Predictive In Vitro Safety Profiling of ADCs Using a Fully iPSC-Derived Human Multi-Tissue Platform

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Background

Antibody–drug conjugates (ADCs) are a rapidly expanding class of targeted cancer therapies designed to increase the therapeutic index of cytotoxic payloads. Despite this promise, clinical development remains frequently limited by dose-limiting or off-target toxicities. Severe treatment-related adverse events occur in up to 58% of Phase I ADC trials[1] and approximately 50% of biologics fail in late-stage development, with safety concerns a major contributor[2]. Traditional preclinical models often fail to predict human tissue-specific toxicities. The U.S. Food and Drug Administration roadmap for biologics emphasizes the need for mechanistically relevant, human-based functional safety profiling to support IND submissions[3]. To address this gap, Ncardia has developed a fully human, iPSC-derived multi-cellular safety screening platform designed to identify on-target/off-tumor and off-target liabilities early in development.

Platform overview

Ncardia's safety platform:

- Uses exclusively human iPSC-derived cell types
- Covers seven physiologically relevant tissues
- Enables real-time functional toxicity assessment
- End-point is non-invasive allowing further optional analysis (i.e. omics)
- Provides mechanistic insight into tissue-specific susceptibility
- Builds upon prior IND-supporting experience in cell therapy programs

This unified system enables early de-risking of ADCs, antibodies, cell therapies, and other biologics.

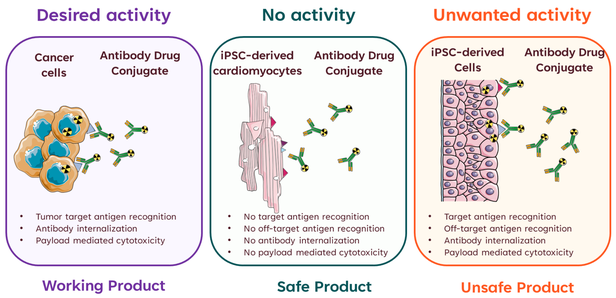

Figure 1. *Overview of ADC activity profiles across target and non-target human cell types* **(Left)** Cancer cells express the intended antigen, enabling specific binding of the ADC, internalization, and payload-driven cytotoxicity, representing the desired therapeutic effect. **(Center)** iPSC-derived cardiomyocytes lack expression of the target antigen, resulting in no ADC binding, no uptake, and absence of cytotoxic response, consistent with a safe profile. **(Right)** A panel of iPSC-derived healthy human cells shows unintended interactions including off-target antigen recognition, ADC internalization, and payload-mediated cell damage, reflecting potential safety risks associated with off-tumor toxicity.

Case Studie: Cetuximab-MMAE safety profile

To demonstrate proof-of-concept, we generated an in vitro safety profile for Cetuximab-MMAE — an EGFR-targeting monoclonal antibody conjugated to monomethyl auristatin E (MMAE). Here we wanted to determine whether our iPSC-derived tissue panel can recapitulate clinically observed toxicity patterns.

Workflow

In silico mRNA expression EGFR

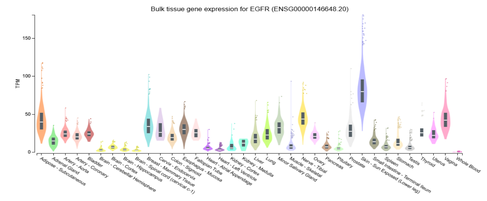

Figure 2. *In silico profiling of EGFR expression across human tissues (GTEx)*

To evaluate potential on-target/off-tumor risk, we first performed an *in silico* analysis of EGFR expression across human tissues using publicly available transcriptomic data from the GTEx database. This analysis combined with known high-risk organs for toxicity, resulted in a representative 7-tissue panel covering major organ systems (Figure 3).

Selected human seven-tissue iPSC derived panel

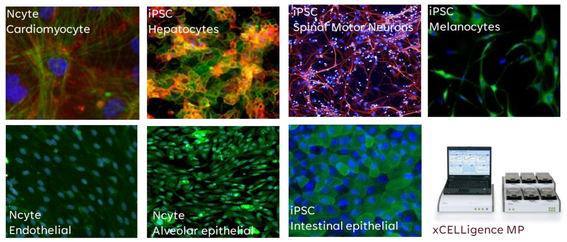

Figure 3. *Characterization of the seven iPSC-derived human cell models selected for ADC safety assessment.*

All cells representing a tissue derived from human iPSCs, providing:

- Lineage-appropriate receptor expression
- Physiological functional properties
- Human-specific biology

Cytotoxicity was measured using real-time impedance-based analysis on the xCELLigence MP platform.

In vitro functional safety profile

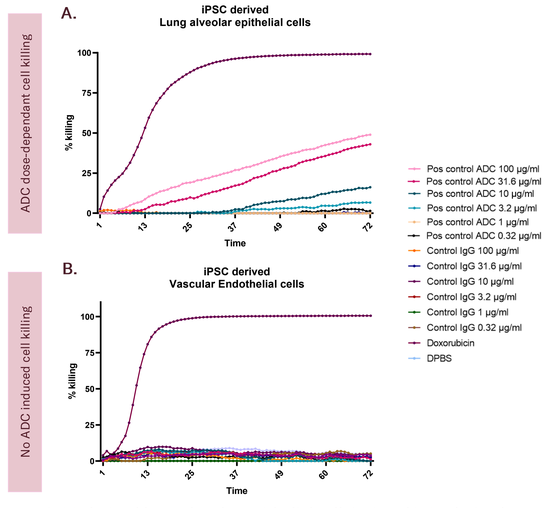

Figure 4. *iPSC-derived lung alveolar epithelial cell (A.) and iPSC derived vascular endothelial cell (B.) susceptibility to Cetuximab-MMAE over 72 hours.*

Impedance allowed for a clear, over time measurement of viability / % killing for all cell types with doxorubicin as toxicity control and quantifiable dose dependent effects where a response was observed (Figure 4).

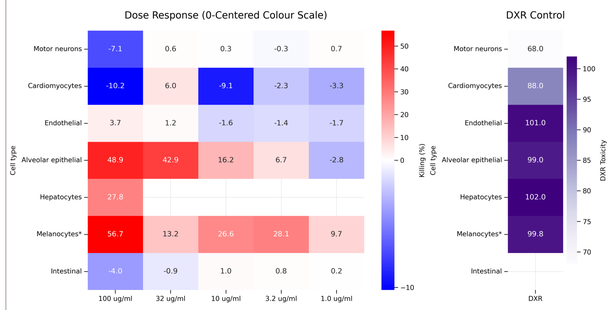

Figure 5. *Cetuximab-MMAE cytotoxicity across a fully iPSC-derived multi-tissue panel.*

* Due to confluency the iPSC derived Melanocytes were measured at 62 hours

At non-clinically relevant dose (100 µg/mL, Supratherapeutic)

- High cytotoxicity in melanocytes (skin-derived cells)
- Moderate effects in lung and liver models
- Minimal to no toxicity in cardiac, CNS, vascular, and intestinal models

This pattern aligns with known EGFR expression distribution.

Clinically Relevant Dose (1 µg/mL)

- Toxicity was largely reversed across tissues
- Low residual sensitivity observed only in melanocytes

This demonstrates the platform's ability to distinguish between supratherapeutic hazard and clinically meaningful exposure risk — supporting translational relevance rather than nonspecific cytotoxicity detection.

Clinical Correlation

Clinically, cetuximab therapy is strongly associated with dermatologic adverse events (>80% of patients)[4]. Large real-world pharmacovigilance datasets from FAERS and VigiAccess consistently identify skin as the dominant toxicity class[5]. Our in vitro findings mirror this clinical signature.

Tissue	EGFR Expression	In Vitro Toxicity (100 µg/mL)	In Vitro Toxicity (1 µg/mL)	Clinical AE Signal
Skin (Melanocytes)	High	High	Low residual	High
Liver (Hepatocytes)	Moderate	Moderate		Low–Moderate
Lung (Alveolar epithelial)	Moderate	Moderate	None	Moderate
Heart (Cardiomyocytes)	Low	Minimal	None	Minimal
CNS (Motor neurons)	Low	Minimal	None	Minimal
Vascular (Endothelial)	Low	None	None	Minimal
Intestine (Epithelial)	Low	None	None	Minimal

Notably, absence of intestinal epithelial toxicity aligns with clinical evidence that gastrointestinal events during cetuximab therapy are typically inflammation-driven rather than due to direct epithelial injury[6]. Moderate cytotoxicity observed in lung and liver at supratherapeutic exposure reflects tissue susceptibility associated with EGFR expression and MMAE payload sensitivity. Importantly, these effects were not observed at clinically relevant concentrations, consistent with regulatory expectations for hazard identification in human in vitro safety models.

Conclusions

Importantly, the use of a fully iPSC-derived, multi-tissue panel provides a unique advantage by enabling mechanistically relevant, human-specific interrogation of ADC off-target or on-target liability within a unified platform. iPSC-derived tissues recapitulate key physiological features—including lineage-appropriate receptor expression, barrier properties, and functional responsiveness—offering a biologically coherent system that surpasses traditional immortalized cell lines. Together, these findings highlight the value of an iPSC-based approach for early safety de-risking, enabling more accurate prediction of normal-tissue susceptibility for ADCs and supporting more informed therapeutic design and development.

Predictive iPSC-based safety profiling of your immunotherapies

Questions? Contact us at: support@ncardia.com, www.ncardia.com

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