identified several potential new directions for ADC research. One is the use of combination ADC therapies, the use of two or more ADCs targeting different antigens. This trend was especially prevalent in the pre-clinical animal experiments. Several of our interviewees doing clinical trials indicated this might be a good idea, but cautioned against potential toxicities of using two different ADCs (each causing their own set of side-effects). So, toxicity would have to be monitored carefully using this approach. In one interesting case, our interviewee used two different antibodies (against HER2 and HER3 in breast cancer) and showed that both antibodies are required for fully blocking growth-inducing signal transduction pathways. So, in this case the dual ADC approach might strongly block the growth-inducing signal transduction plus kill the tumor cells with the payload drug.

Another potential future direction investigated in our interviews was the use of non-internalizing ADCs. Most ADC drugs developed to date target antigens on the surface of tumors, but these must be internalized to kill the cell. This approach does not always work well with large solid tumors with limited surface access to the ADC. Non-internalizing ADCs target antigens in the extracellular matrix (ECM) outside cells. A particularly interesting application of non-internalizing ADCs is to target special angiogenesis markers in the sub-endothelial ECM (surrounding new capillaries) to block the formation of new blood vessels during tumor expansion. Since most solid tumors require an expanding blood supply, these ADCs would have the advantage of being active against a variety of tumors. Our interview with a scientist using this approach indicated that these ECM markers are more stable than antigens on the cell surface, so the ADC lasts longer in position. He also indicated his lab just published their work on an ADC targeting tenasin-C, a glycoprotein present in the ECM. If this class of ADC could be developed, it might provide a treatment for a variety of tumors that all share the need for increased vasculature.
Another direction for future research is immunotoxins (ITs). ITs are a type of cancer therapy consisting of an antibody against a tumor antigen conjugated to a cytotoxic bacterial toxin (like diphtheria toxin) that kills the tumor cell. ITs are different than ADCs, the latter are conjugated to cytotoxic drugs instead of bacterial toxins. ITs initially were ineffective, as they were inactivated by the host immune response against the toxin. But new research focused on altering the toxin to make it less immunogenic. Our interview with a scientist using this approach indicated he felt it was important to develop both ADCs and ITs for fighting cancer, as each has advantages and disadvantages.

Our review of the literature also identified site-specific attachment as a future direction for ADC research. New chemical bonds allow researchers to control the number of cytotoxic drugs attached to each antibody, and to control the exact site of attachment. Our interview with scientists using this technology indicated that the number of drugs per antibody, and their locations, strongly affect ADC activity. So, we conclude that these parameters must be optimized for each ADC drug.

Overall, we conclude that ADCs represent an interesting method for fighting cancer that in most cases more efficiently kill tumor cells than “naked” antibodies alone. The few ADCs already FDA approved likely did so because of their excellent choice of target antigen and their long history of research prior to approval. Although all ADCs currently have adverse side-effects, they appear to be manageable in most cases, and are far better than the patient’s poor prognosis without treatment. Future areas for ADC research include the use of combination therapies, non-internalizing ADCs that target the tumor vasculature, the use of immunotoxins and radioimmune therapies to complement ADCs, and site-specific conjugations to control the number and locations of drug attachments to the antibodies.
APPENDIX

Example Questions for Academic and Industry Scientists Who Helped Develop or Test ADC drugs:

1. Pre-Clinical Experts:
   A. In your opinion, which cancers are the best candidates for ADC treatments?
   B. How strong is the evidence for antigen specificity? For example, how strong is the evidence that CD30 is strongly upregulated in Hodgkin lymphoma, but not in normal tissue?
   C. Do you agree that more research should be funded to identify a wider variety of tumor-specific antigens, for example to treat patients that have down-regulated the initial target antigen?
   D. Which ADC drugs do you think have the strongest data?
   E. Have you observed any off-target killing in any of your mouse experiments?
   F. Do you think that new chemical technologies that allow the drug to be added to a specific location on the antibody will allow a more uniform drug production, and allow the most optimum site to be determined?

2. Clinical Trial Experts:
   A. Do you agree that most ADCs seem to produce manageable side-effects that are mild compared to the relapsed cancer itself, or compared to the side-effects of current chemotherapy treatments?
   B. What is your opinion on what causes the side-effects? Are they caused by the expression of the target antigen in normal tissues, or due to release of the cytotoxic drug prematurely into the surrounding tissue?
   C. What is your opinion on why more ADC drugs have not received FDA approval? If your ADC drug has not yet been approved, what data is needed to gain approval?

3. Combination Treatments:
   A. Some clinical trials have been successful using an ADC drug followed by a traditional chemotherapy agent. Should this combination approach be used more often?
   B. If two different types of tumor-specific antigens could be identified for a given tumor, would a combination approach using two different ADCs improve efficacy?

Example Questions for FDA Legal Experts

1. New Drug Approvals:
   A. For the two ADC drugs that have been FDA-approved, what was so enticing about their data to warrant approval? What data was most crucial in the approval?
   B. Since only two ADC drugs have been approved so far, are the laws regulating new drugs too stringent? If so, what changes do you think should be implemented?
   C. Are ADC experiments not providing strong enough data to warrant more ADC approvals? Do the clinical trials show side-effects that are too severe?
D. Are combination drug approaches more difficult to get FDA approval?

2. Cost:
   A. Do you think pharmaceutical companies have set too high a price for ADC drugs? In England Roche lowered their price for Kadcyla to fall underneath the threshold limit for drugs paid by the government.
   B. Do you think the cost of ADCs will come down as we get better at manufacturing?

3. Compassionate Use Protocols:
   A. To your knowledge, have “compassionate use” protocols been used for an ADC drug when it does not have full FDA approval but it was needed for a dying patient and no other alternative drugs are available?
   B. Do you think this approach could provide more badly needed data on patients treated with ADC drugs?

**INTERVIEW PREAMBLE**

We are a group of students from the Worcester Polytechnic Institute in Massachusetts, and for our research project we are conducting a series of interviews to investigate problems associated with antibody-drug conjugates for specifically targeting cancer cells.

Your participation in this interview is completely voluntary, and you may withdraw at any time. During this interview, we would like to record our conversation for later analysis. We will also be taking notes during the interview on key points. Is this okay with you?

Can we also have your permission to quote any comments or perspectives expressed during the interview? This information will be used for research purposes only, and we will give you an opportunity to review any materials we use prior to the completion of our final report, which will be published on-line in WPI’s archive of projects.

If the subject does not agree to be quoted, we will respond as follows: “Since you would not like to be quoted during this interview, we will make sure your responses are anonymous. No names or identifying information will appear in any of the project reports or publications.”

Your participation and assistance is greatly appreciated, and we thank you for taking the time to meet with us. If you are interested, we would be happy to provide you with a copy of our results at the conclusion of our project.