An Integrated Human Assay Cascade for ADC Evaluation: from Target Engagement to Complex Tumour Microenvironment Activity

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BACKGROUND

The candidate-selection gap

The Problem: ADC candidates are often selected using binding and tumour-cell cytotoxicity alone. This can miss meaningful differences in intracellular delivery, antigen-density dependence, bystander activity, Fc-mediated immune mechanisms, and performance in a human tumour microenvironment—leading to incorrect lead selection.

The Solution: Modular, human-relevant assay cascade connects target engagement to intracellular payload delivery, direct killing, primary immune-cell activity, and complex tumour microenvironment response. By integrating SPR and epitope binning, FcRn recycling, internalisation, IndEx-2 target-density models, ADCC/ADCP/complement assays, and patient-derived co-cultures, we generate a mechanism-linked profile that supports more confident ADC candidate ranking.

METHODS: MODULAR TESTING CASCADE

Each stage answers a distinct decision question and carries the strongest candidates into the next module.

1 — Target biology
ENGAGEMENT
Antigen expression, surface density, affinity and avidity assessment.

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2 — Cellular delivery
PAYLOAD ACCESS
Surface binding, internalization, lysosomal routing and degradation kinetics.

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3 — Target-density window
SELECTIVITY
IndEx-2 titratable antigen models define the density required for response.

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4 — Direct activity
KILLING
Dose response, tumur-cell death and mixed target-positive/negative bystander models.

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5 — Fc and immune activity
HUMAN IMMUNITY
Primary NK ADCC, macrophage ADCP and complement activation/deposition where relevant.

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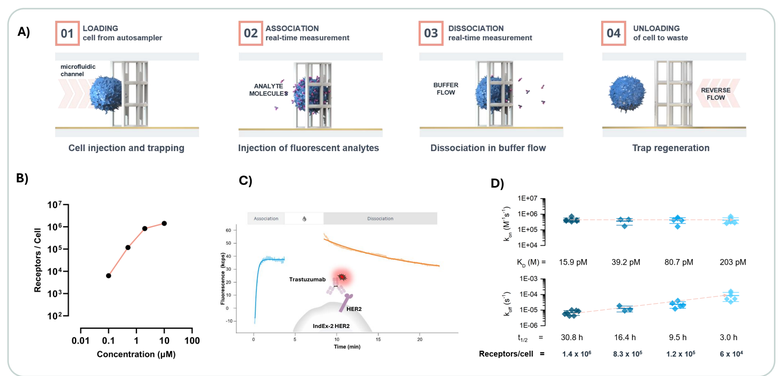
6 — Complex human TME
TRANSLATION
Patient-derived tumor models with autologous fibroblasts and matched immune cells.

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7 — Integrated lead selection
DECISION
Multiparameter flow, imaging, cytokines and viability generate a mechanism-linked rank.

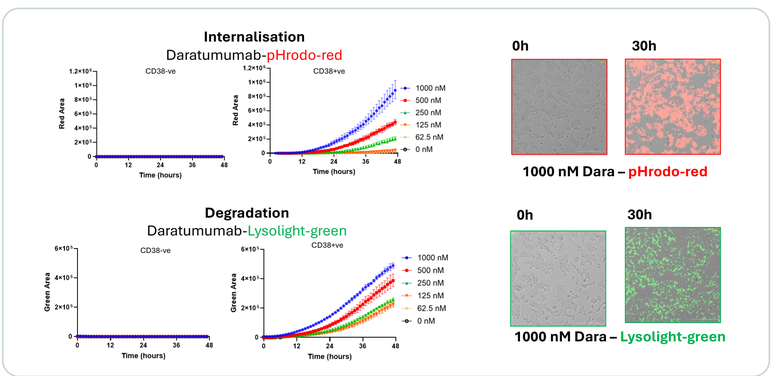
RESULTS: PLATFORM MODULES FOR ADC DECISIONS

The same candidate can move from a conventional binding and viability profile to a mechanistically differentiated profile as biological complexity increases.



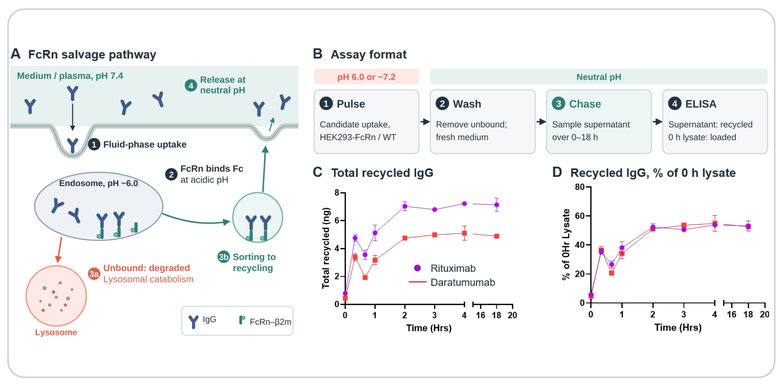
1. Characterising ADC target engagement across antigen levels

Single-cell interaction cytometry (scIC) measures antibody binding in real time (A). Combining titratable HER2 expression (B) with trastuzumab binding measurements (C) revealed slower dissociation at higher receptor abundance, with similar association rates (D). This is consistent with increased avidity, highlighting the relevance of antigen abundance to ADC targeting.



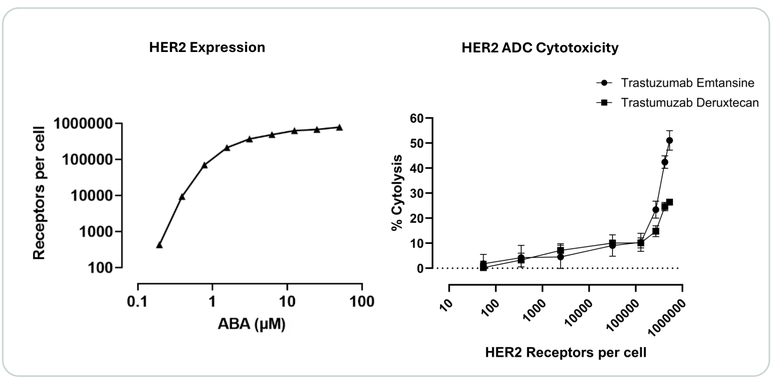
2. Live-cell antibody internalisation and degradation

Time-resolved imaging characterises intracellular trafficking relevant to ADC payload delivery, using pHrodo Red to track entry into acidic compartments and LysoLight Green to report cathepsin B-mediated proteolysis. Controlled receptor expression in HEK293 IndEx2 cells enables assessment of concentration- and receptor-dependent kinetics over 48 hours.



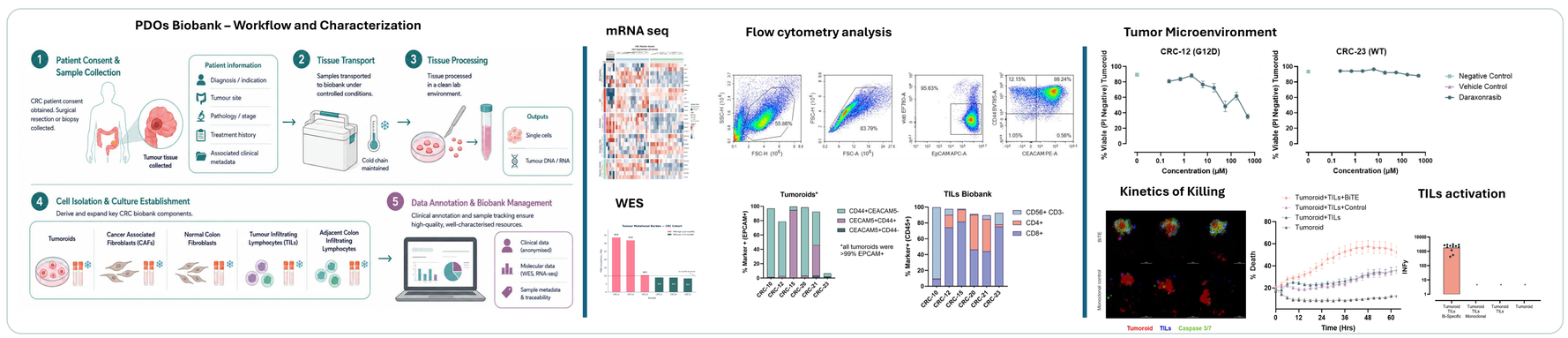
3. Fc Recycling kinetics

(A) Internalized IgG that binds FcRn in acidic endosomes is recycled and released at neutral pH; unbound IgG is routed to lysosomes and degraded. **(B)** Candidates are pulsed onto HEK293-FcRn cells, washed, and recycled IgG is quantified by ELISA in neutral-pH supernatant over 18 h. **(C)** Recycled rituximab and daratumumab (uptake at ~pH 7.2) plateaued within 2–4 h and were stable to 18 h. **(D)** Normalised to cell-associated IgG at 0 h, both converged at ~50–55% recycled, indicating that the difference in (C) reflects uptake rather than recycling efficiency. Concentration 500 nM.



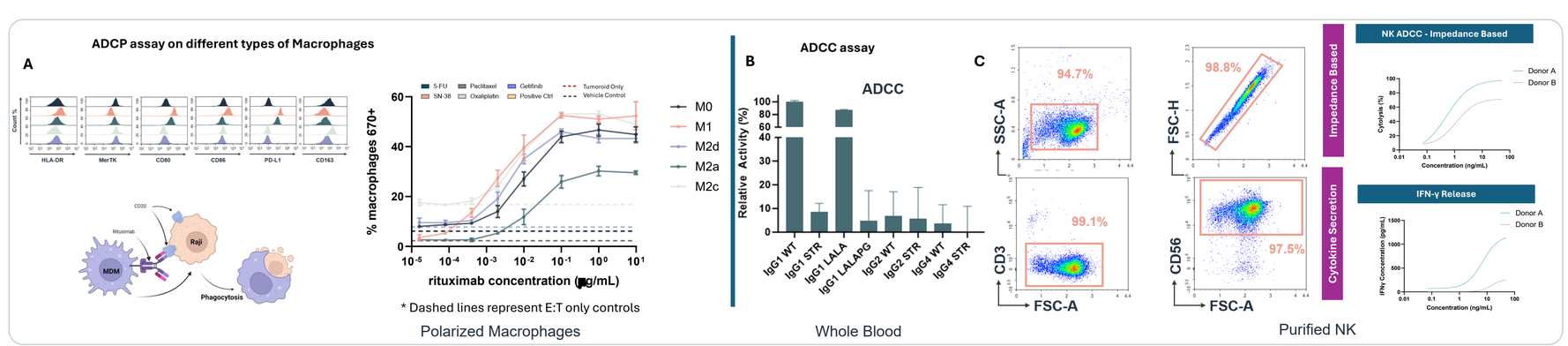
4. Target-density threshold for ADC-mediated killing

By varying receptor density within an otherwise identical cellular background, IndEx-2 enables precise evaluation of antigen density requirements for therapeutic response. Using this approach, differences in anti-HER2 ADC activity were readily distinguished, with Trastuzumab Emtansine reaching ~50% killing at the highest HER2 density tested, compared to less than 30% killing for Trastuzumab Deruxtecan.



5. Testing ADCs in primary patient material - Human tumour microenvironment

CRC PDO Biobank: A molecularly and clinically characterized collection of patient-derived CRC tumoroids with matched TILs (Tumor infiltrated T cells), CAFs (cancer associated fibroblasts) and healthy tissue counterparts. Integrated WES, RNA-seq protein-expression and clinical data enable biomarker-driven patient selection, while mono- and autologous co-culture models support kinetic assessment of tumour killing, payload sensitivity, bystander activity and TME-dependent therapeutic response.



6. Fc-dependent primary immune-cell activity

Fc-dependent immune activity was characterised using primary human ADCP and ADCC assays. **(A)** ADCP was assessed using eFluor670-labelled target cells and M0, M1, M2a, or M2d monocyte-derived macrophages. **(B)** ADCC was measured in PBMC or whole-blood effector-cell formats. Fc-engineered variants can be compared directly to quantify the impact of Fc enhancement or silencing on immune-cell engagement. Customer-specific targets can be incorporated to support selection of Fc designs that retain, enhance, or eliminate effector function. Studies are supported by a large bank of recallable cryopreserved PBMC donors with known Fcγ receptor genotypes.

Working hypothesis

A sequential, human-relevant assay cascade can identify the biological features that distinguish ADC candidates before lead selection.

Poster objective

Connect molecular design to the target-density window required for intracellular payload delivery, killing and activity in a complex human TME.

Integrated output

Candidate-specific differences in internalisation kinetics, antigen-density dependence, bystander activity, primary immune engagement and complex-TME activity provide a mechanism-linked basis for ranking candidates that otherwise appear comparable in standard assays.

Take-home message

A human-relevant ADC cascade links candidate design to the biological conditions required for a durable response.

The strongest lead is supported by evidence across the complete biological path, rather than a single endpoint.

WHAT TO MEASURE
Target density
Internalisation and lysosomal delivery
Bystander activity
Primary-cell immune mechanisms
TME activity and biomarker context

WHAT IT ENABLES
Mechanism-linked candidate ranking
A defined target-density window
Early off-tumour and immune-risk signals
A translational package for lead nomination

THE ADC QUESTION
How does each candidate behave as we move from a controlled antigen-density experiment to the complexity of human biology?

CONCLUSION

A decision framework for ADC lead selection

- **Separate target engagement from productive intracellular delivery.**
- **Quantify the target-density window before extrapolating efficacy to patient biology.**
- **Use human immune and TME modules to identify the mechanism that drives efficacy and differentiation.**

Lead-selection question

Which candidate retains the most favourable integrated profile as the model moves from controlled target density to human biological complexity?

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