

MULTICELLULAR HUMAN INTESTINAL NAM FOR DIARRRHEA RISK STRATIFICATION ACROSS SMALL MOLECULES and ANTIBODY DRUG CONJUGATES

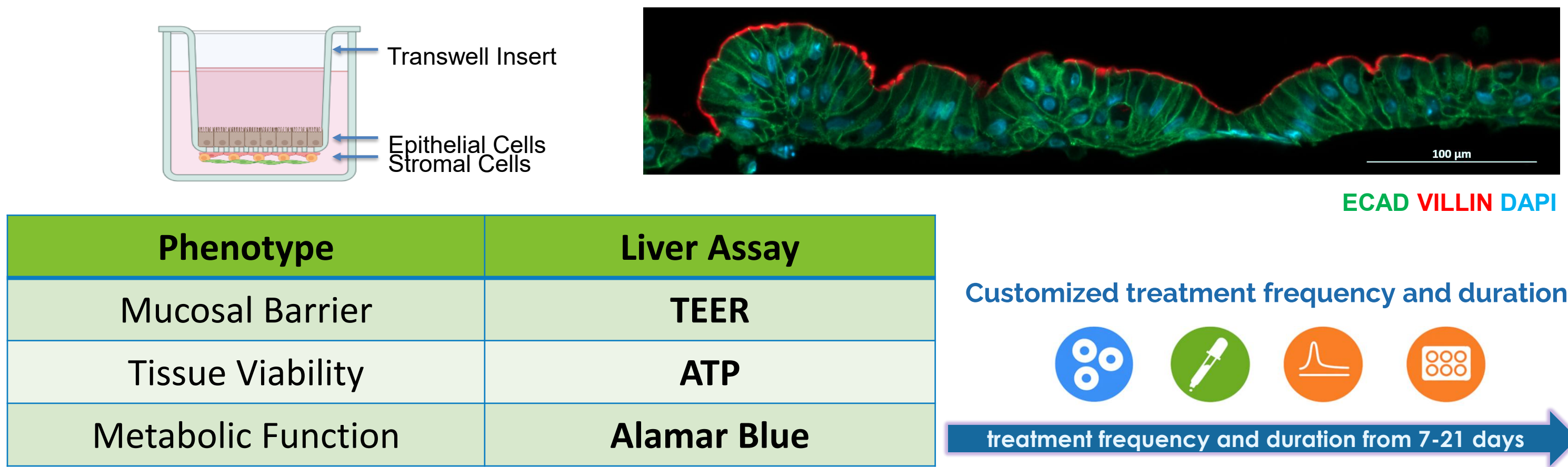
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INTRODUCTION

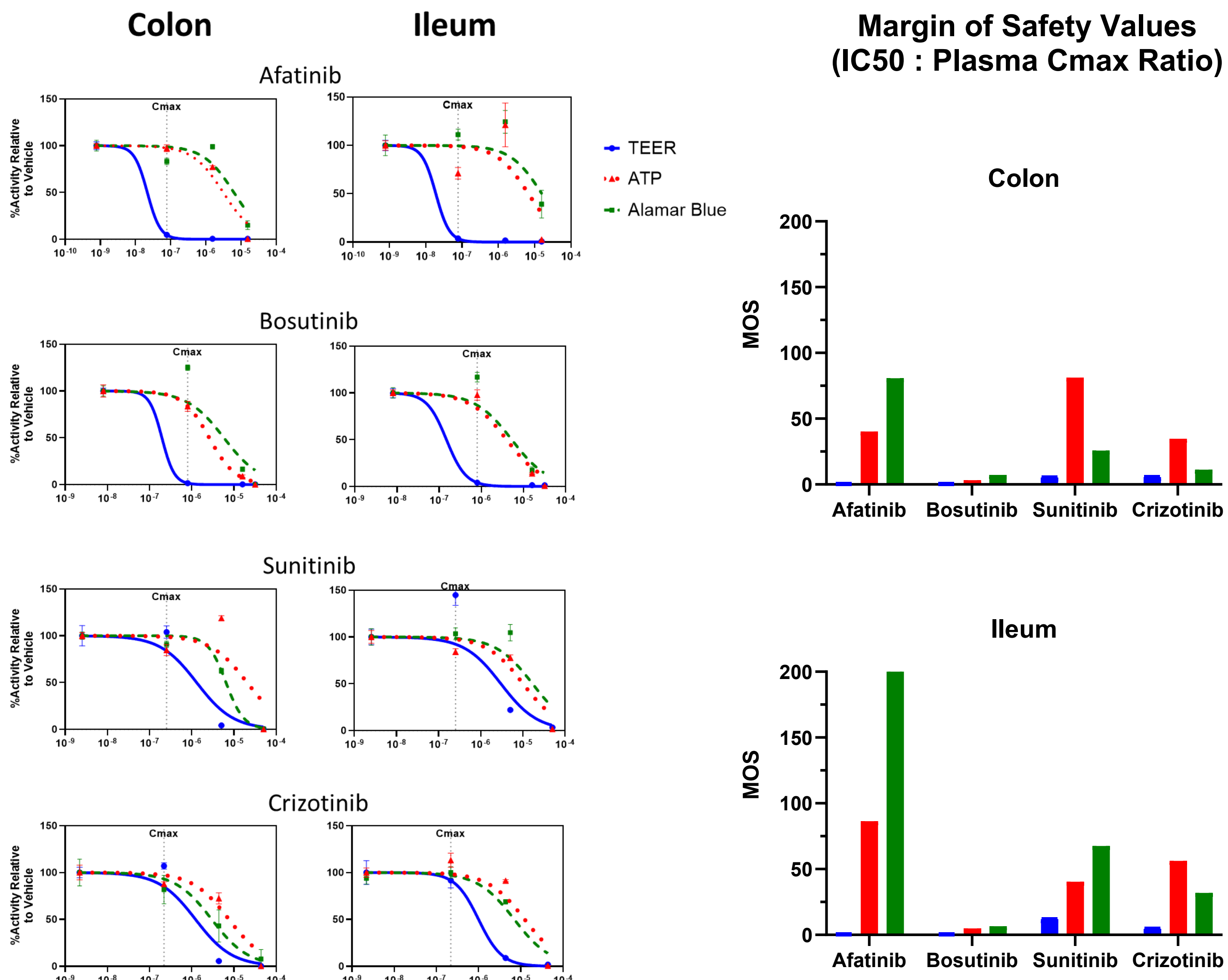
Drug-induced diarrhea is a frequent dose-limiting toxicity driven by diverse mechanisms, including epithelial barrier dysfunction, ion transport dysregulation, and tissue injury. However, current preclinical models often fail to capture the mechanistic complexity and human relevance needed to inform early GI risk decisions, particularly across emerging modalities such as antibody drug conjugates (ADCs) as well as small-molecule kinase inhibitors. To address this gap, we developed a multicellular human intestinal barrier NAM (NAMkind™ GI) that integrates barrier function (TEER), tissue viability (ATP), and metabolic activity (Alamar Blue) under clinically relevant exposure conditions. We applied a margin of safety (MOS) framework (IC₅₀/C_{max}) to generate endpoint-resolved signatures that enable direct translation to human exposure. Using this approach, we aimed to (1) differentiate mechanisms of drug-induced diarrhea across small molecules, including TKIs with distinct clinical phenotypes, and (2) deconvolute ADC-associated GI toxicity, separating the contributions of payload, antibody, and exposure-dependent effects.

METHODS



A 3D human intestinal barrier model (NAMkind™ Intestine) was established for ileum and colon using epithelial and stromal components that maintain polarity and tight-junction integrity. Under repeat-dose conditions, barrier function (TEER) and tissue health/redox state (ATP, Alamar Blue) were measured in a clinically anchored reference set of small-molecule drugs with known diarrhea risk. ADCs were evaluated using matched exposure schedules with intact ADC, antibody-only, and payload-only.

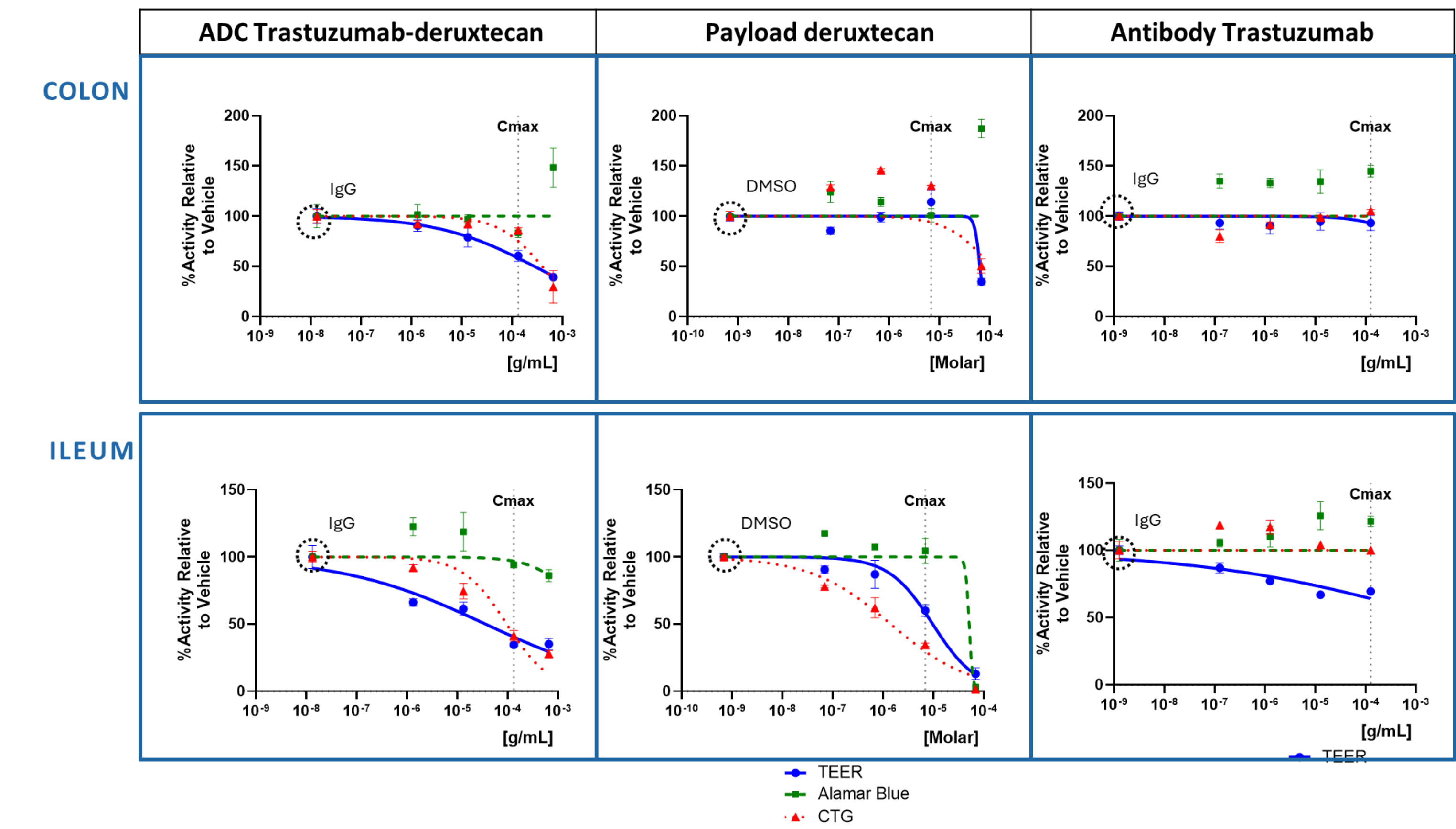
NAMkind™ GI DISTINGUISHES DIFFERENTIAL INJURY OF TYROSINE KINASE INHIBITORS



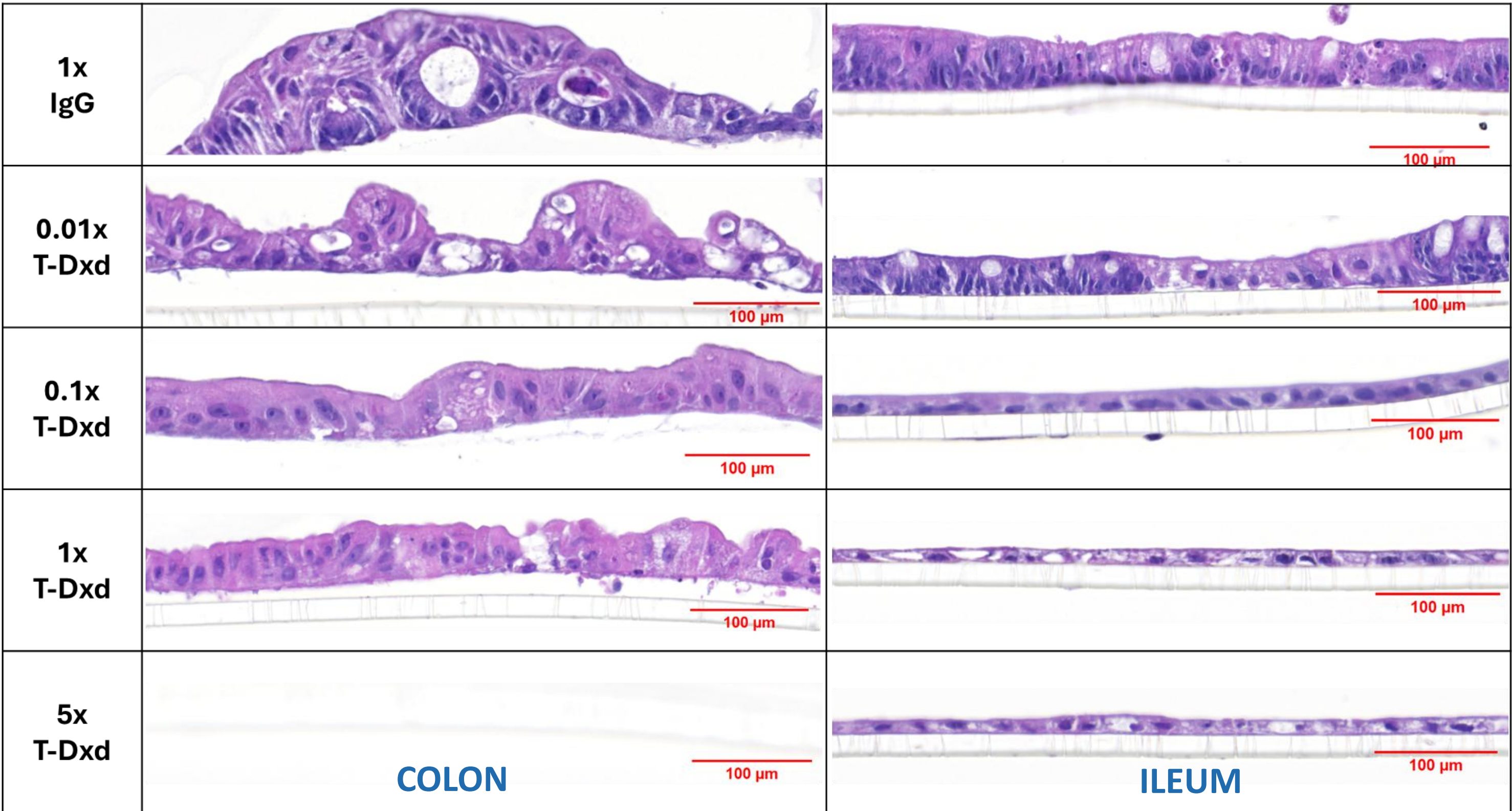
All 4 TKIs have low MOS values for TEER (0.2 – 11). However, the differential effects on viability and metabolic function are consistent with their diarrhea MOA.

Drug	Diarrhea Type and MOA	MOS Pattern of TEER, ATP, Alamar Blue
Afatinib	classic secretory diarrhea via chloride channel activation	Functional epithelial dysregulation, not cytotoxic injury; colon viability & metabolic function more sensitive than ileum
Bosutinib	early-onset, permeability-driven diarrhea	Combined barrier disruption and global epithelial injury (permeability + cytotoxicity); colon & ileum equally affected
Sunitinib	ischemia + motility + mucosal injury	Barrier dysfunction with secondary epithelial stress and injury (consistent with vascular disruption rather than primary cytotoxicity)
Crizotinib	off-target GI epithelial effects / motility changes	Barrier dysfunction with moderate epithelial metabolic stress (colon-predominant), without overt cytotoxicity

NAMkind™ GI DIFFERENTIATES ANTIBODY DRUG CONJUGATE DIARRRHEA RISK



- Trastuzumab-deruxtecan induces dose-dependent toxicity in the self renewing model
- Improved polarization and tight junction integrity of the self renewing model make cells less sensitive to the naked antibody. Toxicity is still observed with payload
- Due to the higher proliferation rate in the self renewing model, toxicity is observed at 1x C_{max}, suggesting an additive effect of the antibody and payload



- The IgG-treated tissues maintained intact epithelial morphology, with preserved architecture and continuous epithelial lining, consistent with a non-toxic baseline condition.
- In contrast, increasing concentrations of trastuzumab deruxtecan resulted in progressive epithelial alterations, characterized by gradual disruption of tissue structure and loss of epithelial integrity with increasing dose.
- This is consistent with barrier compromise observed in the functional TEER readouts.

CONCLUSIONS

- Barrier disruption is a conserved and early driver of drug-induced diarrhea across modalities, with uniformly low TEER MOS revealing a shared liability not captured in traditional models.
- Integrated TEER, ATP, and Alamar Blue metabolic profiling resolves distinct toxicity classes (from secretory dysfunction to epithelial injury), providing direct mechanistic translation to clinical diarrhea phenotypes.

- NAMkind™ GI uniquely deconvolutes ADC toxicity, demonstrating that trastuzumab deruxtecan-mediated GI injury is payload-driven and exposure-dependent, with linker-controlled delivery modulating epithelial damage and producing dose-dependent barrier disruption confirmed histologically.
- This platform enables a paradigm shift from detection to prediction, delivering mechanism-based, human-relevant GI risk stratification that directly informs design, prioritization, and de-risking of small molecules and ADCs.