ARTICLE

Quantitative systems pharmacology model-based investigation of adverse gastrointestinal events associated with prolonged treatment with PI3-kinase inhibitors

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Abstract

Several PI3K inhibitors are in clinical development for the treatment of various forms of cancers, including pan-PI3K inhibitors targeting all four PI3K isoforms (α, β, γ, and δ), and isoform-selective inhibitors. Diarrhea and immune-mediated colitis are among the adverse events observed with PI3K inhibition which limits the maximal tolerated dose. A quantitative systems pharmacology model was developed to investigate PI3K-inhibitor-induced colitis. The effects of individual PI3K isoforms on relevant cellular pathways were incorporated into a mechanistic representation of mucosal inflammation. A virtual clinical population captures the observed clinical variability in the onset timing and rates of diarrhea and colitis for seven clinically tested PI3K inhibitors. Model-based analysis suggests that colitis development is governed by both the inhibition of PI3Kδ, which drives T cell differentiation and proliferation, and PI3Kα, which regulates epithelial barrier integrity. Specifically, when PI3Kα is inhibited below a given threshold, epithelial barrier dysfunction precipitates an exaggerated T effector response due to PI3Kδ-inhibition, leading to risk of diarrhea and colitis. This synergy explains why the lowest diarrhea and colitis rates are seen with the weakest PI3Kδ inhibition (alpelisib), and higher rates are seen with strong PI3Kδ inhibition if PI3Kα is even mildly inhibited (e.g., idelalisib), whereas strong PI3Kδ inhibition in the absence of PI3Kα inhibition does not result in high colitis rates (umbralisib). Thus, the model-based analysis suggests that PI3Kα and δ inhibition play unique but synergistic roles in driving colitis. Finally, we explore if and how dose-regimen might influence colitis rates for molecules that inhibit both PI3Kα and PI3Kδ.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Several PI3K inhibitors are in clinical development and a few have been approved for treatment of different cancer indications. A number of serious class-related as

well as drug-specific adverse effects (AEs) have been reported for various PI3K inhibitors, including severe diarrhea with or without colitis. These inflammatory AEs are likely due to the suppression of the different PI3K isoforms and more specifically to inhibition of PI3Kδ.

WHAT QUESTION DID THIS STUDY ADDRESS?

The focus of this study was to develop and apply a mechanistic quantitative systems pharmacology model to investigate PI3K-inhibition-driven colitis, the mechanistic contribution of different PI3K isoforms to gastrointestinal (GI) toxicity, and strategies to minimize GI adverse events.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

This work quantifies the unique and synergistic role of α and δ inhibition in driving colitis risk.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

The mechanistic model and prospective simulations significantly clarifies the biological interactions leading to colitis under extended PI3K inhibition and supports optimization of future molecule design and dosing strategies.

INTRODUCTION

Phosphoinositidylinositol 3-kinases (PI3Ks) regulate intracellular signaling pathways involved in cell-cycle progression and survival in response to extracellular stimuli.[1] Three classes of PI3Ks have been identified (I, II, and III) and mutations in the class IA PI3K genes are common across various cancer types.[2] Class IA PI3Ks (PI3Kα, β, and δ isoforms) are heterodimeric enzymes composed of a regulatory subunit (p85), and one of three catalytic subunits p110α, p110β, or p110δ.[3] The class IB PI3K (PI3Kγ isoform) consists of one catalytic subunit (p110γ) and one regulatory subunit (p101). Class I PI3Ks play an important role in immune regulation, and the four isoforms differ in terms of tissue distribution and function.[4] PI3Kα is ubiquitously expressed and regulates specific immune cell functions, angiogenesis, and insulin signaling. PI3Kβ is also ubiquitously expressed and plays a critical role in Fcγ receptor-dependent phagocytosis and reactive-oxygen species production in macrophages and neutrophils. Epithelial cells express PI3Kα and β, which regulate their proliferation and survival.[5,6] PI3Kδ and PI3Kγ are expressed mostly on leukocytes, with levels and function that vary depending on cell type and activation conditions. PI3Kδ is critical for effector T cell and regulatory T cell (Treg) differentiation and function.[3,7,8]

Several PI3K inhibitors are in clinical development and or have been approved for treatment of hematological malignancies (idelalisib and copanlisib) and breast cancer (alpelisib). Taselisib (also known as GDC-0032) is a potent and selective PI3K inhibitor with excellent bioavailability and greater sensitivity for the activated PI3Kα form found in cancer cells than wild-type PI3Kα.[9,10] Phase I and II studies investigating the safety and tolerability of taselisib showed promising early clinical activity in patients with advanced or metastatic solid tumors.[11-13]

Although PI3K inhibition is generally well-tolerated in a controlled clinical setting, serious class-related and drug-specific adverse effects (AEs) have been reported.[14] Immune-mediated AEs reported for different PI3K inhibitors include cutaneous reactions, severe diarrhea with or without colitis, hepatotoxicity, and pneumonitis.[15] These inflammatory AEs are likely mediated by PI3K inhibition, particularly PI3Kδ,[16] on different lymphocyte subsets including Tregs.[2] Tregs are critical for the maintenance of immune tolerance and the control of inflammation.[7] Tregs appear to be involved in PI3K-associated immune AEs, such as diarrhea and colitis.[17,18] In a phase II trial of idelalisib in combination with rituximab, 46% of enrolled patients experienced diarrhea, and T-cell infiltrates were identified in colonic biopsies from most patients with diarrhea.[19] In addition, the PI3Kδ isoform plays a role in the differentiation of T-helper cells into Th1 and Th2 lineages.[20] Idelalisib now carries a black-box warning regarding hepatotoxicity, diarrhea/colitis, pneumonitis, and intestinal perforation.[16] Surprisingly, copanlisib, which has similar PI3K receptor selectivity and indicated use, does not have such warnings and the prevalence of grade 3 diarrhea is less than 5%.[21] However, it is noted that copanlisib is dosed once weekly as compared to daily dosing of idelalisib.

Diarrhea typically precedes colitis in patients, and aggressive pharmacological management of diarrhea can lessen its severity and mitigate severe colitis.[22-24] Moreover, dose reduction and alternate dosing schedules may also improve tolerability for specific PI3K

inhibitors. Finding an optimal dose and schedule limiting these AEs while still demonstrating clinical anti-tumor efficacy is of paramount importance for existing PI3K inhibitors. The present study describes the development of a mechanistic quantitative systems pharmacology (QSP) model to investigate PI3K-inhibitor-induced colitis. With this model, we aimed to: (1) explore the mechanistic contribution of specific PI3K isoforms to colitis; (2) evaluate if and how dosing regimen can mitigate colitis rates; and (3) inform the design of next-generation PI3K inhibitors and drug combinations with improved safety profiles.

METHODS

Model development

A mechanistic QSP model was constructed to investigate the possible causes of PI3K-inhibitor-induced colitis and estimate the relative risks of gastrointestinal (GI)-related AEs (diarrhea and colitis) for various PI3K inhibitors. The effects of various PI3K isoforms on relevant cellular pathways (Figure 1) were integrated into a mechanistic mathematical representation of mucosal inflammation and epithelial damage. The model includes two mechanistic compartments, a "blood" compartment representing the systemic circulation and a "mucosa"

compartment representing a cross-section of colon, including the epithelial layer, lamina propria, and contributions from gut-associated lymphoid tissues. The blood compartment serves as a source for immune cells. The mucosa compartment is the site of PI3K-induced inflammation leading to colitis. Based on a comprehensive literature survey, mechanisms and processes relevant for the onset of colitis were included in the model with a focus on cells and pathways regulated by the various PI3K isoforms (Figure 1). The following cells and functions are represented:

- Epithelial cell proliferation, maturation, activation, and apoptosis.
- Antigen-presenting cell (APC) recruitment, activation, and apoptosis.
- Naïve T cell recruitment and differentiation into Tregs and effector T cells.
- Treg and effector T cell proliferation, activation, and apoptosis.
- Neutrophil recruitment and turnover.
- Cytokine production: IL-10, IL-12, inflammatory cytokines, and T effector cytokines.
- Regulation of cellular processes by other cell types, cytokines, and PI3K isoforms.
- Modulation of PI3K isoform activities by PI3K inhibitors.
- Pharmacokinetics (PKs) for PI3K inhibitors.
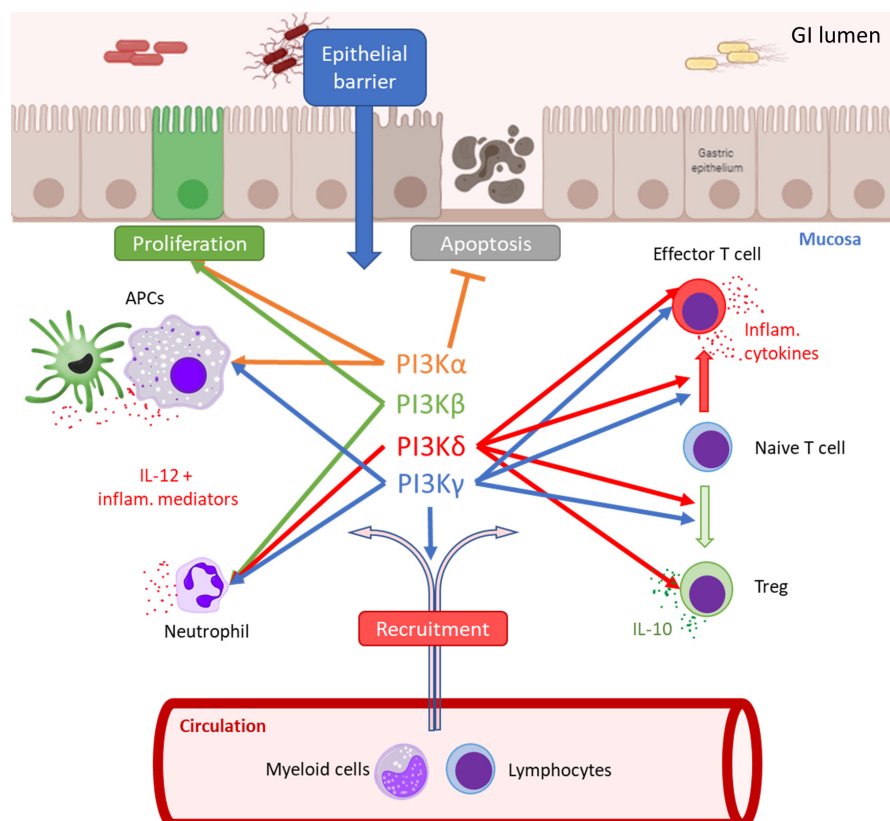
FIGURE 1 Effect of PI3K isoforms on mucosal biology leading to inflammation and colitis risk. APC, antigen-presenting cell; GI, gastrointestinal

The clinical readouts of the model include: (1) an epithelial quality score normalized between 0 and 1. It is a composite score of epithelial cell damage and impairment and leakiness of the junctions. It is driven by the number of healthy epithelial vs damaged epithelial cells. A score of 1 indicates a healthy epithelium and drops toward 0 with increasing damage; and (2) a GI risk score which is primarily driven by tissue inflammation as indicated by T cells, neutrophil infiltration, and tissue cytokine levels, and to a lesser extent by epithelial quality. The GI risk score is normalized from 0 to 1 and is used to estimate two clinical end points in the model: (i) the time of onset of diarrhea is defined as the time at which the GI risk score crosses a defined threshold value; and (ii) the time of onset of colitis is calculated as the area under the curve of the GI risk score (Table 1). These mathematical representations reflect the hypothesis that diarrhea results from an acute GI dysfunction, whereas colitis arises with sustained dysfunction and persistent inflammation, consistent with the observations that diarrhea precedes colitis and that aggressive management of diarrhea can mitigate colitis incidence or severity. Model development, calibration, and qualification against additional data sets were conducted as described in previous publications.[25,26]

The representation of the biology was informed by an extensive survey of the literature, with a particular focus on the role of PI3K in the relevant cellular processes (Table S1). Individual processes, such as epithelial cell proliferation, maturation, and apoptosis, were calibrated based on published preclinical and clinical studies, including inflammatory bowel diseases. Individual processes were then integrated and whole system-level behaviors were tested. The impact of the PI3K inhibitor on these pathways is determined by the contribution of each PI3K isoform to a particular pathway or effect (Table S2) and the drug half-maximal inhibitory concentration (IC_{50})s for inhibition of each of the PI3K isoforms. The IC_{50}s reported in the literature (Table S3) were used in the model. Based on the drug PK and isoform IC_{50}, the PI3K drugs' inhibition of the relative fractional activity of the pathways varies from 1 (no drug related inhibition) to 0 (complete inhibition of pathway).

At the whole-system level, the model simulations were consistent with expectations based on public data and broader scientific understanding. These include data from preclinical colitis models and clinical studies in healthy individuals or patients with cancer before and after PI3K treatment and are broadly categorized as follows:

1. Untreated baseline with a stable healthy epithelium: no inflammation, and all cell numbers and mediator concentrations at a dynamic equilibrium; the colon epithelium is initially healthy and only becomes inflamed upon extended PI3Ki dosing.
2. Damage and recovery from an acute immunological stimulus simulating a self-limiting chemically induced colitis model.[27] Qualitatively, the immunological stimulus should initiate early recruitment and activation of immune cells, production of inflammatory mediators, followed by healing of the damaged epithelium, and return to the prestimulus healthy state. Capturing this behavior ensures that the model representation is physiologically plausible (i.e., that the system is able to manage an acute challenge and return to stable conditions during the chronic phase; Table S4).
3. Increase in mucosal cell counts in response to PI3K-inhibitor treatment should be within the range reported in patients with colitis compared to healthy patients (Table S4).
4. Responses to the different PI3K inhibitors in a virtual population match those reported in clinical trials with respect to frequency and onset of diarrhea and colitis (Table 2).

PI3K inhibitors and compound dynamics

Seven different PI3K inhibitor molecules with different specificities for the four PI3K isoforms were represented in the model (Table S3). The GI AEs for these molecules have been reported in the literature in terms of diarrhea severity (grades 1–4) and/or occurrence and severity of colitis. As AE end points differed among studies, we broadly classified these PI3K inhibitors into two groups: low risk (<5% patients with colitis) and high risk (5–15% of patients with colitis; Table 2).

For the analyses in this work, the average PK profile was used for the drugs. The population variability in the simulated outcomes is due to mechanistic differences in

TABLE 1 Evaluation of epithelial quality, GI risk score, and AE onset times

EQ (normalized units)	Healthy epithelial cells + 0.2 * activated epithelial cells
GI risk score (GI risk: normalized units)	0.2*(1−EQ) + 0.8 * (activated-Tcells/Tcell$_{ref}$ + activated-APC/APC$_{ref}$ + neutrophils/neutrophils$_{ref}$)/3
Diarrhea onset (day)	GI risk crosses threshold on 0.6 [normalized units]
Colitis onset (day)	AUC of GI risk crosses threshold of 120 [normalized units-day]

Abbreviations: AE, adverse event; APC, antigen-presenting cell; EQ, epithelial quality; GI, gastrointestinal.

TABLE 2 Percentage of patients with colitis risk: virtual population comparison to clinical risk category

Compound	Clinical GI risk		Percent of virtual population with colitis risk	Model-based rationale for risk profile: α and δ inhibition	
	Risk category	References		α-inhibition	δ-inhibition
Taselisib	5–15%	11,12,28	10.2%	Strong (>90%)	Strong (>90%)
Idelalisib	5–15%	16,29	9.5%	Weak (10–25%)	Strong (>90%)
Duvelisib	5–15%	30	11.4%	Weak (~5%)	Strong (>90%)
Umbralisib	<5%	31	1.1%	Negligible (~0%)	Strong (>90%)
Alpelisib	<5%	32,33	2.4%	Strong (>90%)	Mod/weak (15–55%)
Pictilisib	5–15%	34	16.3%	Strong (75–90%)	Strong (80–95%)
Copanlisib	<5%	35,36	1.5%	Strong->weak (90–>15%)	Strong->weak: (90–>15%)

Abbreviation: GI, gastrointestinal.

the underlying biology. Pathway pharmacodynamic (PD) effects for the PI3K inhibitors were determined by the tissue drug concentration relative to the IC_{50} values (Table S3). PK parameters for the PI3K inhibitor molecules are provided in Table S5. These temporal inhibition profiles are shown in Figure 2.

Virtual population

A virtual population (VPop), comprised of multiple virtual patients, was developed using the methodology outlined in Gadkar et al.[26] to capture variability in the contribution of the different cell types and cytokines to epithelial damage and inflammation. Further, variability in the degree to which each PI3K isoform influences various cellular processes, and thus the relative impact of inhibiting that particular isoform on the GI risk profile, was also represented in the VPop (details included in Supplementary Material S6). All virtual patients in the VPop were required to satisfy the criteria that cell counts pre- and post-therapy were consistent with the literature data (Table S4).

Colitis and diarrhea clinical trial data for six of the compounds (Table 2) were used to calibrate the model and assign prevalence for individual virtual patients in the VPop. Copanlisib clinical data were used for subsequent model validation. In addition to published population level statistics for GI risk profiles, individual patient data for diarrhea and colitis onset were available for taselisib (6 mg q.d. dose, proprietary clinical trial data). During this clinical trial, ~50% of the patients experienced diarrhea and 8–10% developed colitis. The onset time for diarrhea ranged from 3 to 200 days and colitis onset ranged from 80 to 200 days. The range of diarrhea onset times for other PI3K inhibitors has not been reported and hence data for taselisib was used to represent

population variability. The available clinical response data for all the different PI3K inhibitor compounds were represented within this single mechanistic model of mucosal inflammation and epithelial barrier damage. Model calibration and VPop development was performed for all the data simultaneously.

The Supplementary Material includes the Simbiology model file and associated MATLAB code to simulate the model (SimBiology version: version 5.10, 2020a; MATLAB version: version 9.8, 2020a).

RESULTS

The model captures colon homeostasis and epithelial recovery from an acute insult

Ensuring the model can capture both (1) equilibrium in the healthy colon and (2) transient immune response and epithelial recovery after a forced acute epithelial insult, is critical to ensuring appropriately stable dynamic behaviors of the epithelial and immune cells in the model. In the absence of PI3K inhibition, the model and VPop represents a stable healthy colon. Acute perturbation or tissue insult (e.g., transient epithelial damage, chemical injury, or bacterial infection) should lead to recruitment and activation of immune cells and production of inflammatory mediators, causing an increase in inflammation that should resolve after the infection is subdued or damage is repaired. Figure S1 shows the response of the VPop to an immunogenic antigen challenge that results in acute epithelial damage. Simulations show a transient increase in neutrophils and APCs with a corresponding increase in the GI risk score. The increase in immune cell infiltration is resolved within 20–30 days consistent with chemically induced colitis in preclinical models.[27]
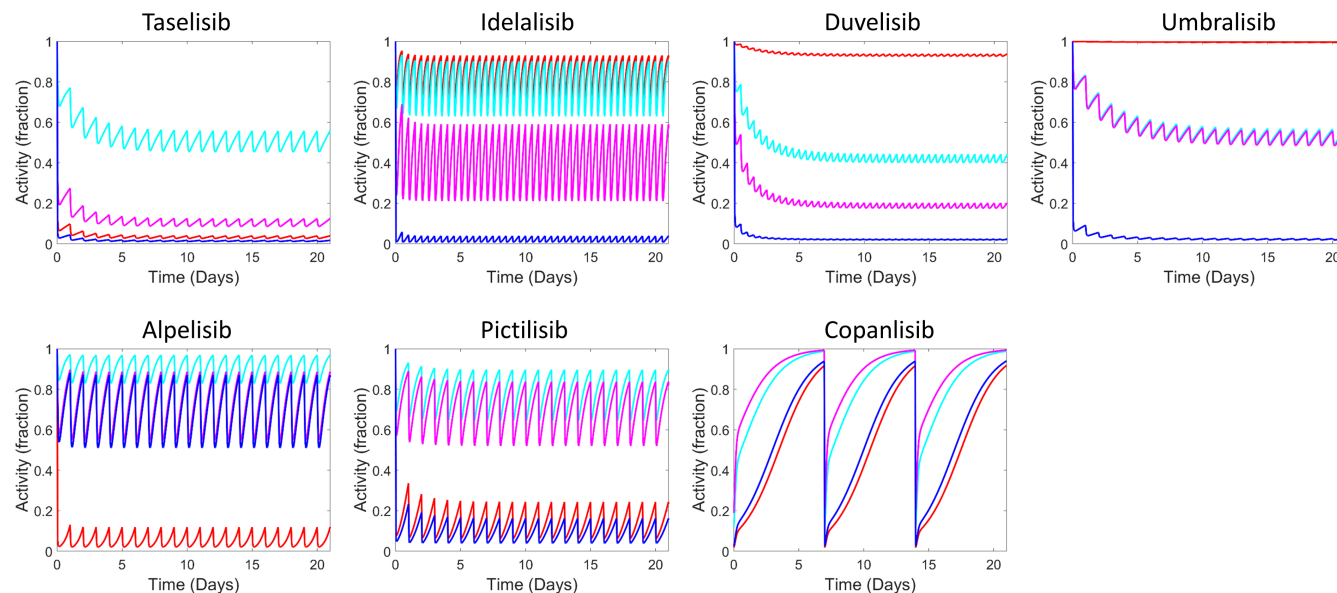
FIGURE 2 Normalized activity of the PI3K pathways for the four isoforms of PI3K; red: PI3Kα, cyan: PI3Kβ, magenta: PI3Kγ, and blue: PI3Kδ for the PI3K inhibitor molecules included in this work: taselisib, idelalisib, duvelisib, umbralisib, alpelisib, pictilisib, and copanlisib

TABLE 3 Percentage of diarrhea and colitis risk for alternate dosing regimens with 4 mg of taselisib

Dosing regimen	Percentage of virtual population with diarrhea onset	Percentage of virtual population with colitis onset
Daily dose	52%	10%
5 days on, 2 days off	53%	15%
14 days on, 7 days off	58%	15%

The virtual population captures clinical GI risk for different PI3K inhibitors

The prevalence of individual virtual patients in the VPop was calculated to match the percentage of patients experiencing diarrhea and colitis at 4 mg taselisib q.d. treatment[11,12]; 52% of the VPop patients are predicted to have diarrhea and 10% progress to colitis (Table 3). The clinical variability in the onset time for diarrhea (3–200 days) and colitis (80–200 days) with taselisib is also captured accurately by the VPop (Figure 3).

The relative specificities of the PI3K inhibitors evaluated in this model for the four PI3K isoforms contribute significantly to their risk profiles. Table 2 shows the prediction for all the PI3K inhibitors in terms of percentage of virtual patients that exhibit high GI risk scores and demonstrates that the VPop captures the differential colitis incidence for the PI3K molecules. Due to lack of consistency of risk categorization in reported clinical studies across the different PI3K inhibitor molecules, the molecules are classified as low colitis incidence (<5% population with colitis) and high colitis incidence (5–15% of population with colitis). The AE profiles for all the PI3K
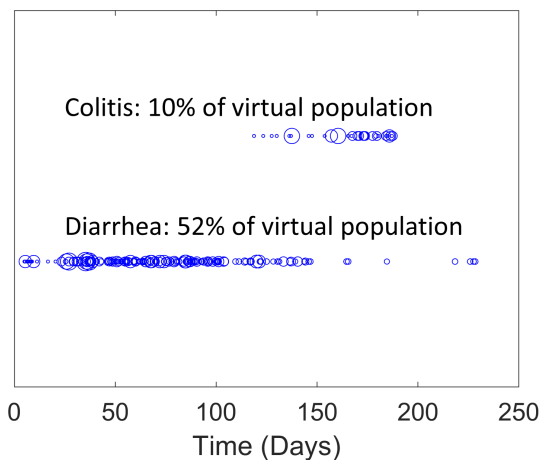
FIGURE 3 Onset time for diarrhea and colitis in virtual patient population on taselisib 4 mg q.d.; each open circle represents a virtual patient, with a diameter proportional to its weighted prevalence; diarrhea is manifested in 52% and colitis in 10% of the virtual population

compounds are shown in Figure S7. These simulations demonstrate the risks with PI3K inhibition alone and do not explicitly include effects of diarrhea managing comedications. The dynamic time profiles of changes in the

cellular dynamics and the risk scores in response to PI3K inhibition treatment is shown in Figure S8.

Sensitivity of GI risk to IC_{50} for different isoforms

To understand how the isoform specificities of the different drugs result in differential risk profiles, we performed a sensitivity analysis to explore the impact of the different isoforms on the epithelial health, inflammatory immune response, and GI risk. Table S3 shows the IC_{50} for taselisib for the four PI3K isoforms. The different isoforms drive multiple pathways, which are differentially impacted by PI3K inhibition depending on the IC_{50} of taselisib to the relevant isoform. In this analysis, we evaluate the sensitivity of the GI risk to the drug IC_{50} for the α and δ isoforms to elucidate the effects on epithelial damage and T-cells, respectively. Both the IC_{50} values are varied independently over two orders of magnitude and the impact on the inflammatory cell response, epithelial damage, and GI risk is predicted for simulated taselisib q.d. treatment. These sensitivity analyses of the IC_{50} for PI3Kα and PI3Kδ are described below:

Epithelial damage due to PI3Kα-inhibition is permissive for progression to colitis

Figure 4a shows the PI3Kα activity at four different IC_{50} against PI3Kα: 0.29 nM (nominal value for taselisib),

2.9 nM (10×), 8.7 nM (30×), and 29 nM (100×). The IC_{50} values for the other isoforms are kept constant at the nominal values for taselisib. The percent inhibition of the PI3Kα is reduced as the IC_{50} is increased, such that at a 100-fold increase over the nominal IC_{50} value, the PI3Kα inhibition is reduced from greater than 95% to ~20% on average for the dosing period. The inhibition for the other isoforms remains unchanged. Figure 4b,c shows the diarrhea and colitis onset times for the VPop for these four PI3Kα IC_{50} values. There is a marked reduction in the percent of patients experiencing diarrhea (from 52% to 27%) indicating that improved epithelial quality reduces diarrhea rate. However, the proportion of patients experiencing colitis is only moderately reduced from 10% to 7%, indicating that the most colitis-prone virtual subjects require only a small level of epithelial damage to trigger the inflammatory process that leads to inflammation and colitis.

Immune response and GI risk are very sensitive to PI3Kδ inhibition

Figure 4d shows the inhibition of the PI3Kδ activity for four different IC_{50} for PI3Kδ: 0.12 nM (nominal value for taselisib), 1.2 nM (10×), 3.6 nM (30×), and 12 nM (100×). The IC_{50} values for the other isoforms are kept constant at the nominal values for taselisib. The percent inhibition of the PI3Kδ is reduced as the IC_{50} increases, such that at 100-fold increase over the nominal IC_{50}
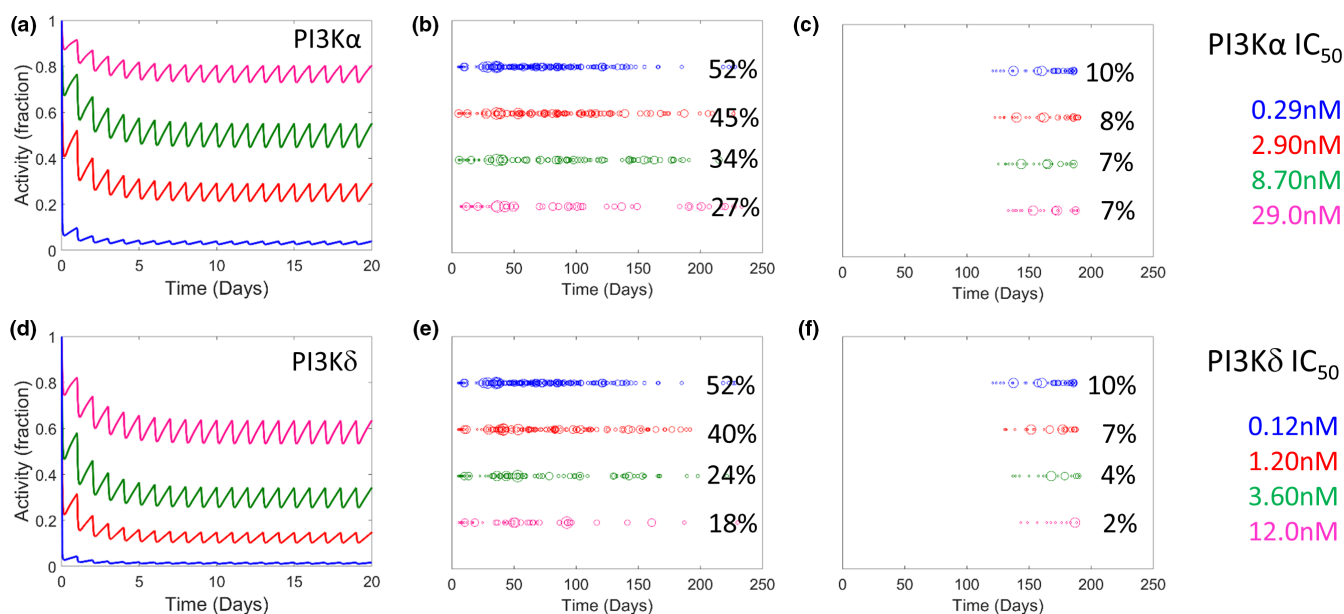
FIGURE 4 (a) Normalized activity of the PI3Kα pathways for varying PI3Kα IC_{50}; onset time for (b) diarrhea and (c) colitis with varying levels of PI3Kα IC_{50}; (d) normalized activity of the PI3Kδ pathways for varying PI3Kδ IC_{50}; onset time for diarrhea (e) and (f) colitis with varying levels of PI3Kδ IC_{50}; (percentage values in the figures indicate percentage of the virtual population with diarrhea or colitis risk). IC_{50}, half-maximal inhibitory concentration

value, the PI3Kδ inhibition decreases from greater than 95% to ~40%. The inhibition for the other isoforms remains unchanged. Figure 4e,f shows the diarrhea and colitis onset times for the VPop for these four PI3Kδ IC$_{50}$ values. The rates of diarrhea and colitis are both diminished with weaker PI3Kδ inhibition, with a reduction from 52% to 18% in a proportion of patients experiencing diarrhea and a reduction of 10% to 3% in patients experiencing colitis as the PI3Kδ IC$_{50}$ is increased by 100-fold.

Permissive epithelial damage and T cell skewing by α and δ isoform inhibition, respectively, together drive GI risk

Figure 5 shows the modulation of the underlying modeled biology with changes in the IC$_{50}$ for PI3Kα and PI3Kδ. There is a marked improvement in epithelial quality with weaker PI3Kα inhibition, resulting in reduced APC activation and a reduction in inflammatory T cells. When altering only the PI3Kδ related pathways, there is no significant change in the epithelial quality, which is primarily driven by PI3K α and β. However, with weaker PI3Kδ inhibition, effector T cells are reduced whereas the Tregs are increased, illustrating that PI3Kδ inhibition alters the balance between effector and regulatory T cells toward a more inflammation-prone state. As reported in Table S2, the proliferation of both effector T cells and Tregs is altered by PI3Kδ inhibition and the simulations show an increase in the pro-inflammatory response in the gut mucosa with greater PI3Kδ inhibition. The modeled synergy between α and δ isoform inhibition explains the differential risk profiles of the various PI3K inhibitors, as outlined in Table 2 based on the temporal isoform inhibition profiles in Figure 2. Even a weak (but non-negligible) PI3Kα inhibition causes enough epithelial damage to create a permissive condition, which when combined with T cell skewing due to strong PI3Kδ inhibition leads to inflammation and colitis in susceptible virtual patients.

Effect of PI3K inhibitor dose schedule on GI risk

Infrequent dosing of copanlisib leads to low predicted colitis frequency despite combined α,δ-inhibition

Seemingly at odds with the model finding that cooperation of α and δ inhibition enable and drive progression to colitis, emergent data indicate low colitis rates in clinical trials of the PI3Kα,δ-inhibitor copanlisib. We applied our model to simulate the copanlisib dose regimen (q.w. dosing) in the virtual population and compared model predictions to the reported results, which were not used in model development. Interestingly, the simulations indeed predicted low colitis incidence (1.5%) in the VPop. Figure 2 shows the profiles for the effect on copanlisib on the activity for the four PI3K isoforms with once a week dose of 60 mg. Due to the relatively infrequent (q.w.) dosing of copanlisib used for the treatment of adult patients with relapsed follicular lymphoma, the inhibition of the PI3K isoforms and the subsequent downstream effects responsible for colitis are not sustained enough to drove progression; specifically, δ-isoform inhibition is not sustained to a strong extent between doses, resulting in the predicted low risk in the VPop and consistent with the clinical observation.

Dosing holidays are not predicted to alter colitis rates significantly for taselisib

The model was also used to evaluate whether dosing holidays from the normal daily dosing regimen of taselisib could reduce colitis rates. Three taselisib dosing regimens are considered for this evaluation: (i) daily dosing, (ii) 5 day on-2 days off, and (iii) 14 days on-7 days off dosing. These regimens illustrate the effects of different lengths of dosing holidays on the risk. Figure 6a shows the activity of the four PI3K isoforms for the three taselisib dosing regimens. As expected, the inhibition of the PI3K-dependent pathways is diminished during the dosing holidays. However, even during the 7-day drug holiday, the levels of the α, γ, and δ isoforms do not return to their baseline untreated values of 1, suggesting that the underlying pathways for epithelial cells and inflammatory pathways of T cell and neutrophils are still impacted. In keeping with the hypothesis that even a weak inhibition of PI3Kα combined with a strong inhibition of PI3Kδ drive colitis progression, all dose regimens maintain moderate-strong inhibition of α and strong inhibition of δ over the entire dosing window. Table 3 shows the percentage of subjects in the VPop experiencing diarrhea and colitis. The AE profiles for these alternate dosing regimens are shown in Figure S9. For the scenarios with dosing holidays, the predictions for both the diarrhea and colitis risks do not improve compared to daily dosing, suggesting that the dosing holidays evaluated do not reduce taselisib-induced colitis frequency. The modest increase in the colitis frequency with the dosing holidays is due to the differential effect of the PI3Kδ effect on T cells and Tregs, whereas in a small fraction of the VPop reducing the PI3Kδ inhibition during the dosing holiday reduces the protective effects of Tregs.
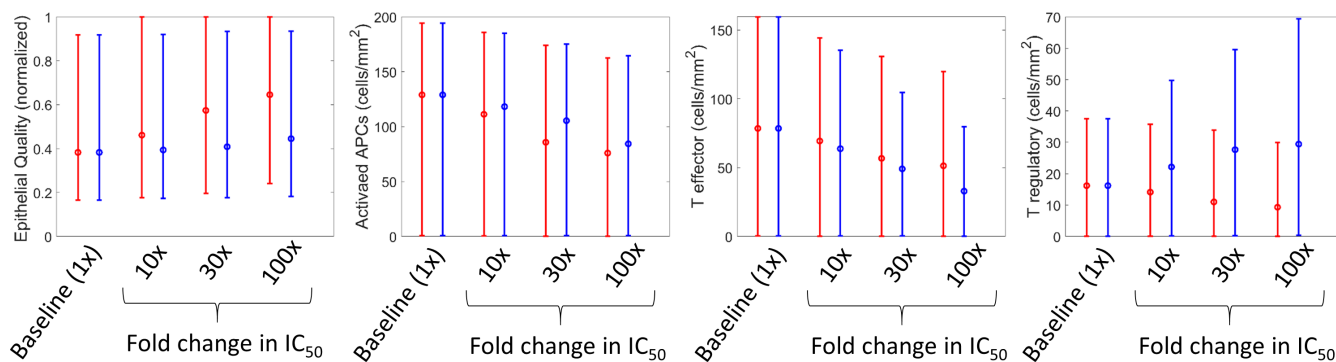
FIGURE 5 Numbers of activated APCs, T effector cells, regulatory T cells, and epithelial quality for varying levels of PI3Kα IC_{50} (red) and PI3Kδ IC_{50} (blue); the baseline (1×) refers to the default IC_{50} for PI3Kα and PI3Kδ and these are independently varied by 10×, 30×, and 100× of baseline; open circle: median; error bars: 5–95 percentile of virtual population. APC, antigen-presenting cell; IC_{50}, half-maximal inhibitory concentration
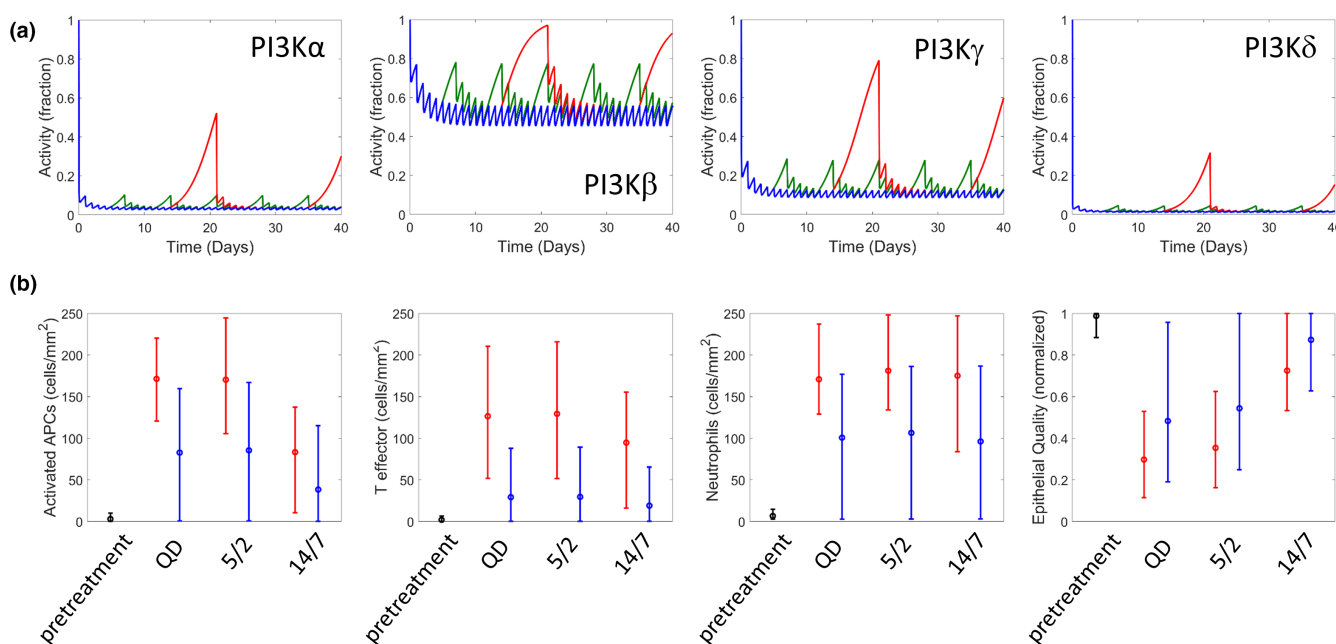
FIGURE 6 (a) Normalized activity of the four PI3K isoforms for the dosing regimens with 4 mg of taselisib; blue: daily dosing; green: 5 days on, 2 off; red: 14 days on, 7 off (b) numbers of activated APCs, T effector cells, neutrophils, and epithelial quality prior to PI3K treatment (black) and in response to different dosing regimens of 4 mg taselisib (red: virtual patients with diarrhea risk, blue: virtual patients without diarrhea); open circle: median; error bars: 5–95 percentile of virtual population

Figure 6b shows the numbers of APCs, T cells, neutrophils and the epithelial quality for the VPop stratified into two groups of virtual patients, those with high and low diarrhea susceptibility. The group with high susceptibility is the virtual patients that experience diarrhea in the simulations and the low susceptibility group are diarrhea-free with the taselisib dosing regimens evaluated. The less susceptible group shows a lesser increase in inflammatory cells and better epithelial quality with taselisib treatment compared to the high susceptibility group. For the high susceptibility group, dosing holidays result only in a modest reduction in effector T cell infiltration and have no effect on neutrophils. The epithelial

damage is lower with taselisib dose holidays but is still sufficient to enable immune cell activation and subsequent inflammation.

DISCUSSION

PI3K inhibitor compounds differ in their PI3K isoform selectivity and affinity profiles and have been associated with different degrees of GI risk. The PI3Kδ isoform in particular has been implicated in GI inflammation, due to its modulation of immune activity and emphasized by the significant GI risk associated with the δ-selective

inhibitor idelalisib. However, trials of other δ-selective inhibitors (umbralisib) have encountered lower incidences of colitis. The hypothesis that PI3K isoform affinities collectively determine a compound's risk profiles is intuitively attractive; however, the relationship between affinities and safety profiles is not straightforward. We investigated the mechanistic connections between PI3K isoforms and GI outcomes by constructing a QSP model that integrates publicly available data and pathological processes involved in drug-induced colitis and the role of PI3K isoforms in these processes. This mechanistic representation explicitly accounts for the role that each PI3K isoform (and inhibition thereof) plays in inflammatory responses in the colon and can thus be used to investigate the likely mechanistic impact of compounds with different isoform specificities on biological processes and associated GI risk outcomes. The PKs of the drugs also influence these outcomes as drug exposures relative to its IC_{50} values for the four PI3K isoforms determine the extent of inhibition effect on each isoform. The model and associated VPop accurately captured population-level diarrhea and colitis timing and incidence upon extended PI3K inhibitor dosing. The mathematical formulation of epithelial quality (EQ) and GI risk score is based on a mechanistic understanding of the drivers of these processes and calibrated with clinical data. Risk data for six PI3K inhibitor molecules was used for model calibration and data from one molecule was used for validation. Additional model testing can be performed as GI risk data emerge for new PI3K inhibitor molecules. Further, alternate mathematical representation of EQ and GI risk score can also be explored but is outside the scope of the current work.

The mechanistic model enabled investigation of the complex immunological interactions that lead to increased GI risk. For example, systematically altering the IC_{50} values for PI3Kα versus PI3Kδ led to insights about the interplay between epithelium and immune cells. The most significant biological impact of PI3Kα inhibition is a reduction in epithelial quality, whereas PI3Kδ inhibition is associated with a shift in the T effector versus Treg balance. Model-based investigations suggest that some minimal amount of epithelial damage (e.g., due to PI3Kα inhibition) is required to initiate the inflammatory process, beyond which GI risk increases with inhibition of PI3Kδ. The simulations further indicate that the degree of epithelial damage required to trigger this process likely varies among patients. This hypothesis thus reconciles the risk profiles of the different inhibitors. For example, although both idelalisib and umbralisib most potently inhibit the δ-isoform, this hypothesis proposes that the α-inhibition achieved by

idelalisib is sufficient to initiate the inflammatory process, whereas this is not the case for umbralisib. An important consideration in these calculations is the dose, PK, and regimen of the different drug. With sufficient drug concentrations, a δ-selective inhibitor, such as idelalisib, is still predicted to surpass the low in silico threshold of roughly 15% α-isoform inhibition sufficient for increased population risk of colitis. Interestingly the α-δ dual-selective inhibitor copanlisib has not reported comparably high rates of colitis. When we tested our model for this copanlisib, we accurately predicted this result in silico, with the model results suggesting that, despite dual inhibition, infrequent dosing of this molecule allowed the epithelium to recover and inflammation to resolve between doses, thus limiting the progression to colitis in the virtual patients.

Given the critical role of dose/regimen, we also investigated mitigation strategies for the high-risk molecule, taselisib, simulating the impact of dosing holidays on predicted GI AE incidence. No significant reduction in incidence was predicted under any of the dosing holiday scenarios tested. A similar approach of simulating protocols under consideration can be taken to optimize protocols for future compounds. However, to fully explore the value of such alternate dosing regimens, their impact on efficacy must also be assessed in parallel. Exploration of models of efficacy models would be specific to the intended indication and was beyond the scope of this investigation.

A critical caveat of this work is that the combination therapy of PI3K with estrogen-modulating therapies, such as letrozole or fulvestrant, or concomitant medications that may be administered to manage diarrhea were not included in the current simulations. For example, lymphocyte-depleting chemotherapy could impact immune activation and thereby colitis risk or severity, and an anti-diarrheal may counteract the epithelial damage that contributes to colitis. Risk factors, such as hyperglycemia, are also not currently addressed. Despite these limitations, the mechanistic model and prospective simulations provide insights into the biological interactions leading to PI3K-inhibitor induced colitis and can be used to support optimization of future compounds and dosing strategies.

CONFLICT OF INTEREST

K.G., L.D., L.S., J.J., J.A.W., and S.R. were full-time employees at Genentech Inc. when this work was conducted. M.K.J. was a summer intern at Genentech when this work was conducted. C.F. and V.H. were full-time employees at Rosa & Co. when this work was conducted. M.L.R. was a contractor for Rosa & Co when this work was conducted.

AUTHOR CONTRIBUTIONS

All authors wrote the manuscript, designed the research, performed the research, and analyzed the data.

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