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Infliximab not Associated With Increased Risk of Malignancy or Hemophagocytic Lymphohistiocytosis in Pediatric Patients With Inflammatory Bowel Disease

Jeffrey S. Hyams[1*], Marla C. Dubinsky[2*], Robert N. Baldassano[3], Richard B. Colletti[4], Salvatore Cucchiara[5], Johanna Escher[6], William Faubion[7], John Fell[8], Benjamin D. Gold[9], Anne Griffiths[10], Sibylle Koletzko[11], Subra Kugathasan[12], James Markowitz[13], Frank M. Ruemmele[14,15], Gigi Veereman[16], Harland Winter[17], Nicholas Masel[18], Chu Ri Shin[19], Kezhen L. Tang[19], Meena Thayu[19]

Affiliations

1. Connecticut Children's Medical Center, Hartford, CT, USA
2. The Icahn School of Medicine at Mount Sinai, NY, USA
3. The Children's Hospital of Philadelphia, Philadelphia, PA, USA
4. University of Vermont Children's Hospital, Burlington, VT, USA
5. Sapienza University of Rome/University Hospital, Rome, Italy
6. Erasmus Mc-Sophia Children's Hospital, Rotterdam, Netherlands
7. Mayo Clinic, Rochester, MN, USA
8. Chelsea and Westminster Hospital, London, United Kingdom
9. Children's Center for Digestive Health Care, LLC, Atlanta, GA, USA
10. Hospital For Sick Children, Toronto, Canada
11. Dr von Hauner Children's Hospital, Ludwig Maximilians University München, Munich, Germany
12. Emory University, Atlanta, GA, USA
13. Cohen Children's Medical Center Of New York, NY, USA
14. Assistance-Publique, Hôpitaux de Paris (APHP), Hôpital Necker-Enfants Malades, Service de Gastroentérologie pédiatrique, Paris, France
15. Université Paris Descartes –Sorbonne Paris Cité, Faculté de Médecine, Paris, France
16. Universitair Ziekenhuis ,Vrije Uniuversiteit Brussel, Brussels, Belgium
17. MassGeneral Hospital for Children, Boston, MA, USA
18. DOCS, Durham, NC, USA
19. Janssen Scientific Affairs, LLC, Horsham, PA, USA

*** Contributed equally to this manuscript.**

Corresponding Author:

Meena Thayu, MD MSCE

Janssen Research and Development, LLC

1400 McKean Road

Spring House, PA, USA 19477

☎ 215-793-7078

mthayu@its.jnj.com

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Author Contributions

	study concept and design	acquisition of data	analysis and interpretation of data	drafting of the manuscript	critical revision of the manuscript for important intellectual content	statistical analysis
Hyams	X	X	X	X	X	
Dubinsky	X	X	X	X	X	
Baldassano	X	X			X	
Colletti	X	X			X	
Cucchiara	X	X			X	
Escher	X	X			X	
Faubion	X	X			X	
Fell	X	X			X	
Gold	X	X			X	
Griffiths	X	X			X	
Koletzko	X	X			X	
Kugathasan	X	X			X	
Markowitz	X	X			X	
Ruemmele	X	X			X	
Veereman	X	X			X	
Winter	X	X			X	
Masel					X	X
Shin					X	
Tang			X	X	X	X
Thayu	X		X	X	X	

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James Markowitz has served as a consultant for AbbVie, Celgene, Eli Lilly, Janssen, Soligenix, and UCB.

Frank M. Ruemmele serves as a board member for Johnson & Johnson; has received speaker fees from AbbVie, Centocor, Ferring, Johnson & Johnson, Mead Johnson, MSD, Nestle, and Shering-Plough; and has been invited to AbbVie, Danone, Mead Johnson, Nestle, Nestle Nutrition Institute, MSD France, and Takeda.

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Harland Winter has served as an advisory board member and/or consultant for AstraZeneca, Avaxia, Janssen, Mead Johnson, Nutricia, Paraxel, Pediatric IBD Foundation, Prometheus, Salix, Shire, USB; has served as an expert witness for Mulvey, Ennis, Keefe & Donovan, The Perry Law Firm, Falk, Waas, Hernandez, Cortina, Solomon & Bonner, Peabody & Arnold, and Law Offices of James S. Rogers; has received royalties for UpToDate and UPDATE GERD; has received grants for AbbVie, AstraZeneca, Autism Research Foundation, Janssen, Nestle Nutrition, Nutricia, Pediatric IBD Foundation, Shire and UCB; and has patents pending for biomarkers for IBD and autism.

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Investigators:

US: J Hyams, M Dubinsky, R Baldassano, R Colletti, J Markowitz, W Faubion, B Gold, H Winter, S Kugathasan, K Crissinger, S Rabizadeh, H Naon, J Huang, D Ziring, E Hoffenberg, B Kirschner, P Hyman, A Furr, M El-Baba, R Arumugam B Sudel, M Kappelman, J Rosh, S Baker, J Markowitz, G Wetzler, J Grunow, J Kelsen, M Maksimak, M Tsou, I Leibowitz, G Wahbeh, S Werlin, S Schwarz, N Mcgreal, M Heyman, G Silber, D Walkiewicz-Jedrzejczak, A Patel, S Cohen, J Fong, H Thompson, J Whitworth, E Gleghorn, J Ramakrishna, J Bornstein, R Sockolow, L Prestridge, C Jolley, N Ogunmola, F Daum, Y Al-Tawil, J Lavine, U Pisharody, A Chawla, D Pashankar, M Integlia

Canada: A Griffiths, H Huynh, A Otley

Belgium: G Veereman E Sokal, I Hoffman, P Bontems

Denmark: L Folmer Hansen

France: F Ruemmele, JP Hugot, A Lachaux,

Germany: S. Koletzko, M Classen, S Buderus, U Baumann, M Radke,

Italy: S Cucchiara, A Barabino, G De'Angelis, AM Staiano,

Netherlands: J Escher, L de Ridder, A Kindermann, P Van Rheenen, J.J. Schweizer, G Damen

UK: J Fell, R Muhammed, S Chong, M Auth

Abstract:

Background and Aims: Immunosuppressive therapy for inflammatory bowel disease (IBD) in pediatric patients is thought to increase risk of malignancy and lymphoproliferative disorders, including hemophagocytic lymphohistiocytosis (HLH). We compared unadjusted incidence rates and of malignancy and HLH in pediatric patients with IBD exposed to infliximab compared with patients not exposed to biologics and calculated standardized incidence ratios (SIRs).

Methods: We collected and analyzed data from 5766 participants in a prospective study of long-term outcomes of pediatric patients with IBD (NCT00606346), from 2007 through 30 June 2016. Patients were 17 years old or younger and had Crohn's disease, ulcerative colitis, or IBD unclassified with 24,543.0 patient-years of follow-up. We estimated incidence rates for malignancy and HLH as events/1000 patient-years of follow-up. We calculated age-, sex-, and race-adjusted SIRs, with 95% CIs, using the Surveillance, Epidemiology, and End Results Program (SEER) database.

Results: Thirteen of the 15 patients who developed a malignancy and all 5 of the patients who developed HLH had been exposed to thiopurine; 10 patients with malignancy patients had also been exposed to a biologic agent. Unadjusted incidence rates showed no increased risk of malignancy (0.46/1000 patient-years) or HLH (0.0/1000 patient-years) in patients exposed to infliximab as the only biologic vs those unexposed to biologics (malignancy: 1.12/1000 patient-years; HLH: 0.56/1000 patient-years). SIRs did not demonstrate an increased risk of malignancy among patients exposed to infliximab (SIR; 1.69; 95% CI, 0.46–4.32) vs patients not exposed to a biologic agent (SIR, 2.17; 95% CI, 0.59–5.56), even when patients were stratified by thiopurine exposure.

Conclusions: In determination of age-, sex- and race-adjusted SIRs using data from a large clinical trial and the SEER database, we found that infliximab exposure did not associate with increased risk of malignancy or HLH in pediatric patients with IBD. Thiopurine exposure is an important precedent event for the development of malignancy or HLH in pediatric patients with IBD.

Keywords: DEVELOP Registry; cancer risk; tumor necrosis factor antagonist; anti-TNF

Introduction

Crohn's disease (CD) and ulcerative colitis (UC), which comprise inflammatory bowel disease (IBD), are characterized by chronic inflammation of the gastrointestinal tract. Nearly 25% of cases are diagnosed during childhood, and the incidence of IBD is increasing in children.[1, 2] The associations between IBD and intestinal malignancies, including colorectal carcinoma, small bowel adenocarcinoma, and intestinal lymphoma are well established; extra-intestinal malignancies related to IBD include lymphoma, leukemia, and skin cancers.[3, 4] Risk factors for malignancy associated with IBD include local and systemic inflammation leading to immune dysregulation with impaired tumor surveillance; [5] the carcinogenic effects of immunosuppressive therapy, particularly with respect to extra-intestinal malignancies; [6] as well as exposure to environmental factors including ionizing radiation. [7]

Assessment of the comparative effects of different therapies upon malignancy risk in IBD is complicated by multiple factors including confounding by indication. Patients with more severe disease may have an increased risk of malignancy due to ongoing inflammation, but due to disease severity are also more likely to receive certain medications, including immunomodulators and biologic agents, such anti-tumor necrosis factor-alpha (anti-TNF-α) therapies.[8] Furthermore, serial, combination, and intermittent treatment involving thiopurines (6-mercaptopurine and azathioprine), the most widely used immunomodulator agents in IBD, and anti-TNF-α therapies may have additive or synergistic risks. The relative impact of duration of exposure versus cumulative dose of thiopurines and anti-TNF-α therapies is not well characterized.[9] Recent studies suggest that in patients with IBD who discontinue thiopurines,

the risk of lymphoma may decrease to the risk in patients with IBD never exposed to thiopurines; however, it is not clear when this risk potentially diminishes. [10-12] Although not considered a true malignancy, but rather a disorder of immune hyperstimulation and dysregulation, hemophagocytic lymphohistiocytosis (HLH) is also associated with IBD and may have catastrophic consequences, including death. Similar to IBD-associated malignant lymphoproliferative disorders, such as lymphoma, the risk of HLH in patients with IBD is increased in the setting of systemic inflammation and exposure to immunosuppressive therapy, particularly thiopurines, with concurrent primary infection with Epstein-Barr virus (EBV). [13]

Prior reports on the risk of malignancy and HLH associated with therapy for pediatric patients with IBD have been limited to largely retrospective studies with small sample sizes and/or limited duration of follow-up (F/U), systematic reviews of data pooled across multiple studies, or case series. [13-16] Utilizing data from the DEVELOP Registry (An Inflammatory Bowel Disease Multicenter, Prospective, Long-term Registry of Pediatric Patients, NCT00606346), the objectives of this study in pediatric patients with IBD were (1) to compare unadjusted incidence rates of malignancy and HLH in infliximab (IFX)-exposed patients vs. those unexposed to biologics and (2) to calculate standardized incidence ratios (SIRs) with 95% CIs using the Surveillance, Epidemiology, and End Results (SEER) database, in order to compare the incidence of malignancy in pediatric patients with IBD exposed to infliximab vs. those unexposed to biologics.

Methods

Study design

DEVELOP is a multicenter, prospective, cohort study of long-term safety and clinical outcomes in pediatric patients with CD, UC or IBD-unclassified (IBD-U) treated with REMICADE® (infliximab) and/or other medical therapies for IBD. Enrollment commenced in 2007 and is currently ongoing, and patients are to be followed for 20 years (see Figure 1 for study design). As of 30 June 2016, 5766 patients with CD, UC, or IBD-U (North America) or with CD and UC [(European Union (EU)] have been enrolled at 82 academic and community-based pediatric gastroenterology practices, including 56 sites in North America and 26 sites in the EU (United Kingdom, Belgium, the Netherlands, France, Germany, Denmark, and Italy). At each investigative site, participation in the study was offered to successive patients. The Sponsor or its designee monitored enrollment on an ongoing basis to ensure that approximately equal numbers of patients exposed to infliximab as well as patients exposed only to other treatments at the time of study entry were enrolled at each site. The design of DEVELOP (NCT00606346) was approved by the institutional review board or ethics committee at each participating site. DEVELOP was conducted in accordance with current Food and Drug Administration regulations and guidelines, including Good Pharmacovigilance Practices and Pharmacoepidemiologic Assessment, the European Clinical Trials Directive and associated guidelines, International Conference on Harmonisation guidelines on Good Clinical Practices, and the principles of the Declaration of Helsinki, as well as all other applicable national and local laws and regulations.

Each patient's parent/legal guardian had to be capable of providing written informed consent, and assent was to be obtained from the child according to local regulations. Patients reaching the age of 18 years or the legal age of maturity according to local regulations during study participation were required to provide written informed consent to continue participation in the study. Janssen Biotech, Inc. (Horsham, PA), the manufacturer of infliximab is the sponsor of DEVELOP.

Study participants/evaluations

Eligible participants included pediatric patients with a confirmed diagnosis of IBD (i.e., CD or UC or IBD-U) for at least 2 months, and at the time of enrollment were less than 17 years of age in North America, and 6 years to less than 17 years of age in the EU. Patients were ineligible to participate in the study if they had a history of other IBD-like conditions associated with genetic diseases (i.e. glycogen storage disease 1b), were participating in any clinical trial for an investigational agent that was not commercially available, or were currently participating in another study supported by the sponsor.

Patient data were collected at enrollment and then approximately every six months. Patient demographics; disease characteristics; medication history, including a comprehensive review of medication exposures prior to study entry; and disease activity, including the modified Pediatric Crohn's Disease Activity Index (PCDAI) for CD patients and Partial Mayo score for UC and IC patients were collected at enrollment. [17-19] Interval medical and surgical history, hospitalizations, changes in medications, disease activity measures, and adverse event

history were collected at F/U visits. All malignancy and HLH cases were validated with supporting documentation, including pathology reports.

Statistical analysis

Medication exposure was defined as "ever-exposed," which includes exposure at any time prior to the malignancy or HLH event. The study population was divided into the following cohorts: infliximab (exposure to infliximab as the only biologic in the presence or absence of exposure to non-biologics), biologics (exposure to any biologic, including infliximab, in the presence or absence of exposure to non-biologics), and non-biologics (exposure to 5-aminosalicylates, corticosteroids, thiopurines, methotrexate, antibiotics, or other non-biologic IBD therapies, in the absence of exposure to any biologics). The infliximab cohort is a subgroup of the biologics cohort, which includes exposure to anti-TNF-α and/or non-anti-TNF-α biologics. Each of these cohorts was further stratified by thiopurine and methotrexate exposure. At baseline, patients were included in a treatment cohort based on cumulative medication exposure at the time of study enrollment. Given this is a long-term study in which study participants may have had changes in medication regimens, total patient-years of exposure for a single participant may include contributions to more than one cohort, and a malignancy or HLH event was assigned to a specific cohort based on cumulative exposure at the time of the event. For example, a patient exposed to 1 year of non-biologic immunomodulator therapy in the absence of biologics followed by 2 years of infliximab therapy (either in the presence or absence of an immunomodulator) at the onset of a malignancy or HLH event would have contributed a total duration of 3 patient-years of follow-up, which would include 1 patient-year in the non-biologics cohort and 2 patient-years in the infliximab cohort. The malignancy or HLH

event would be assigned to the infliximab cohort, since infliximab exposure preceded the onset of the event. For a patient who developed a malignancy or HLH event after discontinuation of infliximab (either in the absence or presence of an immunomodulator) without the initiation of other biologics, the event was assigned to the infliximab cohort. Finally, for a patient who developed a malignancy or HLH event after exposure to an immunomodulator, infliximab, and another anti-TNF-α or non-anti-TNF-α biologic, the malignancy was assigned to the biologics cohort.

Baseline demographic and disease characteristics were evaluated for the entire study population as well as by exposure cohort using descriptive statistics, i.e., means and standard deviations for continuous variables and frequencies and percentages for categorical outcomes. For the comparisons among the infliximab, biologics, and non-biologics cohorts, the Wilcoxon rank sum test was used for continuous variables and Chi-square test was used for categorical variables. The rates of malignancy events per 1000 patient-years of follow-up were calculated for each medication exposure cohort as the quotient of the total number of malignancy events and cumulative patient-years of exposure, then multiplied by 1000, and expressed with a corresponding 95% CI. The incidence rates of malignancy events for each exposure cohort, as well as for the entire study population, were also stratified by thiopurine exposure; similar analyses were performed using stratification by methotrexate exposure. The incidence of HLH was calculated for the entire study population and then stratified by thiopurine and methotrexate exposure. Standardized incidence ratios (SIRs) with 95% CI using the SEER program database [20] were also used to compare the incidence rates for malignancy. SIRs were calculated as the quotient of the observed number of malignancy events in an exposure cohort

and the expected number of malignancy events in the general US pediatric population in the SEER database, with adjustments for age, gender, and race. A SIR with the lower bound of 95% CI greater than 1.0 indicates a statistically significantly increased risk for the development of malignancy, compared to the SEER reference population. Similar to the unadjusted analyses, SIRs were also calculated stratified by thiopurine and methotrexate exposure. Of note, the SEER database does not include non-melanoma skin cancers (NMSC). As such, the 2 cases of basal cell carcinoma reported in this study were excluded from the SIR analyses. Statistical comparisons between the infliximab and biologics cohorts and the respective non-biologics cohorts as well as the entire study population stratified by thiopurine exposure were made by calculation of the ratio of the SIRs with 95% confidence intervals; a confidence interval including 1.0 indicated no statistically significant differences between SIRs. Data were analyzed using SAS software version 9.2 (SAS Institute, Cary, NC).

Results

Patient characteristics and treatment

Baseline patient characteristics are summarized in Table 1. Between 31 May 2007 and 30 June 2016, 5,766 patients, median age 13 years, (interquartile range 10-15 years) were enrolled in the study. The infliximab and biologics cohorts were approximately 1 year older ($p<0.0001$, both) and had greater duration of disease ($p<0.0001$, both) compared to the non-biologics cohort at enrollment, but no differences for gender or race were observed. Mean modified PCDAI and partial Mayo scores demonstrated greater disease activity in CD and UC patients, respectively, in the infliximab and biologics cohorts versus the non-biologics cohort

(p<0.005, both). The biologics cohort (n=2824) predominantly was comprised of patients with anti-TNF-α biologic exposure, including: infliximab (94.6%), adalimumab (34.0%), certolizumab (5.1%), golimumab (0.6%), and etanercept (0.2%); note that these categories are not mutually exclusive as patients may have received more than one anti-TNF-α therapy. A total of 232 patients in the biologics cohort were exposed to non-anti-TNF-α biologic agents for a total of 404.2 patient-years; 15 of these patients were exposed to non-anti-TNF-α biologic agents in the absence of TNF-α biologic agents. Specific exposures for the 232 patients exposed to non-anti-TNF-α therapies included the following: vedolizumab (78.9%), ustekinumab (18.1%), and natalizumab (9.1%), with 15.5% of patients exposed to at least one other non-anti-TNF-α biologic therapy; these categories are not mutually exclusive as patients may have been exposed to more than one non anti-TNF-α biologic agent.

Clinical History of Malignancies and HLH

Table 2 summarizes clinical history for the fifteen malignancy events and table 3 shows the five HLH events observed in the study. Medication and infectious exposures prior the reporting of malignancy and HLH events are noted. Among the malignancies, 8/15 events were either leukemia or lymphoma; and the remaining 7 cases included adenocarcinoma of the parotid gland (n=1), basal cell carcinoma (n=2), malignant melanoma (n=1), renal cell carcinoma (1), cholangiocarcinoma (1), and mycosis fungoides (1). Ten patients were exposed to infliximab, and among them, five patients were also exposed to adalimumab; 6 patients were exposed to methotrexate; and nine of ten infliximab-exposed patients were also exposed to thiopurines. Four patients were exposed to thiopurines in the absence of biologics. Thus, 13 of the 15 patients with malignancies were exposed to thiopurines; and the majority of these

patients developed malignancy after less than 5 years of thiopurine exposure. Specific therapies at the time of malignancy diagnosis are detailed in Table 2. Two of the five lymphoma cases and one of the two basal cell carcinoma cases did not have exposure to any biologics, including infliximab. Three patients reported a family history of malignancy. In addition, infectious exposures, if available, prior to malignancy or HLH diagnosis are also summarized in Tables 2 and 3. All cases of HLH occurred during active thiopurine therapy; none had exposure to infliximab, adalimumab, or methotrexate. Four of the five cases of HLH were associated with primary EBV infection, and one case was associated with presumed primary cytomegalovirus (CMV) infection; serological data confirming primary infection for the HLH case associated with CMV infection was not available. Twelve out of 15 malignancy events and all HLH events occurred in CD patients; additionally, all patients except for the male with renal cell carcinoma were alive as of the data cut-off.

Incidence of Malignancy and HLH

Table 4 summarizes unadjusted incidence rates of malignancy for the study population and also classifies the individual malignancy diagnoses by cohort. Median F/U for the study population was 4.7 years; with a total of 24543.0 patient-years of follow-up. With stratification by thiopurine exposure, there were no evidence of increased incidence rates of malignancy (events/1000 patient-years; 95% CI) in the infliximab cohorts vs. the respective non-biologic cohorts: with thiopurines, (0.53; 0.14, 1.35) vs. (0.69; 0.19, 1.76) and without thiopurines, (0.31; 0.01, 1.75) vs. (0.32; 0.01, 1.79), respectively, as the incidence rate point estimates for the infliximab cohorts were lower than the non-biologics cohorts with highly overlapping 95% confidence intervals. Additionally, without stratification by thiopurine exposure (Supplemental

Table 1), there was no increased incidence of malignancy in the infliximab (0.46; 0.15, 1.08) or the biologics cohort (0.64; 0.31, 1.18) versus the non-biologics cohort (0.56; 0.18, 1.31).

Table 5 summarizes unadjusted incidence rates of malignancy and HLH for the entire study population, stratified by thiopurine exposure. The data indicated a trend toward higher incidence rates (events/1000 patient-years; 95% CI) of both malignancy (0.75; 0.40, 1.29 vs. 0.27; 0.03, 0.99) and HLH (0.29; 0.09, 0.68 vs. 0.00; 0.00, 0.41) in the thiopurine exposed vs. respective thiopurine unexposed cohorts. The combined incidence rate (events/1000 patient-years; 95% CI) of malignancy and HLH was also greater in the thiopurine cohort (1.05; 0.62, 1.65) vs. the thiopurine-unexposed cohort (0.27; 0.03, 0.99), indicating a trend toward an increased risk for both of these events in thiopurine-exposed patients. Similar findings were observed for the biologics cohorts versus the non-biologics cohorts for all of the above unadjusted analyses, including analyses stratified by thiopurine exposure.

Figure 2 displays SIRs of the individual exposure cohorts as well as for the entire study population. Figure 2, Panel A indicates no increased risk of malignancy (SIR, 95% CI) in the infliximab cohorts, stratified by thiopurine exposure (1.77; 0.36, 5.17 and 1.49; 0.04, 8.28), compared to the SEER reference population as well as to the respective non-biologics cohorts (2.47: 0.51, 7.22 and 1.59; 0.04, 8.86). Although the biologics cohorts, stratified by thiopurine exposure did not show an increased malignancy risk compared to the respective non-biologic cohorts, patients with biologic plus thiopurine exposure had a significantly increased risk of malignancy compared to the SEER reference population. The lower limit of the 95% confidence interval for the SIR suggested the standardized incidence rate is at least 1.32 fold for the

biologic plus thiopurine exposed compared to the SEER reference population. Patients exposed to non-biologics, stratified by thiopurine exposure, had no evidence of increased risk of malignancy compared to the SEER reference population. Panel B indicates that stratification of the entire study population by thiopurine exposure demonstrated a statistically significantly increased malignancy risk in patients exposed to thiopurines compared to SEER database, with or without biologic exposure. The ratio of the SIRs for the thiopurine-exposed patients compared with thiopurine non-exposed patients indicated no statistically significant difference. Additionally, SIRs without stratification by thiopurine exposure (Supplemental Table 3), indicated no increased risk of malignancy in the infliximab or the biologics cohorts versus the non-biologics cohort.

In comparison to the number of patients exposed to thiopurines, far fewer were exposed to methotrexate; 3857 patients had 17224.1 patient-years of exposure to thiopurines, and 1509 patients were exposed to methotrexate with 5710.0 patient-years of follow-up. Of the 1509 methotrexate-exposed patients, 977 also had exposure to thiopurines, with only 532 exposed only to methotrexate in the absence of thiopurines for a duration of 1630.1 patient-years. Comparison of unadjusted incidence rates of malignancy by exposure cohorts, additionally stratified by methotrexate exposure demonstrated no increased rates in the methotrexate-exposed cohorts vs. the methotrexate-unexposed cohorts. Additionally, stratification of the entire study population by methotrexate exposure showed no increased incidence of malignancy, HLH, or both malignancy and HLH in the methotrexate-exposed cohort vs. the methotrexate-unexposed cohort. (Supplemental Table 2)

Discussion

Clinicians caring for children with IBD face important challenges as they outline the benefits and risks of therapeutic alternatives for patients and families. Studies have shown the efficacy of anti-TNF-α agents in inducing and maintaining remission in pediatric Crohn's disease [21, 22] and ulcerative colitis, [23] as well as improved one year clinical outcomes, including growth and development, in children with new onset Crohn's disease following early anti-TNFα therapy.[24-26] Nonetheless, many families, as well as clinicians, remain reluctant to use anti-TNF-α therapy secondary to the fear of malignancy until a patient has failed conventional non-biologic therapies, including thiopurines. This "step up approach" often does not take into account the risks of disease progression due to suboptimal treatment. Missing from this deliberation are robust data on the risk of malignancy in pediatric patients with IBD exposed to the wide range of available therapies.

Data from DEVELOP, the largest prospective cohort study of long-term safety outcomes in pediatric patients with IBD to date, indicate that infliximab therapy is not associated with an increased risk of malignancy. Furthermore, the data demonstrate a trend toward an increased risk of malignancy in thiopurine-exposed patients, irrespective of biologic exposure. Prior reports on malignancy risk in both adult and pediatric IBD have focused upon lymphoma and NMSC, the most prevalent extra-intestinal malignancies associated with IBD.[10-12, 27-30] Beaugerie et al., reported a hazard ratio for lymphoproliferative disorders, including lymphoma, of 5.28 (95% CI, 2.01–13.9) in patients with IBD exposed to thiopurines. Other studies have suggested that there is an increased risk of lymphoma in patients with IBD both on thiopurines alone and in combination with an anti-TNF-α agent.[28, 31, 32] In a systematic analysis, Dulai, et. al.

demonstrated that among pediatric patients with IBD, the lymphoma risk was no greater in patients who received anti-TNF-α therapy compared to those treated with other therapies.[15] Multiple studies have demonstrated an association between thiopurines and NMSC risk in patients with IBD; [30] of note, one case of basal cell carcinoma in our study was exposed to thiopurines in the absence of biologics. Although consistent with prior reports, this is the first study in pediatric patients with IBD to include both infliximab-exposed and infliximab- and biologic-unexposed cohorts with significant patient years of F/U for the purposes of robust comparisons using the SEER database. Comparisons between infliximab monotherapy vs. infliximab/thiopurine combination therapy were also possible, due to the extensive patient-years of follow-up for each cohort. The prospective nature of the study allowed for the collection of detailed medical history, including duration of medication exposure prior to and during study participation.

In our study, we also observed 5 cases of HLH, a potentially fatal lymphoproliferative disorder that more commonly occurs in immunocompromised individuals. Though not considered a true malignancy secondary to the lack of monoclonality of the proliferating cell population, the associations between EBV infection, thiopurine use, and lymphoma as well as non-malignant lymphoproliferative disorders such as HLH in adult and pediatric patients with IBD are well-established. [11, 13, 31, 33] Our study also highlights the role of thiopurines in the pathogenesis of HLH, as all five patients who developed HLH were exposed to thiopurines but not exposed to biologics or methotrexate. It is postulated that chronic lymphopenia due to thiopurine exposure suppresses the host cellular-mediated immune response via suppressor T-lymphocytes during primary EBV infection; this impaired response leads to hyperproliferation

of infected lymphocytes, with ensuing complications. [13] The Cancers Et Surrisque Associé aux Maladies inflamma toires intestinales En France (CESAME) study reported that over 52% of the 23 cases of lymphoproliferative disorders were EBV positive, [11] with an incidence rate of 0.1/1000 patient-years for primary EBV infection-related post-mononucleosis lymphomas, a phenomenon observed in EBV naïve young men less than 35 years of age. [34] Given EBV exposure does occur in the majority of individuals as children or young adults, the pediatric age group is particularly vulnerable to repercussions of primary infection. Although the cases of HLH reported in this study represent a related but different diagnostic and pathologic entity and are very rare, it should be noted that when limited to the primary EBV-associated cases, a higher incidence rate of 0.2/1000 patient-years compared to the CESAME rate of EBV-related post-mononucleosis lymphoma, was observed. Also different about the HLH cases reported here are three of the five patients who developed HLH were adolescent females and that one case of HLH was associated with CMV infection (Table 3). As such, both male and female pediatric patients with IBD exposed to thiopurine therapy should be considered at risk for HLH, and viral infections such as EBV and CMV infection should be considered in the differential diagnosis or as potential triggers for disease when patients are diagnosed with HLH. Notably, given the infrequency of these cases, it has not been possible to compare incidence rates for HLH in pediatric patients with IBD by specific exposures prior to the conduct of this study.

A key limitation of the study is related to the fact that malignancy events in this patient population are rare. Based upon an event rate of .61/1000 patient-years (15 events/24543 patient-years), the study has the power to detect a 4.2 fold or higher risk between any pair-wise categorization of this cohort (e.g., thiopurine-exposed vs thiopurine non-exposed,

biologic-exposed vs biologic-non-exposed). As such, 65 events with 106,000 patient-years would be required to detect a 2 fold or higher difference. Due to this fact, the study was not able to demonstrate statistically significant differences in malignancy risk between cohorts in the entire population or individual cohorts stratified by thiopurine exposure. Immortal time bias may also have confounded our results, as there may not have been sufficient follow-up time for events to occur. Although the data demonstrate increased malignancy risk in patients exposed to biologics and thiopurines compared to the respective SEER reference populations, this observation may be due to confounding by indication; patients in the biologics cohort may have a higher likelihood of adverse events due to greater disease activity and may have been exposed to more than one biologic. Additionally, these patients may have had a longer duration of follow-up. Also, the analyses assume that patients in the SEER database do not have IBD or are not exposed to these classes of medications. If this assumption were not true, the results would be biased toward a relative risk of 1. An additional limitation of our study is that 3/15 patients with malignancy were from France, and the SEER reference population is entirely from the US; differences between the SEER reference population and that in the EU could be a potential source of bias. Other limitations of the study include the fact that although prospective in nature, due to the fact that patients were evaluated every 6 months in a non-interventional study versus a clinical trial during which they may have had more frequent visits, there may have been under-reporting of adverse events, including malignancy and HLH. However, if there were under-reporting of adverse events, there is no evidence to suggest that it would have been differential across the cohorts. Loss to follow-up is also a limitation of long-term safety registries.; the attrition rate of 20.1% for the total study population, although

comparable to other registries, was relatively high compared to administrative databases. [27, 35] However, comparison of attrition rates across the different cohorts showed no significant differences (Table 1). Additionally, despite the fact that consecutive patients at every site were offered participation in the study, there may have been selection bias, which could have potentially contributed to under-reporting of malignancy or HLH events. The study was not able to comment on the impact of different IBD therapies upon the risk of the development of hepatosplenic T-cell lymphoma, as no cases have been identified in our patient cohort as of the data cut-off. The six methotrexate-exposed patients who developed malignancy also had prior exposure to thiopurines and infliximab. Our data on the effect of methotrexate exposure on risk of malignancy are limited because of the relatively small numbers of patients exposed to methotrexate. Additional long term data from DEVELOP will more clearly define the potential malignancy risk potential of methotrexate therapy alone or in combination with anti-TNF-α agents. Finally, our study did not collect detailed data on ionizing radiation, which may be associated with increased malignancy risk. [36] Consequently, we cannot draw meaningful conclusions regarding the impact of this exposure.

Our findings may inform the decision-making process in constructing treatment plans for children with IBD and communicating risks of different therapies to patients and families. The SIRs presented in Figure 2 present the risk of malignancy (excluding NMSC) in different treatment cohorts, relative to the SEER reference population. Presentation of these data as incidence rates (events/10,000 patient-years) may provide clinicians with another format in which to present these risks, relative to the incidence rate of 2.2/10,000 patient-years in the general US pediatric population (Supplemental Table 4). Recent studies have suggested that cessation of thiopurines in patients with IBD may reduce lymphoma risk[10-12]

but not the risk of NMSC. [30] Therefore, a sensitivity analysis was performed to evaluate whether or not the risk of malignancy (excluding NMSC) would change after thiopurine cessation for at least 1 year. Four patients with malignancy (3 with solid tumors and one with malignant melanoma) discontinued thiopurine therapy prior to malignancy diagnosis; the patient with malignant melanoma had discontinued thiopurines for a period under 1 year. Supplemental Table 5 indicates that patients with ongoing thiopurine exposure or discontinuation of therapy within one year of malignancy diagnosis had an SIR of 4.45 (CI: 1.92, 8.77). Those who discontinued thiopurine therapy for 1 or more years prior to malignancy diagnosis has an SIR 1.48 (CI: 0.30, 4.32), which approached the SIR for the thiopurine non-exposed group: 1.30 (CI: 0.16, 4.71). These results suggest that cessation of thiopurines for 1 year or greater may reduce malignancy risk (excluding NMSC), approaching the baseline risk observed in patients not exposed to thiopurines.

In summary, this study demonstrates that pediatric patients with IBD exposed to biologics in combination with thiopurines or thiopurines in the presence or absence of biologics have significantly increased risks of malignancy, compared to the respective SEER reference populations. The analyses establish an association between thiopurine exposure and the development of malignancy, which previously has only been shown in adult studies or systematic reviews which have pooled data from multiple pediatric studies. Furthermore, this study provides additional evidence for the association between thiopurine exposure and HLH, for which there are even more limited data. Given the recent data in adults suggesting the minimal effect of thiopurines in changing the natural history of CD, [37, 38] as well as pediatric data demonstrating no change in growth deficits in children with incident pediatric CD treated with thiopurines, [39] our robust safety data should give clinicians pause as they weigh the potential benefits and risks of thiopurines in the treatment of pediatric patients with IBD.

Figure legend

Figure 1. Study Diagram

Figure 2. Standardized incidence ratios (SIR) for the development of malignancy using the Surveillance, Epidemiology, and End Results (SEER) database. (A) includes the 3 main exposure cohorts, further stratified by thiopurine exposure (the IFX cohort is a subset of the biologics cohort), and (B) includes the total study population stratified by thiopurine exposure. An SIR with the lower bound of 95% CI < 1.0 indicates no evidence of increased risk for the development of malignancy, compared to the SEER reference population, adjusted for age, gender, and race. Comparisons between the infliximab and biologics cohorts and the respective non-biologics cohorts as well as the entire study population stratified by thiopurine exposure were made by examination of ratios of the SIRs and revealed no statistically significant differences. Additionally, the SEER database does not include non-melanoma skin cancers (i.e., basal cell carcinoma); therefore, the 2 cases of basal cell carcinoma were excluded from the SIR analyses.

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Figure 1.

* in North America, patients were eligible for enrollment in the DEVELOP study with either CD, UC or IBD-U while patients in the EU were eligible for enrollment only with either CD or UC

† in North America, patients were eligible for enrollment in the DEVELOP study < 17 years of age while patients in the EU were eligible for enrollment ≥ 6 and < 17 years of age

IBD=inflammatory bowel disease, CD=Crohn's disease, IBD-U=inflammatory bowel disease-unclassified, IFX=infliximab, UC=ulcerative colitis

Figure 2

thio=thiopurine, bio=biologic, IFX=infliximab, No.=number, pts=patients, F/U=follow-up, IQR=interquartile range, SIR=standardized incidence ratio

Table 1. Patient demographics and baseline disease characteristics

	Infliximab[#]	Biologics	Non-Biologics	Total	p-value
Total patients at enrollment, N	2396	2824	2942	5766	
Age (years)					<0.0001[*/**]
Mean (SD)	12.6 (2.83)	12.7 (2.82)	11.9 (3.12)	12.3 (3.01)	
Median	13.0	13.0	12.0	13.0	
Age at IBD Diagnosis (Mean) SD	10.3 (3.26)	10.1 (3.28)	9.8 (3.57)	9.9 (3.43)	
IBD Diagnosis					
CD	1766 (73.7%)	2113 (74.8%)	1934 (65.7%)	4047 (70.2%)	
UC	517 (21.6%)	575 (20.4%)	857 (29.1%)	1432 (24.8%)	
IBD-U	113 (4.7%)	136 (4.8%)	151 (5.1%)	287 (5.0%)	
Geographic location					
EU	375 (15.7%)	448 (15.9%)	475 (16.1%)	923 (16.0%)	
NA	2021 (84.3%)	2376 (84.1%)	2467 (83.9%)	4843 (84.0%)	
Gender, N (%)					0.0948[*] / 0.0789[**]
Male	1307 (54.5%)	1540 (54.5%)	1672 (56.8%)	3212 (55.7%)	
Female	1089 (45.5%)	1284 (45.5%)	1270 (43.2%)	2554 (44.3%)	
Race[***]					0.2826 [*] / 0.5374 [**]
White	1852 (77.3%)	2188 (77.5%)	2325 (79.0%)	4513 (78.3%)	
Non-White	439 (18.3%)	501 (17.7%)	510 (17.3%)	1011 (17.5%)	
Not Collected[***]	105 (4.4%)	135 (4.8%)	107 (3.6%)	242 (4.2%)	
Median Duration of disease, (years)	1.7	1.9	1.3	1.6	<0.0001*/**
Number of patients that discontinued, N (%)	471 (19.4%)	707 (18.3%)	451 (23.8%)	1158 (20.1%)	
Modified PCDAI (CD)					0.0019[*] / 0.0009 [**]
N	1688	2029	1904	3933	
Mean (SD)	11.3 (10.88)	11.4 (11.03)	9.8 (9.30)	10.6 (10.25)	
Inactive Disease (0-8)	739 (43.8%)	882 (43.5%)	870 (45.7%)	1752 (44.5%)	0.0001[*] / <0.0001[**]
Mild Disease (9-24)	707 (41.9%)	845 (41.6%)	849 (44.6%)	1694 (43.1%)	
Moderate to Severe	242 (14.3%)	302 (14.9%)	185 (9.7%)	487 (12.4%)	

Disease (>24)					
Partial Mayo (UC)					<0.0001*/**
N	427	472	775	1247	
Mean (SD)	1.8 (2.04)	1.8 (2.02)	1.1 (1.65)	1.3 (1.83)	
≤2 points	292 (68.4%)	321 (68.0%)	641 (82.7%)	962 (77.1%)	<0.0001*/**
3-4 points	81 (19.0%)	93 (19.7%)	87 (11.2%)	180 (14.4%)	
≥5 points	54 (12.6%)	58 (12.3%)	47 (6.1%)	105 (8.4%)	
Partial Mayo (IBD-U)					0.0077* / 0.0140**
N	68	76	117	193	
Mean (SD)	1.6 (2.10)	1.5 (2.04)	0.9 (1.72)	1.1 (1.87)	
Median	1.0	1.0	0.0	0.0	
IQ range	(0.0; 3.0)	(0.0; 3.0)	(0.0; 1.0)	(0.0; 2.0)	
Range	(0; 8)	(0; 8)	(0; 8)	(0; 8)	
≤2 points	49 (72.1%)	56 (73.7%)	103 (88.0%)	159 (82.4%)	0.0212* / 0.0326**
3-4 points	11 (16.2%)	12 (15.8%)	7 (6.0%)	19 (9.8%)	
≥5 points	8 (11.8%)	8 (10.5%)	7 (6.0%)	15 (7.8%)	

Infliximab cohort includes patients who were exposed to infliximab as the only biologic
P-values are from Wilcoxon rank sum test for continuous variables and from chi-square test for categorical variables. P-value for race compares distributions of white vs. non-white.
*p-value is comparing infliximab vs. non-biologics.
**p-value is comparing biologics vs. non-biologics.
***Not collected due to local French regulations and therefore not included in the p-value calculation
SD=standard deviation, IBD=inflammatory bowel disease, EU=Europe, NA=North America, PCDAI=Pediatric Crohn Disease Activity Index, CD=Crohn's disease, UC=Ulcerative colitis, IBD-U=Inflammatory Bowel Disease-Unclassified

Table 2. Clinical History for malignancy cases

Patient	Diagnosis	Age at malignancy diagnosis, Gender, Race, Country	IBD Diagnosis, Duration between IBD diagnosis and malignancy or HLH event onset (years)	IBD treatment prior to malignancy or HLH event diagnosis(Y/N) Duration of therapy (years)				Current therapy at the time of malignancy or HLH diagnosis	Family history of Malignancy	Infectious Exposures*
				IFX	ADA	6MP /AZA	MTX			
Lymphoid malignancies										
1	Acute monocytic leukemia	15, M, W, USA	CD, 9.2	Y 4	Y <1	Y 6	Y 3	ADA, MTX	N	Unknown
2	Acute lymphocytic leukemia†	16, M, H, USA	UC, 4.5	N	N	N	N		N	EBV negative
3**	B-cell lymphoma	16, F, W, USA	CD, 1.6	N	N	Y 2	N	6MP	N	Primary EBV Infection
4	B-cell lymphoma	14, M, A, USA	CD, 7.3	Y 5	Y 3	Y <1	Y 6	IFX, AZA	N	Unknown
5	B-cell lymphoma	17, M, H, USA	UC, 5.1	Y 2	N	N	N	IFX	N	EBV negative
6	B-cell lymphoma	22, M, NC, FRA	CD, 14.1	Y 13	Y 8	Y 6	Y 6	ADA, AZA	N	EBV positive
7	Chronic myeloid leukemia	14, M, NC, FRA	CD, 3.2	Y 2	N	Y 3	Y <1	IFX, 6MP	N	EBV negative
8	Hodgkin's lymphoma	16, M, NC, FRA	CD, 4.1	N	N	Y 4	N	6MP	N	Unknown
9	Mycosis fungoides (CTCL)	13,M, AA, USA	CD, 4.1	N	N	Y 3	N	6MP	lymphoma	EBV negative
Solid malignancies										
1	Parotid gland adenocarcinoma	17, F, W, USA	CD, 3.6	Y <1	Y 2	Y <1	N	ADA	N	unknown
2	Renal papillary cell carcinoma *	20, M, W, USA	UC, 4.4	Y 4	Y 3	Y 3	N	ADA	N	Unknown
3	Cholangiocarcinoma	18, F, AA, USA	CD, 13	Y 12	N	Y 4	Y 12	IFX	N	EBV positive
Skin malignancies										
1	Basal cell carcinoma	13, F, W, USA	CD, 1.1	Y <1	N	Y <1	N	IFX	lymphoma	Unknown
2	Basal cell carcinoma	16, F, W, USA	CD, 3.2	N	N	Y 3	N	6MP	prostate	Unknown
3	Malignant melanoma	14, M, W, USA	CD, 2.0	Y 2	N	Y 1	Y 1	IFX	N	Unknown

† This patient was exposed only to 5-ASAs and steroids

6MP=6-mercaptopurine, ADA=adalimumab, AZA=azathioprine, CD=Crohn's disease, CTCL=Cutaneous T-cell lymphoma, EBV=Epstein Barr virus, HLH=haemaphagoyctic lympohistiocytosis, IBD= inflammatory bowel disease, IFX=infliximab, MTX=methotrexate, N=No, Y=Yes
All patients had the diagnosis of CD and received steroids and/or 5-aminosalicylates. W=White, AA=Black or African American, A=Asian, H=Hispanic or Latino, UC=ulcerative colitis, USA=United States of America, FRA=France, M=male, F=female
* Infectious exposures noted if available ** One patient experienced both a malignancy event and an HLH event.

Table 3. Clinical History for HLH cases

Patient	Diagnosis	Age at HLH diagnosis, Gender, Race, Country	IBD Diagnosis, Duration between IBD diagnosis and malignancy or HLH event onset (years)	IBD treatment prior to malignancy or HLH event diagnosis(Y/N) Duration of therapy (years)				Current therapy at the time of malignancy or HLH diagnosis	Family history of HLH	Infectious Exposures*
				IFX	ADA	6MP /AZA	MTX			
Hemophagocytic Lymphohistiocytosis (HLH)										
1	HLH	16, F, W, USA	CD, 1.2	N	N	Y 1	N	6MP	N	Primary EBV infection
2	HLH	16, M, W, USA	CD, 11	N	N	Y 11	N	6MP	N	Primary EBV infection
3**	HLH	16, F, W, USA	CD, 1.6	N	N	Y 2	N	6MP	N	Primary EBV infection
4	HLH	15, F, AA, USA	CD, 1.2	N	N	Y 1	N	6MP	N	CMV infection***
5	HLH	19, M, W, GER	CD, 6.8	N	N	Y 7	N	AZA	N	Primary EBV infection

6MP=6-mercaptopurine, ADA=adalimumab, AZA=azathioprine, CD=Crohn's disease, CMV=Cytomegalovirus, EBV=Epstein Barr virus, HLH=hemophagoyctic lympohistiocytosis, IBD=inflammatory bowel disease, IFX=infliximab, MTX=methotrexate, N=No, Y=Yes, M=male, F=female
All patients had the diagnosis of CD and received steroids and/or 5-aminosalicylates. W=White, AA=Black or African American, USA=United States of America, GER=Germany. * Infectious exposures noted if available ** One patient experienced both a malignancy event and an HLH event. ***Unknown if primary CMV infection or reactivation

Table 4. Unadjusted Incidence Rates of Malignancy

	Infliximab and thiopurines	Infliximab without Thiopurines	Biologics and thiopurines	Biologics without Thiopurines	Non-Biologics and thiopurines	Non-Biologics without thiopurines	All patients*
Total patients, N	1603	824	2723	1146	1134	763	5766
Total patient years of follow-up	7613.0	3189.5	11416.2	4202.2	5808.0	3116.6	24543.0
Median patient years of follow-up (Interquartile range)	3.3 (1.45; 5.05)	2.7 (0.90; 4.63)	4.4 (2.49; 5.68)	3.4 (1.42; 5.02)	2.3 (1.06; 4.83)	1.7 (0.70; 4.34)	4.7 (2.36; 5.72)
Events per 1000 patient-years [Number of events]	0.53 [4]	0.31 [1]	0.79 [9]	0.24 [1]	0.69 [4]	0.32 [1]	0.81 [20]
95% CIs	[0.14, 1.35]	[0.01, 1.75]	[0.36, 1.50]	[0.01, 1.33]	[0.19, 1.76]	[0.01, 1.79]	
Neoplasms benign, malignant and unspecified (including cysts and polyps)	0.53 [4]	0.31 [1]	0.79 [9]	0.24 [1]	0.69 [4]	0.32 [1]	0.61 [15]
Basal cell carcinoma	0.13 [1]		0.09 [1]		0.17 [1]	-	0.08 [2]
Chronic myeloid leukaemia	0.13 [1]		0.09 [1]				0.04 [1]
Malignant melanoma	0.13 [1]		0.09 [1]				0.04 [1]
Adenocarcinoma			0.09 [1]				0.04 [1]

Acute monocytic leukaemia			0.09 [1]				0.04 [1]
B-cell lymphoma		0.31 [1]	0.09 [1]	0.24 [1]	0.17 [1]		0.04 [1]
Diffuse large B-cell lymphoma stage IV			0.09 [1]				0.04 [1]
Renal cancer metastatic			0.09 [1]				0.04 [1]
Hodgkin's disease nodular sclerosis					0.17 [1]		0.04 [1]
Mycosis fungoides					0.17 [1]		0.04 [1]
Cholangiocarcinoma	0.13 [1]		0.09 [1]				0.04 [1]
T-cell type acute leukaemia						0.32 [1]	0.04 [1]

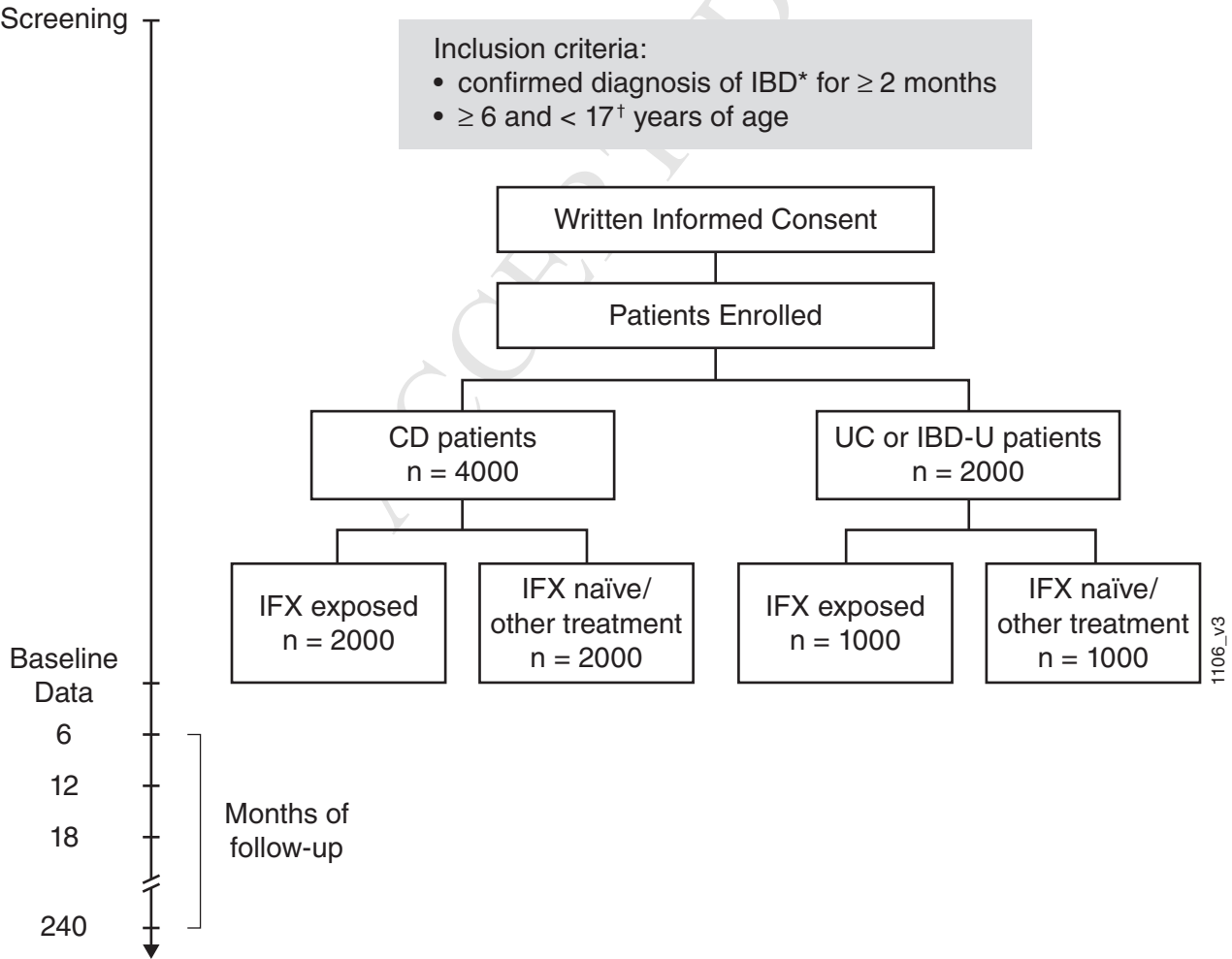
* ”All patients” includes the entire study population, as well as the sum of thiopurine-exposed and thiopurine non-exposed patients, and the sum of the biologics ± thiopurines and non-biologics ± thiopurines cohorts. The infliximab ± thiopurines cohort is a subset of biologics ± thiopurines cohort. The biologics cohorts include primarily exposure to anti-TNF-α biologics; 137 patients were exposed to non-anti-TNF-α biologics, either with or without thiopurines for a total of 210.1 patient-years of exposure. TNF=Tumor necrosis factor

Table 5. Unadjusted incidence rates of malignancy and HLH by thiopurine exposure

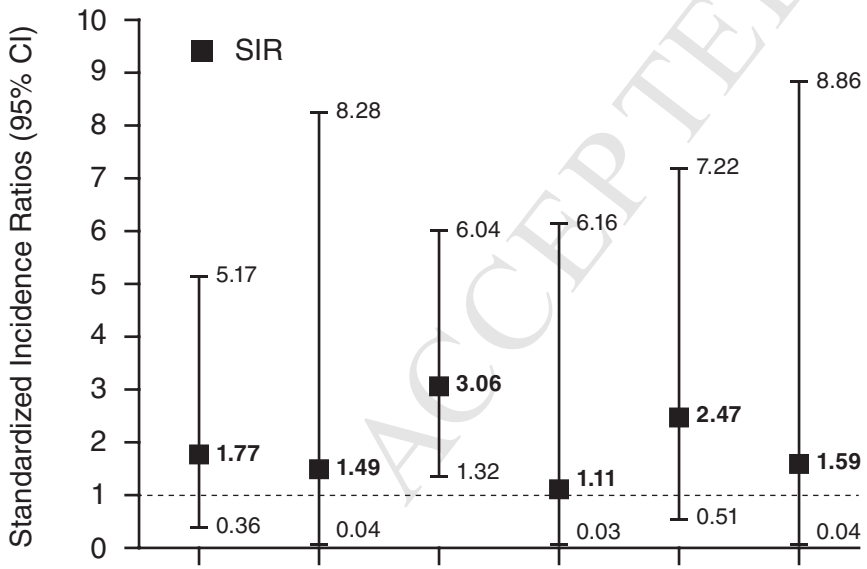
	Thiopurine exposed ± Biologics	Thiopurine non-exposed ± Biologics	All patients
Total patients, n	3857	1909	5766
Total patient years (patient-years of follow-up	17224.1	7318.8	24543.0
Median patient-years of follow-up	4.8	3.0	4.7

Malignancy events/1000 patient-years [N]; 95% CI	0.75 [13] [0.40, 1.29]	0.27 [2] [0.03, 0.99]	0.61 [15] [0.34, 1.01]
HLH events/1000 patient-years [n]; 95% CI	0.29 [5] [0.09, 0.68]	0 [0.00,0.41]	0.2 [5] [0.066, 0.475]
Malignancy + HLH events/1000 patient-years [N]; 95% CI	1.05 [18] [0.62, 1.65]	0.27 [2] [0.03, 0.99]	0.81 [20] [0.50, 1.26]

CI=Confidence interval, HLH=hemophagocytic lymphohistiocytosis



A



	IFX + thio	IFX – thio	Bio + thio	Bio – thio	Non-bio + thio	Non-bio – thio
No. of pts	1603	824	2723	1146	1134	763
Total patient-years of F/U (IQR)	7613.0 (1.45; 5.05)	3189.5 (0.90; 4.63)	11416.2 (2.49; 5.68)	4202.2 (1.42; 5.02)	5808.0 (1.06; 4.83)	3116.6 (0.70; 4.34)
No. of pts with events	3	1	8	1	3	1

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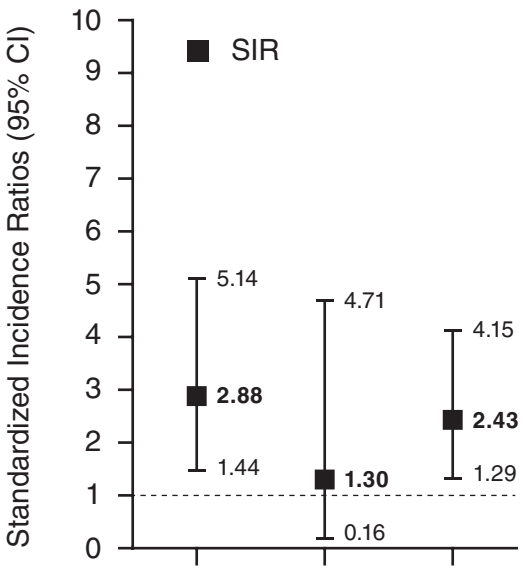
B



	Thio-exposed ± bio	Thio non-exposed ± bio	**Total**
No. of pts	3857	1909	5766
Total patient-years of F/U (IQR)	17224.1 (2.69; 5.94)	7318.8 (1.17; 5.02)	24543.0 (2.36; 5.72)
No. of pts with events	11	2	13

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Supplemental Material

Table S1. Cumulative summary of malignancies

	Infliximab	**Biologics**	**Non-Biologics**	**Total**
Number of Patients at Baseline or Through Registry follow-up	2427	3869	1897	5766
Total Patient-years of follow-up	10802.5	15618.4	8924.6	24543.0
Number of patients/1000 Patient-years of follow-up [Number of patients with any new malignancy]	0.46 [5]	0.64 [10]	0.56 [5]	0.61 [15]
Events per 1000 Patient-years of follow-up [Number of events]	0.46 [5]	0.64 [10]	0.56 [5]	0.61 [15]
95% CIs	[0.15, 1.08]	[0.31, 1.18]	[0.18, 1.31]	[0.34, 1.01]

Table S2. Unadjusted incidences of malignancy and HLH by methotrexate (MTX) only exposure

	All patients	Methotrexate Only	Non-Methotrexate Only
Number of Patients at Baseline or Through Registry	5766	532	5234
Total Patient Years of follow-up	24543.0	1630.1	22912.9
Malignancy events Number per 1000 patient-years of follow-up [Number of events]	0.61 [15]	0	0.65 [15]
95% CIs	[0.34, 1.01]	[0.00, 1.84]	[0.37, 1.08]
HLH events per 1000 patient-years of follow-up [Number	0.2 [5]	0	0.22 [5]
95% CIs	[0.07, 0.48]	[0.00, 1.84]	[0.07, 0.51]

HLH=Hemophagocytic Lymphohistiocytosis

Table S3. Standardized Incidence Ratios for the development of malignancy using the Surveillance, Epidemiology, and End Results (SEER)* database

	Infliximab	**Biologics**	**Non-Biologics**	**Total**
Number of patients	2427	3869	1897	5766
Total patient-years of follow-up	10802.5	15618.4	8924.6	24543.0
Median patient-years of follow-up	3.1	4.3	2.3	4.7
Observed number of patients with events	4	9	4	13
Incidence per 1000 patient-years	0.37	0.58	0.45	0.53
95% CIs	(0.10, 0.95)	(0.26, 1.09)	(0.12, 1.15)	(0.28, 0.91)
Expected number of patients	2.37	3.52	1.84	5.36
Standard Incidence Ratio (SIR)	1.69	2.56	2.17	2.43
SIR 95% confidence interval	0.46-.4.32	1.17-4.86	0.59-5.56	1.29-4.15
*The SEER database does not include non-melanoma skin cancers (i.e., basal cell carcinoma); therefore, the 2 cases of basal cell carcinoma were excluded from the SIR analyses.				

Table S4. Comparison of malignancy rates (excluding non-melanoma skin cancers) by exposure

	Rate of Malignancy/10,000 Patient-years
Expected Rate in General US Pediatric Population	2.2
IBD Patients treated with biologics	5.8
Biologics with thiopurines	7.0
Biologics without thiopurines	2.4
IBD Patients treated with non-biologics	4.5
Non-biologics with thiopurines	5.2
Non-biologics without thiopurines	3.2
IBD=inflammatory bowel disease, US-United States	

Table S5. Standardized incidence ratios (SIR) for the development of malignancy using the Surveillance, Epidemiology, and End Results (SEER) database with thiopurine-exposed patients stratified by ongoing exposure at malignancy diagnosis

	All Patients	**Thiopurine-exposed**		**Thiopurine non-exposed**
		Discontinuation ≤ 1 year prior to or concomitant exposure at time of malignancy diagnosis*	Discontinuation > 1 year prior to malignancy diagnosis**	
Observed number of patients with events	13	8	3	2
Total patient-years of follow up	24543.0	9005.4	8218.7	7318.8
Mean (SD)	4.26 (2.122)	2.55 (1.886)	3.11 (2.000)	3.21 (2.199)
Median	4.65	1.86	3.03	3.00
IQ range	(2.36; 5.72)	(1.00; 3.94)	(1.35; 4.75)	(1.17; 5.02)
Range	(0.0; 9.0)	(0.0; 9.0)	(0.0; 8.0)	(0.0; 8.9)
Observed number of patients with events	13	8	3	2
Incidence per 1000 patient-years	0.53	0.89	0.37	0.27
95% CIs	(0.28, 0.91)	(0.38, 1.75)	(0.08, 1.07)	(0.03, 0.99)
Expected number of patients	5.36	1.80	2.03	1.53
Standard Incidence Ratio (SIR)	2.43	4.45	1.48	1.30
SIR 95% CIs	(1.29, 4.15)	(1.92, 8.77)	(0.30, 4.32)	(0.16, 4.71)

*Patient with malignant melanoma discontinued thiopurines 213 days prior to malignancy diagnosis

** Patients with parotid adenocarcinoma, renal cell carcinoma, and cholangiocarcinoma had discontinued thiopurines for > 2 years prior to malignancy diagnosis

SD=standard deviation, IQ=interquartile range