

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

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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) preselection 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 “neoplasm*”, combined with “phase 1” or “phase I” 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

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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. Spreafico, **Table 1**; refs. 4–12; K. Vashisht, **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 (FR α), 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 FR α 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 FR α 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-5T4 MMAF ADC (PF-06263507) in solid tumors. While 5T4 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.

ADC	Target	Warhead	Tumor type	Trial design	Patients, N	Target expression ^a	Clinical activity
Enrichment							
MLN2704 (4)	PSMA	DM1	Prostate	Phase I escalation	23	PSMA highly expressed in prostate cancers	4% ORR, 1 PR 2/23 (8.6%) had \geq 50% decline in PSA
Pinatuzumab vedotin (5)	CD22	MMAE	DLBCL, INHL, CLL	Phase I escalation	75	Expressed on mature B cells and $>$ 95% of B-NHL and CLL	32% ORR; 3 CR, 5 PR DLBCL (n = 29): 52% ORR; 7 CR, 8 PR CLL (n = 10): 0% ORR
Mirvetuximab soravtansine (6)	Folate receptor α	DM4	Advanced solid tumors ^b	Phase I escalation	44	Overexpressed in epithelial cancers, including ovarian (80%)	5% ORR, 2 PR
Intermediate enrichment							
BMS-936561 (7)	CD70	Duocarmycin	RCC, B-NHL	Phase I escalation	26	Highly expressed in leukemia, gliomas, and RCC	0% ORR
CMB-401 (8)	Mucin	Calicheamicin	Platinum-insensitive ovarian	Phase I escalation	34	Highly expressed in epithelial cancers, including breast and ovarian ($>$ 90%)	0% ORR 4/34 (11.8%) had $>$ 50% reduction in CA125
CMB-401 (9)	Mucin	Calicheamicin	Platinum-sensitive ovarian	Phase II single arm	21	Highly expressed in epithelial cancers, including breast and ovarian ($>$ 90%)	0% ORR 4/21 (19%) had $>$ 50% reduction in CA125
MEDI-547 (10)	EphA2	MMAF	Advanced solid tumors	Phase I escalation	6	Highly expressed in selected cancers	Study terminated early due to AE
Labetuzumab govitecan (11)	CEACAM5	SN38	CRC	Phase II expansion	86	Highly expressed in $>$ 80% CRC	1% ORR; 1 PR
No enrichment							
PF-06263507 (12)	5T4	MMAF	Advanced solid tumors	Phase I escalation	26	Highly expressed in many solid tumors, including lung, breast, gastric, pancreatic, and esophageal cancer	0% ORR

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 α , folate receptor α ; 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.

^aTarget expression from literature or preclinical information as cited by publication.

^bEnrollment without prior documentation of FR α positivity allowed for patients with the following tumor types: epithelial ovarian cancer, primary peritoneal cancer, fallopian tube cancer, endometrial cancer, NSCLC, and renal cell cancer.

Table 2. ADC clinical trials with preselection of patients based on target expression.

ADC	Target	Warhead	Indication	Trial design	Patients, N	Target expression ^a	Test method • Cutoff • Part of diagnosis (Y/N)	Clinical activity	Clinical expression	TRR
TRR showed correlation or trend of association between response and target expression										
Brentuximab vedotin (13)	CD30	MMAE	CD30+ CTCL	Phase II single arm	48	Expressed on activated/malignant T cells	<ul style="list-style-type: none"> IHC in fresh biopsies Any positivity on tumor cells Yes 	ORR = 54%; 13 PR, 2 CR	Low: 10/28 (36%) Med: 12/28 (43%) High: 5/28 (18%)	ORR did not appear to correlate with expression; however, CR limited to patients with med/high expression A trend observed in 6 DLBCL patients Needs to be confirmed with a larger number of patients
Coltuximab ravtansine (14)	CD19	DM4	CD19+ R/R B-NHL	Phase I escalation	69	Ubiquitously expressed on B cells, except plasma cells	<ul style="list-style-type: none"> IHC or flow cytometry Unknown cutoff Yes 	ORR = 29%; 2 CR, 7 CRu	All positive at diagnosis In retest: 7/11 (64%) \geq 2+ intensity	A trend observed in 6 DLBCL patients Needs to be confirmed with a larger number of patients
DMOT4039A (15)	Mesothelin	MMAE	Pancreatic and platinum-resistant ovarian cancer	Phase I escalation	71	Highly expressed in pancreatic (86%-100%) and ovarian (77%) cancers	<ul style="list-style-type: none"> IHC Any expression in escalation and \geq2 intensity in expansion No 	Pancreatic (n = 40): ORR = 8%; 2 PR Ovarian (n = 31): ORR = 30%; 3 PR	\geq 2 intensity Pancreatic: 27/30 (77%) Ovarian: 24/25 (96%)	5 of 6 PR (83%) had \geq 2 intensity
Glembatumumab vedotin (16)	gpNMB	MMAE	gpNMB+ breast cancer	Phase II randomized	83	Overexpressed in breast cancer (40%-60%)	<ul style="list-style-type: none"> IHC by central lab in archival tissue \geq5% stromal or epithelial cells No 	All (n = 83): ORR = 12%; 10 PR \geq 25% gpNMB+ tumor cells (n = 23); ORR = 30%; 7 PR	326/328 (99%) screening patients met cutoff for positivity \geq 25% of tumor cells: 21% in all breast cancer; 40% of TNBC	Higher ORR in patients with \geq 25% positive tumor cells
Mirvetuximab soravtansine (17)	Folate receptor α	DM4	FR α + platinum-resistant ovarian, fallopian tube, and peritoneal cancer	Phase I expansion	27	Overexpressed in epithelial cancers, including ovarian (80%)	<ul style="list-style-type: none"> IHC by central lab in archival tissue \geq25% cells with \geq2+ intensity No 	ORR = 22%; 4 PR, 2 CR \geq 25% cells with \geq 2+ intensity	Low = 6/27 (22%) Med = 5/27 (19%) High = 16/27 (59%)	All responders had med/high expression Both CR were observed in high expressers
T-DM1 (18)	HER2	DM1	2/3L HER2+ Breast cancer	Phase II single arm	73	Overexpressed in ~20% of breast cancers	<ul style="list-style-type: none"> Local and central laboratory IHC and/or FISH 3+ (>10% staining) by Herceptest or ratio \geq2.0 by FISH Yes 	HER2+: ORR = 42%; 27 PR, 0 CR HER2-: No responders	All previously HER2+ by local lab In central lab retest: HER2+: 64/72 (88%) HER2-: 8/72 (12%)	All responders were confirmed HER2+
T-DM1 (19)	HER2	DM1	2/3L HER2+ Breast cancer	Phase I imaging study	56	Overexpressed in ~20% of breast cancers	<ul style="list-style-type: none"> Local laboratory IHC and/or FISH 3+ (>10% staining) by Herceptest or ratio \geq2.2 by FISH Yes 	HER2+: ORR = 70%; 28 PR, 0 CR HER2-: ORR = 12.5%; 2 PR, 0 CR	All previously HER2+ by local lab By imaging: HER2+: 40/56 (71%) HER2-: 16/56 (29%)	Greater proportion of responders were HER2+ by imaging
T-DM1 (20)	HER2	DM1	2L+ HER2+ Breast cancer	Phase II single arm	110	Overexpressed in ~20% of breast cancers	<ul style="list-style-type: none"> Local and central laboratory IHC or FISH 3+ (>10% staining) by Herceptest or ratio \geq2.2 by FISH Yes 	HER2+: ORR = 41%; 33 PR HER2-: ORR = 20%; 3 PR	All previously HER2+ by local lab In central lab retest: HER2+: 80/95 (84%) HER2-: 15/95 (16%)	Greater proportion of responders were confirmed HER2+

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Table 2. ADC clinical trials with preselection of patients based on target expression. (Cont'd)

ADC	Target	Warhead	Indication	Trial design	Patients, N	Target expression ^a	Test method • Cutoff • Part of diagnosis (Y/N)	Clinical activity	Clinical expression	TRR
T-DMI (21)	HER2	DMI	2L+ HER2+ Breast	Phase II single arm	112	Overexpressed in ~20% of breast cancers	<ul style="list-style-type: none"> Local and central laboratory IHC or FISH 3+ (>10% staining) by Herceptest IHC or ratio ≥ 2.2 by FISH Yes 	HER2+: ORR = 34%, 25 PR HER2-: ORR = 5%, 1 PR	All previously HER2+ by local lab In central lab retest: HER2+: 74/95 (78%) HER2-: 21/95 (22%)	Greater proportion of responders were confirmed HER2+
Vadastuximab talirine (22)	CD33	PBD	CD33+ AML	Phase I escalation	131	Expressed in leukemic myeloblasts of most AML	<ul style="list-style-type: none"> Flow cytometry by local laboratory Any positivity Yes 	At RP2b: 44% CR + CRi rate	Baseline CD33+ on blasts by central laboratory flow cytometry on BM and blood	Increased potential for blast clearance with high CD33; however, no correlation between expression and CR rate
Negative TRR (no relationship between response and expression)										
Brentuximab vedotin (23)	CD30	MMAE	CD30+ R/R DLBCL	Phase II single arm	49	Expressed on 14%-25% of DLBCL	<ul style="list-style-type: none"> IHC by central lab ≥1% positivity on tumor cells No 	ORR = 44%, 13 PR, 8 CR	Median 25% positivity	No correlation CR observed in patients with undetectable CD30
Brentuximab vedotin (24)	CD30	MMAE	R/R CD30+ PTCL	Phase II single arm	35	Highly variable expression	<ul style="list-style-type: none"> IHC by local and central labs ≥1% positivity on tumor cells No 	ORR = 41%, 6 PR, 8 CR	≥1% positivity: Local lab: 34/34 (100%) Central lab: 25/31 (81%)	No correlation CR observed in patients with undetectable CD30
Mirvetuximab soravtansine (25)	Folate receptor α	DM4	FRα+ platinum-resistant ovarian, fallopian tube, peritoneal cancer	Phase I expansion	46	Overexpressed in epithelial cancers, including ovarian (80%)	<ul style="list-style-type: none"> IHC by central lab in archival tissue ≥25% cells with ≥2+ intensity No 	ORR = 26%, 11 PR, 1 CR	124 of 154 (81%) patients at screening met cutoff for positivity In 46 treated: Low = 9/46 (20%) Med = 14/46 (30%) High = 23/46 (50%)	No significant trend Responders observed in low, med, high expressers
TAK-264 (MLN0264) (26)	GCC	MMAE	GCC+ gastric cancer	Phase II single arm	38	Expressed in 60%-70% of gastric cancers	<ul style="list-style-type: none"> IHC in archival tissue H score ≥10 No 	ORR = 5%, 2 PR	In 38 treated: Low = 14/38 (37%) Med = 15/38 (39%) High = 9/38 (24%)	No correlation Neither of the two PR were high GCC expressers
T-DMI (27)	HER2	DMI	1L HER2+ breast	Phase II randomized	67	Overexpressed in ~20% of breast cancers	<ul style="list-style-type: none"> Local and central laboratory IHC and/or FISH 3+ (>10% staining) by Herceptest or ratio ≥ 2.0 by FISH Yes 	65% ORR 36 PR, 7 CR Median PFS = 14.2 mo in all; median PFS in confirmed HER2+ = 14.2 mo	All previously HER2+ by local lab In central lab retest: HER2+ = 52/67 (77.6%)	No correlation No difference in PFS in all enrolled patients compared with reconfirmed HER2+ (14.2 mo in both)
TRR could not be evaluated										
Brentuximab vedotin (28)	CD30	MMAE	R/R CD30+ PMBCL	Phase II single arm	15	High, heterozygous expression on majority (80%) of PMBCL	<ul style="list-style-type: none"> IHC or flow cytometry Unknown cutoff Yes 	Trial terminated due to inactivity ORR = 13%, 2 PR	NA; used pathology report	Cannot be evaluated No stratification and low clinical activity
Brentuximab vedotin (29)	CD30	MMAE	R/R CD30+ hematologic malignancies	Phase I escalation	44	High expression in HL (100%) and SALCL	<ul style="list-style-type: none"> IHC or flow cytometry Unknown cutoff Yes 	ORR = 59%, 10 PR, 14 CR	NA; used pathology report	Cannot be evaluated No stratification
Brentuximab vedotin (30)	CD30	MMAE	R/R CD30+ HL SALCL	Phase I/II escalation-expansion	20 (14 HL; 6 SALCL)	Expressed on Reed-Sternberg cells in HL and ALCL	<ul style="list-style-type: none"> IHC or flow cytometry Unknown cutoff Yes 	HL: ORR = 67%, 5 CR, 1 PR SALCL: ORR = 100%, 4 CR, 1 PR	NA; used pathology reports	Cannot be evaluated No stratification

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Table 2. ADC clinical trials with preselection of patients based on target expression. (Cont'd)

ADC	Target	Warhead	Indication	Trial design	Patients, N	Target expression ^a	• Test method			TRR
							Cutoff	Part of diagnosis (Y/N)	Clinical activity	
BR96-doxorubicin (31)	Lewis Y	Doxorubicin	LewisY+ solid tumors	Phase I escalation	66	Expressed on majority of epithelial tumors, including breast, GI, NSCLC	• IHC • ≥20% tumor cells • No	ORR = 3.4%; 2 PR	≥20% tumor cells: CRC: 148/164 (90%) Lung: 38/46 (83%) Breast: 30/40 (75%) Cannot be evaluated No stratification and low clinical activity	
Cantuzumab mertansine (32)	CanAg (MUC1)	DMI	CanAg+ advanced solid tumors	Phase I escalation	20	Highly expressed in most pancreatic, biliary, CRC, and other solid tumors	• IHC • ≥2+ intensity • No	ORR = 0%	≥2+ intensity: 18/20 (90%) Cannot be evaluated No clinical activity	
Coltuximab ravtansine (33)	CD19	DM4	CD19+ R/R ALL	Phase II single arm	36	Expressed by 90% ALL	• IHC or flow cytometry • >30% cells • Yes	ORR = 22%; 3 PR, 2 CR, 3 CR	NA; used pathology report Cannot be evaluated No stratification	
DS-8201a (34)	HER2	Topo1	HER2+ breast cancer	Phase I escalation	115	Overexpressed in ~20% of breast cancers	• Local laboratory IHC or FISH • 3+ (>10% staining) by Herceptest IHC or ratio ≥2.2 by FISH • Yes	ORR = 57%; 2 CR, 63 PR	NA; used previous diagnosis Cannot be evaluated No stratification	
Inotuzumab ozogamicin (35)	CD22	Calicheamicin	CD22+ ALL	Phase I/II escalation-expansion	72	Expressed on >90% ALL	• IHC or flow cytometry by local laboratory • ≥20% positive blasts • Yes	49/72 (68%) CR/CRi	NA; used pathology report Cannot be evaluated No stratification	
SGN-75 (36)	CD70	MMAF	R/R CD70+ NHL and RCC	Phase I escalation	58 (19 NHL, 39 RCC)	Expressed in 60% lymphomas and 72% RCC	• IHC by central lab in archival or fresh tissue • >1% cells • No	RCC: ORR = 5%; 2 PR NHL: ORR = 5%; 1 CR	In screened patients with RCC: 111 of 127 (87%) positive In screened patients with NHL: 82 of 107 (77%) positive Cannot be evaluated Low clinical activity	
TAK-264 (MLN0264) (37)	GCC	MMAE	GCC+ pancreatic cancer	Phase II single arm	43	Expressed in 60%-70% of pancreatic cancers	• IHC in archival tissue • H score ≥10 • No	ORR = 2%; 1 PR	In 43 treated: Low = 11/43 (25%) Med = 15/43 (35%) High = 17/43 (40%) Cannot be evaluated Low clinical activity	
T-DM1 (38)	HER2	DMI	2L+ HER2+ Breast	Phase I escalation	24	Overexpressed in ~20% of breast cancers	• Local laboratory IHC and/or FISH • 3+ (>10% staining) by Herceptest IHC or ratio ≥2.2 by FISH • Yes	ORR = 25%; 6 PR, no CR	NA; used previous diagnosis Cannot be evaluated No stratification	

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; CRC, colorectal cancer; CRi, complete remission with incomplete hematologic recovery; CRu, unconfirmed complete response; CTCL, cutaneous T-cell lymphoma; DLBCL, diffuse large B-cell lymphoma; FRα, 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; PR, partial response; PTCL, peripheral T-cell lymphoma; RCC, renal cell carcinoma; R/R, relapsed/refractory; sALCL, systemic anaplastic large cell lymphoma; TNBC, triple-negative breast cancer; TRR, target-response relationship.

^aTarget expression from literature or preclinical information as cited by publication.

Table 3. ADC clinical trials using retrospective target expression strategy.

ADC	Target	Warhead	Indication	Trial design	Patients, N	Target expression ^a	Clinical activity	Target expression frequency	TRR
TRR showed correlation or trend of association between response and expression									
Brentuximab vedotin (39)	CD30	MMAE	Mycosis fungoides	Phase II single arm	30	Expressed in 10%-15% of mononuclear infiltrate	ORR = 70%; 21 PR	Low: 14/32 (44%) Intermediate: 14/32 (44%) High: 4/32 (13%)	Patients with low (<5%) CD30 had significantly lower likelihood of clinical response (17% vs. 83%, <i>P</i> = 0.0046)
DS-8201a (40)	HER2	Deruxtecan	Breast or gastric (regardless of HER2 status)	Phase I escalation	24	Highly expressed in 15%-20% of breast cancer and 20% of gastric tumors	All (<i>n</i> = 23): ORR = 43.5%; 10 PR HER2 2 ⁺ or 3 ⁺ (<i>n</i> = 18): ORR = 50%; 9 PR HER2 neg or 1 ⁺ (<i>n</i> = 5): ORR = 20%; 1 PR	0/1+ = 5/24 (22%) 2+ = 4/24 (17%) 3+ = 15/24 (61%)	Increased response in HER2-IHC 3 ⁺ patients, although there were some responders with HER2 low expression. No responders in HER2-negative patients
Lifastuzumab vedotin (41)	Nap12b	MMAE	Platinum-resistant ovarian cancer	Phase II randomized	47	Highly expressed by ~90% ovarian cancer	14 PR, 1 CR	Low = 6/95 (7%) High = 85/95 (93%)	Trend of association with clinical activity
Rovalpituzumab tesirine (42)	DLL3	PBD	SCLC and NEC	Phase I escalation	82 (74% SCLC, 8 NEC)	Expressed in ~80% SCLC	At RP2D (<i>n</i> = 60): ORR = 18%; 11 PR In DLL3 high (<i>n</i> = 26): ORR = 38%; 10 PR	Low = 10/39 (26%) High = 29/39 (74%)	All responders had high DLL3 expression
Sactuzumab govitecan (43)	Trop2	SN38	TNBC	Phase II single arm	69	Highly expressed in many epithelial cancers	ORR = 30%; 2 CR, 19 PR	Low = 4/48 (8%) Med/High = 42/48 (88%)	Trend of association between response and expression; however, majority of patients had med/high Trop2 expression
Sactuzumab govitecan (44)	Trop2	SN38	SCLC	Phase II single arm	50	Highly expressed in many epithelial cancers	ORR = 14%; 7 PR	Low = 9/25 (40%) Med = 13/25 (52%) High = 2/25 (8%)	Trend of association between response and expression; however, majority of patients had med/high Trop2 expression
TRR evaluated in separate study									
Glembatumumab vedotin (45)	gpNMB	MMAE	Locally advanced or metastatic breast cancer	Phase I/II escalation/expansion	42	Overexpressed in breast cancer (40%-60%)	At RP2D (<i>n</i> = 33): ORR = 12%; 5 PR In gpNMB ⁺ at RP2D (<i>n</i> = 11): ORR = 18%, 2 PR	<i>n</i> = 19 Most 16/19 (84%) had stroma staining (>5%-85%) Only 4/19 (21%) had epithelial cell staining (all ≤20%)	Associations between response and intensity/distribution of target expression were not evaluated.
TRR could not be evaluated									
Bivatuzumab mertansine (46)	CD44v6	DM1	HNSCC	Phase I escalation	31	Highly expressed by >95% HNSCC	ORR = 10%; 3 PR	<i>n</i> = 23 All ≥2+ intensity On average, ≥95% tumor cells positive	Could not be evaluated Low clinical activity
Cantuzumab mertansine (47)	CanAg (MUC1)	DM1	CanAg ⁺ advanced solid tumors	Phase I escalation	39	Highly expressed in most pancreatic, biliary, colorectal, and other solid tumors	ORR = 0%	1+ : 1/39 (3%) 2+ : 5/39 (13%) 3+ : 32/39 (82%)	Could not be evaluated Low clinical activity
Cantuzumab mertansine (48)	CanAg (MUC1)	DM1	CanAg ⁺ advanced solid tumors	Phase I escalation	37	Highly expressed in most pancreatic, biliary, colorectal, and other solid tumors	ORR = 0%	1+ : 0/34 (0%) 2+ : 6/34 (18%) 3+ : 28/34 (82%)	Could not be evaluated Low clinical activity
Glembatumumab vedotin (49)	gpNMB	MMAE	Advanced (stage III and IV) melanoma	Phase II	62	Highly expressed in melanoma (~60%)	ORR = 11%; 6 PR, 1 CR	58/59 (98%) of tumors gpNMB-positive (>5% tumor epithelial cell staining) 45/59 (76%) of tumors had 100% gpNMB-positive epithelial cells	Differentiation between responders and nonresponders based on IHC scoring was not determined as the majority of patients (76%) had high levels of gpNMB.
Sactuzumab govitecan (50)	Trop2	SN38	Epithelial cancers	Phase I basket	178	Highly expressed in many epithelial cancers	ORR = 17%; 20 PR	Low = 16/150 (11%) Med/High = 123/150 (82%)	Differentiation between responders and nonresponders based on IHC scoring was not determined; the majority (82%) of patients had medium/high levels of Trop2.

Note: In 50% of the trials analyzed, there was a trend of association between response and expression.

Abbreviations: CR, complete response; gpNMB, glycoprotein NMB; HNSCC, head and neck squamous cell carcinoma; Nap12b, sodium-dependent phosphate transport protein 2B; NEC, neuroendocrine cancer; ORR, objective response rate; PBD, pyrrolbenzodiazepine dimer; PR, partial response; RP2D, recommended phase 2 dose; SCLC, small cell lung cancer; SN38, active metabolite of irinotecan; Top-1, topoisomerase 1 inhibitor; TNBC, triple-negative breast cancer; TRR, target-response relationship.

^aTarget expression from literature or preclinical information as cited by publication.

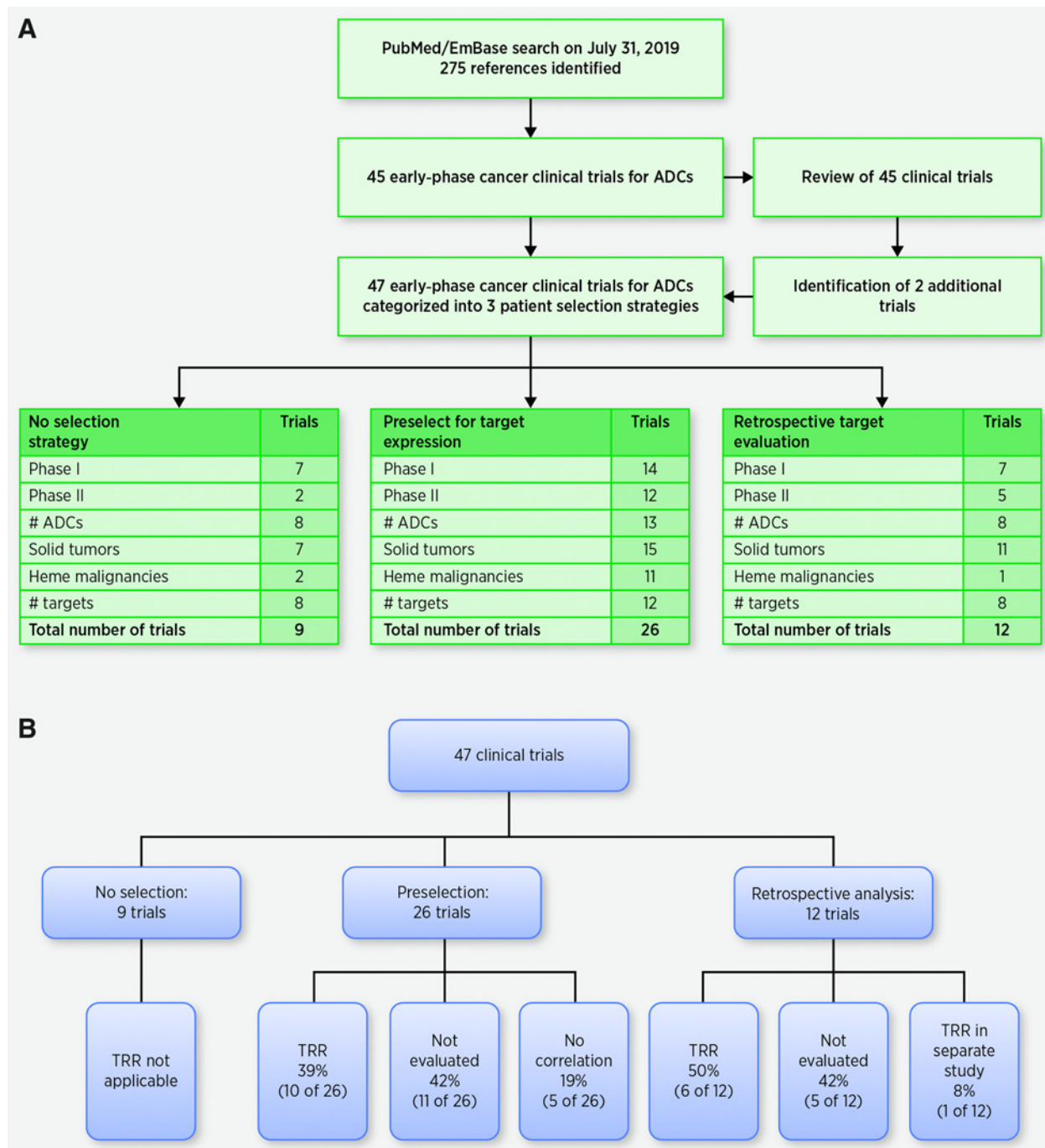


Figure 1.

Early (phase I and II) clinical trials used to evaluate the target–response relationship for ADCs. **A**, Trial characteristics. A total of 47 early-phase clinical trials were reviewed during this analysis and categorized into three patient selection strategies. **B**, Summary of target–response relationships. Approximately half of the trials employed a patient preselection strategy and just over 80% utilized preselection or retrospective analysis.

(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 pre-selected 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 2** (refs. 13–38). Of these, 9 trials involved targets being tested in a range of solid tumors, including anti-FR α 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 ($\geq 25\%$ cells positive) was only found in 27% 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 FR α 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.

The TRR for 11 of the trials in this category could not be assessed, due either to low clinical activity or lack of stratification. For SGN-75, an anti-CD70 MMAF ADC, ORR in RCC, and NHL was only 5% in each disease indication despite preselecting patients based on target expression. In this case, development of the molecule was terminated early due to lack of clinical activity. Moreover, no stratification of target expression was reported. Because all patients met the cutoff of >1% tumor cells, if patients are not stratified by target expression (e.g., low/med/high expression), the TRR cannot be assessed. This demonstrates the importance of retrospectively evaluating patients according to stratified categories to interpret the data.

In 5 of the 26 trials (19%), there was no apparent relationship between target expression and response. These include brentuximab vedotin in CD30⁺ DLBCL and PTCL, mirvetuximab soravtansine in FR α ⁺ ovarian, TAK-264 in GCC⁺ gastric cancer, and one trial with T-DM1 in HER2⁺ breast cancer. While the cause could not be determined, it was thought to be tumor heterogeneity and/or bystander activity.

In 6 of 26 trials (23%), a positive TRR was observed with either a higher ORR or responses (CR and PR) limited to patients with highest target expression. This included coltuximab ravtansine in CD19-positive B-NHL, DMOT4039A in patients with mesothelin-positive pancreatic and ovarian, glembatumumab vedotin in gpNMB-positive breast cancer, vadastruximab talirine in CD33-positive AML, mirvetuximab soravtansine in FR α -positive ovarian, and brentuximab vedotin in CD30-positive CTCL.

In the remaining 4 trials (15%), a very strong TRR was observed in 4 of 6 trials involving T-DM1 in patients with HER2-positive breast cancer. Although all patients were enrolled based on documented HercepTest positivity, accurate analysis of TRR was feasible in these studies due to retesting with HercepTest. In all T-DM1 trials, patients were diagnosed as HER2 positive and had received prior trastuzumab. However, most of this testing was done in local laboratories. To be eligible for the T-DM1 trial, patients were required to resubmit archival samples to a central laboratory for retesting. Despite having received a positive diagnosis using the FDA-approved HercepTest, the rate of HER2-negative patients assessed by the central read ranged from 12%–22% (18, 20, 21, 27). Similar discordance between testing results done locally versus centrally was observed with mirvetuximab soravtansine (17), demonstrating the risks of false positives and negatives that occur with diagnostics. Moreover, in these studies, HER2-positive status based on central retrospective assessment was associated with significantly higher ORR and longer progression-free survival (PFS) compared with HER2-negative status which points to the importance of understanding the limitations of assays for patient safety and efficacy reasons. This very strong relationship could be due to the relatively high HER2 expression levels (IHC2+/FISH+, IHC 3+) required for HercepTest positivity, and the well-characterized role of HER2 in tumor proliferation.

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 TRR 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 $\geq 2+$ 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 5 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 lifastuzumab 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 quantitate 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 TRR. However, as with preselection strategies, in several trials (5 of 12 trials; 42%), TRR could not be evaluated due to limited clinical activity or lack of stratification. For cantuzumab-mertansine (CanAg) and bivatuzumab 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 bivatuzumab 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 TRR was not reported (45). For gemtatumumab 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

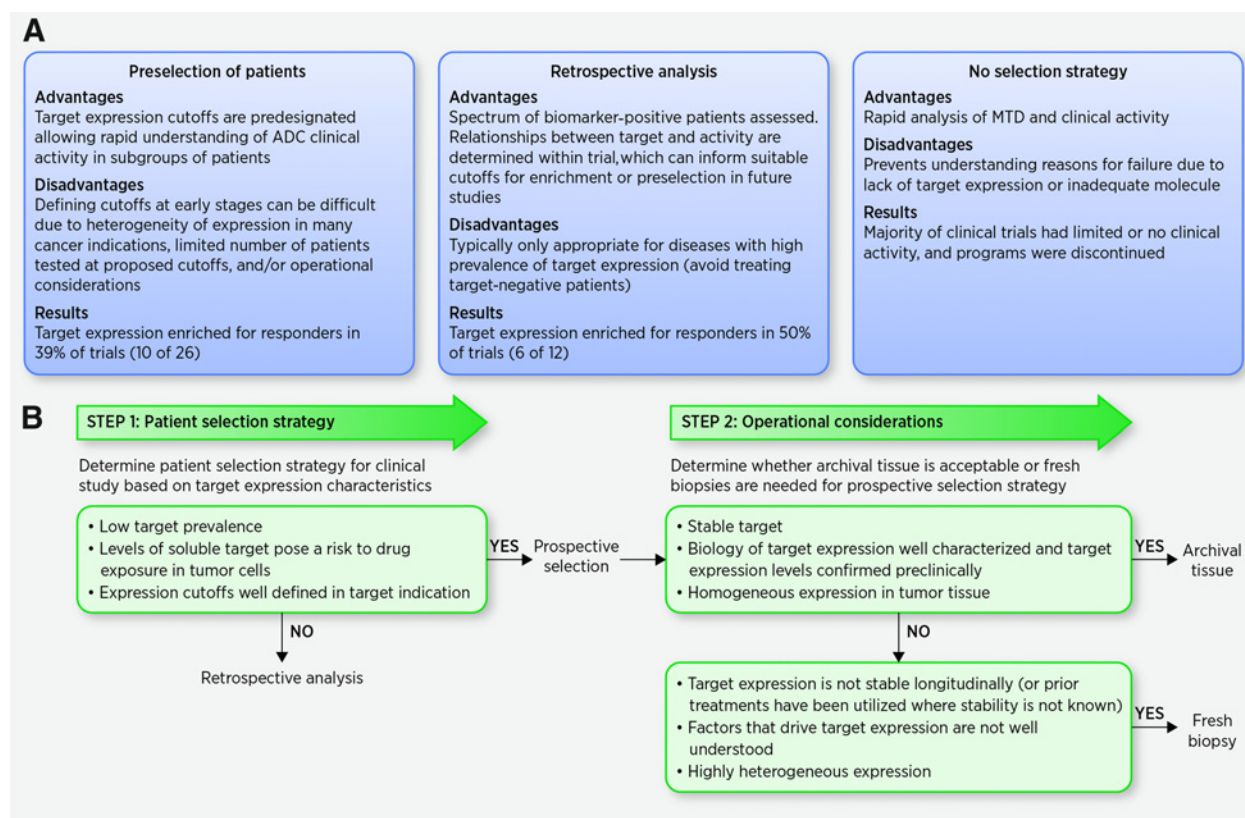


Figure 2.

Clinical development of ADCs. **A**, Patient selection strategies. The advantages, disadvantages, and TRR results are summarized for the three patient selection strategies. Decisions regarding selection strategy should be based on the advantages and disadvantages for each specific ADC program. **B**, Key translational decisions. Several factors should be considered when determining whether to preselect patients or employ retrospective strategies in early-phase clinical studies. Prospective selection of patients is recommended when there is a low prevalence of target or when target expression cutoffs have been well characterized in the disease indication for clinical studies. Levels of soluble target must also be considered as this may affect ADC exposure in patients. For retrospective analysis, the key risk is treating patients with insufficient target expression to mediate tumor cell killing. Thus, initial clinical development should focus on disease 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.

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. Initial trials can be conducted in tumor types with the highest prevalence, and relationships between target expression and clinical activity can be established in early stages of clinical development. If clinical activity is consistently higher in patients with elevated expression, enrichment or prospective selection of patients can be considered in subsequent trials.

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

compared with DLL3-positive patients (57–60). The phase III trial was placed on hold 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 FR α -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 FR α 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 FR α 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 anticipated 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 accomplished 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. Furthermore, levels of ADC target antigens per cell can vary significantly between ADC targets (62, 63) such that “low” expression of one target may 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 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 vedotin, and in some studies with mirvetuximab soravtansine and rovalpituzumab 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, relationships 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. Spreafico 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.

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