

Beyond Conventional Adcs: Engineering The Next Generation of Antibody–Drug Conjugates For Cancer Therapy

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Abstract

Antibody–drug conjugates (ADCs) are often optimized one component at a time even though clinical performance emerges from interactions among target density, internalisation, linker stability, payload mechanism, drug-to-antibody ratio (DAR), tumour penetration and resistance. This paper introduces the Multi-Parameter Adaptive ADC Engineering Framework (MAAEF), a prospective, falsifiable design-and-verification strategy for solid tumours with heterogeneous antigen expression. MAAEF combines dual-target recognition, tumour-responsive release, orthogonal dual payloads and site-specific DAR control in a seven-group factorial architecture. It pre-specifies construct-quality gates, antigen-stratified two-dimensional and three-dimensional assays, 12-cycle resistance evolution, molecular profiling and locked multi-objective model validation. Five normalized indices quantify linker selectivity, heterogeneity coverage, penetration, resistance suppression and safety; a composite score is permitted only after weight sensitivity and external validation. The framework's immediate output is not a claim of therapeutic superiority, but an auditable route for determining whether integrated co-engineering produces synergistic benefit beyond component-level gains. By separating design targets from observations and requiring efficacy and developability to pass independent advancement gates, MAAEF addresses a recurrent source of overclaiming in preclinical ADC research. The protocol is intended to support reproducible candidate ranking, transparent failure analysis and clinically relevant go/no-go decisions before in-vivo translation.

Keywords: antibody–drug conjugate; bispecific ADC; dual payload; tumour heterogeneity; linker engineering; site-specific conjugation; resistance; multi-objective optimization.

1. Introduction

ADCs couple antibody selectivity to a potent payload, yet their therapeutic window is governed by a system rather than by three isolated parts. Target abundance and internalisation affect delivered dose; conjugation and hydrophobicity shape aggregation and clearance; linker chemistry controls premature and intracellular release; payload permeability determines bystander killing; and tumour architecture limits distribution [1–6]. Recent reviews therefore describe next-generation ADC development as a coordinated engineering problem, not a search for maximal cytotoxicity alone [7–10].

Solid tumours expose the weakness of component-by-component optimization. Antigen expression varies within and between lesions, low-perfusion regions restrict macromolecular penetration, and selective pressure can promote antigen loss, altered trafficking, drug efflux or payload-specific resistance [11–15]. Bispecific targeting may preserve uptake across mixed antigen states, while dual payloads can reduce dependence on one lethal mechanism [16–19]. These features also create new liabilities: molecular complexity, uncertain payload stoichiometry, increased hydrophobicity and manufacturing heterogeneity [20–22].

The central gap is methodological. Published work richly describes targets, linkers, payloads and conjugation platforms, but provides no widely used, pre-specified framework that isolates their main effects, tests their interactions under controlled heterogeneity, and links efficacy to developability and safety [23–27]. MAAEF addresses that gap. The primary research question is whether coordinated dual targeting, responsive release, orthogonal payload activity and site-specific DAR control improve tumour coverage and resistance suppression without sacrificing product quality or off-target safety. The primary hypothesis is that the fully integrated construct will exceed the conventional control on both an efficacy/resistance domain and a safety/developability domain; superiority on only one domain is insufficient.

Table 1. Translational limitations and corresponding MAAEF design responses

Observed limitation	Design response	Falsifiable implication
Spatial antigen heterogeneity	Dual-target/bispecific recognition	Activity is preserved in A-low/B-high and mixed spheroids.
Premature systemic release	Tumour-responsive linker	Tumour-to-physiological release ratio increases without aggregation.
Single-mechanism escape	Orthogonal dual payloads	Resistance fold-change and surviving clones decline across serial exposure.
DAR heterogeneity	Site-specific conjugation	DAR variance, free payload and high-molecular-weight species decrease.
Potency–safety trade-off	Two-domain advancement rule	A candidate fails if either efficacy or developability gate is missed.



Figure 1. MAAEF architecture. Tumour biology constrains four coupled engineering layers that converge on therapeutic-index improvement.

2. Framework and Methods

2.1 Design logic and variables

MAAEF treats the ADC as a multi-objective system. Inputs include antigen density and heterogeneity, internalisation, trafficking, linker release, DAR, payload potency and permeability, penetration, off-target viability and resistance markers. The design does not assume that every maximum is desirable. Strong bystander activity, for example, may improve coverage but reduce normal-cell separation; high DAR may increase nominal potency while worsening hydrophobicity and clearance [2,5,28]. Candidate selection therefore uses constrained optimization rather than a single efficacy rank.

Table 2. Core variables and their decision roles

Domain	Variables	Decision role
Tumour biology	Antigen A/B density; mixed-cell fraction; internalisation; spheroid radius	Defines strata and prevents a high-antigen model from masking failure.
Product quality	Mean DAR and distribution; monomer; aggregate; free payload; plasma integrity	Mandatory developability gate before biological interpretation.
Release	Physiological and tumour-condition release; half-life; LSI	Separates circulation stability from intended activation.
Activity	IC50/IC90; apoptosis; clonogenic survival; bystander killing	Quantifies potency, depth and durability rather than IC50 alone.

Domain	Variables	Decision role
Resistance	RFC; antigen loss; ABC transporters; trafficking and death markers	Tests whether benefit persists under repeated selection.
Safety	Normal-cell viability; cytokine signal; target/off-target selectivity	Prevents efficacy-only advancement.

2.2 Comparative construct architecture

Seven constructs estimate the contribution of each intervention and selected two-way combinations (Figure 2). G1 is the conventional single-target, conventional-linker, single-payload, randomly conjugated control. G7 contains all four interventions. A matched isotype or non-binding ADC is included as a procedural control but is not ranked as an engineering group. Payload molar exposure is balanced across comparisons, and all constructs are produced in independent batches to avoid confounding architecture with one manufacturing lot.

Table 3. Seven-group comparative architecture

Group	Targeting	Linker	Payload	Conjugation	Purpose
G1	Single	Conventional	Single	Random	Reference control
G2	Single	Responsive	Single	Site-specific	Linker effect
G3	Dual	Conventional	Single	Site-specific	Target effect
G4	Single	Responsive	Dual	Site-specific	Payload effect
G5	Dual	Responsive	Single	Site-specific	Target × linker
G6	Dual	Conventional	Dual	Site-specific	Target × payload
G7	Dual	Responsive	Dual	Site-specific	Integrated candidate

Controlled Contribution and Interaction Design

Group	 Dual target	 Responsive linker	 Dual payload	 Site-specific conjugation
G1	Baseline	Baseline	Baseline	Baseline
G2	Baseline	✓ Included	Baseline	✓ Included
G3	✓ Included	Baseline	Baseline	✓ Included
G4	Baseline	✓ Included	✓ Included	✓ Included
G5	✓ Included	✓ Included	Baseline	✓ Included
G6	✓ Included	Baseline	✓ Included	✓ Included
G7	✓ Included	✓ Included	✓ Included	✓ Included

Figure 2. Factor-coverage matrix for G1–G7. Site-specific conjugation is held constant in engineered groups to reduce DAR-distribution confounding.

2.3 Biological models, replication and blinding

A solid-tumour system is eligible only if both targets are measurable, internalising and clinically relevant, and if resistant or slow-cycling models are available. Four isogenic antigen states are established: A-high/B-high, A-high/B-low, A-low/B-high and A-low/B-low. Mixed spheroids are assembled with pre-specified cellular proportions

and verified by quantitative flow cytometry before dosing. Treatment allocation, image analysis and endpoint extraction are blinded to construct identity. Randomisation is blocked by experiment date and spheroid size.

Table 4. Replication and analysis populations

Experiment	Independent units	Technical structure	Primary contrast
Product quality	3 manufacturing batches/group	Duplicate analytical injections	G7 vs G1; batch random effect
2D binding and activity	6 biological experiments/state	3 wells/experiment/dose	Architecture × antigen state
3D spheroids	10 spheroids/group/experiment; 3 experiments	Whole-spheroid imaging	G7 vs G1 in mixed models
Resistance evolution	6 independent lineages/group; 12 cycles	Duplicate dose–response curves	Treatment × cycle
Molecular profiling	6/group targeted; 5/group omics	Predefined QC and blinded pipeline	G1-resistant vs G7 cycle-12
External model validation	Locked independent dataset	No retuning after lock	Calibration and prediction error

2.4 Assays and quality gates

Constructs are characterised by orthogonal methods for DAR, aggregation, charge variants, hydrophobicity, free payload and plasma integrity. Binding and receptor occupancy are measured by quantitative flow cytometry; internalisation and lysosomal colocalisation use time-resolved microscopy. Release is assessed at pH 7.4 and under one or more mechanism-matched tumour conditions, such as acidic, proteolytic or reducing environments. Biological evaluation proceeds from 2D viability and apoptosis to clonogenic survival, bystander assays and mixed-antigen spheroids [29–31]. A failed product-quality gate stops biological ranking because apparent potency from unstable or free payload is not interpretable.

Table 5. Assays, endpoints and advancement requirements

Stage	Primary endpoints	Pre-specified requirement
Quality	DAR distribution, monomer, aggregate, free drug, plasma integrity	No critical out-of-specification result; batch CV acceptable.
Targeting	Kd, receptor occupancy, internalisation, lysosomal colocalisation	No collapse in either single-low state relative to dual-high.
Linker	Physiological release, tumour-condition release, LSI	Selective activation increases without premature release.
2D activity	IC50/IC90, apoptosis, clonogenic survival	Improvement is consistent across antigen states, not one cell line.
3D activity	Penetration depth, TPI, core viability, residual resistant cells	Coverage and killing improve in mixed spheroids.
Resistance	RFC, antigen change, transporter and pathway response	Cycle interaction favours G7 with lower clone survival.
Safety	Normal-cell viability, cytokine signal, selectivity ratio	Safety/developability gate passes independently of efficacy.

2.5 Pre-specified indices and advancement rule

Indices are calculated per biological unit before aggregation. Each is scaled to [0,1] using limits defined from controls and assay capability, not from the best-performing test construct. The ADC Therapeutic Engineering Score (ATES) is exploratory until weights and normalization limits are frozen and tested by sensitivity analysis. A high composite cannot compensate for a failed safety or product-quality gate (Figure 3).

Table 6. Operational indices

Index	Definition	Interpretation
LSI	Tumour-condition release ÷ physiological-condition release	Higher values indicate more selective activation.
HCI	1 – CV of normalized killing across antigen states	Higher values indicate robust heterogeneity coverage.
TPI	Median penetration depth ÷ spheroid radius	Higher values indicate deeper normalized distribution.
RFC	Cycle-12 IC50 ÷ baseline IC50	Lower values indicate slower resistance amplification.
RSI	1 – normalized RFC relative to G1	Higher values indicate greater resistance suppression.
SI	Normalized separation between tumour and normal-cell viability	Higher values indicate a wider cellular safety margin.
ATES	$0.20\text{HCI} + 0.20\text{LSI}^* + 0.20\text{TPI} + 0.15\text{potency}^* + 0.15\text{RSI} + 0.10\text{SI}$	Exploratory composite; starred terms are normalized.



Figure 3. Illustrative, pre-specified acceptance profile. Values are design gates, not experimental observations.

2.6 Statistical and computational analysis

Continuous endpoints are summarized with means and 95% confidence intervals when residual assumptions are tenable, otherwise with medians and bootstrap intervals. Two-way mixed models test architecture, antigen state and interaction; experiment and batch are random effects. Serial resistance uses treatment, cycle and their interaction with lineage-specific random intercepts and slopes. Dose–response curves use four-parameter nonlinear models. Omics analyses control the false-discovery rate at $q < 0.05$. Effect sizes and compatibility intervals are primary; exact p-values are secondary. Missingness, exclusions and outliers are reported by group.

For prediction, molecular and biological features are processed inside nested cross-validation. Candidate algorithms include elastic net, gradient boosting and random forest, compared with a prespecified linear baseline. The final pipeline is locked before independent validation. Regression performance includes R^2 , RMSE, MAE and calibration slope; classification includes AUROC, precision–recall AUC, sensitivity, specificity, F1 and calibration.

Permutation testing assesses signal beyond chance. Explainability is used to audit model behaviour, not to infer causality [32–35].

Table 7. Analysis plan and reporting standards

Question	Model/analysis	Minimum report
Does targeting resist heterogeneity?	Mixed model: architecture × antigen state	Interaction estimate, 95% CI, adjusted contrasts
Is release selective?	Mixed model on log release ratio	LSI, CI, physiological and tumour release separately
Is 3D performance improved?	Hierarchical model by spheroid and experiment	TPI, core viability, residual cells, effect size
Is resistance delayed?	Repeated-measures mixed model	Treatment × cycle, RFC, lineage trajectories
Are pathways altered?	Predefined contrasts and enrichment	Effect size, FDR q, pathway direction
Does the model generalize?	Nested CV plus locked external validation	Calibration, error/discrimination, uncertainty
Is ranking robust?	Weight and normalization sensitivity	Rank stability and gate failures

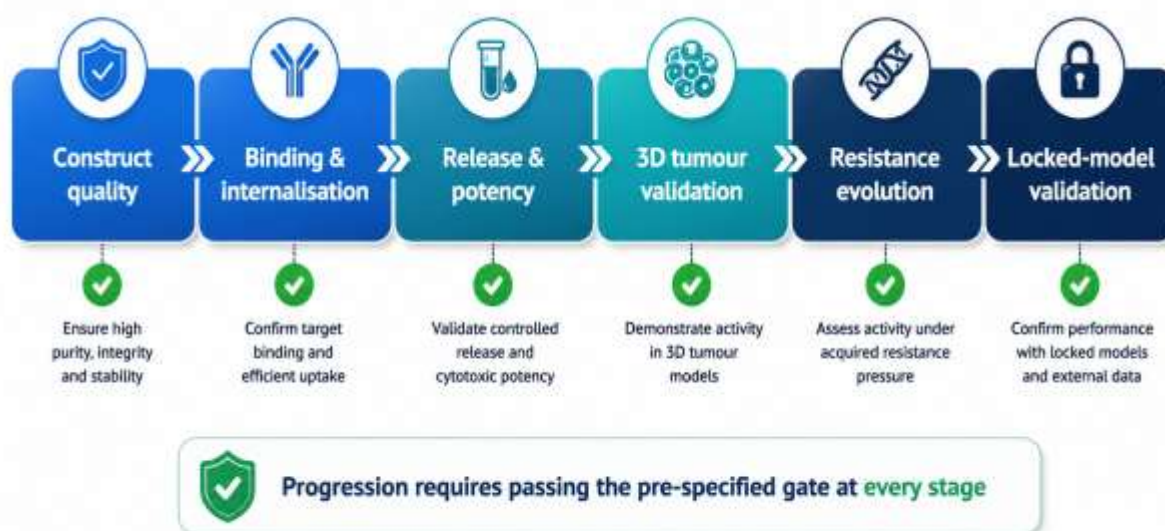


Figure 4. Stage-gated experimental and computational verification workflow.

3. Framework Outputs and Verification Logic

The framework yields three auditable outputs. First, it identifies whether an apparent G7 advantage is attributable to one dominant component or to interaction. G2–G6 are therefore mechanistic comparators, not decorative controls. Second, it distinguishes broad activity from selective potency: a construct that performs only in A-high/B-high cells fails the heterogeneity objective even if its pooled IC₅₀ is low. Third, it integrates development risk into candidate ranking. Figure 5 formalizes this principle: advancement requires evidence in both efficacy/resistance and safety/developability domains.

The strongest confirmatory pattern would include: reproducible site-specific product quality; preserved binding and internalisation in both single-low antigen states; low physiological but high tumour-condition release; deeper 3D distribution with reduced core viability; and a smaller increase in RFC across serial exposure. These are expected signatures, not results. Conversely, premature release, loss of normal-cell separation, unstable dual-payload stoichiometry or model failure in external validation would refute the integrated architecture regardless of potency.

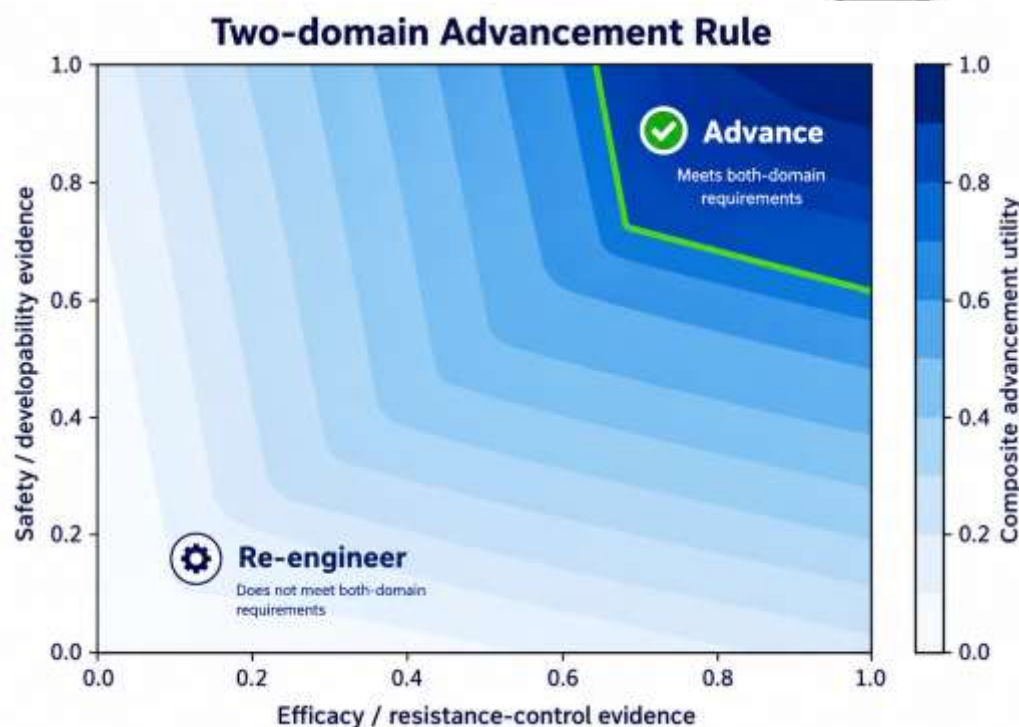


Figure 5. Two-domain decision surface. The advancement contour is conceptual and must be populated with observed, uncertainty-aware evidence.

Table 8. Claim boundaries and evidence required for a full results paper

Claim	Current status	Evidence required before assertion
G7 improves potency	Hypothesis	Raw dose–response data across all antigen states and independent repeats.
Responsive release is selective	Hypothesis	Matched physiological/tumour release kinetics with mass balance.
Resistance is suppressed	Hypothesis	Independent lineage trajectories through 12 cycles and molecular corroboration.
Therapeutic index is widened	Hypothesis	Efficacy plus normal-cell, PK and in-vivo tolerability evidence.
ATES predicts candidate quality	Method proposal	Frozen weights, sensitivity analysis and independent validation.
MAAEF enables rational ranking	Framework contribution	Prospective use with transparent failures and replication.

4. Discussion

MAAEF converts a broad next-generation ADC concept into a testable program. Its main contribution is not the addition of more assays, but the alignment of each claim with a comparator, a unit of replication and a failure rule. This is important because dual targeting, responsive linkers, dual payloads and site-specific conjugation each have independent supporting evidence [16–22], yet combining them can introduce non-additive manufacturing and safety risks. The seven-group structure makes those trade-offs visible.

The framework also narrows the common gap between 2D potency and solid-tumour performance. Antigen-stratified models and mixed spheroids prevent averaging from concealing a vulnerable subpopulation. Normalized penetration, core viability and residual resistant cells connect spatial delivery to function. Serial exposure then asks whether early potency remains durable. These features respond directly to current concerns about heterogeneity, bystander effects and acquired resistance [11–15,29–31].

There are limitations. A seven-group program is resource intensive, and not every target pair permits clean isogenic modelling. Tumour-associated linker triggers can overlap with normal physiology. In-vitro safety indices cannot substitute for pharmacokinetics, tissue cross-reactivity or toxicology. Composite scores may create false precision if normalization and weighting are fitted after results are known. MAAEF therefore treats ATES as exploratory, requires weight sensitivity, and prevents compensation across independent gates. External validation is essential for any predictive claim.

A complete empirical paper should replace every planning threshold with raw-data-derived estimates, archive code and analysis decisions, and report deviations from this protocol. Negative findings remain informative: they can locate failure in target coverage, release control, payload compatibility, conjugation quality or resistance biology. That diagnostic value is a practical advantage over a single optimized-versus-control comparison.

5. Conclusion

MAAEF provides a rigorous route for evaluating next-generation ADCs as integrated therapeutic systems. It combines controlled component comparisons, heterogeneity-aware models, stage-gated product quality, 3D and resistance validation, and locked multi-objective analysis. The framework supports a strong Q2-level methodological contribution because it is specific, falsifiable and transparent about the boundary between design targets and observations. Its central proposition remains to be tested: coordinated engineering should advance only when gains in tumour coverage and resistance control are accompanied by acceptable safety and developability.

Declarations

Ethics approval and consent to participate: Not applicable to this methodological framework; future animal or patient-derived work requires prospective institutional approval. Consent for publication: Not applicable. Data availability: No experimental dataset was generated for this framework paper. Competing interests: None declared. Funding: No specific funding was reported. Author contributions: Withheld from the blinded manuscript.

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