Recapitulating Alveolar–Tumor Crosstalk *In Vitro* to Predict Lung Toxicity and Efficacy of Antibody-Drug-Conjugates (ADCs)

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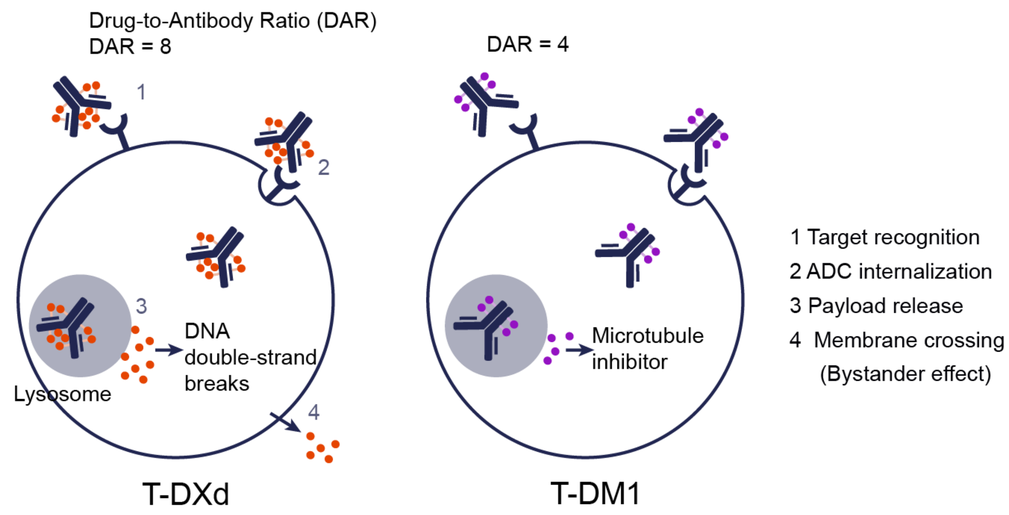
INTRODUCTION

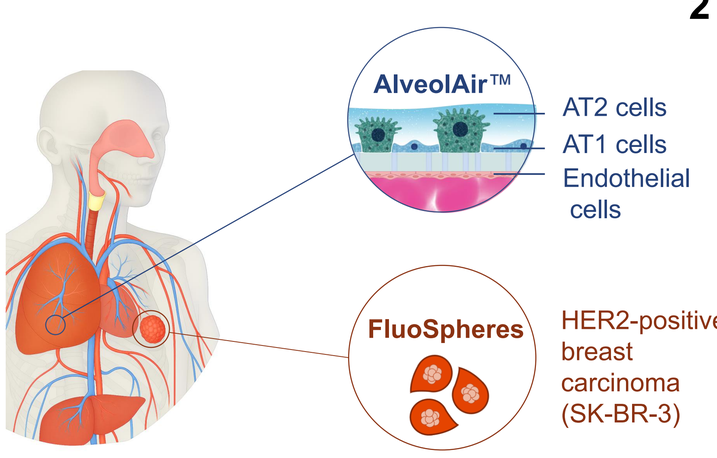
ADCs are an expanding class of targeted cancer therapies that combine a monoclonal antibody, a potent payload, and a linker. Once bound to their antigen, the ADC is internalized, and the payload is released to exert its therapeutic effect. However, premature release or diffusion from tumor cells can lead to toxic bystander effects in healthy tissues.

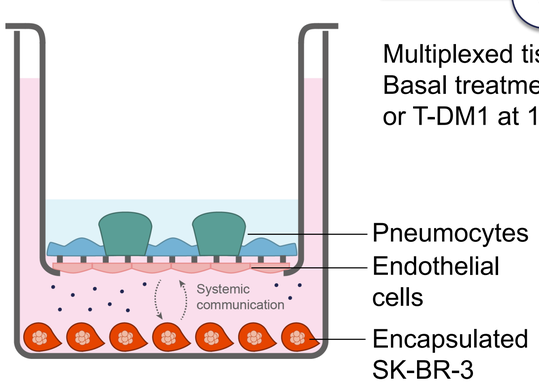
Trastuzumab deruxtecan (T-DXd), a HER2-targeting ADC, shows strong efficacy but is associated with interstitial lung disease (ILD) in up to 15% of patients—a serious effect involving injury of the alveolar tissue. Because toxicities are often missed in animal models, the FDA now recommends using advanced *in vitro* systems based on human biology.

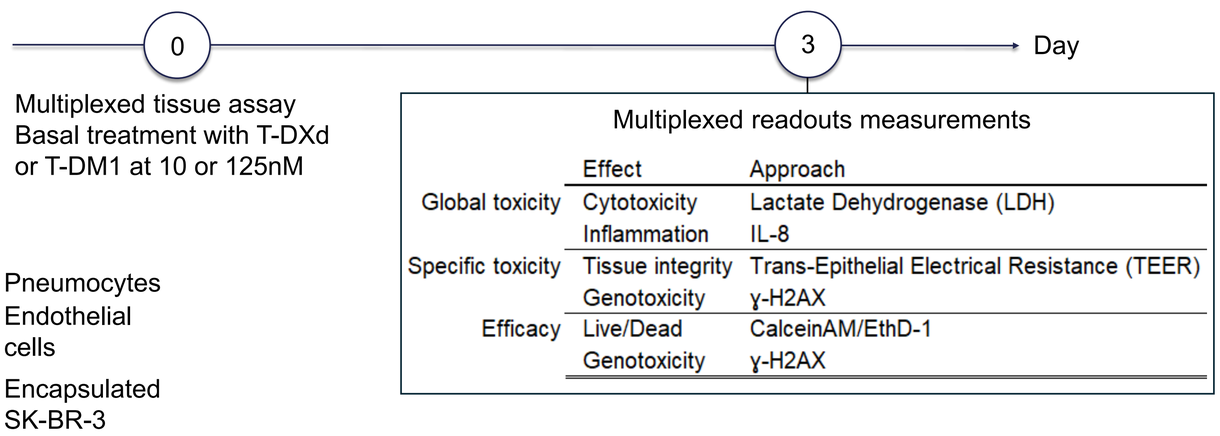
To meet this need, Epithelix and FluoSphera co-developed a systemic *in vitro* platform combining AlveolAir™, a reconstituted human alveolar tissue, with encapsulated HER2+ tumor spheroids. This model enables lung–tumor communication. Tested with T-DXd and trastuzumab emtansine (T-DM1) — with a lower ILD risk, the system captured anticancer effects and distinct lung responses.



2 Technologies, 1 Integrated Strategy for Predictive Testing



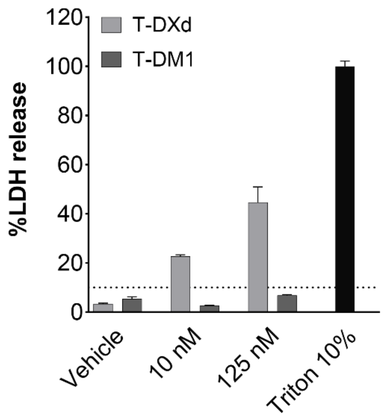




	Effect	Approach
Global toxicity	Cytotoxicity	Lactate Dehydrogenase (LDH)
	Inflammation	IL-8
Specific toxicity	Tissue integrity	Trans-Epithelial Electrical Resistance (TEER)
	Genotoxicity	ɣ-H2AX
Efficacy	Live/Dead	CalceinAM/EthD-1
	Genotoxicity	ɣ-H2AX

The bloodstream plays a central role in connecting distant organs throughout the body. In the context of ADCs, effects can occur not only at the target site (tumors) but also in off-target organs such as the lungs. To address this, Epithelix and FluoSphera have jointly developed a co-culture platform that replicates inter-organ communication in vitro. **Epithelix** provides a functional alveolar model (AlveolAir™) using primary human type I/II pneumocytes and endothelial cells cultured at the air–liquid interface[1]. **FluoSphera** brings systemic capability through cell encapsulation, allowing multiple human tissues to be co-cultured in permeable, color-coded capsules[2]. In this study, HER2-positive SK-BR-3 breast carcinoma cells (disease model) are encapsulated in hollow alginate capsules.

Global Cytotoxicity on the AlveolAir™ Model

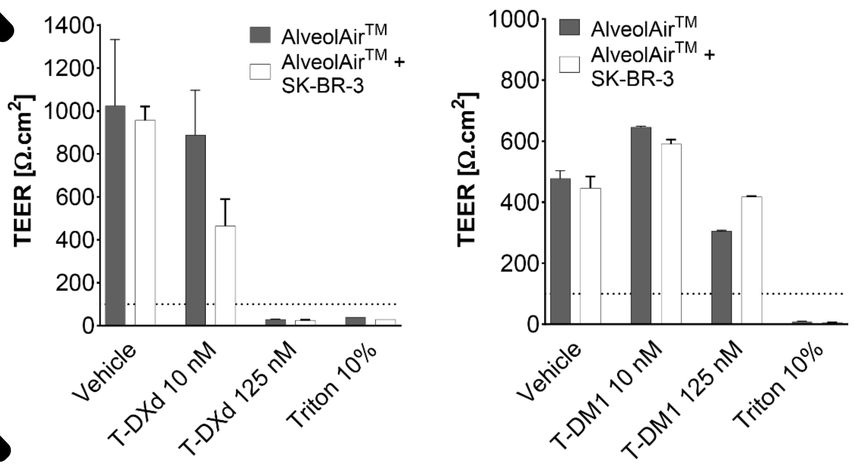


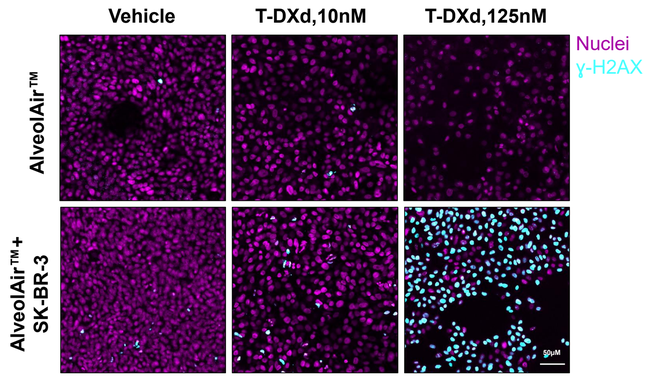
In the absence of SK-BR3 spheroids, T-DM1 does not show cytotoxicity.

In contrast, T-DXd induces dose-dependent toxicity, even though the ADC has not yet been internalized by the tumor.

(n=3, mean ± SEM)

Tissue Integrity of the AlveolAir™ Model





(Up) Only T-DXd affects the alveolar epithelium. At low doses, the effect is potentiated in the presence of SK-BR-3 spheroids (n = 3, mean ± SEM).

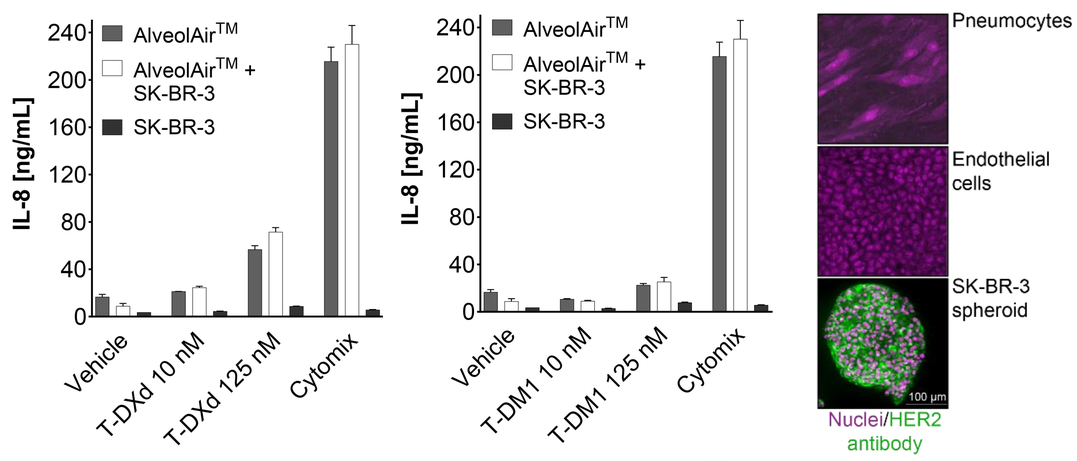
(Down) T-DXd also causes DNA double-strand breaks in alveolar endothelial cells, in a dose-dependent manner and in co-culture.

CONCLUSION

Epithelix and FluoSphera developed a robust, miniaturized human *in vitro* model that recapitulates inter-organ communication. This model enables simultaneous functional and structural assessment of both the anticancer effects and ILD risk of ADCs.

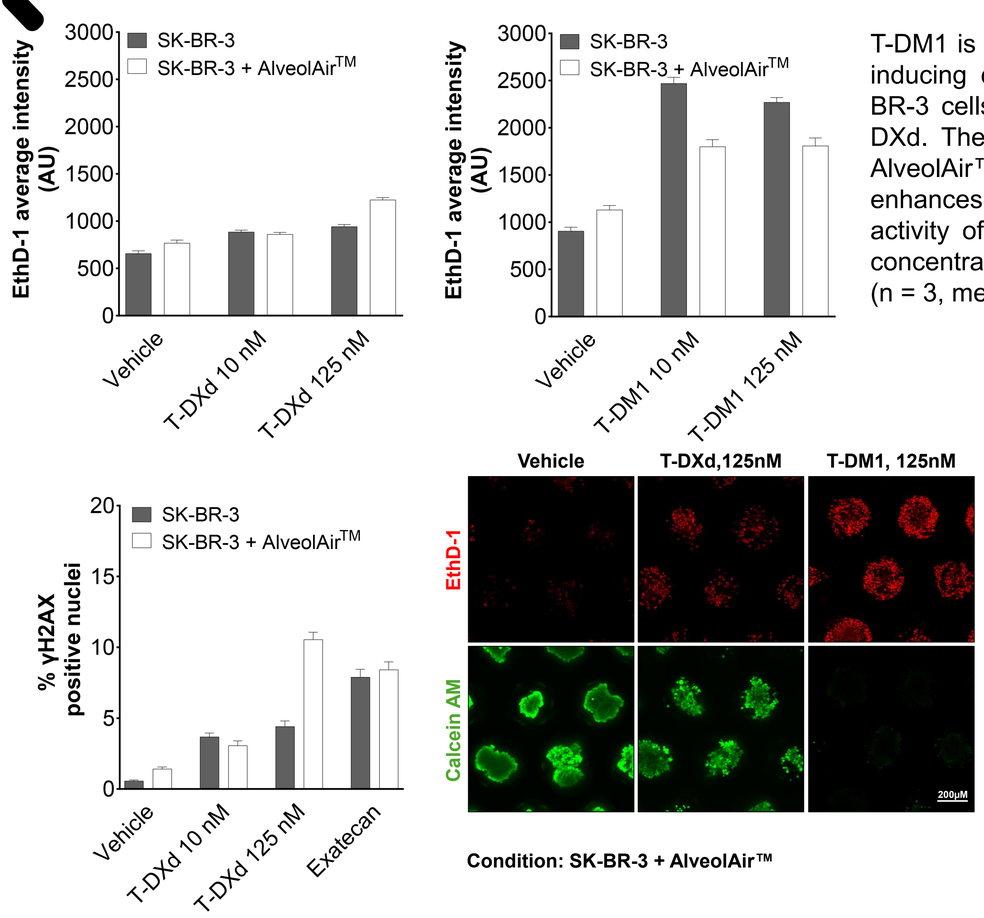
The study shows several mechanisms may underly these effects: (1) **Bystander effect**: T-DXd, internalized by SK-BR-3 spheroids, releases its payload, which damages lung tissue integrity and induces DNA breaks in the AlveolAir™ model. (2) **Direct lung toxicity**: Through non-specific internalization or extracellular cleavage[3], T-DXd acts directly and triggers inflammation as previously reported[4]. (3) **Differential efficacy**: T-DXd has a cytostatic effect, while T-DM1 is cytotoxic. Interestingly, the anticancer effect of T-DXd is enhanced in the presence of the AlveolAir™ model.

Inflammation on the Co-Culture Model



Unlike T-DM1, T-DXd increases IL-8 secretion in the AlveolAir™ model in a dose-dependent manner, even in absence of SK-BR-3 spheroids (n = 3, mean ± SEM).
However, HER2 is only expressed in SK-BR-3 cells, not in the AlveolAir™ model.

Anticancer effect on SK-BR-3 spheroids



T-DM1 is highly effective at inducing cell death in SK-BR-3 cells compared to T-DXd. The presence of the AlveolAir™ model enhances the anticancer activity of T-DXd at higher concentrations.
(n = 3, mean ± SEM)

The potentiation, observed with T-DXd treatment, is particularly striking when evaluating DNA double-strand breaks, in line with the mode of action of its payload.
Calcein-AM, which fluoresces only in metabolically active cells, shows that T-DXd acts more as a cytostatic agent, while T-DM1 is cytotoxic.

References: [1] Ferreira Lopes et al., *Microorganisms* **2025**, *13*(3), 572, [2] Patent: WO2021058557A1, JP7672717B2, [3] Tsao et al., *Nature Communications* **2025**, 13:3 167, [4] Mc Cray et al., *Toxicology and Applied Pharmacology* Volume 485 **2024**, 116886