WHITE PAPER

Preclinical screening platforms for Antibody-Drug Conjugate therapeutics.

Antibody Drug Conjugates (ADCs) represent one of the most rapidly expanding oncology therapeutic categories. Hundreds of candidates are undergoing active clinical development, with about two hundred companies engaged in ADC development[1]. Recent regulatory adjustments within the pharmaceutical industry in the US have begun to influence drug developers' research strategies worldwide[2]. This has led to a heightened focus on expediting the discovery and screening processes for ADC therapeutic candidates.

In their simplest form, ADCs fuse monoclonal antibodies with cytotoxic drugs tailored for targeted cancer therapy. Traditionally, ADCs exhibit specific binding to tumor cell epitopes, undergo internalization, and release the payload within the cell, facilitating precise delivery of cytotoxic drugs directly to cancer cells. A crucial element of ADCs is the chemical linker utilized for conjugation, which determines the cleavage properties of the conjugate, dictating the site and timing of payload release.

Consequently, companies are actively designing not only the antibody but also the linker and payload to tailor the properties of ADCs to their intended applications and increase their efficacy. More complex designs involve strategies targeting multiple antigens, employing diverse payloads, and utilizing various linker cleavage mechanisms, which can occur intracellularly or extracellularly[3]. The intricate interplay among these elements significantly impacts the efficacy and safety of ADCs in cancer treatment, emphasizing the necessity for meticulous screening to optimize ADC design[4].

Generation of cell models

The efficacy of ADCs relies on the antibody's specific binding to surface markers expressed on the target cell. These conjugates allow for precise drug delivery to cancer cells while minimizing effects on healthy tissues. Researchers employ robust models such as engineered cell lines to optimize antibody specificity. These models enable experimental assessment of potential targets using knockout cell lines where specific genes are disrupted and knock-in models where genes can be overexpressed or mutated to recapitulate a disease state. By studying engineered cell models, researchers can effectively screen and evaluate ADC candidates' specificity and efficacy during early development[5].

Cell panel screening for ADC-based combination therapies

In the landscape of cancer treatment, the efficacy of traditional therapies often hinges on the challenge of delivering potent doses of chemotherapy to tumors without inducing severe side effects. This limitation has led to the emergence of ADCs, which leverage antibodies' specificity to target cancer cells precisely while delivering potent therapeutic payloads akin to traditional chemotherapy agents. The essential advantage lies in their ability to achieve higher doses with reduced side effects, a feat primarily attributed to their targeted delivery mechanism. Moreover, while traditional therapies encounter hurdles in solid and liquid tumors, ADCs can offer a versatile solution for various cancer types. Here, combination screens are crucial in developing ADCs by evaluating their interaction with existing treatments. These tools enable researchers to test ADCs with current therapies, identify synergistic effects, and optimize regimens, enhancing efficacy while minimizing side effects. This approach paves the way for more effective and personalized cancer medicine.

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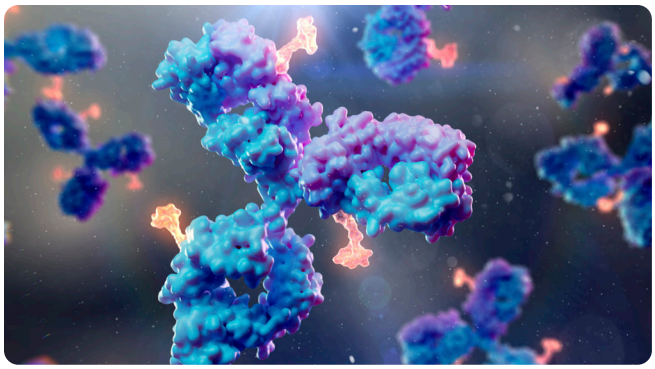


In addition to engineered models, cell panels across solid and hematological indications can be employed to test the efficacy of ADCs. For instance, a combination screen was conducted using a library of 104 compounds, including small molecules and antibodies, in a 6x6 dose matrix format across 20 non-Hodgkin lymphoma cell lines, with naratuximab conjugated to the anti-microtubule agent DM1. The results indicate a novel mechanism wherein the potency of the ADC can be augmented through CD20 binding. These findings facilitated the advancement of combination therapy into clinical stages for B cell lymphoma[6].

High-throughput screens of ADC combinations with various enhancer partner compounds across multiple solid tumor cancer cell lines identify the most susceptible cell lines. This approach efficiently reveals robust synergies between the ADC and small-molecule intracellular pathway inhibitors to impair cancer growth. Important mechanistic insights about the relative contribution of the antibody versus the warhead subunits of an ADC to driving synergy can be further interrogated using three-way combination screens[7].

Screening of genetic drivers and mechanisms of action and resistance

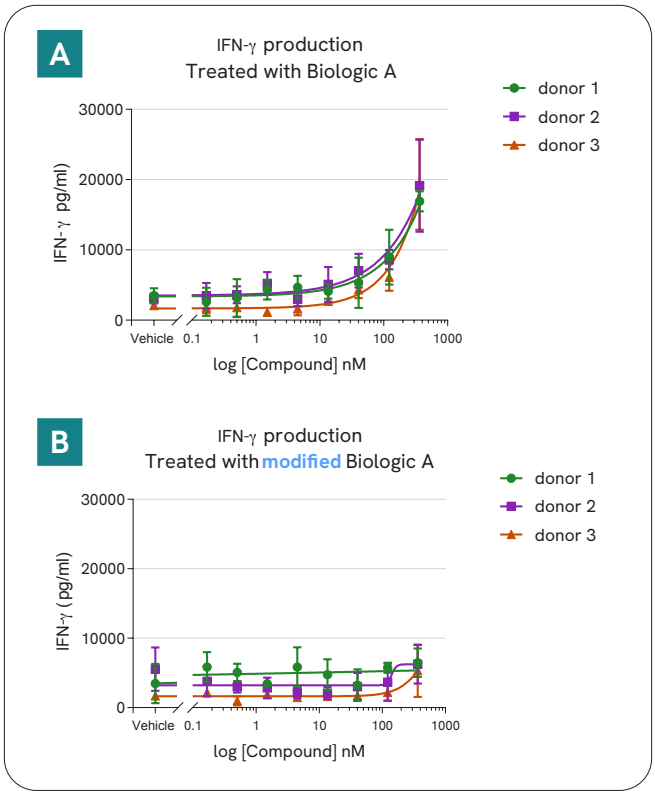
Despite their promise, patients often develop resistance to ADCs over time. Understanding the underlying mechanisms is crucial for improving treatment options. One key resistance mechanism involves changes in antigen expression, where cancer cells downregulate or mutate the targeted antigen, reducing ADC binding and internalization. Research indicates that alterations in antigen expression can significantly impact ADC efficacy. Additionally, ADC processing and resistance changes, particularly in intracellular trafficking, are critical. For instance, regulators of endolysosomal trafficking, such as C18orf8/RMC1, play essential roles in ADC toxicity through endosomal maturation[8].

CRISPR-based genome-wide screens have emerged as valuable tools for understanding ADC resistance. Researchers can identify those modulating ADC toxicity and resistance by systematically knocking out specific genes. Comparative analysis of screens with ADCs bearing different linkers offers insights into processing and resistance mechanisms. Moreover, CRISPR screens uncover genes that enhance or inhibit ADC toxicity, offering potential sensitization strategies to overcome resistance.

For instance, inhibiting sialic acid biosynthesis has sensitized cells to ADC treatment by increasing ADC internalization[9]. These findings highlight the importance of ongoing research into ADC resistance mechanisms and the potential of CRISPR screening to inform sensitization strategies.

Immune cell-based screening for the optimization of ADC structure

Recent insights in the literature propose new perspectives on the mechanism of action for ADCs, suggesting their efficacy may stem from their ability to recruit the immune system for tumor clearance. This research opens avenues for engineering ADCs, highlighting the potential to manipulate their design to enhance or minimize immune involvement based on therapeutic objectives. This offers a strategic pathway for tailoring treatment approaches to optimize therapeutic outcomes. Here is where immune cell-based screening is indispensable for optimizing and refining the structure of ADCs. Firstly, it ensures selective targeting and minimizes systemic toxicity, validates the specificity of ADC binding to intended targets, and enhances tumor-selective properties while reducing off-target effects.



Immunogenicity assessment of biologics by ImmuSignature™ MLR assay. This study assessed the immunogenicity of biologics linked to cytotoxic agents. Figure B illustrates the ImmuSignature mixed lymphocyte reaction in evaluating the modification of biologic A. The results demonstrate a significant reduction in immunogenicity compared to the initial proinflammatory effects, as indicated by IFN-g secretion in Figure A measured using the HTRF™ assay technology..

Furthermore, immune cell-based assays can support the evaluation of linker stability and payload effectiveness, which is crucial for controlled drug release and cancer cell killing. Finally, by uncovering resistance mechanisms and assessing immunogenicity, these studies inform strategies to overcome resistance and mitigate immune responses that may neutralize ADC effects[10].

Cell panel screening for payload evaluation

Efflux pumps can actively remove ADC payloads from cancer cells, reducing intracellular concentration and efficacy. In this context, large cell panel screen platforms can be essential to guide payload selection for efficient drug payload delivery analysis and conjugate/linker design evaluation. In payload selection, diverse cytotoxic agents are weighed across a spectrum of cancer cell lines to identify potent and efficacious payloads. Screening can also uncover unconventional payloads with differentiated mechanisms of action, as evidenced by recent ADC approvals[11].

Furthermore, comparing ADC activity in 3D tumor models versus traditional 2D cell cultures yields valuable insights into drug penetration and efficacy within complex tumor environments[8]. In the design of conjugation and linkers, screening across cell panels can assist in optimizing both the site and the number of linker/drug molecules conjugated to the antibody for homogeneity and therapeutic consistency. It can also support the evaluation of cleavable and non-cleavable linkers, assess physicochemical properties, and refine bioconjugation methods.

In summary, systematically evaluating antibody-drug conjugate therapeutics through in vitro screening across diverse cell lines is essential for improving therapeutic efficacy while reducing off-target effects.

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940 Winter Street
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