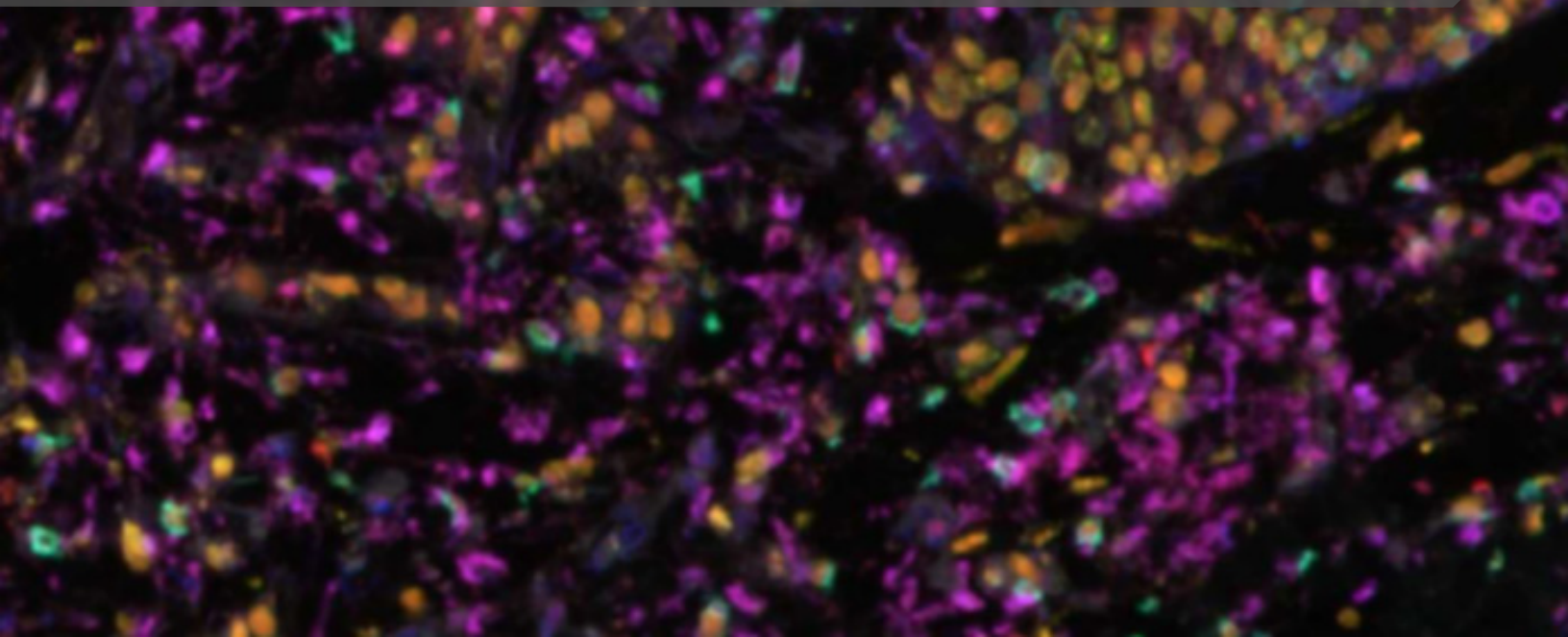
Aliri BIOANALYSIS

WHITE PAPER

Rethinking Biomarker Strategies for Antibody–Drug Conjugates

Using Spatial Biology to Decode Productive ADC Activity in Solid Tumors



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EXECUTIVE SUMMARY

Antibody–drug conjugates (ADCs) have transformed oncology and represent one of the fastest-growing therapeutic modalities in cancer drug development. Advances in antibody engineering, linker chemistry and payload design have expanded the clinical success of ADCs, leading to numerous approvals and a rapidly growing development pipeline. Yet despite these advances, predicting which patients will respond remains a major challenge.

Historically, biomarker strategies have focused on target expression. While antigen expression is essential for ADC binding, recent studies consistently show that it does not fully explain therapeutic outcome. Tumors with similar antigen expressions frequently exhibit markedly different responses, suggesting that additional biological mechanisms determine productive ADC activity.

These observations suggest that ADC efficacy depends on a broader biological context in which target biology, delivery and intracellular processing, payload susceptibility, and the tumor microenvironment interact. Drug molecules must reach the tumor, penetrate heterogeneous tissue architecture, access target-expressing cells, internalize and traffic productively, release an active payload, and encounter tumor cells that are susceptible to its mechanism of action. At the same time, vascular organization, stromal barriers, immune composition, macrophage interactions, hypoxia, and local cell states can influence ADC penetration, processing, and response. These processes are interdependent, yet they are rarely evaluated together.

The key translational question is therefore not simply whether the target is present, but whether the sequence of biological events required for productive ADC activity occurs within the same tumor regions.

This white paper explores how biomarker strategies for ADCs are evolving from isolated measurements toward integrated biological evidence. Rather than presenting individual technologies, it illustrates how spatially connecting drug distribution, target engagement, pharmacodynamic response, and tumor microenvironment can identify the biological bottlenecks driving sensitivity or resistance and ultimately support development decisions across preclinical and clinical programs.

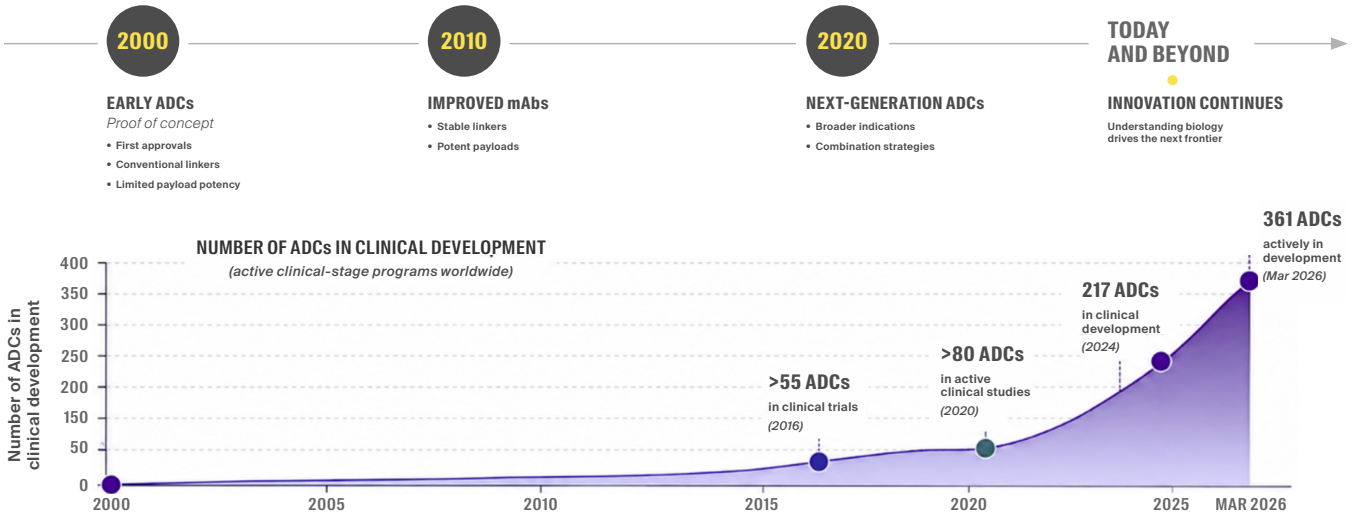

FIGURE 1. The evolution of ADC development: Continuous innovation in antibody engineering, linker chemistry and payload design has transformed ADCs into one of the fastest-growing therapeutic classes in oncology. While engineering continues to improve molecule design, understanding the biological determinants of response has emerged as the next major challenge for translational biomarker development.

The ADC Revolution: Engineering Has Outpaced Biology

Few therapeutic modalities have evolved as rapidly as antibody–drug conjugates. Over the past decade, improvements in antibody specificity, linker stability, conjugation technologies, and highly potent payloads have significantly expanded both the number of approved ADCs and the range of tumor indications under investigation.

These advances have fundamentally changed expectations for targeted therapies. New-generation ADCs have demonstrated clinical benefit in patient populations previously considered unlikely to respond, challenging traditional assumptions regarding target expression and patient selection. At the same time, pharmaceutical companies continue to develop increasingly sophisticated molecules incorporating novel payloads, dual mechanisms of action, and improved pharmacological properties.

Despite these achievements, one important challenge remains.

Engineering innovation has progressed remarkably, yet our understanding of biology governing therapeutic response has not advanced at the same pace. Clinical experience consistently demonstrates that apparently similar tumors may respond very differently to the same ADC. Understanding why this occurs has become one of the most important questions in translational oncology.

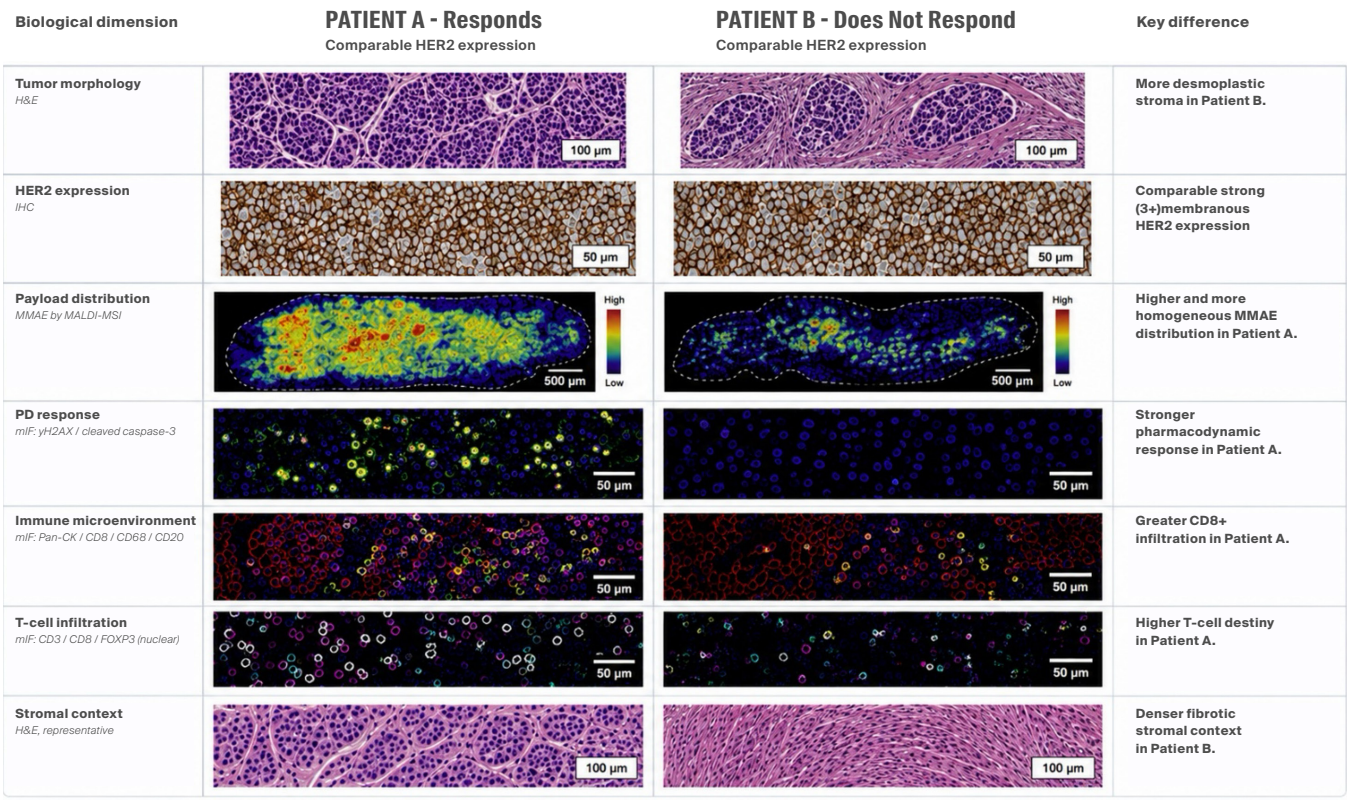

FIGURE 2. Target expression alone rarely explains therapeutic response. Tumors with comparable antigen levels may differ substantially in tissue architecture, drug penetration, target accessibility, internalization and trafficking, payload release and susceptibility, pharmacodynamic activation, and immune organization. The tumor microenvironment can influence several of these steps and may itself be remodeled by ADC-induced tumor-cell death. These biological differences contribute to heterogeneous responses observed clinically.

Are We Measuring the Right Biology?

Current biomarker strategies provide valuable information. However, they were largely developed to answer individual biological questions rather than explain the complete mechanism underlying therapeutic response.

Immunohistochemistry determines whether the therapeutic target is expressed. Pharmacokinetic analyses describe systemic drug exposure. Transcriptomic profiling characterizes gene expression, while pharmacodynamic biomarkers confirm that biological activity has occurred. Each measurement contributes important insight into ADC biology.

The limitation is not the quality of these biomarkers; it is that they are typically interpreted independently. ADC activity depends on multiple biological processes occurring within the same tumor. Measuring only one component provides an incomplete understanding of why treatment succeeds or fails.

An additional challenge is that these processes operate across different spatial scales. Target binding and intracellular processing occur at the cellular and subcellular level, while payload diffusion, stromal barriers and bystander effects operate across cellular neighborhoods. Antigen heterogeneity, vascular organization, immune composition and stromal architecture extend across larger tumor regions. Importantly, the tumor microenvironment is not simply a downstream consequence of ADC activity: it can influence tissue penetration, local processing and payload susceptibility, while ADC-induced cell death can in turn remodel immune and stromal organization. Understanding ADC activity therefore requires not only measuring these processes but resolving how they interact spatially within the same tissue.

Rather than asking whether a single biomarker predicts response, the more relevant question becomes: How do these biological processes work together to determine therapeutic outcome? This represents an important evolution in biomarker thinking. The objective is no longer simply to measure biology, but to connect biological evidence in a way that better explains clinical response.

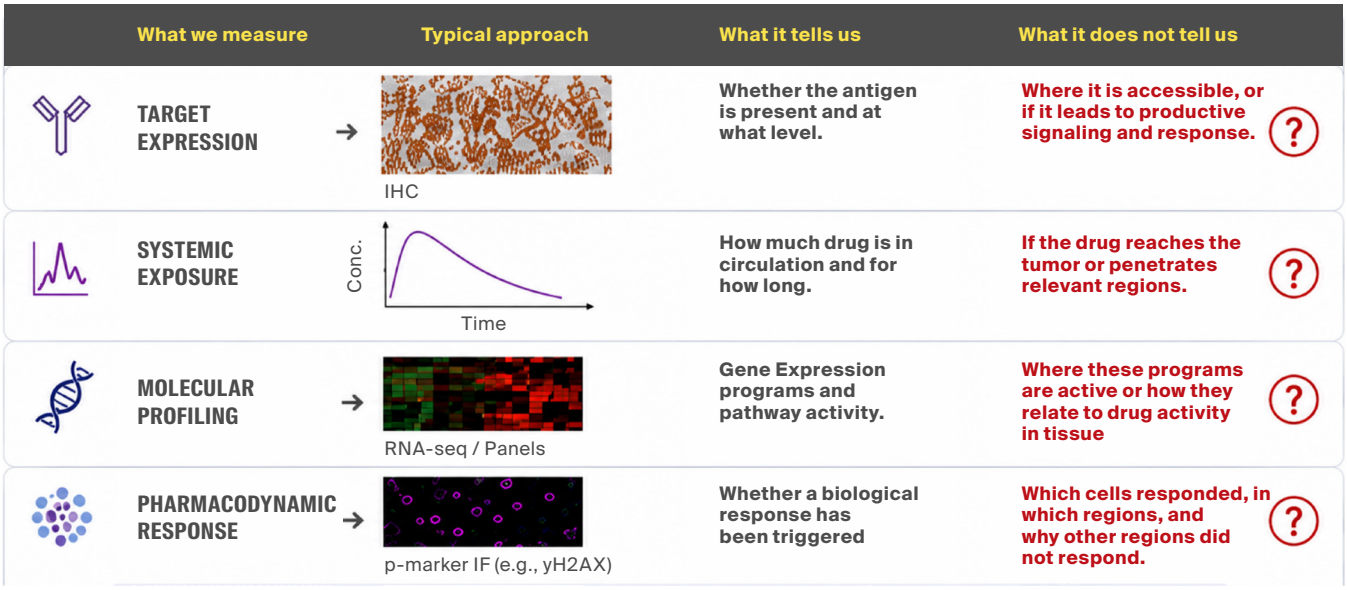

FIGURE 3. Conventional biomarkers each provide valuable but partial information. Integrating these complementary observations offers a more comprehensive understanding of the biological processes underlying ADC efficacy.

Connecting the Biological Dot

Therapeutic response is not determined by a single biological event. For an ADC to be effective, multiple processes must align within the same tumor regions. These can be considered across four interacting dimensions: target biology (expression, accessibility and heterogeneity); delivery and processing biology (binding, internalization, trafficking, linker processing and payload release); payload biology (mechanism of action, intrinsic susceptibility, proliferation state, drug efflux and resistance pathways); and tumor and immune context (vasculature, stromal barriers, immune composition, macrophage interactions, hypoxia and spatial architecture).

A weakness in any one of these dimensions can become a bottleneck. Importantly, the tumor microenvironment interacts with the ADC process throughout rather than occupying a single downstream position. It can shape regional exposure and cellular susceptibility, while ADC-induced tumor-cell death may subsequently alter immune and stromal organization. Likewise, ADC resistance does not necessarily indicate delivery failure: a tumor may receive and process an ADC efficiently yet remain intrinsically insensitive to the released payload. Connecting these complementary layers of evidence therefore provides a more complete picture of tumor biology than interpreting individual measurements in isolation.

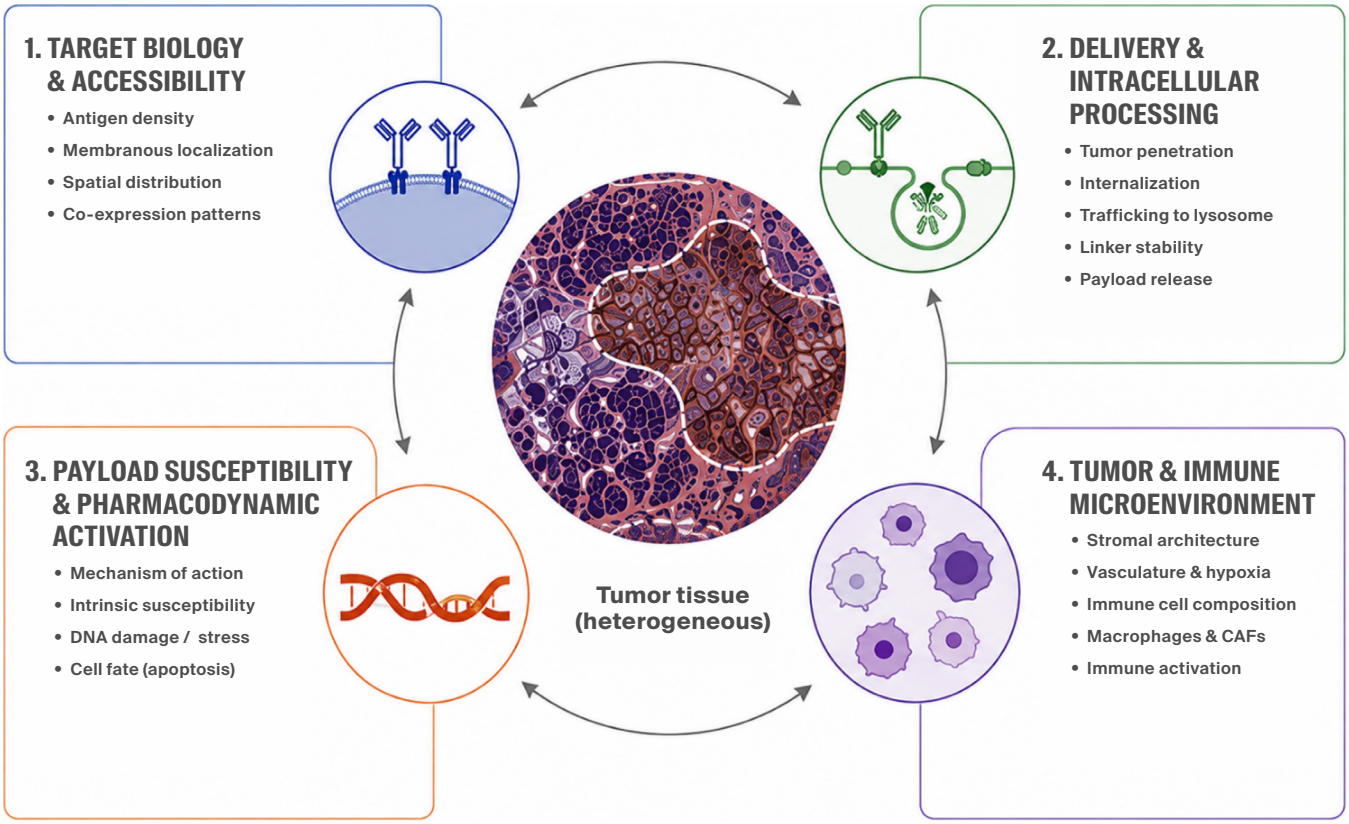

FIGURE 4. ADC response emerges from the spatial interaction of four biological dimensions within intact tumor tissue: target biology, delivery and intracellular processing, payload susceptibility, and tumor and immune context. The tumor microenvironment can influence ADC penetration, processing and response, while ADC activity can in turn remodel local immune and stromal organization. Integrating these dimensions provides a more comprehensive understanding of response and resistance than any individual biomarker alone.

From Biological Questions to Development Decisions

Recent spatial technologies do more than generate additional biological data they enable researchers to answer specific translational questions at the tissue level.

Each technology contributes evidence about a different step of productive ADC activity. Mass spectrometry imaging identifies whether the payload reaches tumor regions. Multiplex spatial imaging determines whether target-positive cells are accessible and whether pharmacodynamic activity has occurred. Spatial transcriptomics reveals pathway activation, cellular states, and resistance programs, while AI-driven spatial analytics integrates these measurements into functional tissue neighborhoods.

The value of these approaches lies in their combination. Instead of producing independent biomarker readouts, they help identify where the biological process breaks down: insufficient delivery, limited target accessibility, ineffective payload activity, or an unfavorable tumor microenvironment. These spatial bottlenecks provide actionable evidence for patient selection, mechanism confirmation, combination strategies, and Go/No-Go development decisions.

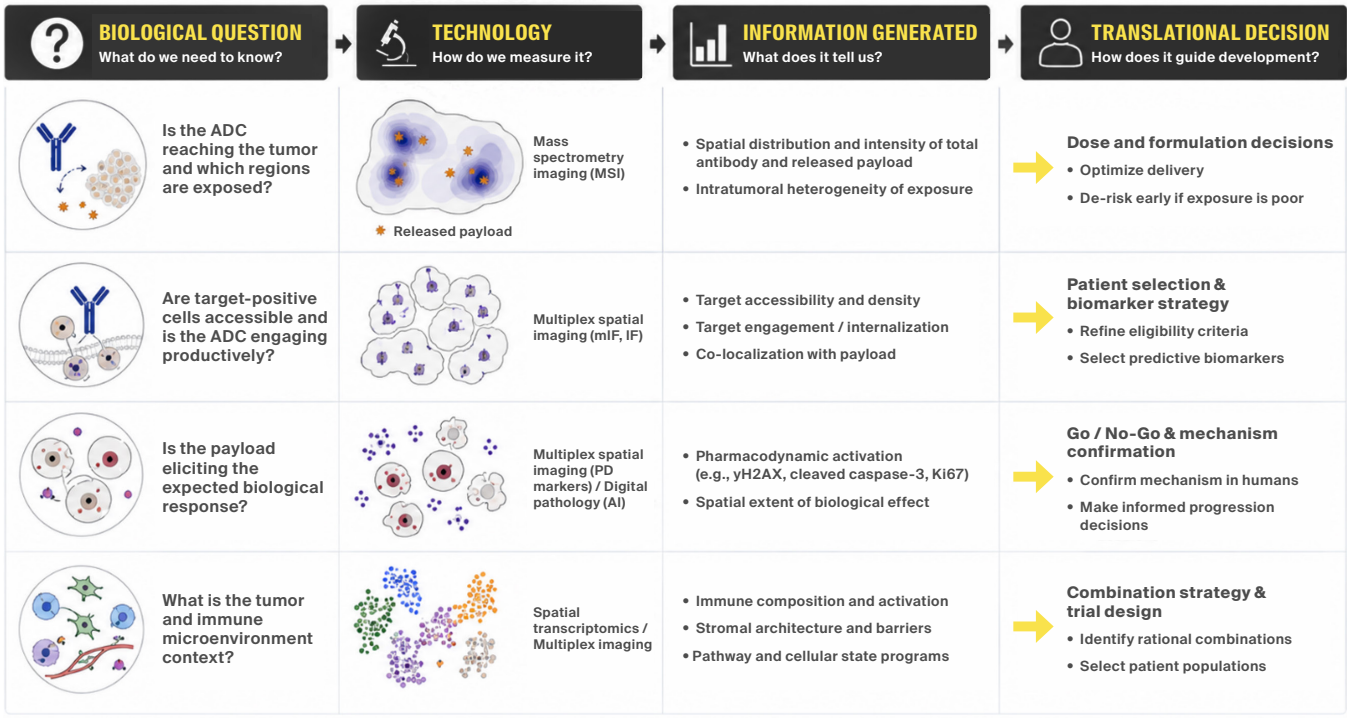

FIGURE 5. From Biological questions to development decisions: How spatial technologies generate actionable evidence across ADC development.

From Multimodal Data to Integrated Spatial Evidence

The value of spatial biology for ADC development lies not in any single measurement, but in the ability to connect multiple biological events within the same tissue context. Productive ADC activity requires a sequence of events - tumor exposure, target accessibility and engagement, intracellular processing and payload release, followed by the expected pharmacodynamic response that must occur within the same tumor regions. These events are further influenced by the surrounding stromal and immune microenvironment.

Multimodal spatial approaches provide complementary information across this sequence. Mass spectrometry imaging can map regional payload exposure, multiplex spatial imaging can resolve target accessibility and pharmacodynamic response, and spatial transcriptomic or proteomic approaches can characterize cellular states and the tumor microenvironment. The critical step is therefore not simply to generate these measurements, but to integrate them spatially so that their relationships can be evaluated within corresponding tissue regions.

This integrated view makes it possible to distinguish regions in which the biological sequence is aligned from those in which it is interrupted. Target-positive regions with limited payload exposure may indicate a delivery limitation, whereas regions with adequate exposure but weak pharmacodynamic activation may suggest intrinsic payload resistance. Other regions may reveal stromal or immune contexts associated with restricted penetration or response. Importantly, these mechanisms may coexist within the same heterogeneous tumor.

Spatial integration therefore moves biomarker interpretation from parallel measurements toward mechanistic evidence. By identifying where key biological events co-occur - and where they diverge - integrated spatial analysis can provide a stronger basis for mechanism confirmation, patient stratification, combination strategies and informed development decisions.

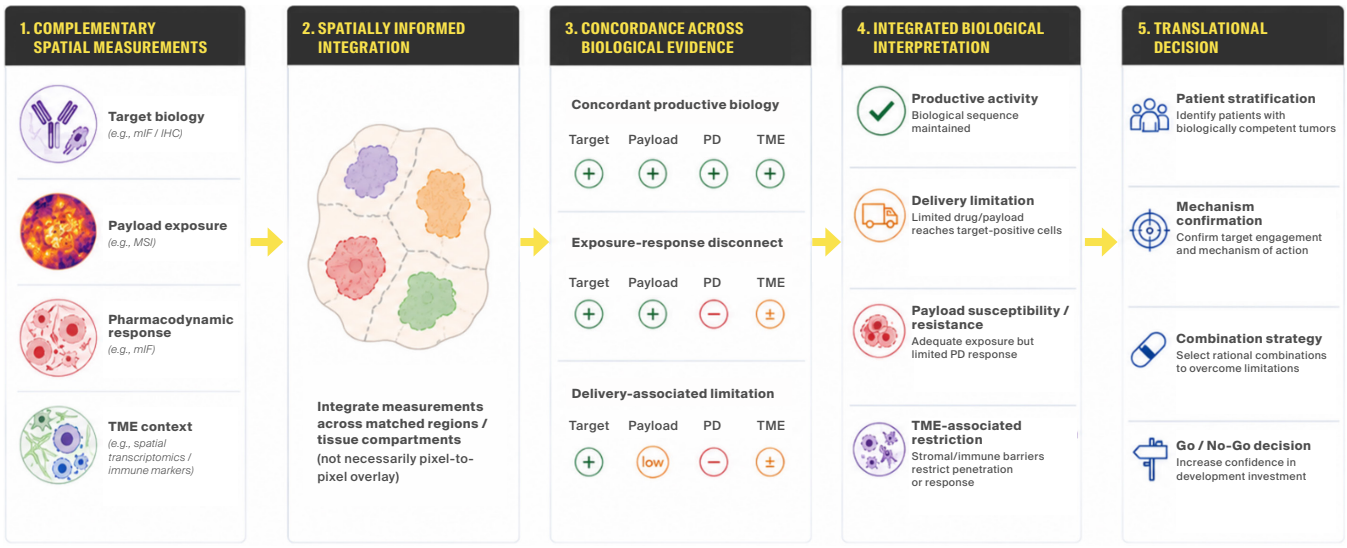

FIGURE 6. Integrating complementary spatial evidence across ADC biology. Spatial assays do not necessarily need to be directly overlaid to provide integrated biological evidence. Depending on assay resolution and tissue availability, complementary measurements can be related through image registration, serial-section analysis, or matched tissue regions and compartments. Evaluating concordance between target biology, payload exposure, pharmacodynamic response and tissue context can reveal where productive ADC activity is maintained or disrupted and support mechanism-informed development decisions.

CONCLUSION

Antibody–drug conjugates continue to redefine the landscape of targeted oncology therapies. As molecular engineering advances, the next opportunity for innovation lies in improving our understanding of the biology that determines therapeutic response.

Future biomarker strategies are unlikely to rely on a single predictive measurement. Instead, they will increasingly integrate complementary sources of biological evidence to understand how target biology, ADC delivery and intracellular processing, payload susceptibility, tissue architecture, and the tumor microenvironment collectively influence treatment outcome. Importantly, the microenvironment should be considered both as a determinant of ADC activity and as a compartment that may be remodeled by treatment.

Ultimately, the objective is not to generate more biomarkers, but to identify the spatial biological bottlenecks that determine whether an ADC can produce productive activity within individual tumors. By connecting complementary layers of evidence, spatial biology provides mechanistic insight that can improve patient stratification, strengthen translational confidence, and support more informed development decisions from early discovery through clinical development.

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