Response letter to the reviewers

Reviewer 1:

Thank you for your expert opinion and your appreciation.

Reviewer 2:

1) Please comment association between duration of Crohn's disease (CD) and cancer.

Dear reviewer,

Thank you for your comments. Carcinogenesis under chronic inflammatory conditions, particularly cancer in inflammatory bowel disease is an important issue in long-term surveillance of patients suffering from (stricturing) CD. Whereas several studies focus on the association and risk of cancer in ulcerative colitis (UC), there are hardly any data on incidental cancer in CD. Therefore, recommendations are often extrapolated from data we receive from UC. To date, there are only 2 trials with a limited number of patients that investigate incidental cancer in patients undergoing resections for Crohn's disease. Maykel et al.[1] investigated 222 patients that were diagnosed with dysplasia or cancer after colonic resections for CD. They identified long disease duration, older age at diagnosis and disease extent as independent risk factors. Furthermore, Yamazaki et al.[2] analyzed 132 patients that developed colonic strictures and reported a high rate of 6.8% patients with malignancies. Again, duration of disease was associated with cancer, particularly when disease extended over 20 years. These findings are consistent with our cohort, where cancer was diagnosed after a substantial history of CD. Chronic pro-inflammatory environment and consecutive cellular damage have been associated with an increased risk of colonic cancer in patients with CD involving the colon[3]. As a consequence, localized, diffuse or multifocal genomic changes precede cancer in inflammatory bowel disease[4] and lead to point mutations of p53 and malignant transformation in UC. Therefore, endoscopic surveillance is crucial especially in patients with risk factors.

Association between duration of CD and cancer was integrated in the discussion section.

2) How about (anal) fistula in your case?

Although stricturing phenotype is dominated by stenosis, penetrating behaviour may additionally occur. Nowadays, potent targeted and immunomodulatory therapy are highly effective in treatment of CD. Therefore, persistent anal fistula is an indicator for failed or low medical response, which again leads to prolonged inflammation. Interestingly, four patients in our cohort were diagnosed with incidental cancer in the rectum. In case 4 and 6 the histological report did not reveal any fistula formation. In contrast, the other 2 patients had fistula. Histological analysis reported fistula formation for one patient, which clinically was not manifest. Besides rectal stenosis, which led to surgical intervention the other patient, he also had documented fistula formation for about 18 years, which did not respond to medical therapy. Failed treatment led to long-standing massive fistula and a chronic abscess in the supralevator space. Furthermore, in this case carcinoma was also present when fistula where examined. Taken together, long-standing fistulating anal disease not responding to conventional therapy is an expression of aggressive disease beviour, which should be subject to periodic exploration and histological analysis[5]. Importantly, intestinal fistula should be discriminated from perianal fistulizing disease, given that they differ in management and outcome[6]. Although there are hardly any data about cancer originating from extra-anal fistula formation in CD, one has to be aware of the possibility of incidental cancer clinically manifesting in penetrating CD[7].

(Anal) fistula formation was integrated in the discussion section and changes were highlighted in red.

Reviewer 3:

Thank you for your remarks.

1) In the introduction line 8, the authors raise the question about "endoscopic surveillance and cancer prevention". In their discussion, the authors must clearly identify how their present results may change this statement.

Our results highlight that personalized endoscopic surveillance is not feasible in all patients with stricturing CD. We currently put further emphasis on the risk of incidental carcinoma especially in older patients with long disease duration that cannot be fully explored by endoscopic means. This strategy may lead to better long-term survival in patients with incidental carcinoma in future.

This paragraph was added to the discussion and is highlighted in red.

2) In their discussion, the authors could add a table summarizing their results and prior results showing the number of patients, % of patients with incidental adenocarcinoma and identified risk factors.

Thank you for your good point. The table was added as Table 3 in the discussion and will hopefully provide a better overview.

3) Do the authors know how long the patients had symptoms of stricture from Crohn's disease prior to the surgical resection that either did or did not identify adenocarcinoma?

We discriminated surgical interventions into acute or elective procedures that somehow also reflect severity of stricturing disease. Unfortunately, we did not explore duration of symptoms that led to surgical interventions. We were discussing this interesting issue designing this trial but due to the retrospective nature of the study and the poor long-term patient survival we were unable to consider this variable.

To the authors knowledge there are clear associations between duration of disease and the detection of incidental cancer but no data on symptom duration and its link to incidental cancer. The authors hypothesize that rapid worsening of symptoms could be a link to cancer in CD due to aggressive tumor progression. Nevertheless, this is one the questions that should be raised and answered in future clinical trials.

4) The authors need to discuss in their results the finding with regards to medication use (which is in their table).

Changing paradig in medical treatment and its impact on carcinogenesis are still under debate. For example, monoclonal anti-TNF antibodies have established anti-cancer effects, whereas it is also secreted by tumors to enhance neoplastic proliferation. Additionally, antimetabolites generate mutagenic oxidative DNA damage and may promote carcinogenesis. Axelrad et al.[8] analysed 333 patients with IBD and history of cancer and concluded that anti-TNF alpha or antimetabolites were not associated with an increased risk of recurrent or new cancer.

These findings are comparable with our results, where patients with strictures and no cancer had even higher rates of medical therapy compared to patients with strictures and cancer. One could even speculate that novel potent therapeutic options may break the chain of chronic inflammation and even have beneficial results as far as incidental cancer is concerned. However, our number of cancers was so small that a very large multicenter trial would be needed to power such a hypothesis.

The discussion was adapted and marked in red.

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5) Malignant lesion of the ileum Please identify the lesion as we can not tell whether the authors believe it arose in a terminal ileal stricture or in a cecal stricture.

The malignant lesion arose in the terminal ileum 6cm before the ileocecal valve. Table 2 was changed to be more specific and the term ileocolon was changed to ileum. These corrections were also made in the results section and also highlighted in red.

6) In table the result for Body Mass Index under "stricturing CD and incidental cancer" is empty.

Thank you for your careful review of our manuscript. The mean BMI in the group stricture and incidental cancer was 22.4 with a standard deviation of ±4.4. Table 1 was corrected and the change highlighted in red.

1 **Maykel JA**, Hagerman G, Mellgren AF, Li SY, Alavi K, Baxter NN, Madoff RD. Crohn's colitis: the incidence of dysplasia and adenocarcinoma in surgical patients. *Dis Colon Rectum* 2006; **49**: 950-957 [PMID: 16729218 DOI: 10.1007/s10350-006-0555-9]

2 **Yamazaki Y**, Ribeiro MB, Sachar DB, Aufses AH, Jr., Greenstein AJ. Malignant colorectal strictures in Crohn's disease. *Am J Gastroenterol* 1991; **86**: 882-885 [PMID: 2058631]

3 **Ekbom A**, Helmick C, Zack M, Adami HO. Increased risk of large-bowel cancer in Crohn's disease with colonic involvement. *Lancet* 1990; **336**: 357-359 [PMID: 1975343]

4 **Axelrad JE**, Lichtiger S, Yajnik V. Inflammatory bowel disease and cancer: The role of inflammation, immunosuppression, and cancer treatment. *World J Gastroenterol* 2016; **22**: 4794-4801 [PMID: 27239106 PMCID: PMC4873872 DOI: 10.3748/wjg.v22.i20.4794]

5 **Zefelippo A**, Costa S, Caprioli F, Contessini-Avesani E. Perianal Crohn's disease and fistula-associated carcinoma: challenges in diagnosis. *Int J Colorectal Dis* 2015; **30**: 1589-1591 [PMID: 25643850 DOI: 10.1007/s00384-015-2140-y]

6 **Kiani QH**, George ML, Carapeti EA, Schizas AM, Williams AB. Colovesical fistula: should it be considered a single disease? *Ann Coloproctol* 2015; **31**: 57-62 [PMID: 25960973 PMCID: PMC4422988 DOI: 10.3393/ac.2015.31.2.57]

7 **Harisankar CN**. Incidentally detected sigmoidovesical fistula in a case of rectosigmoid cancer detected on FDG PET-CT. *Clin Nucl Med* 2015; **40**: e143-144 [PMID: 24999684 DOI: 10.1097/RLU.0000000000000518]

8 **Axelrad J**, Bernheim O, Colombel JF, Malerba S, Ananthakrishnan A, Yajnik V, Hoffman G, Agrawal M, Lukin D, Desai A, McEachern E, Bosworth B, Scherl E, Reyes A, Zaidi H, Mudireddy P, DiCaprio D, Sultan K, Korelitz B, Wang E, Williams R, Chen L, Katz S, Itzkowitz S, New York Cs, Colitis O. Risk of New or Recurrent Cancer in Patients With Inflammatory Bowel Disease and Previous Cancer Exposed to Immunosuppressive and Anti-Tumor Necrosis Factor Agents. *Clin Gastroenterol Hepatol* 2016; **14**: 58-64 [PMID: 26247164 DOI: 10.1016/j.cgh.2015.07.037]