

Understand ADC Performance with CellCarta's Precision Assays

Addressing the Challenges of ADC Development

Antibody-drug conjugates (ADCs) are a class of targeted cytotoxic therapies that are changing the landscape of cancer treatment. They have revitalized well-established cancer biomarkers, like HER2, and unleashed the potential of any cancer-associated antigen as an ADC target.

While establishing the effectiveness and safety profile of ADC drugs, researchers and clinicians must address critical and challenging questions as their clinical trials progress.

- How and to what extent must the ADC target be expressed on cancer cells?
- How effectively does the ADC kill target cells?
- Does the ADC have on-target, off-tumor effects?
- Does the ADC trigger immune activation?

By addressing these, and other questions, developers can optimize target recognition and engagement, refine patient stratification, and mitigate safety risks — ultimately improving ADC efficacy.

CellCarta offers a wide range of platforms and assays that provide a systematic, data-driven approach to assess and understand ADC behaviour in the clinic. Our services can help you build a comprehensive picture of the factors that impact ADC performance, from target expression level and density, to characterization of the tumor microenvironment and immune status in the periphery, so you can streamline development and understand clinical outcome.

Optimizing Target Engagement

» Confirming Target Expression

Confirmation of ADC target expression enables you to determine potential ADC engagement, which is a prerequisite for efficacy. ADC target expression analysis is an essential element of patient selection and stratification, companion diagnostic (CDx) development, and the evaluation of off-target effects.

A multiplatform approach for reliable target expression measurement

CellCarta provides a comprehensive suite of solutions to deliver precise and reliable target expression data for a range of sample types. Our team can support the development of your biomarker strategy, from exploratory endpoint all the way to CDx.

- **Target expression in solid tumor** using immunohistochemistry (IHC) and immunofluorescence (IF)
- **Target expression assessment in the periphery, or biopsy samples**, using flow cytometry
- **Quantitative, reagent-free target detection** using mass spectrometry, supporting quantification in the early stages of development where affinity reagents might be unavailable
- **Evaluation of gene and target expression correlation** using PCR
- **Deep transcriptomic analysis** for a better understanding of all pathways triggered by the ADC, using bulk and single-cell RNA sequencing.

» Measuring Target Density

While the presence of an ADC target is indicative of ADC therapy suitability, variability in the density and pattern of expression can impact drug internalization, retention, and overall therapeutic response.

The advent of ADC therapies is shifting traditional scoring paradigms. IHC staining and scoring of HER2 has been used as a surrogate for *in situ* hybridization (ISH) staining to identify HER2 gene amplification. These same HER2 IHC assays are now being deployed to determine the presence of HER2 protein on tumor cells, which requires a new and different approach to scoring designed to detect any expression of the HER2 ADC target.

Advanced quantification of target density

CellCarta has developed and validated both visual pathologist scoring methods and semi-quantitative HALO image analysis algorithms that measure the expression of ADC targets, like HER2, on a continuous scale, as opposed to traditional ASCO CAP categories. Both the pathologist and AI algorithms have been developed to assess:

- The percentage of tumor cells that express the ADC target
- The heterogeneity of this expression within the tumor area
- The sub-cellular compartment where the target is expressed (nucleus, cytoplasm, and cell membrane)
- The overall intensity of expression, and/or the intensity of expression in each sub-cellular compartment

Depending upon the study requirements, our pathologists' analysis and image analysis algorithms for detecting ADC targets can be validated to support exploratory, secondary and primary endpoints, patient stratification, and patient selection. Our mIF and mIHC assays can also be used to help detect the downregulation of ADC targets as a potential resistance mechanism.

Our unified approach to assay development and global quality management system ensures that clients receive consistent reproducible scoring results regardless of where the IHC staining and algorithms are deployed.

» Assessing Drug-Target Binding

Even when target density is sufficient, ADCs may not be as efficient as expected. Through several techniques, including measuring downstream consequences of ADC binding, we indirectly assess binding efficiency, internalization, and payload activity.

Custom solutions for binding assessment

We can assess:

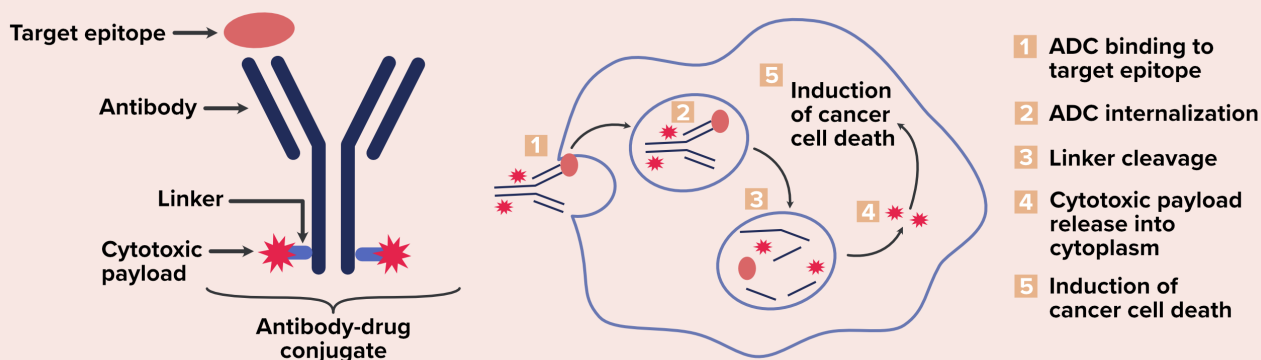
- **The extent of ADC target engagement** through flow cytometry receptor occupancy (RO) assays for non-solid tumors.
- **Downstream analysis** of transcriptional changes at the bulk (RNAseq) and/or single-cell levels (CITE-seq) to enable a better understanding of ADC binding and activity.

» Assessing ADC Presence and Stability

When it comes to assessing the pharmacokinetics of ADCs, affinity-based capture methods are the gold standard. However, they might be unavailable, due to a lack of capture reagent, or deliver inadequate sensitivity and specificity, due to the structural complexity of the ADC.

Our approach for sensitive detection

We developed a **mass spectrometry**-based method that is affinity reagent-free, making it easy to deploy and suitable for all types of molecules in pre-clinical study. This method also matches the sensitivity and specificity of hybrid ligand-binding assays (LBA) and liquid chromatography-mass spectrometry (LC-MS) assays.



Investigating Beyond the Target

» Monitoring the Tumor Microenvironment (TME)

Profiling the TME is crucial to understanding why some patients, and some tumor types, may or may not respond to ADCs. The TME is a highly complex environment, with interactions between stromal barriers, myeloid-derived suppressor cells (MDSCs), and hypoxic regions impacting therapeutic response.

In-depth TME monitoring for ADC troubleshooting

CellCarta offers expertise across multiple platforms for comprehensive TME analysis:

- **Evaluating the TME immune cell infiltration**, including the measurement of T-cell exhaustion

and activation by flow cytometry, identification of broad immune cells by multiplex IF and multiplex IHC, and in-depth transcriptomic and protein expression of immune response with CITE-seq and bulk RNAseq.

- **Investigating the spatial distribution of immune cells** as well as cells expressing the target of interest in the TME through advanced spatial biology technologies (Visium, GeoMx, COMET). The comprehensive analysis includes both transcriptomic and proteomic profiling, as well as high-resolution mapping.

Our IHC biomarkers allow for efficient assessment of ADC relevant targets

B7H3	CD19	CD79b	cMET	FOLR1	Nectin-4
B7H4	CD22	CEACAM5	DLL3	FOLRa	TROP2
BCMA	CD30	Claudin - 18	EGFR	HER2	

Assessing ADC Safety

» Through Targeted Toxicity Assessment

To understand, and eventually minimize, unintended effects on healthy tissues, developers must be able to precisely assess **on-target, off-tumor ADC toxicity**.

Our mIF and mIHC platforms can **evaluate target expression in normal tissues**, helping to identify potential safety concerns early.

If cells in the periphery express the target, flow cytometry can be used to **monitor absolute counts of circulating target-expressing cells**, and the read-out can be used as a surrogate for off-target killing if there is a decrease in target-expressing healthy cell counts.

Toxicity pathways can also be investigated by bulk RNA sequencing and downstream gene set enrichment analysis (GSEA), which will show if some of these pathways are up or down regulated.

CellCarta conducts all safety assessments using **CAP/CLIA-accredited laboratories**, ensuring the highest standards for regulatory compliance.

Partner with CellCarta to Streamline your ADC Development

In addition to our extensive ADC-specific expertise and capabilities, we also offer several additional benefits to further support your ADC development:



Comprehensive sample logistics services

We can take care of the handling, transport, and storage of blood samples and fresh biopsies, ensuring a worry-free sample management process.



A global footprint

With facilities in Canada, USA, Belgium, Australia and China, we have a truly global presence, strengthened by local expertise.



Companion diagnostic expertise

We have extensive experience in developing companion diagnostics (CDx), supported by deep regulatory knowledge, so can aid the transition of assays into CDx applications.



CellCarta ADC Precision Assays

Target Expression	
Target expression in solid tumor	IHC, IF
Target expression in the periphery, or biopsy samples	Flow cytometry
Reagent-free target detection	Mass spectrometry
Transcriptomic analysis of target pathways	Bulk and single-cell RNA sequencing
Target Density	
Measuring patterns of expression with advanced quantification	IHC, pathologist scoring and AI image analysis algorithms
Drug-target Binding	
Assessing ADC target engagement	Flow cytometry (RO assays)
Assessing downstream transcriptomics	Single-cell RNA sequencing
ADC presence and stability	
Pharmacokinetics (PK)	Mass spectrometry
Tumor Microenvironment (TME)	
Evaluating the immune response in the TME (presence and activity)	Flow cytometry, CITE-seq
Spatial distribution of the immune cells in the TME	IHC, Visium, GeoMx, COMET
ADC safety	
Evaluating on-target, off-tumor toxicity	mIF, mIHC, flow cytometry, RNA sequencing

Ready to streamline your ADC development?

Get in touch today to discuss your project and how our tailored solutions can support clinical success.

BR_ADC_2025-14-04



ABOUT CELLCARTA

Leading provider of specialized precision medicine laboratory services to the biopharmaceutical industry. Leveraging its integrated analytical platforms in immunology, histopathology, proteomics and genomics, as well as related specimen collection and logistics services, CellCarta supports the entire drug development cycle, from discovery to late-stage clinical trials. The company operates globally with 9 facilities located in Canada, USA, Belgium, Australia and China.

For more information on how CellCarta can partner with you, please contact us:

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