Patient Selection Strategies to Maximize Therapeutic Index of Antibody–Drug Conjugates: Prior Approaches and Future Directions

Marna Williams, Anna Spreafico, Kapil Vashisht, and Mary Jane Hinrichs

ABSTRACT

Antibody–drug conjugates (ADC) are targeted agents that have shown promise in treating cancer. A central challenge in development of ADCs is the relatively narrow therapeutic index observed in clinical studies. Patient selection strategies based on expression of the target in tumors have the potential to maximize benefit and provide the best chance of clinical success; however, implementation of biomarker-driven trials can be difficult both practically and scientifically. We conducted a survey of recent clinical experience from early-phase ADC trials completed between 2000 and 2019 to evaluate the different approaches to patient selection currently being used and assess whether there is evidence that target expression is associated with clinical activity. Our analysis of patient selection strategies indicates that optimal trial design for early-stage trials should be based on multiple factors, including prevalence and heterogeneity of target expression among intent-to-treat patients, as well as biological factors influencing expression of cell surface and soluble target. To ensure a high probability of success, early implementation of patient selection strategies centered around target expression are pivotal to development of ADCs. In this review, we propose a strategic approach that can be applied for optimization of trial design.

Introduction

Antibody–drug conjugates (ADC) are targeted therapies that are designed to deliver highly potent cytotoxic agents to tumor cells while sparing normal tissue. Therefore, by design, it is critical to maximize the potential of this therapy by using biomarker-driven clinical trials. In addition, it is important to limit exposure of ADCs to patients who will benefit from treatment due to the significant off-target toxicities associated with these molecules (1, 2). However, implementation of biomarker-driven trials where patients are selected on the basis of tumor target expression can be challenging in early drug development due to multiple factors including: tumor heterogeneity, assay sensitivity, and accuracy, potential changes in target expression after multiple therapies, and difficulties in determining threshold levels for target expression that correlate with efficacy (3). To date, early-phase trials with ADCs have focused primarily on target expression as a patient selection strategy, either by guiding prospective enrollment, or retrospectively evaluating biomarkers associated with clinical benefit. However, there have been no thorough reviews of the various selection strategies and whether they correlate with clinical efficacy.

To determine whether a relationship exists between target expression and clinical activity, we comprehensively examined patient selection strategies that have been used in 47 phase I and II clinical trials of ADCs. We compared the advantages and disadvantages of each strategic approach, as well as outcomes with respect to whether levels of target expression enriched for clinical response. On the basis of this analysis, we propose a strategic approach that can be applied to early-stage clinical trial design to maximize the probability of observing clinical activity with ADCs.

Study Identification and Categorization

Early-phase studies were defined as all phase I and II clinical trials conducted with ADCs in both hematologic and solid tumor patients. ADCs were defined as full-length monoclonal antibodies (mAb) stably conjugated to cytotoxic small-molecule agents, irrespective of tumor target antigen and warhead mechanism of action. Antibodies conjugated to radioisotopes were excluded since clinical trial design for these agents is often conducted under specialized conditions. Ongoing studies that are not completed, reported only as conference abstracts, and combination studies using multiple agents were excluded.

Each study was categorized into one of the following three classes based on its overall patient selection strategy regarding target expression in tumor tissue: (i) no selection strategy, (ii) preselction based on tumor target expression, (iii) retrospective evaluation. To provide a comprehensive analysis, we performed a search on PubMed/EmBase using appropriate search terms and report publication dates from January 1, 2000 to July 30, 2019. Search terms utilized were: “antibody drug conjugate” or “antibody-drug conjugate” or “immunoconjugate” or “immune-conjugate”, combined with “cancer” or “oncol” or “metastat” or “neoplasmin”, combined with “phase I” or “phase II” or “phase 2” or “phase II”, combined with “clinical trial”. The search for PubMed/EmBase publications was limited to articles published in the English language after January 2000 (with no limitation on congress abstracts in Insightmeme). Data collection was based only on the contents of the publications. The following data were collected: name of therapeutic, target, warhead, tumor type, trial design, number of patients, observed clinical activity, and number of patients treated. Preclinical understanding of tumor target expression (i.e., literature or preclinical assessment) was also obtained. The
methods used to quantify target expression were captured, as well as the cut-off value(s) used to define positivity when provided in the publication.

Data were reported in an Excel spreadsheet and categorized by patient selection strategy by a first reviewer (M.J. Hinrichs). Each category was assigned a second reviewer, who performed a cross-check of the data (A. Spreadico, Table 1; refs. 4–12; K. Vashish, Table 2; refs. 13–38; M. Williams, Table 3; refs. 39–50). A positive association between target expression and clinical activity (target-response relationship; TRR) was based on findings where there was higher frequency of objective response rates (ORRs) in target-expressing patients. A negative TRR was defined as no correlation between ORR and target expression. In addition, TRR was deemed not evaluable if there was no clinical activity. Any discrepancies among the entries were resolved by consensus.

This review includes evaluation of all available studies that met the criteria stated here and differences in results reported from the three classes may be due to differences in clinical study design elements, methods used within the study for evaluation of biomarkers, the size of clinical studies, patient indications evaluated in studies, or differences in evaluable data including but not limited to relationships between biomarker expression and clinical activity. No statistical comparisons were evaluated because of the small sample size.

Clinical Trial Identification, Review, and Analysis

Among 282 abstracts identified through the PubMed/EmBase search, a total of 45 fulfilled the prespecified inclusion criteria. Review of these 45 references led to the identification of two additional trials that were not detected by the PubMed/EmBase search. Therefore, a total of 47 references were reviewed and categorized into three different classes according to patient selection strategy: (i) no selection strategy, (ii) preselection based on target expression, (iii) retrospective analysis of target expression (Tables 1–3; refs. 4–50). The characteristics of the early-phase clinical trials used to evaluate the TRRs for ADCs are presented in Fig. 1A. TRRs for each of the three patient selection strategies are outlined in Fig. 1B.

ADC clinical trials without a defined patient selection strategy

The trials included in this class were defined as those involving ADCs that did not evaluate target expression either retrospectively or prospectively. A total of 9 trials were identified (Table 1; refs. 4–12). Within this class, we further subdivided each trial into 3 categories based on target expression as reported in the literature (enrichment) within different tumor types.

Enrichment (n = 3)

This subcategory refers to trials that enrich for target expression by limiting enrollment to tumor types with preestablished high target expression. In this case, lack of a selection strategy poses minimal risk, but does not exclude the potential of dosing patients who do not express or may express different levels of target expression if the tumor were to evolve over time and expression patterns or levels changed. We identified three trials, including pinatuzumab vedotin, mirvetuximab soravtansine, and MLN2704, the targets of which are cluster of differentiation-22 (CD22), folate receptor alpha (FRα), and prostate-specific membrane antigen (PSMA), respectively. For pinatuzumab vedotin and MLN2704, these targets are very well-characterized and have almost 100% expression in the respective cancer indications (5, 51). Some clinical activity was observed with pinatuzumab vedotin in indolent non-Hodgkin lymphoma (iNHL) and particularly in diffuse large B-cell lymphoma (DLBCL); however, there were no responses in chronic lymphocytic leukemia (CLL). The sponsor discontinued pinatuzumab vedotin following head-to-head comparison in a phase II study with another B-cell targeting ADC, polatuzumab vedotin (52). Similarly MLN2704 also showed some signals of efficacy, but the sponsor discontinued due to toxicity (4). In the case of mirvetuximab soravtansine (6), high target expression is well characterized within certain tumor types. Therefore, the strategy was to allow patients with these tumor types on study without documentation of Frα expression to enrich for target expression. At higher doses (>3.3 mg/kg), 2 partial responses (PR) were observed in tumor subtypes (endometrial and epithelial ovarian cancer) with known Frα expression.

Intermediate enrichment (n = 5)

This subcategory refers to trials that enrich for target expression by selecting tumor types with preestablished higher prevalence of target expression. In this case, lack of preselection involves slightly more risk than in the enriched subcategory, as target expression is generally moderately variable (>75% positivity) or heterogeneous in certain tumor types. Five trials in solid tumors were identified, of ADCs targeting CEACAM (11), CD70 (7), MUC1 (8, 9), and Epha2 (10). In all the trials, limited or no anti-tumor activity was detected, despite moderate to high target expression; consequently, development of these ADCs was discontinued due to lack of clinical activity (53, 54). Moreover, in the case of anti-Epha2 MMAF (MEDI-547), significant safety concerns (bleeding) led to discontinuation (10).

No enrichment (n = 1)

This subcategory refers to trials that enroll all tumor types despite limited or no information on target expression in disease indications selected for phase I. This type of trial poses the highest risk, as patients who do not express target will likely be enrolled. Only 1 trial was identified in this category: anti-ST4 MMAF ADC (PF-06263507) in solid tumors. While ST4 has been reported as highly expressed in a large study of over 700 patients with ovarian cancer (12), expression is not well-characterized in other tumor types (12). Development of this ADC was discontinued after no ORRs occurred in 26 patients treated in the first-in-human trial (12).

While this strategy enables rapid enrollment and minimizes time to evaluate safety and maximum tolerated dose (MTD), it also poses a significant risk to the overall development plan if no therapeutic activity is observed. This risk is specific to trials that do not involve enrichment strategies, such as PF-062635207, where no ORRs occurred and there was no information on target expression. Some reviewed publications cited evidence for prevalence of target expression; however, reported prevalence may not be representative of patients enrolled in studies, particularly for estimates based primarily on preclinical data; where the methodology to evaluate expression may not have been well validated; and/or where prior exposure to therapy could have altered target expression levels and freshly obtained tumor samples were not analyzed for target expression.

ADC clinical trials with preselection of patients

This class was defined as clinical trials in which patients were prospectively selected on the basis of target expression. A total of 26 trials were identified (Table 2; refs. 13–38). There were two types of clinical trials in this class: (i) trials in which patient selection was based on test results collected at time of clinical disease diagnosis and (ii) trials in which Clinical Laboratory Improvement Amendments-grade
### Table 1. ADC clinical trials without a defined patient selection strategy.

<table>
<thead>
<tr>
<th>ADC Enrichment</th>
<th>ADC Target</th>
<th>Warhead</th>
<th>Tumor type</th>
<th>Trial design</th>
<th>Patients, N</th>
<th>Target expression(^a)</th>
<th>Clinical activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enrichment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLN2704 (4)</td>
<td>PSMA</td>
<td>DMI</td>
<td>Prostate</td>
<td>Phase I escalation</td>
<td>23</td>
<td>PSMA highly expressed in prostate cancers</td>
<td>4% ORR, 1 PR, 2/23 (8.6%) had ≥50% decline in PSA</td>
</tr>
<tr>
<td>Pinatuzumab vedotin (5)</td>
<td>CD22</td>
<td>MMAE</td>
<td>DLBCL, iNHL, CLL</td>
<td>Phase I escalation</td>
<td>75</td>
<td>Expressed on mature B cells and &gt;95% of B-NHL and CLL</td>
<td>32% ORR, 3 CR, 5 PR DLBCL (n = 29): 52% ORR, 7 CR, 8 PR iNHL (n = 25): 0% ORR CL (n = 10): 0% ORR, 2 PR</td>
</tr>
<tr>
<td>Mirvetuximab soravtansine (6)</td>
<td>Folate receptor α</td>
<td>DM4</td>
<td>Advanced solid tumors(^b)</td>
<td>Phase I escalation</td>
<td>44</td>
<td>Overexpressed in epithelial cancers, including ovarian (80%)</td>
<td>5% ORR</td>
</tr>
<tr>
<td><strong>Intermediate enrichment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMS-936561 (7)</td>
<td>CD70</td>
<td>Duocarmycin</td>
<td>RCC, B-NHL</td>
<td>Phase I escalation</td>
<td>26</td>
<td>Highly expressed in leukemia, gliomas, and RCC</td>
<td>0% ORR</td>
</tr>
<tr>
<td>OMB-401 (8)</td>
<td>Mucin</td>
<td>Calicheamicin</td>
<td>Platinum-insensitive ovarian cancer</td>
<td>Phase I escalation</td>
<td>34</td>
<td>Highly expressed in epithelial cancers, including breast and ovarian (&gt;90%)</td>
<td>0% ORR, 4/34 (11.8%) had ≥50% reduction in CA125</td>
</tr>
<tr>
<td>OMB-401 (9)</td>
<td>Mucin</td>
<td>Calicheamicin</td>
<td>Platinum-sensitive ovarian cancer</td>
<td>Phase II single arm</td>
<td>21</td>
<td>Highly expressed in epithelial cancers, including breast and ovarian (&gt;90%)</td>
<td>0% ORR, 4/21 (19%) had ≥50% reduction in CA125, Study terminated due to AE</td>
</tr>
<tr>
<td>MEDI-547 (10)</td>
<td>EphA2</td>
<td>MMAF</td>
<td>Advanced solid tumors</td>
<td>Phase I escalation</td>
<td>6</td>
<td>Highly expressed in selected cancers</td>
<td></td>
</tr>
<tr>
<td>Labetuzumab govitocan (11)</td>
<td>CEACAMS</td>
<td>SN38</td>
<td>CRC</td>
<td>Phase II expansion</td>
<td>86</td>
<td>Highly expressed in &gt;80% CRC</td>
<td>1% ORR; 1 PR</td>
</tr>
<tr>
<td><strong>No enrichment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF-06263507 (12)</td>
<td>ST4</td>
<td>MMAF</td>
<td>Advanced solid tumors</td>
<td>Phase I escalation</td>
<td>26</td>
<td>Highly expressed in many solid tumors, including lung, breast, gastric, pancreatic, and esophageal cancer</td>
<td>0% ORR</td>
</tr>
</tbody>
</table>

Note: Target expression was not evaluated either retrospectively or prospectively in these publications. Abbreviations: AE, adverse event; B-NHL, B-cell non-Hodgkin lymphoma; CA125, cancer antigen 125; CLL, chronic lymphocytic leukemia; CR, complete response; CRC, colorectal cancer; DLBCL, diffuse large B-cell lymphoma; FR\(\alpha\), folate receptor alpha; iNHL, indolent non-Hodgkin lymphoma; NSCLC, non-small cell lung cancer; ORR, objective response rate; PR, partial response; PSA, prostate-specific antigen; RCC, renal cell carcinoma; SN38, active metabolite of irinotecan; TRR, target-response relationship.

\(^a\)Target expression from literature or preclinical information as cited by publication.

\(^b\)Enrollment without prior documentation of FR\(\alpha\) positivity allowed for patients with the following tumor types: epithelial ovarian cancer, primary peritoneal cancer, fallopian tube cancer, endometrioid cancer, NSCLC, and renal cell cancer.
Table 2. ADC clinical trials with preselection of patients based on target expression.

<table>
<thead>
<tr>
<th>ADC</th>
<th>Target</th>
<th>Warhead</th>
<th>Indication</th>
<th>Trial design</th>
<th>Patients, N</th>
<th>Target expressiona</th>
<th>Part of diagnosis (Y/N)</th>
<th>Clinical activity</th>
<th>Clinical expression</th>
<th>TRR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brentuximab vedotin (12)</td>
<td>CD30</td>
<td>MMAE</td>
<td>CD30+ CTCL</td>
<td>Phase II single arm</td>
<td>48</td>
<td>Expressed on activated/ malignant T cells</td>
<td>• IHC in fresh biopsies</td>
<td>ORR = 54%; 13 PR, 2 CR</td>
<td>Low: 10/28 (36%)</td>
<td>ORR did not appear to correlate with expression; however, CR limited to patients with med/high expression</td>
</tr>
<tr>
<td>Coltuximab ravtansine (14)</td>
<td>CD39</td>
<td>DM4</td>
<td>CD39+ R/R B-NHL</td>
<td>Phase I escalation</td>
<td>69</td>
<td>Ubiquitously expressed on B cells, except plasma cells</td>
<td>• IHC of flow cytometry</td>
<td>ORR = 29%; 2 CR, 7 CRu</td>
<td>All positive at diagnosis in relapse: 7/9 (78%)</td>
<td>A trend observed in 6 DLBCL patients; Needs to be confirmed with a larger number of patients</td>
</tr>
<tr>
<td>DM174 (15)</td>
<td>Mesothelin</td>
<td>MMAE</td>
<td>Pancreatic and platinum resistant ovarian</td>
<td>Phase I escalation</td>
<td>71</td>
<td>Highly expressed in pancreatic (86%–100%) and ovarian (77%) cancers</td>
<td>• IHC</td>
<td>Pancreatic (n = 40): ORR = 8%; 2 PR</td>
<td>Pancreatic: 27/30 (77%)</td>
<td>5 of 6 PR (83%) had ≥ 2 intensity</td>
</tr>
<tr>
<td>Gemcitabine vedotin (16)</td>
<td>gPMB1</td>
<td>MMAE</td>
<td>gPMB1 = breast cancer</td>
<td>Phase II randomized</td>
<td>88</td>
<td>Overexpressed in breast cancer (40%–60%)</td>
<td>• IHC by central lab in archival tissue</td>
<td>All (n = 88): HER2+ = 12%; 10 PR</td>
<td>326/328 (99%) screening patients not evaluable for positivity</td>
<td>Higher ORR in patients with ≥ 25% positive tumor cells</td>
</tr>
<tr>
<td>Mirezumab soravtansine (17)</td>
<td>Folate receptor α</td>
<td>DM4</td>
<td>Ptx+ platinum-resistant ovarian, fallopian tube, and peritoneal cancer</td>
<td>Phase I expansion</td>
<td>27</td>
<td>Overexpressed in epithelial cancers, including ovarian (80%)</td>
<td>• IHC by central lab in archival tissue</td>
<td>ORR = 22%</td>
<td>HER2+: 64/72 (88%)</td>
<td>All responders had med/high expression</td>
</tr>
<tr>
<td>T-DM1 (18)</td>
<td>HER2</td>
<td>DM1</td>
<td>2/3 HER2+ Breast</td>
<td>Phase II single arm</td>
<td>73</td>
<td>Overexpressed in ~20% of breast cancers</td>
<td>• Local and central laboratory (IHC and/or FISH) &gt; 10% staining by Herceptest or ratio ≥ 2.0 by FISH</td>
<td>HER2+ = 27 PR, 0 CR</td>
<td>All previously HER2+ by local lab in central lab retest: HER2+ = 87/2 (93%)</td>
<td>All responders were confirmed HER2+</td>
</tr>
<tr>
<td>T-DM1 (19)</td>
<td>HER2</td>
<td>DM1</td>
<td>2/3 HER2+ Breast</td>
<td>Phase I imaging study</td>
<td>96</td>
<td>Overexpressed in ~20% of breast cancers</td>
<td>• Local laboratory (IHC and/or FISH) &gt; 10% staining by Herceptest or ratio ≥ 2.2 by FISH</td>
<td>HER2+: ORR = 27%</td>
<td>All previously HER2+ by local lab in central lab retest: HER2+ = 87/2 (93%)</td>
<td>Greater proportion of responders were HER2+ by imaging</td>
</tr>
<tr>
<td>T-DM1 (20)</td>
<td>HER2</td>
<td>DM1</td>
<td>2/3 HER2+ Breast</td>
<td>Phase II single arm</td>
<td>100</td>
<td>Overexpressed in ~20% of breast cancers</td>
<td>• Local and central laboratory (IHC and/or FISH) &gt; 10% staining by Herceptest or ratio ≥ 2.2 by FISH</td>
<td>HER2+: ORR = 41%</td>
<td>All previously HER2+ by local lab in central lab retest: HER2+ = 82/95 (86%)</td>
<td>Greater proportion of responders were confirmed HER2+</td>
</tr>
</tbody>
</table>

(Continued on the following page)
Table 2. ADC clinical trials with preselection of patients based on target expression. (Cont’d)

<table>
<thead>
<tr>
<th>ADC</th>
<th>Target</th>
<th>Warhead</th>
<th>Indication</th>
<th>Trial design</th>
<th>Patients, N</th>
<th>Target expression*</th>
<th>Test method</th>
<th>Cutoff</th>
<th>Part of diagnosis (Y/N)</th>
<th>Clinical activity</th>
<th>Clinical expression (Y/N)</th>
<th>TRR</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-DM1 (21)</td>
<td>HER2</td>
<td>DM1</td>
<td>2L+ HER2+</td>
<td>Breast</td>
<td>Phase II single arm, N2</td>
<td>Overexpressed in ~20% of breast cancers</td>
<td>Local and central laboratory IHC or FISH 3+ (&gt;10% staining) by Herceptest IHC or ratio ≥ 2.2 by FISH</td>
<td>Yes</td>
<td>HER2+; ORR = 34% (25 PR)</td>
<td>All previously HER2+ by local lab</td>
<td>In central lab retest; HER2+ = 74/95 (78%); HER2+ = 21/35 (62%)</td>
<td>Greater proportion of responders were confirmed HER2+</td>
</tr>
<tr>
<td>Vadilatuzumab tateine (22)</td>
<td>CD33</td>
<td>PB5</td>
<td>CD33+ AML</td>
<td>Phase I elicitation, N131</td>
<td>Expressed in leukemia myeloblasts of most AML</td>
<td>Flow cytometry by local laboratory; Any positivity</td>
<td>Yes</td>
<td>ATIP2D; 44% CR + CRi rate</td>
<td>Baseline CD33+ on blasts by central laboratory flow cytometry on BM and blood</td>
<td>Data not shown</td>
<td>Increased potential for blast clearance with high CD33; however, no correlation between expression and CR rate</td>
<td></td>
</tr>
</tbody>
</table>

Negative TRR (no relationship between response and expression)

<table>
<thead>
<tr>
<th>ADC</th>
<th>Target</th>
<th>Warhead</th>
<th>Indication</th>
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<th>Patients, N</th>
<th>Target expression*</th>
<th>Test method</th>
<th>Cutoff</th>
<th>Part of diagnosis (Y/N)</th>
<th>Clinical activity</th>
<th>Clinical expression (Y/N)</th>
<th>TRR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brentuximab vedotin (23)</td>
<td>CD30</td>
<td>MMAE</td>
<td>CD30+ R/R DLBL</td>
<td>Phase II single arm, N49</td>
<td>Expressed in 14%-25% of DLBL</td>
<td>IHC by central lab; ≥10% positivity on tumor cells</td>
<td>No</td>
<td>ORR = 44% (26 PR, 8 CR)</td>
<td>Median 25% positivity</td>
<td>No correlation</td>
<td>CR observed in patients with undetectable CD30</td>
<td></td>
</tr>
<tr>
<td>Brentuximab vedotin (24)</td>
<td>CD30</td>
<td>MMAE</td>
<td>R/R CD30+ RTPC</td>
<td>Phase II single arm, N35</td>
<td>Highly variable expression</td>
<td>IHC by local and central labs; ≥10% positivity on tumor cells</td>
<td>No</td>
<td>ORR = 41% (6 PR, 8 CR)</td>
<td>≥3% positivity</td>
<td>No correlation</td>
<td>CR observed in patients with undetectable CD30</td>
<td></td>
</tr>
<tr>
<td>Minetuximab sarelatane (25)</td>
<td>Folate receptor α</td>
<td>DM4</td>
<td>FRAa+ platinum-resistant ovarian, fallopian tube, peritoneal cancer</td>
<td>Phase I expansion, N46</td>
<td>Overexpressed in epithelial cancers, including ovarian (80%)</td>
<td>IHC by central lab in archival tissue; ≥25% cells with ≥2+ intensity</td>
<td>No</td>
<td>ORR = 26%; 11 PR, 1 CR</td>
<td>124 of 154 (81%) patients at screening met cutoff</td>
<td>No significant trend</td>
<td>Responders observed in low, med, high expressions</td>
<td></td>
</tr>
<tr>
<td>TAK-264 (MLN0264) (26)</td>
<td>GCC</td>
<td>MMAE</td>
<td>GCC+ gastric cancer</td>
<td>Phase II single arm, N38</td>
<td>Expressed in 60%-70% of gastric cancers</td>
<td>IHC in archival tissue; H score ≥ 210</td>
<td>No</td>
<td>ORR = 5%; 2 PR</td>
<td>In 38 treated</td>
<td>No correlation</td>
<td>Neither of the two PR were high GCC expression</td>
<td></td>
</tr>
<tr>
<td>T-DM1 (27)</td>
<td>HER2</td>
<td>DM1</td>
<td>1L HER2+</td>
<td>Breast</td>
<td>Phase II randomized, N67</td>
<td>Overexpressed in ~20% of breast cancers</td>
<td>Local and central laboratory IHC and/or FISH 3+ (&gt;10% staining) by Herceptest IHC or ratio ≥ 2.2 by FISH</td>
<td>Yes</td>
<td>HER2+; ORR = 14.2 mo</td>
<td>All previously HER2+ by local lab</td>
<td>In central lab retest; HER2+ = 52/67 (77.6%); HER2+ = 14.2 mo in both</td>
<td>No correlation</td>
</tr>
</tbody>
</table>

TRR could not be evaluated

<table>
<thead>
<tr>
<th>ADC</th>
<th>Target</th>
<th>Warhead</th>
<th>Indication</th>
<th>Trial design</th>
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<th>Clinical activity</th>
<th>Clinical expression (Y/N)</th>
<th>TRR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brentuximab vedotin (28)</td>
<td>CD30</td>
<td>MMAE</td>
<td>R/R CD30+ PMBL</td>
<td>Phase II single arm, N15</td>
<td>High, heterozygous expression on majority (80%) of PMBL</td>
<td>IHC or flow cytometry; Unknown cutoff</td>
<td>Yes</td>
<td>Trial terminated due to toxicity; ORR = 13%; 2 PR</td>
<td>NA; used pathology report</td>
<td>Cannot be evaluated</td>
<td>No stratification and low clinical activity</td>
<td></td>
</tr>
<tr>
<td>Brentuximab vedotin (29)</td>
<td>CD30</td>
<td>MMAE</td>
<td>R/R CD30+ hematologic malignancies</td>
<td>Phase I elicitation, N44</td>
<td>High expression in HL (100%) and sALCL</td>
<td>IHC or flow cytometry</td>
<td>Unknown cutoff</td>
<td>Yes</td>
<td>ORR = 59%; 10 PR, 14 CR</td>
<td>NA; used pathology report</td>
<td>Cannot be evaluated</td>
<td>No stratification</td>
</tr>
<tr>
<td>Brentuximab vedotin (30)</td>
<td>CD30</td>
<td>MMAE</td>
<td>R/R CD30+ HL sALCL</td>
<td>Phase I/II escalation, expansion, N20 (14 HL; 6 sALCL)</td>
<td>Expressed on Reed-Sternberg cells in HL and sALCL</td>
<td>IHC or flow cytometry</td>
<td>Unknown cutoff</td>
<td>Yes</td>
<td>HL; ORR = 67%; 5 CR, 1 PR</td>
<td>NA; used pathology report</td>
<td>Cannot be evaluated</td>
<td>No stratification</td>
</tr>
</tbody>
</table>

(Continued on the following page)
## Table 2. ADC clinical trials with preselection of patients based on target expression (Cont’d)

<table>
<thead>
<tr>
<th>ADC</th>
<th>Target</th>
<th>Warhead</th>
<th>Indication</th>
<th>Clinical expression</th>
<th>TRR</th>
<th>Clinical activity</th>
<th>Part of diagnosis</th>
<th>Clinical activity</th>
<th>Clinical expression</th>
<th>Test method</th>
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<tr>
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<tr>
<td>BR96-</td>
<td>Lewis</td>
<td>Doxorubicin</td>
<td>LewisY</td>
<td>≥20% tumor cells: doxorubicin (31)</td>
<td>66</td>
<td>Expressed on majority of tumors, including CRC: 148/164 (90%)</td>
<td>Cannot be evaluated</td>
<td>No stratiﬁcation and low clinical activity</td>
<td>ORR 3.4%; 2 PR</td>
<td>IHC</td>
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<tr>
<td>Cantuzumab</td>
<td>CanAg</td>
<td>DM1</td>
<td>CanAg</td>
<td>Advanced solid tumors</td>
<td>20</td>
<td>Highly expressed in ≥2+ intensity</td>
<td>Cannot be evaluated</td>
<td>No stratiﬁcation</td>
<td>ORR 22%; NA; used pathology report</td>
<td>IHC or FISH</td>
</tr>
<tr>
<td>Coltuximab</td>
<td>CD19</td>
<td>DM4</td>
<td>CD19</td>
<td>R/R ALL</td>
<td>36</td>
<td>Expressed by 90% of cells</td>
<td>Cannot be evaluated</td>
<td>No stratiﬁcation</td>
<td>ORR 57%; NA; used previous diagnosis</td>
<td>IHC by central lab or local laboratory</td>
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<tr>
<td>Inotuzumab</td>
<td>CD22</td>
<td>Calicheamicin</td>
<td>CD22</td>
<td>≥2+ intensity: ≥2% positive blasts</td>
<td>72</td>
<td>Expressed on &gt;90%</td>
<td>Cannot be evaluated</td>
<td>No stratiﬁcation</td>
<td>ORR 68%; CR/CRi</td>
<td>IHC by central lab in archival or fresh tissue</td>
</tr>
<tr>
<td>TAK-264</td>
<td>GCC</td>
<td>MMAE</td>
<td>GCC</td>
<td>R/R (MLN0264)</td>
<td>43</td>
<td>H score ≥10</td>
<td>Cannot be evaluated</td>
<td>No stratiﬁcation</td>
<td>ORR 2%; 1 PR</td>
<td>Local laboratory IHC or ratio ≥2.2 by FISH</td>
</tr>
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<tr>
<td>T-DM1</td>
<td>HER2</td>
<td>DM1</td>
<td>2L</td>
<td>HER2</td>
<td>24</td>
<td>Overexpressed in 60% of breast cancer</td>
<td>Cannot be evaluated</td>
<td>No stratiﬁcation</td>
<td>ORR 25%; NA</td>
<td>Local laboratory IHC</td>
</tr>
</tbody>
</table>

Note: In many cases despite TRR evaluation being problematic or low clinical activity, associations between target expression and response were observed in several clinical trials. Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; B-NHL, B-cell non-Hodgkin lymphoma; CR, complete response; CRi, complete remission with incomplete hematologic recovery; CRu, unconﬁrmed complete response; CTCL, cutaneous T-cell lymphoma; DLBCL, diffuse large B-cell lymphoma; FR, folate receptor alpha; GCC, guanylyl cyclase C; GI, gastrointestinal; gpNMB, glycoprotein NMB; HL, Hodgkin lymphoma; NA, not applicable as target expression not re-evaluated in publication; NHL, non-Hodgkin lymphoma; NSCLC, non–small cell lung cancer; ORR, objective response rate; PMBCL, primary mediastinal large B-cell lymphoma; PTCL, peripheral T-cell lymphoma; RCC, renal cell carcinoma; sALCL, systemic anaplastic large cell lymphoma; TNBC, triple-negative breast cancer; TRR, target-response relationship; 20% positive blasts; 30% cells; 5% cells; yes; no; ≥2.2 by FISH.
Table 3. ADC clinical trials using retrospective target expression strategy.

<table>
<thead>
<tr>
<th>ADC</th>
<th>Target</th>
<th>Warhead</th>
<th>Indication</th>
<th>Trial design</th>
<th>Patients, N</th>
<th>Target expression*</th>
<th>Clinical activity</th>
<th>Target expression frequency</th>
<th>TRR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRR showed correlation or trend of association between response and expression</td>
<td>Brentuximab vedotin (39)</td>
<td>CD30</td>
<td>MMAE</td>
<td>Mycosis fungoides</td>
<td>Phase II single arm</td>
<td>30</td>
<td>Expressed in 10%-15% of mononuclear infiltrate</td>
<td>ORR = 70%; 21 PR</td>
<td>Low: 14/32 (44%); Intermediate: 14/32 (44%); High: 4/32 (13%)</td>
</tr>
<tr>
<td></td>
<td>DSM200 (40)</td>
<td>HER2</td>
<td>Derauitinectan</td>
<td>Breast or gastric (regardless of HER2 status)</td>
<td>Phase I escalation</td>
<td>24</td>
<td>Highly expressed in 15%-20% of breast cancer and 20% of gastric tumors</td>
<td>ORR = 35.7%; 2 CR, 1 PR</td>
<td>Low = 6/95 (7%); High = 85/95 (93%)</td>
</tr>
<tr>
<td></td>
<td>Liasitumab vedotin (41)</td>
<td>NaPi2b</td>
<td>MMAE</td>
<td>Platinum-resistant ovarian</td>
<td>Phase II randomized</td>
<td>47</td>
<td>Expressed in ~90% ovarian cancer</td>
<td>ORR = 30%; 2 CR, 19 PR</td>
<td>Low = 4/48 (8%); Med/High = 42/48 (88%)</td>
</tr>
<tr>
<td></td>
<td>Sacituzumab govitecan (42)</td>
<td>Trop2</td>
<td>SN38</td>
<td>TNBC</td>
<td>Phase II single arm</td>
<td>69</td>
<td>Highly expressed in many epithelial cancers</td>
<td>ORR = 14%; 7 PR</td>
<td>Low = 9/25 (40%); Med = 13/25 (52%); High = 2/25 (8%)</td>
</tr>
<tr>
<td></td>
<td>Sacituzumab govitecan (43)</td>
<td>Trop2</td>
<td>SN38</td>
<td>SCLC</td>
<td>Phase II single arm</td>
<td>50</td>
<td>Highly expressed in many epithelial cancers</td>
<td>ORR = 14%; 7 PR</td>
<td>Low = 9/25 (40%); Med = 13/25 (52%); High = 2/25 (8%)</td>
</tr>
<tr>
<td>TRR evaluated in separate study</td>
<td>Gemcitabine vedotin (44)</td>
<td>gemM1</td>
<td>MMAE</td>
<td>Locally advanced or metastatic breast cancer</td>
<td>Phase I/II escalation/ expansion</td>
<td>42</td>
<td>Overexpressed in breast cancer (40%-60%)</td>
<td>ORR = 10%; 3 PR</td>
<td>n = 23</td>
</tr>
<tr>
<td></td>
<td>Blasitumab mertansine (45)</td>
<td>C224v6</td>
<td>DM1</td>
<td>HNSCC</td>
<td>Phase I escalation</td>
<td>31</td>
<td>Highly expressed by &gt;95% HNSCC</td>
<td>ORR = 10%; 3 PR</td>
<td>n = 23</td>
</tr>
<tr>
<td></td>
<td>Carbotumab mertansine (46)</td>
<td>C6A2-2 (MUC1)</td>
<td>DM1</td>
<td>CanAg* advanced solid tumors</td>
<td>Phase I escalation</td>
<td>39</td>
<td>Highly expressed in most pancreatic, biliary, colorectal, and other solid tumors</td>
<td>ORR = 0%</td>
<td>1+: 3/39 (10%) 2+: 5/39 (13%) 3+: 0/39 (0%)</td>
</tr>
<tr>
<td></td>
<td>Carbotumab mertansine (47)</td>
<td>C6A2-2 (MUC1)</td>
<td>DM1</td>
<td>CanAg* advanced solid tumors</td>
<td>Phase I escalation</td>
<td>37</td>
<td>Highly expressed in most pancreatic, biliary, colorectal, and other solid tumors</td>
<td>ORR = 0%</td>
<td>1+: 0/34 (0%) 2+: 6/34 (18%) 3+: 28/34 (82%)</td>
</tr>
<tr>
<td></td>
<td>Gemcitabine vedotin (48)</td>
<td>gemM1</td>
<td>MMAE</td>
<td>Advanced stage III and IV melanoma</td>
<td>Phase II</td>
<td>62</td>
<td>Highly expressed in melanoma (~60%)</td>
<td>ORR = 11%; 6 PR, 1 CR</td>
<td>58/59 (98%) of tumors gemM1-positive (~5% tumor epithelial cell staining)</td>
</tr>
<tr>
<td></td>
<td>Sacituzumab govitecan (49)</td>
<td>Trop2</td>
<td>SN38</td>
<td>Epithelial cancers</td>
<td>Phase I basket</td>
<td>178</td>
<td>Highly expressed in many epithelial cancers</td>
<td>ORR = 17%; 20 PR</td>
<td>Low = 16/150 (11%); Med/High = 123/150 (82%)</td>
</tr>
</tbody>
</table>

Note: In 50% of the trials analyzed, there was a trend of association between response and expression. Abbreviations: CR, complete response; gemM1, glycoprotein NMB; HNSCC, head and neck squamous cell carcinoma; NaPi2b, sodium-dependent phosphate transport protein; PBD, pyrrolobenzodiazepine dimer; PR, partial response; RP2D, recommended phase 2 dose; SCLC, small cell lung cancer; SN38, active metabolite of irinotecan; Top-1, topoisomerase I inhibitor; TNBC, triple-negative breast cancer; TRR, target-response relationship.

*Target expression from literature or preclinical information as cited by publication.
(CLIA-grade) biomarker assays were utilized, with specific cutoffs for target expression prior to enrollment in the trial.

**Target expression evaluated during diagnosis of disease (n = 15)**

This subcategory refers to trials in which patients were preselected for target expression, using existing data generated with a validated assay, often as part of standard diagnosis of disease. A total of 15 trials were identified (Table 2; refs. 13–38). In the case of solid tumors, HER2 expression was assessed in seven trials using the FDA-approved HercepTest as part of the standard diagnosis for patients with breast cancer. The remaining 8 trials were conducted in hematologic malignancies, where standard lineage...
markers [e.g., CD33 in acute myeloid leukemia (AML)] are used during diagnosis and staging. While these biomarkers can be used for preselection in clinical studies, there are limitations and assumptions that must be considered. For example, cutoffs for HER2 target expression based on HercepTest are determined from clinical activity with prior HER2-targeted therapies. Refinement of such cutoffs and exploration of additional biomarkers to explore the levels of target expression associated with response to each ADC should be considered (21). Moreover, the clinical relevance of using the same cutoff for ADCs and mAbs is not well-understood.

Target expression evaluated with CLIA-based biomarker assay (n = 11)

This subcategory refers to trials where patients are preselected for target expression prior to enrollment. All patients must provide archival or fresh tumor tissue at screening for assessment of target expression using a CLIA-based biomarker assay with a defined cut-off. This approach is needed for targets in disease indications with no established diagnostic. A total of 11 trials were identified as outlined in Table 3 (refs. 13–38). Of these, 9 trials involved targets being tested in a range of solid tumors, including anti-FRα DM4 (mirvetuximab soravtansine) in ovarian cancer and anti-gpNMB MMAE (glembatumumab vedotin) in breast cancer.

The major advantage of this approach is that by verifying target expression prior to dosing, the probability of detecting therapeutic activity can be increased. Moreover, preclinical assessment of target expression can be compared to levels of target in the clinical trial (intent-to-treat patients). At the same time, this approach involves many scientific and logistical barriers that can affect the trial. From a scientific perspective, there is a significant risk associated with defining a cutoff for positivity in early-stage trials, given the heterogeneity of target expression in many cancer indications and limited number of patients tested at the cutoff prior to employing preselection of patients. In this case, the cutoff is often defined arbitrarily, or using preclinical models with unknown clinical relevance. Moreover, there is often significant heterogeneity that can make assessing positivity challenging. In the case of glembatumumab vedotin, gpNMB is expressed on both tumor epithelial and stromal cells. Nearly all patients (99%) met the low threshold for eligibility defined as >5% positive epithelial or stromal cells. However, target expression was limited to the stromal compartment in more than half of these patients and epithelial tumor cell staining (≥25% cells positive) was often found in 37% of patients. Moreover, the ORR was 30% (7 PR of 23 patients) in patients with positive epithelial staining, while it was only 12% (10 PR of 83 patients) in all patients meeting the cutoff.

From an operational perspective, preselecting patients can be a significant hurdle due to logistic issues such as tracking down samples and obtaining usable biopsies. In the case of mirvetuximab soravtansine, 22% (6 of 27) collected biopsies were of insufficient quality to assess for target expression (17). Moreover, the decision must be made either to use archival or fresh biopsies. If archival tissue is allowed, then the age of the sample must be considered. This is especially important in cases where target stability over time is poorly characterized. For glembatumumab vedotin, the age of archival tissue ranged from 2 weeks to 20 years (median 3.3 years) before study entry (16). For mirvetuximab soravtansine, the trial design involved collecting both archival and fresh biopsies from a subset of patients (n = 21) to gain understanding of target stability. The concordance of FRα expression in archival and biopsy tissues was 71%, and no major shifts in receptor expression were observed in matched pre- and posttreatment biopsy samples. This type of analysis provides greater confidence for the use of archival tumor tissue in subsequent studies. If fresh biopsies are mandated, patient enrollment, and/or compliance can be challenging.

ADC clinical trials using retrospective target expression strategy

This class was defined as clinical trials in which target expression was retrospectively evaluated. A total of 12 trials were identified (Table 3; refs. 39–50). Retrospective analysis involves post hoc assessment of target expression where patients can enroll in clinical studies without evidence of biomarker status, but archival or fresh tissue specimens are used to assess expression retrospectively. The main
advantage of this strategy is that the relationship between target expression and clinical activity can be obtained during the trial, providing an opportunity to examine efficacy across a spectrum of biomarker-positive patients. However, this approach also increases the risk that patients who are low or negative for target expression may receive the test treatment and, consequently, response rates may be reduced.

When examining the TTR in this category, half of the trials (6/12) demonstrated an association or potential trend of association between response rates (and/or proportion of responders) and target expression. For anti-HER2 deruxtecan ADC (DS-8201a), patients were enrolled regardless of HER2 status; this allowed for analysis of a broad range of expression. Increased responses were observed in patients with HER2-positive status by HercepTest (50% ORR in HER2 with intensity of 2+ or 3+), although some responders had HER2 low expression. Additional work is ongoing to develop a new companion diagnostic test to assess low expression of HER2 by IHC to delineate subgroups of patients who will benefit from treatment with DS-8201a (55). Similarly, for rovalpituzumab tesirine, patients with elevated target expression demonstrated improved clinical activity in phase I. At the recommended phase II dose, patients with high DLL3 expression had substantially better ORR (38%) compared with all-comers (18%; ref. 42). All responders had high DLL3 expression (IHC >50% tumor cells).

For sacituzumab govitecan, a trend of association between target expression and response (CR, PR) was observed in a phase II study in patients with triple-negative breast cancer (TNBC) where all responders had medium/high tumor-associated calcium signal transducer 2 (Trop2) expression (signal by IHC on >10% of cells with ≥2+ intensity). However, since most patients (88%) expressed moderate to high levels of Trop2, it is difficult to draw firm conclusions (43). While there was no statistically significant association between elevated target expression and OS in a phase II study in SCLC, patients with medium/high Trop2 expression had longer duration of survival than patients with low/negative expression (10.5 months vs. 9.2 months; ref. 44). While this could be related to prognostic significance of Trop2, 4 of 5 confirmed PR had elevated Trop2 expression and only 1 of 3 was Trop2 low.

Alternatives to standard IHC scoring of target expression may provide greater dynamic ranges to distinguish differences between responders and nonresponders. This strategy was applied for linstuzumab vedotin where >90% of patients expressed high levels of NaPi2b target. To establish a greater dynamic range, H-scores for IHC and qRT-PCR were used and a trend of increased clinical activity (OR) and benefit (PFS) in patients was observed with increased NaPi2b expression by this alternative scoring (39). Similarly, for brentuximab vedotin, an alternative method to IHC, multispectral imaging (MSI) analysis, was used to quantify CD30 data. In the phase II study, patients with low CD30 by IHC had lower likelihood of clinical response to brentuximab vedotin (39); however, associations between response and expression with the more sensitive MSI assay were not reported.

None of the trials demonstrated a negative TTR. However, as with preselection strategies, in several trials (5 of 12 trials; 42%), TTR could not be evaluated due to limited clinical activity or lack of stratification. For cantuzumab-mertansine (CanAg) and bavituzumab mertansine, associations between target expression and response could not be evaluated due to low clinical activity. Heterogeneity of target expression in the tumor may have contributed to the absence of clinical activity for CanAg (47, 48). For bavituzumab mertansine, high levels of CD44v6 in skin may have contributed to the narrow therapeutic index.

In addition, associations between response and target expression were not evaluated in a phase I basket study with sacituzumab govitecan where retrospective analysis showed that most patients (82%) had med/high expression of Trop2 (50).

Finally, there was a single study (1 of 12 trials; 8%) where the TTR was not reported (45). For gembatumumab vedotin, retrospective analysis verified that patients who responded were gNMB positive; however, analysis of target expression intensity or prevalence and relationships to clinical response were not reported. Instead, relationships between target expression and response were assessed in a subsequent phase II randomized study (see section on preselection strategy; ref. 16).

Together, these data demonstrate that retrospective target evaluation may be a strategically appropriate approach when target expression is enriched in an indication and there is not a need to define a cut-off early in clinical development. Doing so enables a balance between rapid enrollment and the need to obtain data in patients with negative/low expression. Furthermore, this approach provides analysis that can inform suitable cut-offs for preselection and enrichment of patients in future studies, as well as an opportunity to assess the need for a companion diagnostic to support continued development.

### Considerations for Patient Selection Strategies

Each of the patient selection strategies outlined here has its own advantages and disadvantages. Selection of the most appropriate strategy depends on many factors, including but not limited to target prevalence, target biology, patient population, assay limitations, and operational feasibility. We propose several approaches that can be used to help inform patient selection strategies (Fig. 2).

Prospective selection of patients is preferable when target expression cut-offs have been well-established from prior studies and/or target expression is confirmed at disease diagnosis prior to clinical study enrollment (Fig. 2A). This strategy was applied during the development of four of the five currently approved ADCs. While preselection is required for trastuzumab emtansine (Kadcyla) and gemtuzumab ozogamicin (Mylotarg), no additional preselection is needed for inotuzumab ozogamicin (Besponsa) and brentuximab vedotin (Adcetris) as patients diagnosed with acute lymphoblastic leukemia (inotuzumab ozogamicin) and Hodgkin lymphoma or systemic anaplastic large-cell lymphoma (brentuximab vedotin) have uniformly high levels of CD22 and CD30 expression, respectively. For polatuzumab vedotin-piiq (Polivy), a retrospective analysis strategy was utilized, as most patients with lymphoma were predicted to express high levels of CD79b based on preclinical studies (56). In a phase II study in DLBCL and follicular lymphoma (FL) where CD79b expression was assessed retrospectively, there was no correlation between tumor shrinkage and target expression (52).

The key advantage of the retrospective selection strategy is that a range of target expression levels can be assessed during clinical trials and exploratory analysis of associations with clinical activity can be assessed (Fig. 2). Through this approach, an initial understanding of target expression levels required for response can be obtained in early stages of clinical development. Establishment of minimal cutoffs required for response typically requires further studies, as small numbers of patients at the active dose in phase I may make data interpretation difficult. If clinical activity is evident post ADC treatment but cutoffs required for activity have not been determined, preselection, or enrichment of high target expressers may be used to maximize the probability of clinical success at early stages of
development. With this approach, target expression cut-offs can be determined when sufficient clinical activity has been observed in patients with elevated target expression.

The main risk of retrospective analysis is treating patients who do not express sufficient levels of target for ADC internalization and tumor cell killing (Fig. 2B). Thus, the retrospective analysis strategy is recommended for disease indications with high prevalence of target expression. For ADCs where target is expressed at different levels in a variety of tumor types, the retrospective strategic approach is preferable by focusing initially on indications with high target prevalence. Lower limits of target expression required for clinical activity can be determined in subsequent studies. When determining whether archival tissue is appropriate for monitoring target expression in clinical studies, multiple factors that can contribute to variance should be considered, including biological variability (dynamics of expression), intratumoral heterogeneity (spatial and temporal), and stability of expression posttreatment. These factors are central to the success of a biomarker assay particularly when the biomarker is linked to the mechanism of action of therapeutic drug. Each of these factors must be considered when deciding whether to use archival or fresh tumor tissue in clinical studies.

When testing new ADCs with modifications of first-generation therapeutic antibodies (such as new warheads or linkers), retrospective analysis may also be preferable. This will provide a means to determine if lower levels of target are sufficient for response. This strategy was employed with DS-8201a where target expression was assessed retrospectively. Responses were observed in some patients with target expression below the limits of Her2-positive status by the established companion diagnostic assay, HercepTest (American Society of Clinical Oncology Annual Meeting, 2018). This study points to the potential role that various characteristics of the antibody plus warhead [drug-to-antibody ratio (DAR), half-life of ADC, warhead potency, bystander activity, warhead release mechanisms] can play in dictating the level of target required, as both DS-8201a and trastuzumab emtansine use the same anti-HER2 antibody but have different warheads, DARs, and half-lives. Each of these factors can influence optimal dosing and therapeutic index.

While retrospective analysis of target expression is frequently utilized, mixed success has been observed for patient selection for some ADCs. For example, retrospective analysis of rovalpituzumab tesirine demonstrated clinical responses (CR, PR) only in DLL3-high-expressing patients in phase I (42); however, in a phase II study there was no significant difference between the clinical activity in DLL3-high-expressing patients and those with lower DLL3 expression (43). This highlights the importance of considering multiple factors when selecting patients for ADC clinical trials.
Patient Selection Strategies for Antibody–Drug Conjugates

compared with DLL3-positive patients (57–60). The phase III trial was
delayed due to shorter overall survival (OS) reported in the
Rova-T arm compared with topotecan (61). Similar discrepancies
were observed with mirvetuximab soravtansine where patients
with FRα-high expression showed improved benefit in one phase I
study and one expansion cohort (6, 17) but not in a separate phase I
study in the same disease indications (25). In addition, eligibility based
on FRα expression for the phase III FORWARD I study was based on
different scoring methods compared with all prior studies. This
resulted in including patients in the phase III with lower levels of
FRα expression than intended. The phase III did not meet the PFS
primary endpoint and subsequent exploratory analysis with the original
scoring method demonstrated improved outcomes (62). These
examples point to the challenges inherent in understanding the
biology, drivers, and variance of target expression in disease, especially
in intent-to-treat patients.

To ensure maximum probability of success, it is necessary to design
clinical studies so that target expression is either well understood prior
to enrollment or confirmed in the study. Operational challenges such
as compliance with sample collection and/or quality of samples may
impact data interpretation (Fig. 2B). In the trials reviewed here, the
number of samples received was less than the number of patients
enrolled. This may be avoided by narrowing inclusion criteria and/or
managing sample collections from an operational perspective.

Confirmation within a study may require assessment of freshly acquired
tumors from study patients, particularly when expression is antici-
pated to be low or negative in a large proportion of patients. If biopsies
are not defined as mandatory in the clinical study protocol, collection is
unlikely to occur as patients may decline. In these instances, feasibility
of sample collection and potential safety implications must also be
considered. If target expression is stable, preselection can be accom-
plished with archival samples which will improve the efficiency of
study enrollment.

For each ADC target, levels of expression can only be compared
between patients provided the same assay has been utilized. Further-
more, levels of ADC target antigens per cell can vary significantly
between ADC targets (62, 63) such that “low” expression of one target
can equate to “high” expression of another. More quantitative measures
of target expression such as digital imaging may be required for a better
understanding of the relationships between target heterogeneity and
clinical response. In addition, when feasible, absolute quantification of
target expression could help to avoid differences in methods, such as
such as in the phase III FORWARD I study of mirvetuximab soravtansine.

In summary, based on the trials reviewed, there is no clear evidence
that preselecting patients with high levels of target expression on their
tumors consistently associates with increased ADC clinical activity.
For some targets, elevated expression associates with activity (e.g., DS-
8201a and T-DM1) while for others it does not (e.g., brentuximab
edotin, and in some studies with mirvetuximab soravtansine and
royaltipuzumab tesirine). Associations with response may depend on
the target, the specialized engineering of the ADC, and/or disease
indications. Thus, decisions on whether to utilize preselection or
retrospective analysis in clinical studies depend on multiple factors
including target prevalence, whether target expression levels are
affected by patient biology and disease, ability to obtain target expres-
sion data preclinically, and whether levels of soluble target in intent-to-
treat patients influence drug exposure and/or impact clinical response.

If target expression is consistently elevated in patients despite prior
treatments or disease status, preselection may not be needed. On the
other hand, if target prevalence is low, preselection will be needed but
can lead to operational and financial challenges involved in screening
enough patients to accrue trial enrollment.

Conclusions

ADCs are rapidly advancing, with three new approvals in the past
2 years and several promising molecules in phase III; however, most
ADCs in the clinic fail to advance to pivotal studies. A careful
evaluation of the emerging clinical data indicates that target expression
is important for efficacy with these molecules. To advance trial design,
thoughtful approaches to developing patient selection strategies are
needed to maximize the likelihood of observing activity in early-phase
trials.

The optimal strategy to employ in clinical studies depends on
multiple factors, including but not limited to, available preclinical
information on target expression prevalence and heterogeneity, rela-
tionships between expression and disease status/prognosis, and the
ability to obtain target expression data, particularly in intent-to-treat
patients. This review addresses the role of target expression in patient
selection; however, a comprehensive selection strategy would also
include additional aspects of the mechanism of action of ADCs, such as
patient response to linker and payload (warhead-sensitivity; ref. 2).
For this, development and characterization of biomarkers indicative of
warhead sensitivity is required, along with molecular characterization of
patient profiles with genomics and transcriptomics. In addition,
there are mechanisms of resistance to ADCs including but not limited
to upregulation of drug efflux pumps, induction of antiapoptotic
pathways and lysosomal trafficking defects that prevent intracellular
release of warheads (64–66). Refinement of patient selection can
influence dose selection and treatment regimens that enhance
personalized approaches to managing what is often a narrow therapeutic
index of ADCs.

Disclosure of Potential Conflicts of Interest

M. Williams is a director of translational medicine at and has ownership interest
(including patents) in AstraZeneca. A. Spradforo is an advisory board member for
Merck, BMS, Oncorus, and Janssen and reports receiving other commercial research
support from Novartis, BMS, Bayer, Surface Oncology, Northern Biologics,
Regeneron, Symphogen, Janssen, AstraZeneca, MERCK, Roche, Alkermes, and
Array Biopharma. K. Vashisht is an associate director at AstraZeneca. M.J. Hinrichs is a
senior director clinical scientist at Ipsen and clinical scientist at AstraZeneca.

Acknowledgments

The authors would like to thank Steve Coats (AstraZeneca) and Lillian Siu
(University Health Network, University of Toronto) for critical review and comments
on the manuscript, as well as Kyoko Kelly (AstraZeneca) and John O’Boyle
(AstraZeneca) for their assistance in preparation of the manuscript. Editorial support
was provided by Susanne Gilbert of Cirrus Communications (Macclesfield, UK) and
was funded by AstraZeneca.

Received November 8, 2019, revised February 5, 2020; accepted June 11, 2020,

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conjugates: future directions in clinical and translational strategies to improve
Patient Selection Strategies for Antibody–Drug Conjugates

Molecular Cancer Therapeutics

Patient Selection Strategies to Maximize Therapeutic Index of Antibody–Drug Conjugates: Prior Approaches and Future Directions

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