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Insights Brief

Understanding Gastrointestinal Toxicity Risk Through Human-Relevant Intestinal Models

Mechanistic toxicology application through NAMs



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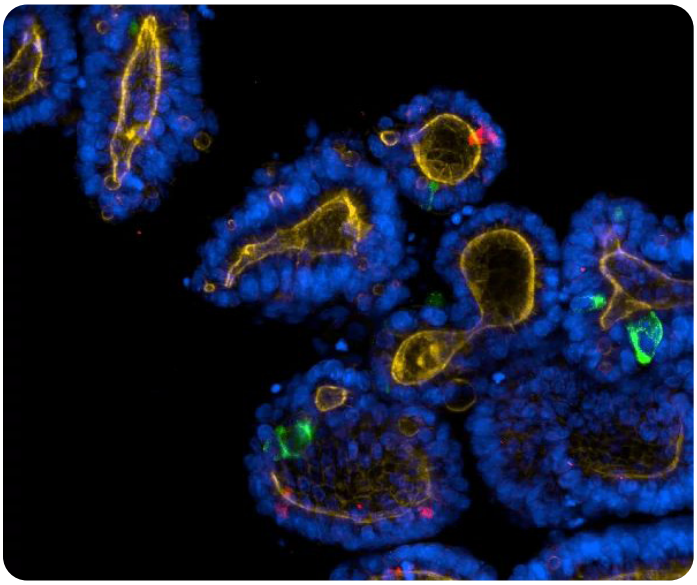
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Executive summary

Gastrointestinal (GI) toxicity remains one of the most common adverse events associated with targeted cancer therapies and can significantly impact treatment adherence, dose intensity and patient quality of life. Among the toxicities observed with epidermal growth factor receptor (EGFR) inhibitors, diarrhea is particularly prevalent and frequently represents a dose-limiting adverse event. While EGFR tyrosine kinase inhibitors (TKIs) have transformed the treatment of EGFR-mutant malignancies, substantial differences in GI toxicity are observed across compounds within the same therapeutic class. For example, the second-generation EGFR inhibitor afatinib is associated with a markedly higher incidence of diarrhea compared with first-generation inhibitors such as gefitinib and third-generation inhibitors such as osimertinib. Understanding the biological mechanisms underlying these differences is critical for compound selection, dose optimization and clinical risk mitigation.

Traditionally, safety assessment of GI toxicity has relied on animal studies and conventional in vitro models. However, these approaches often fail to fully recapitulate human intestinal physiology, limiting their ability to predict clinically relevant toxicities or identify the mechanisms driving adverse responses. Animal models may not accurately reproduce human epithelial turnover, signaling pathways or drug exposure profiles, while simplified two-dimensional cell cultures lack the cellular complexity and tissue architecture necessary to evaluate functional intestinal responses.

Recent advances in New Approach Methodologies (NAMs) provide an opportunity to address these limitations through the use of human-relevant model systems. Human intestinal organoids generated from induced pluripotent stem cells (iPSCs) replicate key structural and functional characteristics of the intestinal epithelium, including cellular diversity, proliferative capacity and epithelial homeostasis. These models enable mechanistic interrogation of drug-induced



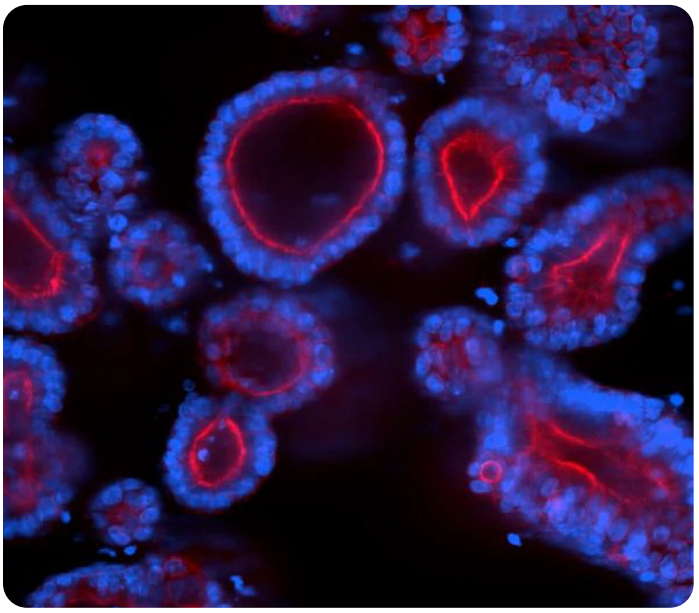
injury and provide translationally relevant insights into compound-specific toxicity liabilities.

In this case study, human intestinal organoids were used to investigate the mechanisms underlying GI toxicity associated with EGFR inhibitors. By integrating organoid viability assays, high-content imaging, proliferation and apoptosis markers, and physiologically based pharmacokinetic (PBPK) modelling, researchers assessed the effects of first-, second- and third-generation EGFR TKIs on intestinal epithelial health. This multiparametric approach enabled direct comparison of compound-specific toxicity profiles while linking in vitro findings to clinically relevant exposure levels.

The study demonstrated that human intestinal organoids successfully reproduced the clinical ranking of EGFR inhibitor-associated GI toxicity. Afatinib induced loss of viability, reduced epithelial proliferation and increased apoptosis at substantially lower concentrations than gefitinib or osimertinib, consistent with its higher clinical incidence of diarrhea. Furthermore, PBPK-informed exposure modelling provided additional translational context by incorporating dynamic intestinal drug concentrations,

strengthening the relevance of the findings for human safety assessment.

Together, these results illustrate how human-relevant intestinal organoid platforms can move beyond traditional hazard identification to deliver mechanistic insights into toxicity pathways. By combining advanced in vitro systems with exposure modelling, sponsors can better characterize GI safety liabilities early in development, support compound prioritization and generate data that are more predictive of clinical outcomes. This approach demonstrates the value of integrating NAMs into nonclinical development programs to improve decision-making and de-risk therapeutic development.



Case study: Understanding EGFR inhibitor-associated gastrointestinal toxicity using human intestinal organoids

Background

Tyrosine kinase inhibitors targeting EGFR have transformed treatment of EGFR-mutant cancers. However, gastrointestinal adverse events, particularly diarrhea, remain common dose-limiting toxicities. Afatinib exhibits substantially higher diarrhea incidence than first- and third-generation EGFR inhibitors. Human intestinal organoids provide a human-relevant platform that recapitulates intestinal structure and function and can be used to investigate toxicity mechanisms.

Approach

Researchers used iPSC-derived human intestinal organoids with viability profiling, multiparametric imaging, proliferation and apoptosis markers and PBPK modelling to assess gastrointestinal toxicity across EGFR TKIs.

Results

Human intestinal organoids reproduced the clinical gastrointestinal toxicity ranking. Afatinib showed markedly greater toxicity (IC_{50} ~2 nM) compared with gefitinib (215 nM) and osimertinib (125 nM) (Figure 1). Imaging demonstrated epithelial injury, reduced proliferation and increased apoptosis (Figure 2). PBPK modelling enabled translation of in vitro findings to clinically relevant intestinal exposure (Figure 3).

Figure 1: Human intestinal organoids can differentiate gastrointestinal toxicity risk across EGFR tyrosine kinase inhibitors

(A) Dose-response viability analysis was performed in human intestinal organoids following exposure to first, second, and third generation EGFR TKIs. Integration of in vitro potency with clinical exposure (Cmax) enabled estimation of gastrointestinal risk, where lower IC_{50}/Cmax ratios were associated with increased toxicity liability. GIT, gastrointestinal tract; TKI, tyrosine kinase inhibitor; Cmax, maximum plasma concentration. (B) Dose-response curves showing the effects of the EGFR inhibitors Gefitinib (1st generation), Afatinib (2nd generation), and Osimertinib (3rd generation) on cell viability. Viability is expressed as a percentage relative to the DMSO-treated control. Data points represent mean ± SD. Nonlinear regression analysis was used to determine half-maximal inhibitory concentrations (IC_{50}), indicated by dashed lines.

A

EGFR TKI	Dose (mg)	Diarrheagenic index*	Diarrheagenic index* Grade>3	C-max (nM)	IC_{50} (nM)	Log10 IC_{50}/Cmax	GIT risk
Gefitinib 1st generation	250	46.6%	3.8%	190	215	0.05	
Afatinib 2nd generation	40	95.2%	14.4%	78	2	-1.6	
Osimertinib 3rd generation	80	40.1%	1.8%	600	125	-0.38	

*Typical clinical dosage

B

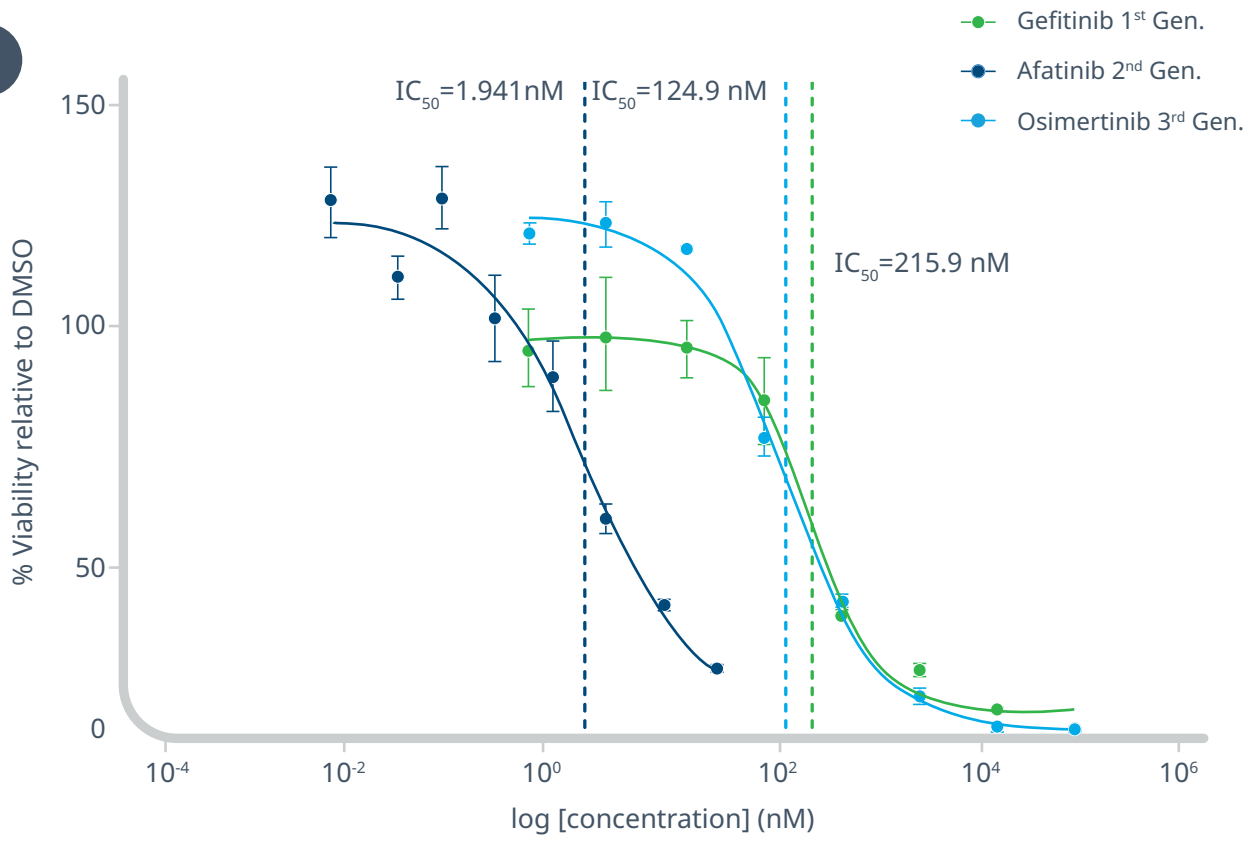
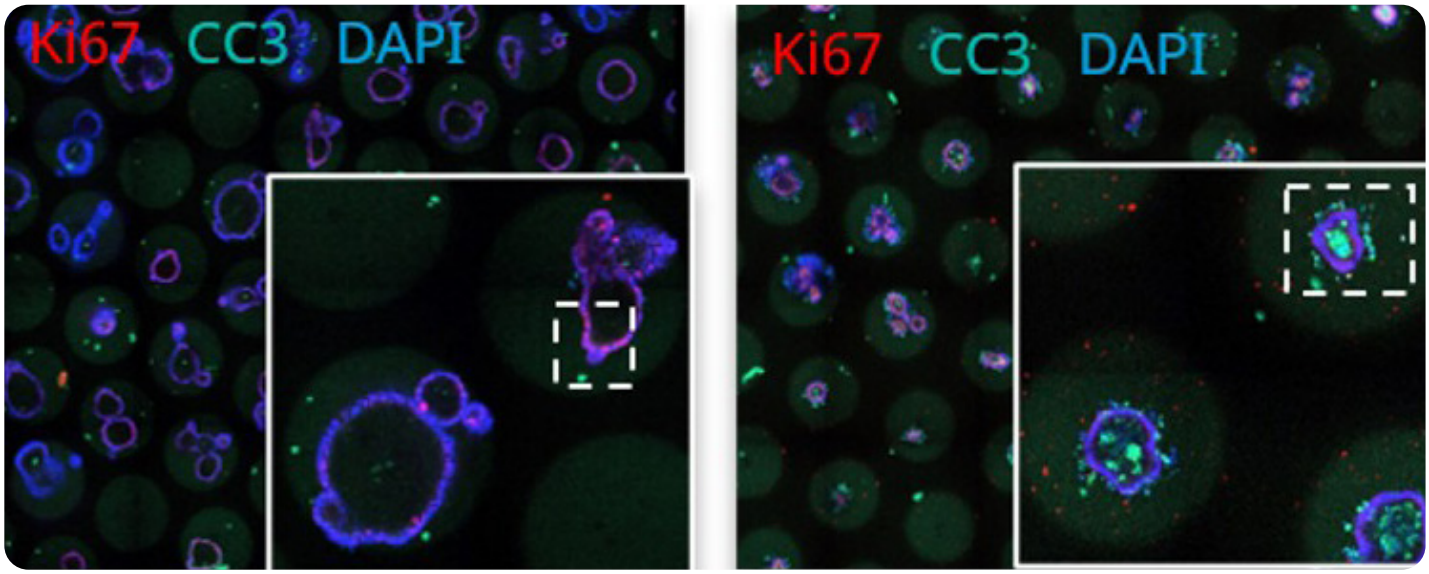


Figure 2: Imaging reveals differential effects of EGFR inhibitors on intestinal epithelial homeostasis

(A) Representative high-content images of human intestinal organoids exposed to afatinib or gefitinib and stained for proliferating cells (Ki67, red), apoptotic cells (cleaved caspase-3, CC3, green), and nuclei (DAPI, blue). (B) Quantitative image analysis demonstrated a concentration-dependent reduction in proliferative Ki67-positive cells accompanied by increased apoptotic activity following EGFR inhibition. Afatinib induced loss of proliferative capacity and apoptosis at substantially lower concentrations than gefitinib. *CC3, cleaved caspase-3; DAPI, 4′,6-diamidino-2-phenylindole*.

A



B

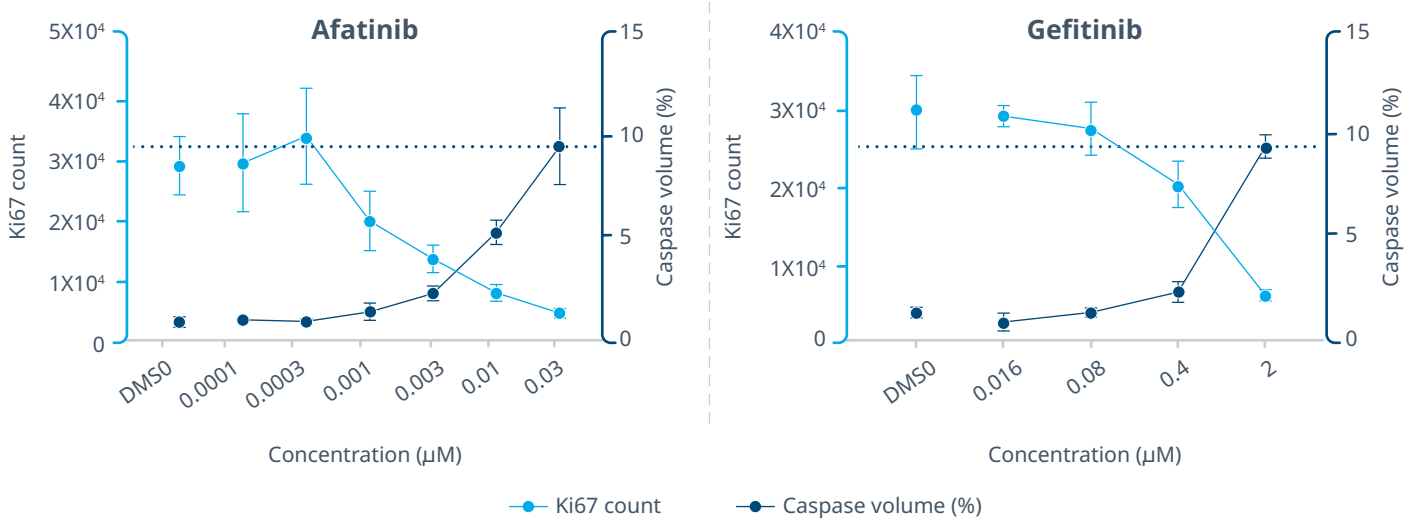
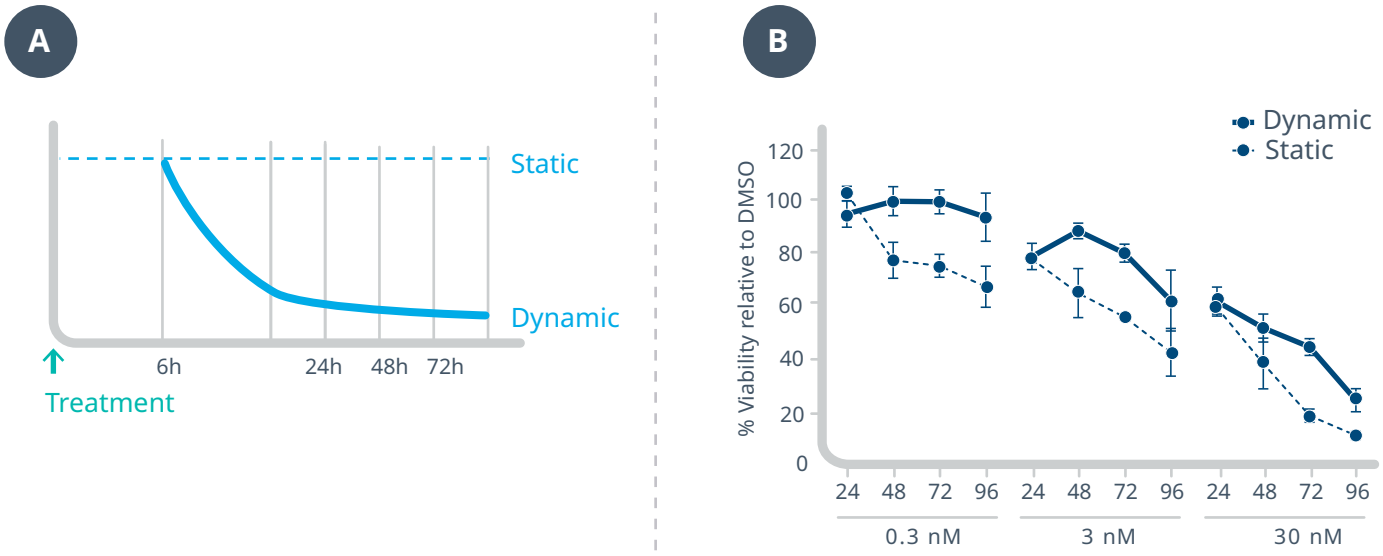


Figure 3: PBPK-simulated dynamic dosing improves the physiological relevance of gastrointestinal toxicity assessment in human intestinal organoids

Prior PBPK modelling predicted that administration of the clinically relevant afatinib dose (40 mg) results in unbound intestinal interstitial concentrations of approximately 3 nM, whereas subclinical exposure scenarios are associated with concentrations of approximately 0.3 nM. These exposure estimates were used to define the concentration range and dynamic dosing regimen evaluated in human intestinal organoids. (A) Under dynamic conditions, compound concentrations were adjusted over time to mimic predicted in vivo intestinal exposure profiles, while static cultures were maintained at a constant concentration. (B) Comparison of static and PBPK-informed dynamic dosing revealed distinct effects on organoid viability, demonstrating the importance of incorporating exposure kinetics into mechanistic toxicology studies.



Insights

Combining viability, imaging, functional assays and PBPK modelling generated translational mechanistic evidence supporting compound prioritization, gastrointestinal risk assessment and development decisions. The study demonstrates how human-relevant NAMs can move beyond hazard identification to mechanistic understanding.

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