Pathological assessment of gastrointestinal biopsies from patients with idelalisib-associated diarrhea and colitis

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Aim: Idelalisib (IDELA) treatment is associated with diarrhea/colitis (incidence of ~15% grade ≥ 3). We performed a retrospective analysis of gastrointestinal biopsies from 29 patients treated with IDELA across nine clinical trials. **Methods:** A central core laboratory performed histopathologic review, immunohistochemistry, and droplet digital PCR viral studies. These results were correlated with tissue immune profiling data and morphologic features per modified Geboes score. **Results:** Out of 29 eligible patients with abdominal pain or diarrhea, 24 (82.8%) had reported adverse event terms of diarrhea and/or colitis. Infectious pathogens were detected in 9/29 samples. Most biopsies presented with mixed/inflammatory infiltrates and contained increased numbers of FOXP3⁺ cells versus normal controls. **Conclusion:** This study revealed evidence of T-cell dysregulation and a substantial infectious component in association with IDELA-related diarrhea/colitis.

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Idelalisib (IDELA) is a selective inhibitor of the delta isoform of phosphoinositide 3-kinase (PI3K δ), indicated for the treatment of relapsed chronic lymphocytic leukemia (CLL) and follicular lymphoma [1]. Nonclinical studies have demonstrated that inhibition of PI3K δ hinders B-cell proliferation, chemotaxis, motility and adhesion, and promotes apoptosis in cell lines derived from B-cell malignancies [2]. IDELA treatment provides significant objective responses in patients with refractory CLL and has demonstrated substantial clinical benefit in three Phase III relapsed-CLL trials [3–5]. Progression-free survival, the primary end point of these three studies, improved significantly after addition of IDELA to rituximab, ofatumumab or combination of bendamustine and rituximab ($p < 0.001$ for all) [3–5]. Substantial improvement was also noted in overall response, compared with the control arm. Across clinical trials, grade ≥ 3 diarrhea and/or colitis were described in approximately 15% of patients who received IDELA [3,6–9]. Mild or moderate (grade 1–2) diarrhea may occur within the first several months of treatment, while a more severe (grade ≥ 3), often watery diarrhea can occur later, in the absence of cramps, blood or mucus [10]. The histologic and clinical features of IDELA-associated colitis have been previously reported in small studies [10–12]; however, the pathophysiology is not fully understood [10,11]. The current study describes the pathological assessment, immune cell profiling from tissue and peripheral blood and detection of viral pathogens using highly sensitive droplet digital PCR (ddPCR) assays performed on specimens from 29 patients who developed abdominal pain or diarrhea during IDELA treatment across nine different clinical trials.

Materials & methods

Patient selection criteria & tissue acquisition

Tissue biopsy specimens and clinical data from 29 patients with relapsed CLL or indolent B-cell non-Hodgkin lymphoma who developed symptoms of diarrhea and/or colitis while enrolled in one of nine clinical studies and treated with IDELA alone or in combination with other agents (NCT01659021, NCT01732913, NCT01539291, NCT01539512, NCT01569295, NCT02136511, NCT01282424, NCT01732926, NCT01980888) (Table 1), were utilized in this analysis. The symptoms indicative of diarrhea and/or colitis were as outlined in the Common Terminology Criteria for Adverse Events version 4.03, and included mild to severe abdominal pain, diarrhea or severe diarrhea associated with fever, dehydration and weight loss, ileus, peritoneal signs, hypovolemia and other signs of colon inflammation. All patients involved in this analysis had signed informed consent allowing the study sponsor to request samples of tissue biopsies that were performed as a standard of care. The patients were initially identified from the study database as having had either upper GI or colon biopsies done for the purpose of evaluating abdominal pain or diarrhea. Any patients with available tissues for pathology review were included in this study. Blocks or mounted sections of formalin-fixed, paraffin-embedded (FFPE) tissue were submitted for study. Details of patient inclusion criteria for each of the trials have been previously described [3,4,8,13–16]. Normal colon or small bowel biopsy tissues obtained from routine screening colonoscopies in 18 individuals without malignancy, and who had not been treated with IDELA, were used as controls.

Methods

Hematoxylin and eosin-stained slides of gut biopsies were reviewed by two pathologists with expertise in gastrointestinal pathology and hematopoietic stem cell transplantation. The grades used for evaluation of disease severity were modified both from the Geboes *K et al.* scoring system [17], and from the study by Masia *et al.* [18]. Histologic features that were graded include overall architecture, presence of attenuated glands, apoptosis, regenerative changes, inflammation (neutrophilic, lymphocytic, eosinophilic), edema, crypt destruction, crypt abscesses, and erosion/ulceration. After review and grading of the individual histologic features, the cases were classified into one of four overall morphologic patterns. Tissue samples demonstrating mainly inflammation with or without ischemic changes were classified as inflammatory or inflammatory/ischemic; samples dominated by apoptotic cells with very little inflammation (less than grade 1) were classified as apoptotic; combinations of histological features, including both inflammation and apoptosis, were categorized as mixed, and those with retained architecture and very little to no inflammation or apoptosis were considered normal (Supplementary Table 1).

Immunohistochemistry (IHC) for CD3, CD8, FOXP3, CD79a, cytomegalovirus (CMV), and adenovirus (AdV) was performed when sufficient tissue sample was available. All IHC assays were standard clinical assays performed at the Seattle Cancer Care Alliance certified laboratory (WA, USA), and all clinical standard operating procedures can be obtained from the laboratory upon request. FOXP3 immunostainings were performed at PhenoPath, PLLC (WA, USA). Sections were incubated with the following commercially available primary antibodies: anti-AdV (MAB805 clones 2/6 and 20/11; EMD-Millipore), anti-CMV (clones DDG9 and CCH2; Abcam), anti-CD3 (2GV6; Ventana), anti-CD8 (SP57; Ventana), anti-CD79a (SP18; Ventana), and anti-FOXP3 (SP97; Thermo Fisher Scientific). B-cell to T-cell ratios were calculated based on the ratio of CD79a to CD3 positive cells. CD4 to CD8 ratios were calculated based on the relative percentage of positive T cells. The FOXP3⁺ T-regulatory cells (Tregs) were scored as a percentage of total CD3⁺ T cells. In addition, we annotated the presence of enlarged FOXP3-positive nuclei.

DNA extractions from FFPE tissues were performed using Maxwell 16 FFPE Plus LEV DNA Purification Kit (Promega; Madison, WI, USA) per manufacturer's protocol. DdPCR assay was performed for CMV, AdV, and human herpesvirus 6 (HHV-6) on FFPE tissues. The ddPCR assay was performed using the QX100 droplet generator and reader (Bio-Rad Laboratories, CA, USA), as previously described [19,20].

Absolute numbers of peripheral blood T cells (CD3⁺/CD4⁺ or CD3⁺/CD8⁺) and NK-cells (CD16⁺/56⁺) were measured by flow cytometry. The T- and NK cells were assessed as described by the manufacturer using Becton Dickinson (BD) Multitest™ 6-color *in vitro* diagnostic kit (BD Biosciences). Briefly, whole blood was collected in Cyto-Chex BCT tubes at clinical sites and shipped to Covance (IN, USA) central labs for analysis.

Tissue samples were collected at multiple time points during the study. The collection of these samples at specific time points was driven by each of the protocol-specified visits that varied from study to study ranging from weekly initially, to once every 3–4 weeks as the study progressed. Given that this is a retrospective analysis, blood samples

Table 1. Clinical summary.

Patient	Study	Diagnosis	Age [†]	Gender	Treatment regimen	Organ site biopsy	Time to onset [‡] (diarrhea/colitis)	Time to resolution [‡]	SAE	AE temporally associated with biopsy
1	1	CLL	69	F	IDELA + OFA	Colon, rectum	265	>74 [§]	Yes	Neutropenic fever, colitis
2	1	CLL	64	M	IDELA + OFA	Colon/TI	300	58	Yes	Hypovolemic shock, diarrhea, colitis
3	2	NHL	82	M	IDELA + RTX	Ileocecal/TI	365	100	Yes	Dehydration, diarrhea
4	1	CLL	64	F	IDELA + OFA	Colon	385	57	Yes	Colitis, diarrhea, fever, dehydration
5	1	CLL	64	M	IDELA + OFA	Colon	411	269	Yes	Abdominal pain, hypotension, fever, worsening colitis
6	3	CLL	76	F	IDELA	Colon	247	>71 [§]	No	Fever, weight loss, diarrhea
7	3, 4	CLL	63	M	IDELA	Colon/TI	427	137	Yes	Colitis, weight loss
8	5	CLL	62	M	IDELA + BR	Colon	208	54	No	Diarrhea
9	6	CLL	67	F	IDELA + RTX	Colon	13	5	Yes	Colitis
10 [¶]	7	NHL	60	M	IDELA	Colon	450	>49 [§]	No	Diverticulitis
11	7	NHL	56	M	IDELA	Colon	106	231	Yes	Acute colitis, hematochezia
12	3, 4	CLL	61	M	IDELA	Colon	322	119	Yes	Pancolitis
13	2	NHL	50	M	IDELA + RTX	Colon	96	271	Yes	Diarrhea
14	1	CLL	73	M	IDELA + OFA	Colon	55	720	No	Colitis
15 [¶]	8	NHL	67	F	IDELA + BR	Colon	171	70	Yes	Fever
16	1	CLL	55	M	IDELA + OFA	Colon	156	275	Yes	Colitis
17 [¶]	8	NHL	70	M	IDELA	Colon	127	146 [#]	Yes	Colonic wall thickening
18 ^{††}	1	CLL	57	M	IDELA + OFA	Duodenum	288	>188 [§]	Yes	Diarrhea
19	3, 4	CLL	81	M	IDELA	Colon	517	224 [#]	Yes	<i>Mycobacterium avium-intracellulare</i> /hemorrhagic colitis
20 [¶]	1	CLL	61	M	IDELA + OFA	Colon, gastroduodenal	375	57	Yes	Abdominal pain
21	1	CLL	67	M	IDELA + OFA	Colon	330	71	Yes	Diarrhea
22	1	CLL	70	M	IDELA + OFA	Colon	334	226	No	Diarrhea, CMV infection
23	3, 4	CLL	81	F	IDELA + RTX	Colon	188	11	Yes	Diarrhea (<i>Norovirus</i>), colitis
24	9	CLL	69	F	IDELA + BR	Colon	225	25	Yes	Colitis
25	1	CLL	77	F	IDELA + OFA	Colon	582	64	Yes	Dyspepsia, colitis
26	1	CLL	61	M	IDELA + OFA	Colon	587	37	No	Diarrhea
27 [¶]	5	CLL	59	F	IDELA + BR	Colon	193	30 [#]	Yes	Acute diverticulitis with bowel perforation, pneumonia
28	1	CLL	59	M	IDELA + OFA	Colon	386	191	Yes	Colitis
29	3	CLL	78	M	IDELA	Colon, rectum	149	65	No	<i>Herpes simplex virus</i> infection and diarrhea

[†]Age of patients in years.[‡]Data presented in days.[§]End of study.[¶]Row represents IDELA-treated patients who did not present with diarrhea/colitis at the time of biopsy.[#]Deceased patient.^{††}Patient tested positive for celiac disease and had anti-gliadin antibodies.

AE: Adverse event; BR: Bendamustine and rituximab; CLL: Chronic lymphocytic leukemia; CMV: Cytomegalovirus; F: Female; IDELA: Idelalisib; M: Male; NHL: Non-Hodgkin lymphoma; OFA: Ofatumumab; RTX: Rituximab; SAE: Serious adverse event; Study 1: NCT01659021; Study 2: NCT01732913; Study 3: NCT01539291; Study 4: NCT01539512; Study 5: NCT01569295; Study 6: NCT02136511; Study 7: NCT01282424; Study 8: NCT01732926; Study 9: NCT01980888; TI: Terminal ileum.

obtained at the time of diarrhea/colitis symptoms were not available; the closest blood collection visits are marked in the Supplementary Figure 1.

50 µl of clinical specimen or a quality control sample was combined with 20 µl of antibody cocktail reagent, mixed by brief vortexing, and incubated for 15 min at room temperature (18–26°C) in the dark. Red blood cells

Table 2. Histological features of patient biopsies.

Patient	Architectural distortion; 0 = no 1 = yes	Ischemic changes; 0 = no 1 = focal 2 = multifocal/ patchy	Glandular changes/ apoptosis; 0 = normal 1 = mild 2 = moderate 3 = marked	Glandular changes/ re-generation; 0 = normal 1 = focal 2 = patchy 3 = marked	Neutrophilic inflammation; 0 = none 1 = mild 2 = moderate 3 = marked	Lymphocytic inflammation; 0-3 none to marked	Eosinophilic inflammation; 0-3 none to marked	Edema; 0-3 none to marked	Crypt destruction or crypt abscess; 0 = none 1 = focal 2 = patchy 3 = frequent	Erosion or ulceration; 0 = none 1 = focal 2 = patchy 3 = large areas of ulcer or granulation tissue	Histologic pattern
9	0	0	0	1	0	0	0	0	0	3	Normal
20	0	0	0	0	0	0	0	0	0	0	Normal
5	1	0	3	3	2	1	0	0	1	3	Apoptotic
8	0	1	1	0	0	0	0	1	0	0	Apoptotic
1	1	1	1	1	2	2	0	1	2	1	Inflammatory/ischemic
4	0	2	1	2	2	2	0	0	2	2	Inflammatory/ischemic
6	0	0	1	1	2	2	0	1	2	1	Inflammatory
7	0	2	1	2-3	2	2	0	0	3	1	Inflammatory/ischemic
11	0	2	1	2	3	2	1	0	3	3	Inflammatory/ischemic
15	0	0	1	0	0	1	1 (LP mainly)	0	0	0	Inflammatory
16	0	2	1	1	3	2	0	1	3	2	Inflammatory/ischemic
18 [§]											Inflammatory/ischemic
19	0	2	1	3	3	1	0	1	2	2	Inflammatory/ischemic
24	0	0	1	0	2	1	0	1	1	0	Inflammatory
26	1	0	Minimal [‡]	0	0	1	2 (increase in LP only)	1	0	0	Inflammatory
28	Minimal [‡]	1	Minimal [‡]	0	0	1	0	0	1	0	Inflammatory/ischemic
2	0	2	2	2	1	1	1	1	3	3	Mixed
3	1	2	2	2	2	2	0	1	1	3	Mixed
10	0	0	1	0	0	2	2	0	0	0	Mixed
12	0	1	3	2	2	3	0	0	2	2	Mixed
13	0	0	2	2	1	3	0	1	1	0	Mixed
14	0	2	3	2	3	1	1	1	3	0	Mixed
17	1	2	2	2	2	1	2 (increase in LP only)	2	3	2	Mixed
21	0	2	2+	1	3	1	0	0	2	0	Mixed
22	Too fragmented [†]	0	2	1	0	2	0	0	0	0	Mixed
23	1	2	3	1	2	1	0	0	3	2	Mixed
25	0	2	3	2	2	1	1	1	1	1	Mixed
29	0	2	2	2	1	2	0	0	1	0	Mixed
27	1	0	3	0	3	0	0	0	3	3	Marked destruction [¶]

†The tissues were in pieces and were too small to evaluate the architecture.

‡Minimal or rare change (between 0 and 1).

§The data for this patient are not presented due to discovery of a celiac disease upon the pathology review.

¶The pattern was not recognized due to the marked destruction of the tissue.

LP: Lamina propria.

were then lysed, and white blood cells fixed with 450 µl of FACS lysing solution. The tubes were vortexed and incubated for 15 min at room temperature in the dark. Samples were analyzed using a BD FACSCanto II flow cytometer. Automated data analysis was performed using BD FACSCanto Clinical Software. Internal calibration beads were used to measure absolute counts (cells/µl) for each of the subsets assessed. Absolute cell counts of NK cells (CD3⁻, CD16⁺/56⁺), helper/inducer T cells (CD3⁺, CD4⁺), and cytotoxic T cells (CD3⁺, CD8⁺) were calculated.

Results

Out of the 29 patients whose tissue biopsies were evaluated, 23 (79.3%) had CLL, and six (20.7%) had indolent B-cell non-Hodgkin lymphoma. Twenty (69%) were male, and the median (range) age was 64 years (50–82 years). Eight patients received IDELA monotherapy and 21 patients received combination therapy with rituximab (*n* = 4), ofatumumab (*n* = 13), or bendamustine plus rituximab (*n* = 4). The dose of oral IDELA was 150 mg twice daily, except for single patients treated at 100 mg twice daily and 200 mg twice daily. The demographic and clinical baseline characteristics of the patients are summarized in Table 1.

The adverse event terms of diarrhea and/or colitis (grade 1–5) were reported in 24 cases (82.8%), including two cases of hemorrhagic colitis. Other adverse event terms temporally associated with endoscopy and biopsy were neutropenic fever, dehydration, colonic wall thickening and abdominal pain (Table 1). The treatment of diarrhea/colitis included systemic corticosteroids, oral budesonide, sulfasalazine, and mesalamine. One patient died due to multiple organ failure after surgery for diverticulitis with perforation, but this was not one of the patients who presented with diarrhea/colitis.

Four distinct morphological patterns were identified (Table 3 & Figure 1). Two cases with normal pattern showed preserved architecture and no significant inflammation or apoptosis; two cases were predominantly apoptotic without significantly increased inflammation. A total of 12 cases had an inflammatory pattern with varying degrees of attenuated glands; 12 cases showed a mixed pattern with a combination of inflammation, apoptosis, and/or attenuated glands (Table 2). The histological pattern could not be evaluated in one patient due to degradation of the sample.

Among the 12 cases with an inflammatory/ischemic pattern architectural distortion was seen in three patients. Attenuated glands, a cardinal feature of ischemia, were seen focally in two tissue samples, and five samples showed patchy or marked changes. Minimal to mild degrees of apoptosis were seen in 11 samples. Regenerative changes were seen in seven cases, and moderate to marked neutrophilic inflammation was observed in eight biopsies. Eleven samples exhibited mild to moderate intraepithelial and lamina propria lymphocytic inflammation, and three samples showed mild to moderate eosinophilic inflammation. Mild edema was evident in six samples and crypt abscess and crypt destruction were seen in nine samples; specifically, two cases showed focal crypt destruction or a rare crypt abscess, four contained patchy crypt abscess and destroyed crypts, and three had frequent areas with crypt abscess and destroyed crypts. Patchy and large areas of erosion or ulceration were seen in four samples (Table 2).

Among the 12 samples with a mixed pattern, architectural distortion was evident in three biopsies. Ischemic changes were seen in nine tissue samples and moderate to marked apoptosis was noted in 11 samples. Regenerative changes were seen in 11 cases. Seven samples showed moderate to marked neutrophilic inflammation, six samples exhibited moderate to marked lymphocytic inflammation, and five samples showed mild to moderate eosinophilic inflammation. Significant edema was apparent in the biopsy from one patient. Crypt abscess and crypt destruction were seen in ten samples, of which four had frequent areas with crypt abscess and destroyed crypts. Patchy and large areas of erosion or ulceration were seen in five samples (Table 2).

All normal controls showed no architectural disturbance or inflammation and had normal tissue morphology (Supplementary Table 2). Examples of tissue morphology seen in biopsies from normal controls are depicted in Figures 1 & 3.

Immunohistochemical staining of the biopsy samples for CMV was performed in 29 patients and revealed two patients with strong/moderate positivity and two patients with weak positivity. Adenovirus IHC was done on 29 specimens, and one case was weakly positive. ddPCR, a highly sensitive detection assay, was performed to expand the infectious panel characterization in the available tissues. The ddPCR assay test on FFPE tissues (Table 3 & Figure 2) revealed that six samples were positive for CMV (including the four IHC-positive cases), and one was positive for HHV-6. Among the CMV-positive samples, mixed histopathological pattern was most frequently observed. The weakly positive IHC for AdV stained a very rare cell, a repeat of this stain showed no

Figure 1. Comparison of cytomegalovirus detection by immunohistochemistry. (A) Shows a normal control with no architectural disturbance; (B) is the accompanying negative Cytomegalovirus (CMV) immunohistochemistry (IHC). (C) shows a patient who reported mild adverse events (AEs) with Idelalisib (IDELA) but pathology of the colon demonstrated a normal pattern without significant architectural disturbance; (D) is the accompanying negative CMV IHC. (E) shows a patient who reported AEs with IDELA and the pathology of the colon revealed a predominantly apoptotic pattern (green triangle points to apoptotic cells in high power inset); (F) is the accompanying CMV IHC showing rare positive cells (blue arrows). (G) shows a patient who reported AEs with IDELA and the pathology of the colon demonstrated an inflammatory pattern; (H) is the accompanying negative CMV IHC. (I) depicts tissue from a patient who reported AEs with IDELA treatment and the pathology of the colon demonstrated a mixed pattern, CMV+; (J) is the accompanying CMV IHC showing rare positive cells (blue arrows) (see previous page).

Figure 2. Droplet digital PCR for human herpesvirus 6, cytomegalovirus, and *RPP30* internal housekeeping gene for reference. (A) Shows results of the droplet digital PCR (ddPCR) for patient 21, the only patient in the series who was positive for HHV-6. Blue dots are droplets positive for HHV-6 (in the blue oval), green dots are positive for *RPP30*, and gray dots are negative for both HHV-6 and *RPP30*. (B) Is a hematoxylin and eosin (H&E) picture taken with a 10× objective, featuring a mixed pattern with inflammation, crypt abscess, attenuated glands, and some apoptotic cells. (C) Shows the ddPCR results for patient 5, who had reactivation of CMV (the H&E images are in Figure 1). As the CMV ddPCR assay is duplexed with adenovirus, in this dot plot, the blue dots are droplets with only CMV amplicons but no *RPP30* amplicons, the orange dots in the circle are droplets that amplified both CMV and *RPP30* gene targets, the green dots at the bottom (arrow) are droplets that are positive for *RPP30* gene target, and the black dots at the bottom are droplets that did not amplify either CMV or *RPP30* targets. CMV: Cytomegalovirus; HHV-6: Human herpesvirus 6.

Table 3. Infectious pathogens detected in patient biopsies.

Patient	CMV IHC	AdV IHC	ddPCR (AdV/CMV/HHV-6)	Pathogens identified	Additional pathogens detected
1	Neg	Neg	Neg/Neg/Neg		
2	Neg	Neg	Neg/Neg/Neg		
3 [†]	Neg	Neg	Neg/Pos/Neg	CMV	
4	Neg	Neg	Neg/Neg/Neg		
5 [†]	Pos	Neg	Neg/Pos/Neg	CMV	
6	Neg	Neg	Neg/Neg/Neg		
7	Neg	Neg	IM		
8	Neg	Neg	Neg/Neg/Neg		
9	Neg	Neg	Neg/Neg/Neg		
10	Neg	Neg	IM		
11 [†]	Neg	Pos	Neg/Neg/Neg	AdV	
12	Neg	Neg	Neg/Neg/Neg		
13	Neg	Neg	IM		
14	Neg	Neg	Neg/Neg/Neg		
15	Neg	Neg	Neg/Neg/Neg		
16	Neg	Neg	Neg/Neg/Neg		
17	IM	IM	IM		
18	Neg	Neg	Neg/Neg/Neg		
19 [†]	Neg	Neg	Neg/Neg/Neg		<i>Mycobacterium avium-intracellulare</i>
20	Neg	Neg	Neg/Neg/Neg		
21 [†]	Neg	Neg	Neg/Neg/Pos	HHV-6	
22 [†]	Neg	Neg	Neg/Pos/Neg	CMV	
23 [†]	Pos	Neg	Neg/Pos/Neg	CMV	<i>Norovirus</i>
24	Neg	Neg	Neg/Neg/Neg		
25	Indeterminable	Neg	IM		
26	Neg	Neg	Neg/Neg/Neg		
27 [†]	Pos	Neg	Neg/Pos/Neg	CMV	
28	Neg	Neg	Neg/Neg/Neg		
29 [†]	Rare pos	Neg	Neg/Pos/Neg	CMV	<i>Herpes simplex</i>

[†] Pathogen-positive samples.
 AdV: Adenovirus; CMV: Cytomegalovirus; ddPCR: Droplet digital PCR; HHV-6: Human herpesvirus 6; IHC: Immunohistochemistry; IM: Insufficient material; Neg: Negative; Pos: Positive.

additional positive cells, and the ddPCR was negative for Adv. Two patients with CMV-positive biopsies also had co-pathogens identified by clinical laboratory testing, revealing one case each of *Norovirus* and *Herpes simplex* virus. One additional patient had *Mycobacterium avium-intracellulare* identified by clinical laboratory testing (Table 3). All normal control samples were negative for infectious pathogens (Supplementary Table 2).

Immune cell profiling on available tissue samples was performed with a set of IHC stainings for CD3, CD8, FOXP3, and CD79a (Table 4). Compared with normal controls, GI biopsies from patients treated with IDELA contained increased proportion of FOXP3⁺ cells, including several FOXP3⁺ cells with enlarged nuclei (Table 4, Figure 3 & Supplementary Table 2). Overall, the FOXP3⁺ cells were detected in samples from 20 patients, and eight of these samples were also positive for infectious pathogens. For nine patients additional unstained slides were not available to perform immune cell profiling, including the patient double-positive for CMV and *Norovirus*. IHC performed on the tissues from the normal controls showed a relatively normal B- to T-cell ratio, with very few cells positive for FOXP3, and those that were present were small and located in the interstitium (Figure 3 & Supplementary Table 2).

When grouping patients based on their histologic patterns, the mixed, apoptotic and ischemic/inflammatory patterns were associated with an immune cell profile characterized by increased numbers of FOXP3⁺ Tregs, compared with both normal controls and tissue samples from patients categorized under normal pattern (Table 4, Figure 3 & Supplementary Table 2).

Figure 3. Immune cell profiling by immunohistochemistry and morphologic examination. Comparing the immune profiling of a normal control colon biopsy to one of the Idelalisib (IDELA) colitis patients with Cytomegalovirus (CMV) reactivation showed a marked difference in the number of FOXP3⁺ T-regulatory cells. The large top panel on the left is a picture of normal control colon biopsy stained with hematoxylin and eosin (H&E). Immunohistochemistry (IHC) for CD3, CD8 and CD79a showed a relatively normal B- to T-cell ratio with a subset of the CD3 cells represented as CD8 T-cell. FOXP3 showed very few T-regulatory cells, and those that are present are primarily small and in the interstitium (high power inset). The H&E picture of patient #6 colon biopsy (right) showed marked morphological changes with a mixed pattern and possibly viral cytopathic changes in some of the glands stained for CD3, CD8 and CD79a showed markedly increased T cells with infiltration into the epithelial glands. The majority of the CD3 cells are CD3⁺/CD8⁺ T cells and the FOXP3 showed comparatively increased T-regulatory cells in addition to forms that are infiltrating the glands, with atypical squiggly nuclei or enlarged nuclei (high power inset).

Longitudinal peripheral blood flow data were available around the time of the biopsy in five patients with CMV detected in the tissue biopsy (data availability was driven by the protocol-specified visits). While no consistent pattern was observed, CD4 and CD8⁺ T cells were decreased from baseline around the time of the biopsy in three patients (Supplementary Figure 1). In addition, there was no common pattern for CD16⁺/CD56⁺ NK cells observed in the CMV-positive patients.

Discussion

Severe diarrhea and/or colitis are important causes of interruption and discontinuation of IDELA treatment in patient with B-cell malignancies. This report is a clinicopathologic review of the largest series of patients with IDELA-associated diarrhea/colitis. The distinctive clinical and histological features presented herein are in accordance with those described previously, which include intraepithelial lymphocytosis, crypt cell apoptosis, and neutrophilic inflammation [10–12]. In our study of biopsy samples from 29 patients, we divided the histology patterns into four distinct morphological categories. The first category included two cases that were predominantly normal in morphological appearance. The second category, apoptotic pattern without intensive inflammatory component, was seen in two cases, one of which was positive for CMV; in both cases there was no concurrent neutropenia to account for the lack of acute inflammatory infiltrate. The third morphological pattern, detected in 12 cases, was most similar to cases described in the literature as infectious colitis and was characterized predominantly by inflammation with varying degrees of attenuated glands; apoptotic cells were not a prominent feature. The final category, seen in 12 cases, was a mixed pattern which encompassed varying degrees of apoptotic cells, mixed inflammation, and/or attenuated glands. These mixed morphologic patterns are similar to those previously described in other studies, with the most striking features of architectural disturbance, mixed inflammation (including intraepithelial lymphocytes and neutrophils, and increased eosinophils), crypt abscess/destruction, and apoptosis. Louie *et al.* described morphologic features of apoptosis and cryptitis as main pathological findings [10].

Table 4. Summary of immune cell profiling, histological pattern and infectious pathogen detection, on available tissue samples.

Patient	Histological pattern	Inflammation	CD79a:CD3 [†]	CD4 [‡] :CD8 [§]	FOXP3 [¶]	Pathogen in tissue IHC/ddPCR
1	Inflammatory/ischemic	Yes	0.01	1.86	6–8%	Neg/Neg
4	Inflammatory/ischemic	Yes	0.17	0.11	1–2%	Neg/Neg
6	Inflammatory	Yes	0.125	0.33	5–6% [#]	Neg/Neg
11	Inflammatory/ischemic	Yes	0.05	0.67	9% [#]	AdV/Neg
15	Inflammatory	Yes	6	0.11	2–3%	Neg/Neg
19	Inflammatory/ischemic	Yes	0.33	1	1%	<i>Mycobacterium avium-intracellulare</i>
24	Inflammatory	Yes	0.1	0.43	<5%	Neg/Neg
28	Inflammatory/ischemic	Yes	0.5	4	10%	Neg/Neg
3	Mixed	Yes	7	0.43	4%	Neg/CMV
12	Mixed	Yes	0.07	0.33	10–15% [#]	Neg/Neg
13	Mixed	Yes	7	0.11	20–25%	Neg/IM
14	Mixed	Yes	0.25	4	15% [#]	Neg/Neg
21	Mixed	Yes	2	4	10% [#]	Neg/HHV-6
22	Mixed	Yes	0.01	1	5%	Neg/CMV
25	Mixed	Yes	0.05	0.25	5%	Neg/IM
29	Mixed	Yes	0.01	1	5%	CMV/CMV, <i>Herpes simplex</i>
5	Apoptotic	Yes	0.25	3	6–8%	CMV/CMV
9	Normal	No	3	3	<1%	Neg/Neg
20	Normal	No	3	9	2%	Neg/Neg
27	Marked destruction	Yes	0.01	0.11	5%	CMV/CMV

[†]B-cell to T-cell ratio.[‡]Inferred from CD3 and CD8.[§]Percentage of CD3.[¶]Percentage of T-regulatory cells within the CD3⁺ cell pool.[#]Some of the cell nuclei were enlarged.

CD: Cluster of differentiation; EP: Epithelium; FOXP3: Forkhead box P3; LP: Lamina propria.

Two patients in our cohort showed mostly normal histology despite clinical complaints of abdominal pain and diarrhea. The description of two cases with milder symptoms and no histologic abnormalities, similar to our cohort, was included in Weidner *et al.* [12]. Of note, some of the histologic differences may be due to the timing of the biopsies, as biopsies of the samples with normal histology were taken at 4 and 15 days after initiation of symptoms. It is possible that the normal architecture may simply reflect that these biopsies were obtained after the patient had been treated.

A comprehensive panel for common viral pathogens (CMV, AdV, and HHV-6) that infect the GI tract of immunocompromised patients was performed. IHC and ddPCR identified a known viral pathogen in 8/29 (28%) of our biopsy samples. The additional patient with *Mycobacterium avium-intracellulare* infection brings the total patients with an infectious agent to 9/29 (31%). Louie *et al.* reported 11 cases of IDELA-associated colitis, nine of which were analyzed by CMV IHC and were negative [10]. Weidner *et al.* found four of 14 (28.6%) patients who were found to have viral infection; one patient with serum PCR positive for CMV, one patient with HHV-6 reactivation, one patient with rhinovirus/enterovirus, and one patient with parainfluenza [12]. The results from Weidner's series and this current study raise the need for heightened awareness of infectious agents in patients who present with IDELA-associated diarrhea.

Preclinical data indicate that mesenteric B-cells in the mouse model are associated with protection against mucosal damage by recruiting Tregs [21]. Although the molecular mechanism for IDELA-associated diarrhea is unknown, similar histologic findings were observed in mice with a targeted inactivating mutation in the p110 δ subunit of PI3K (PI3K p110 $\delta^{D910A/D910A}$) [22]. Mice harboring this kinase-dead PI3K δ mutation develop spontaneous colitis, which increases in severity over time and is dependent on the presence of enteric micro-organisms [22]. Colonic explants and macrophages from PI3K p110 $\delta^{D910A/D910A}$ mice produce significantly lower levels of IL-10 and higher levels of IL-12p40 compared with wild-type mice in response to toll-like receptor (TLR) agonist. Tregs from PI3K p110 $\delta^{D910A/D910A}$ mice showed attenuated suppressor function and impaired IL-10 secretion *in vitro*, suggesting a

critical role for PI3K δ in the function and development of Tregs [22,23]. Furthermore, PI3-kinases are also known to be important regulators of TLR and, therefore, impact downstream induction of inflammatory cytokines [24]. Under normal conditions, the TLR pathway would be responsible for eradication of infectious micro-organisms [25,26].

Apart from diarrhea/colitis, other frequently reported IDELA-related toxicities considered likely to be immune-mediated include pneumonitis and elevated hepatic transaminases [4,5]. Studies reported that patients who experienced hepatotoxicity on IDELA therapy had reduced numbers of peripheral blood regulatory T cells, and some had evidence of lymphocytic infiltrates on liver biopsy [27], but no infectious pathogens were identified. The description of biopsies from patients with IDELA-induced pneumonitis is lacking, and the mechanism of lung injury has not yet been elucidated. However, CT-scans revealed ground-glass opacities and interstitial infiltrates that resolved upon IDELA discontinuation and treatment with steroids [28,29]. Interestingly, germline genetic mutations disrupting function of Tregs – including mutation in the *FOXP3* gene – may cause autoimmune syndromes with hepatitis, enteritis, and pneumonitis [30].

In this study, the majority of samples in which an infectious pathogen was detected also contained FOXP3⁺ Tregs, supporting the mechanism that involves T-cell dysregulation with either an inflammatory pattern, including attenuated glands or a mixed histologic pattern. As several samples with mixed or ischemic/inflammatory morphological tissue pattern and detectable levels of Tregs were negative for infectious pathogens, it is possible that the insufficient testing material and poor quality of FFPE tissue prevented pathogen detection. These results differ from previous reports [10,12] at several points; this largest study thus far retrospectively analyzed individual cases of diarrhea/colitis across nine clinical trials; presence of infectious pathogens was detected in 31% of patients; it is, to our knowledge, the first study of this type that performed an immune profiling and characterized T-cell subsets in the biopsies.

In conclusion, the data presented here suggest that, in a significant number of cases, IDELA-associated diarrhea/colitis may be associated with an infectious etiology secondary to, at least in part, immune dysregulation observed along with histopathological abnormalities. Patients treated with IDELA who develop diarrhea should be evaluated thoroughly and monitored for CMV. If evidence of CMV infection or viremia occurs, administration of IDELA should be interrupted [31,32]. Based on these findings, clinical management of patients with IDELA-associated diarrhea/colitis should include both a complete evaluation for infectious causes and, when clinically indicated, an intestinal biopsy.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/fon-2017-0528

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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Summary points

- To better understand the pathophysiology of idelalisib (IDELA)-related diarrhea and/or colitis, we performed a retrospective analysis of formalin-fixed, paraffin-embedded biopsies from 29 patients who received IDELA treatment and experienced gastrointestinal symptoms in nine different clinical trials.
- The assessments included immunohistochemistry (IHC) and droplet digital PCR (ddPCR) for presence of viral pathogens, morphologic features evaluated based on a modified Geboes score, and immune profiling using IHC for CD3, CD79a, and FOXP3 to assess the B-cell, T-cell, and T-regulatory cell subsets. Colon/small bowel tissues from normal individuals were used for comparison.
- Among the 29 patients included in this analysis, 24 (82.8%) experienced diarrhea and/or colitis.
- Nine of 24 samples (eight from patients with diarrhea/colitis) were positive for infectious pathogens, including cytomegalovirus (CMV), adenovirus, and herpesvirus 6, including three cases of CMV detected only by ddPCR. One patient with CMV-positive biopsies tested positive for *Norovirus* and *Herpes simplex virus*, and one patient tested positive for *Mycobacterium avium-intracellulare*.
- Four distinct morphological patterns were identified: a pattern with inflammatory and attenuated glands (12 cases); a mixed pattern, including morphologic features of apoptotic, inflammatory, and/or attenuated glands (12 cases); an apoptotic pattern (two cases); and a predominantly normal pattern with no architectural changes (two cases).
- Overall, an increased percentage of T cells with positive FOXP3 IHC were detected in samples from 20 patients, eight of which were also positive for infectious pathogens, while very few cells positive for FOXP3 were noted in the normal controls.
- The results of histologic examination and immune profiling support the mechanism that T-cell dysregulation may be associated with the IDELA-related diarrhea/colitis.
- Workup of patients with IDELA-associated diarrhea/colitis should include full evaluation for infectious pathogens, and, when clinically warranted, an intestinal biopsy for histologic examination.

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