Reviewer #1:

Comment: If authors could elaborate on occurrence of PTLD seperately with respect to different solid organs

Answer: We elaborated on the occurrence of PTLD separately with respect to different solid organs. We found several sources and included the findings in the manuscript to provide a range for the occurrence of PTLD in various organs. GI tract (30%), CNS (10-15%), lungs (4%), and liver (5-12%). In another study of 140 patients, the localization was found to occur as follows: GI tract 29%, pulmonary (23%), bone marrow (15%), graft (15%) and CNS (5%). We included another study which included 135 patients and had the following distribution: Nodal disease 21%, extranodal disease 79%-> allograft 19%, CNS 8%, bone marrow/blood 17%, liver 13%, lung/pleura 9%, GI tract 23%

Comment: The details about the immunosuppression drug regimen used for induction, maintenance in each category of transplant patient population

We have provided the details about the immunosuppression drug regimen used for induction and maintenance in each category of transplant patient population according to the most recent OPTN/SRTR 2020 Annual Data which was released in March 2022. We found the following trends for induction: heart transplantation may or may not use induction therapy (50:50). When induction is used, T-cell depleting therapy is most often used. For lung transplantation, induction therapy is used 80% of the time, and IL2RA is used most often (70%). For kidney transplantation, T-cell depleting therapy is most often utilized and induction therapy is provided nearly 90% of the time. Induction therapy is most often not used for liver transplantation. For pancreas transplantation, T cell depleting therapy is used 90% of the time. For intestinal transplantation, T cell depleting therapy is most often used, 27.8% of patients did not receive induction.

The trends for maintenance immunosuppression are as follows: Most often tacrolimus/MMF/steroids are used, nearly 70% of the time. After heart transplantation, tacrolimus, MMF, steroids are used (50%) or tacrolimus and MMF are prescribed (40%). After lung transplantation, tacrolimus, mycophenolate, and steroids are prescribed 80% of the time. After kidney transplantation, tacrolimus, MMF, steroids are used

approximately 54% of the time and tacrolimus & MMF are used 36.8%. After liver transplantation, tacrolimus, MMF, and steroids are used in 65% patients. After intestinal transplantation, tacrolimus is prescribed in 73% of patients and is combined with corticosteroids 37.4% of the time.

Comment: If authors could add on some photographs of pathological slides ,endoscopic appearance of the lesions of PTLD

Answer: We have added original slides of two monomorphic lymphomas. We do not have access to polymorphic pathology at our institution currently. We have attached two endoscopic images of PTLD in the small bowel and rectum. These images have varying findings (one of a mass, and one of ulcerated, nodular mucosa to help shed light on the variable appearance of PTLD). Further endoscopic images are not currently available at our institution.

Reviewer #2:

Comment: Please clearly indicate what type of articles were included, a flowchart (for example according to the PRISMA guidelines) with the total number of articles, how many after removal of duplicates, how many were included for full review assessment, how many of these were excluded and why.

Answer: We have increased the depth of the discussion section. It has been changed from a repeat summary of results to a discussion regarding the risk factors, and why these increased risks may occur with immunosuppression regimens, and after specific transplants. We discuss the importance of clinical questioning regarding pathology involving the small bowel and stomach, two of the most frequently involved GI organs. We discuss the most important aspects of treatment including determining EBV status and RIS. We have clearly indicated which studies are included with the data extraction table (Table 2). We have also attached a PRISMA Flow diagram which outlines the total number of articles, how many after removal of duplicates, how many were included for full review assessment, how many of these were excluded and why.

Comment: Please also provide a table with the data extraction from the included articles. If the authors are not able to provide this information, it would be better to call it a literature review instead of a systematic review.

Answer: We have included a data extraction table describing key results from the 9 studies we have included. We have also included a table (Table 1) with data extraction of the articles which describe localization of PTLD, composition of monomorphic vs polymorphic morphology in GI biopsies positive for PTLD, and time from transplant to transplantation found in each study, (6/9 studies included the aforementioned information).

Comment: Results: - Paragraph 4: please indicate the percentage (56 (11.9%) patients). It would also be interesting to give more info regarding the other locations. - I would suggest moving paragraph 9 after paragraph 5 to have a better transition.

Answer: We have provided the percentage of patients who were previously mentioned in Paragraph 4 of the original manuscript (56 (11.9%) patients). We moved the original paragraph 9 of the results to after paragraph 5 as suggested.

We included information regarding locations of PTLD development in other locations (other solid organs) Figure 1 and page 4 lines 17-20. We have also listed the locations within the GI tract where PTLD was found in the included studies which listed this information (Table 1). We have moved paragraph 9 after paragraph 5 as originally suggested to provide a better transition. We were uncertain on which exact paragraph was in question. We apologize for not having line numbers which would facilitate this process. Line numbers have added to aide. If the flow of these two paragraphs in the updated version is not as envisioned, please let us know.

Comment: Regarding the therapies, first of all I'd mention to check the EBV status, as this is the first thing you do after diagnosis and may affect treatment.

Answer: Regarding the reviewer comment on therapies, we did find the importance of determining EBV status. We found the role of EBV-cytotoxic T lymphocytes (EBV-CTLs) or donor lymphocyte infusion (DLI) as second line therapy when rituximab and RIS aren't working in patients who are EBV-positive. We did find older studies which

mentioned the role of acyclovir; however, we did not find treatment for EBV-PTLD. We found absence of EMA and FDA approved therapies, lack of a global standard of care and most importantly, lack of randomized trials to suggest other treatment differences in EBV-positive or EBV-negative PTLD [16, 17].

Comment: Please clarify supportive therapy: I often do not give IV fluids, antibiotics or blood transfusion. I guess you mean IV fluids in case of dehydration due to diarrhoea, or tumour lysis, blood transfusion in case of anaemia (due to bone marrow involvement or bleeding).

Answer: Regarding clarification of supportive therapy, we have removed the comments regarding supportive therapy as this is vague and not pertinent to direct PTLD treatment.

Comment: Paragraph 14: ATG and alemtuzumab are IS therapies given for SOT, and increase the risk for PTLD, not part of PTLD therapy? Please check. Interferon alpha was used as a therapy for PTLD indeed, although it is now rarely used because of increased risk of acute rejection. I would list rituximab separately as a monoclonal antibody against CD20 and not as chemotherapy.

Answer: Regarding the initial paragraph 14 and ATG and alemtuzumab, we removed the sentence regarding ATG and alemtuzumab as therapies for SOT which maybe conflated with therapy for PTLD.

Regarding interferon alpha, we removed interferon alpha as treatment for PTLD as it is rarely used now. We have removed the comment about acute rejection as a risk factor for PTLD. Acute rejection may occur because of RIS it is not a risk factor for PTLD. (Point 28) We have also provided a figure to demonstrate this distribution (Figure 1).

Regarding our language describing rituximab, we have made our language more precise. Rituximab is listed as "rituximab" not as immunotherapy.

Comment: Paragraph 20: what was the control group? Discussion: I miss some depth in the discussion, now it is more a summary of the results.

Answer: Regarding the control group mentioned in the original paragraph 20, the control group was patients that developed PTLD in non-colorectal sites.

Comment: At the beginning of the discussion I miss a discussion about the impact of the EBV status as a risk factor (especially EBV negative + high dose of IS during first year post-Tx gives increased risk).

Answer: Regarding mentioning EBV as a risk factor, we have described the role of EBV status and emphasized it as a risk factor. Primary EBV infection is a significant risk factor 6-76 times noted by one article. We also include EBV-seronegativity in the recipient as a significant risk factor.

Comment: I don't know if acute rejection itself was a risk factor for PTLD or rather the associated increase in IS.

Answer: We have removed "acute rejection" as a risk factor for PTLD. As suggested by the reviewer, we also found that acute rejection may occur because of RIS, acute rejection is not a risk factor for PTLD.

Comment: The authors should give more information on PTLD and the different subtypes (PTLD is not just one disease entity).

Answer: As requested, we have provided more information on PTLD and the different subtypes of PTLD. We included the WHO classification of PTLD which includes non-destructive PTLDs, monomorphic PTLDs, polymorphic PTLDs, and classic Hodgkin lymphoma PTLDs. We also included further sub-categorization for monomorphic, non-destructive, and classic Hodgkin lymphoma PTLD. We included associations with EBV and describe brief pathology of these subtypes.

Comment: I would elucidate early vs late-onset PTLD (latter associated with worse outcome), monomorphic vs polymorphic, early and polymorphic almost always EBV-associated and with higher response chance of reduction in IS +/- rituximab. While in more advanced disease stages or histology types, you often don't do sequential therapy but start with RIS + ritux + chemo.

Answer: As requested, we have provided information differentiating between early and late-onset PTLD. Early PTLD which is often associated with EBV positivity and graft involvement while less commonly associated with monomorphic morphology and less

often presenting as extranodal disease. We did not find treatment differed based on this categorization. We also did not find a difference in survival between early vs. late PTLD.

Comment: I would give some information on reduction of IS (which is often a reduction of calcineurin inhibitor trough levels and stop of MMF/AZA).

Answer: We provide specific information on RIS. We describe RIS as being the most important therapy and describe its efficacy. We describe standard RIS which consists of more than 50% reduction in immunosuppression and cessation of azithromycin and MMF. Assessment of response should occur in 2-4 weeks. We also provide information on when RIS is usually not sufficient (multi-organ dysfunction, systemic disease, or in patients who may be in need of a quick treatment). Reduction in immunosuppression may not work if the disease burden is bulky, it the cancer stage is severe, or for older ages.

Comment: I would avoid the term immunotherapy, I'm not sure what the authors mean exactly. I would use rituximab.

Answer: Regarding the term immunotherapy, we have removed this term. We standardize our terminology and use "rituximab" instead where indicated.

Comment: In the table with data extraction, it would be nice to give some information on the PTLD types in the included studies (e.g., median time to onset, localizations, % monomorphic/polymorphic).

Answer: In the table with data extraction, we provide information on PTLD types when offered in the included studies. 6/9 studies mention median time to onset, localizations within the GI tract, %monomorphic/polymorphic and we have included this information in Table 1.

Comment: The authors sometimes mentioned radiotherapy. This is not often used for GI PLTD, rather for CNS disease. Can you clarify for which disease localization radiotherapy was used in the studies?

Answer: Regarding radiotherapy as a treatment for GI-PTLD, we reviewed the literature for use of radiotherapy as treatment for PTLD. As mentioned in the reviewer comments, radiotherapy is most often used for CNS disease and infrequently used for solitary PTLD. We did find a favorable effect may be obtained in stage 1 plasmacytoma-like PTLD[26].

Comment: Only 9 articles were included, so I would not call it an extensive literature review.

Answer: We believe this is a systematic review we have focused our language to highlight our findings and provided a PRISMA diagram to show the studies we included and excluded. We have included all studies which incorporated discussion of GI PTLD and excluded studies discussing pediatric PTLD, conference abstracts, and non-English studies.

Comment: Treatment depends on several factors… I miss reduction of IS here (which is the mainstay), I'd again clarify what you mean by immunotherapy (I guess rituximab, and would just use this word). I'd say RIS 'can be' associated with acute graft rejection (often PTLD is considered a consequence of over-immunosuppression and RIS is not always associated with acute rejection)

Answer: Indeed, treatment depends on several factors. We describe RIS as being the most important therapy and describe its efficacy. We describe standard RIS which consists of more than 50% reduction in immunosuppression and cessation of azithromycin and MMF. Assessment of response should occur in 2-4 weeks. We also provide information on when RIS is usually not sufficient (multi-organ dysfunction, systemic disease, or in patients who may need a quick treatment). Reduction in immunosuppression may not work if the disease burden is bulky, it the cancer stage is severe, or for older ages. We have removed "immunotherapy" and replaced this term with "rituximab". We have mentioned RIS can be associated with acute graft rejection in the discussion.

Comment: Please introduce the abbreviations in the main text the first time you use them (intro line 1: gastrointestinal (GI), line 2 post-transplant lymphoproliferative disorder (PTLD)) and please use it consistently afterwards (line 7, 13) Typo's: abbreviations: HSCT instead of SCST, consistency in writing (nonspecific in abstract, non-specific in main text), results paragraph 20 colorectal lowercase.

Answer: Regarding consistency in abbreviations, we have introduced abbreviations in the main test the first time they are used (GI, PTLD) and have used them consistently afterwards. Regarding Typos we have corrected the abbreviation for HSCT and

improved the consistency of our writing (i.e., nonspecific vs non-specific). We adjusted "colorectal" to lower case.

Reviewer #3

Comment: Please add page and line numbers.

Answer: We have added page and line numbers.

Comment: This manuscript lacks descriptives tables and figures.

Answer: We have created a figure regarding organ involvement of PTLD, Figure 1 (original figure).

Comment: Please make a figure regarding organ involvement of PTLD. Please make a figure regarding the workflow of the treatment. Please make a table regarding the possible treatments including the readout of immunotherapies.

Answer: We have made a figure regarding the workflow of treatment (original figure). If the figure of therapies, we have provided does not contain the desired "readout of immunotherapies" please let us know. We are not certain which immunotherapies the reviewer means.

Comment: The keywords that were serached are very limited.

Answer: We have adjusted our keywords to now include "reduction of immunosuppression" and "Epstein-Barr Virus". The initial keywords "PTLD, post-transplant lymphoproliferative disorder; gastrointestinal manifestations; risk factors" have remained.

Reviewer #4

Comment: The authors did not perform a systematic review, or at least this was not performed according to the PRISMA guidelines. They should include a PRISMA diagram, specifying what databases were searched and what was the search criteria. Furthermore for the study included in the review a more detailed analysis, specifying in a table all the relevant information should be presented. In the present way, it does not seem to be very detailed and comprehensive.

Answer: We believe we have completed a systematic review. We have included a PRISMA diagram, specified which databases were searched, and what the search criteria were. We have specified in a detailed table, all the relevant information presented. A table of "high-yield points" (Table 2) and a table describing key findings (localization of PTLD, Time from transplant to PTLD, classification [monomorphic vs. polymorphic]) (Table 1).

Reviewer #5:

Comment: In page 4 line 29, the authors report acute cellular rejection as a well-recognized risk factor for the development of PTLD. It would be interesting to further discuss the underlying physiopathology - whether this increase is associated with the treatment of acute rejection (usually an increase in immunosuppression) or with the immune process of rejection itself.

Answer: After further review of acute cellular rejection and development of PTLD, we did not find acute cellular rejection to be a risk factor. Rather, acute cellular rejection can be associated with treatment (RIS). We have removed the statement describing acute cellular rejection as a risk factor for the development of PTLD.

Comment: In page 4 line 36 oncogenic viral infection is cited as a risk factor for PTLD - the most common types of virus (EBV, etc.) could be cited here.

Answer: Regarding specification of oncogenic viral infection. We have elucidated the role of viral infection in PTLD. The roles of EBV, CMV, Hepatitis C, and HHV8 were all noted.

Comment: The choice of treatment for PTLD is vague in the manuscript, the authors could further detail in what situations the reduction of immunosuppressive drugs is sufficient, and when should chemotherapy and immunotherapy be used. While on page 6 line 37 it is stated that the difference between outcomes in surgery and immunotherapy are not well elucidated, but in page 5 line 21 it is stated that surgical resection is rarely considered in patients with PTLD, this point should be clarified.

Answer: Regarding choice of treatment for PTLD, we describe the treatment of PTLD first emphasizing RIS as the mainstay. We specific standard RIS regimens which have been studied. We describe situations when RIS is sufficient, most of the time. RIS consists of

more than 50% reduction in immunosuppression and cessation of azithromycin and MMF. Assessment of response should occur in 2-4 weeks. We also provide information on when RIS is usually not sufficient (multi-organ dysfunction, systemic disease, or in patients who may need a quick treatment. Reduction in immunosuppression may not work if the disease burden is bulky, it the cancer stage is severe, or for older ages. We created Figure 8 to describe the workflow of treatment. We emphasize how treatment is dependent on morphologic subtype if RIS does not have effect.

Regarding initial stated outcomes in surgery and immunotherapy: the role of surgery is more clearly discussed in the revised version. Comparison between medical and surgical therapy cannot necessarily be made in the acute setting based on the available studies and inherent differences in illness. However, we did find long-term outcomes did not appear to be affected after having surgery. The most common reason to have surgery was intestinal obstruction.

Re-reviewer #1:

Comment: The authors revised according to my suggestions and followed a systematic reviews methodology more accurately.

Answer: Thanks for your comments.

Re-reviewer #2:

Comment: The article has been updated very nicely and now addresses exactly the things that were missing in the first version. The addition of the tables and figures is also an added value. This revision has certainly upgraded the manuscript to one of a good quality level.

Answer: Thanks for your comments.

Re-reviewer #3:

Comment: The authors have addressed my concerns and questions. I have no further comments.

Answer: Thanks for your comments.

We hope you find our response and revisions satisfactory. Thank you for your time and assistance.

Sincerely,
William Reiche