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Non-Invasive Assessment of Acute Graft vs. Host Disease of the Gastrointestinal Tract Following Allogeneic Haemopoietic Stem Cell Transplantation Using ^{18}F-FDG PET

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Short Running Title: FDG PET Graft vs. Host Disease GI Tract

ABSTRACT

Background

Acute graft versus host disease of the gastrointestinal tract (Acute GIT-GVHD) often complicates allogeneic haemopoietic stem cell transplantation (AHSCT). ^{18}F-FDG PET/CT (PET) is known to detect active inflammation and may be a useful non-invasive test for Acute GIT-GVHD.

Objective

To evaluate the diagnostic utility of PET to non-invasively assess patients with clinically suspected Acute GIT-GVHD.

Study Design

51 AHSCT patients with clinically suspected Acute GIT-GVHD prospectively underwent PET scanning followed by upper and lower GIT endoscopy within 7 days. Endoscopic biopsies of 4 upper GIT and 4 colonic segments were obtained for histology to compare with corresponding quantitative segmental PET SUVmax values. Receiver operator characteristic curve (ROC) analysis was performed to determine predictive capacity of PET SUVmax for Acute GIT-GVHD. A separate qualitative visual PET analysis was also performed for comparison.

Results

23/51 (45.1%) patients had biopsy confirmed Acute GIT-GVHD, with 19/23 (82.6%) having upper GIT and 22/22 (100%) colonic involvement. 1/23 did not have colonoscopy. GVHD involved the entire colon contiguously in 21/22 patients. For quantitative analysis, Histology from 4 upper GIT and 4 colonic segments were compared with PET SUVmax values. Colonic segments positive for GVHD had higher SUVmax, 4.1 [3.6-4.5] compared to normal colonic segments 2.3 [1.9-2.7], p=0.006. No difference was demonstrated in upper GIT segments. Quantitative PET yielded 69% Sensitivity, 57% Specificity, 73% Negative and 59% Positive predictive value for detection of GVHD compared to 70%, 76%, 76% and 68% respectively for qualitative analysis

Conclusion

^{18}F-FDG PET is a useful non-invasive diagnostic test for Acute GIT-GVHD which when present always involves the colon and usually in its entirety. This suggests colonic biopsy obtained by sigmoidoscopy is adequate for histological confirmation when Acute GIT-GVHD is suspected. Of note, ^{18}F-FDG PET cannot distinguish Acute GIT-GVHD from non-GVHD inflammatory changes in the colon.

Keywords: ^{18}F-FDG PET/CT, Bone Marrow Transplantation, Graft vs. Host Disease, Gastrointestinal Tract.

INTRODUCTION

Allogeneic haemopoietic stem cell transplantation (AHSCT) offers cure for various life-threatening haematological malignancies and disorders. The number of transplants performed each year continues to increase(*1*). Acute graft-versus-host disease (AGVHD) is a recognized complication occurring in 30-50% of AHSCT recipients(*2*). It carries significant morbidity and a 25% mortality rate within 100 days of AHSCT(*3*). Although AGVHD may affect any organ system, there is a strong predilection for involvement of the skin, gastrointestinal tract (GIT) and liver (*4*).

Acute GIT-GVHD is commonly suspected on the basis of diarrhoea post AHSCT. However, the possible differentials are wide and include GVHD, infectious causes such as Clostridioides difficile and Cytomegalovirus colitis, drug effects and chemoradiation toxicity. The current gold standard for Acute GIT-GVHD diagnosis is histology acquired via endoscopic biopsy, characterized by crypt cell apoptosis and crypt loss (*5*). However, endoscopy is an invasive procedure and not without risk. Anaesthetic risk, bleeding and perforation are all potential complications associated with endoscopy, particularly in post AHSCT patients that are unwell and often thrombocytopenic (*6*).

Currently, there is no established role for conventional imaging in the diagnosis of Acute GIT-GVHD (*7*). It has been observed that ^{18}F-FDG PET/CT is a sensitive and specific biomarker of acute large and small bowel inflammation in Inflammatory Bowel Disease (*8*). Furthermore, two pilot studies have reported that PET has a negative predictive value (81-96%) in the assessment of Acute GIT-GVHD (*9,10*), but data remains sparse in this area.

This prospective study aims to evaluate the diagnostic utility of ^{18}F-FDG PET for Acute GIT-GVHD and to determine its role as a non-invasive test for this condition.

PATIENTS AND METHODS

Study Design and Patient Selection

This study was conducted at the Alfred Hospital Melbourne, Australia. Written signed informed consent was obtained from each participant in accordance with the Declaration of Helsinki and approval from the Alfred Hospital research ethics committee. From December 2009 and November 2014, 51 adult patients with clinically suspected Acute GIT-GVHD within 180 days of AHSCT who had not commenced any treatment for GVHD including steroids were prospectively enrolled into a non-interventional study comparing PET, endoscopy and histology.

Clinically suspected Acute GIT-GVHD symptoms included persistent diarrhoea, abdominal pain, anorexia, nausea, vomiting or any combination of the above symptoms within 180 days of AHSCT with no other apparent cause.

There were no restrictions to entry into study relating to underlying haematological disorder, stem cell source or conditioning regimen.

The stem cell source was peripheral blood (PBSC) in 46 cases (90%) and double umbilical cord blood (DUCB) in the remaining 5 cases (10%). Of the 46 PBSC donors, 2 (4%) were Human Leukocyte Antigen (HLA)-identical sibling donors, a further 13 (28%) were HLA-matched related donors, 29 (63%) were HLA-matched unrelated donors and 2 (4%) were mismatched unrelated donors. The 5 double umbilical cord blood donations showed variable levels of HLA matching.

24 patients (47%) received a standard myeloablative conditioning regimen (total body irradiation based), while 13 patients (25%) received a reduced-intensity conditioning and 14 received non-myeloablative conditioning (27%). 16 patients received equine anti-thymocyte globulin as part of the conditioning regimen. For GVHD prophylaxis, patients who underwent a myeloablative

conditioning received cyclosporin, usually with short-course methotrexate. Patients in the reduced-intensity conditioning or non-myeloablative groups received cyclosporin and mycophenolate mofetil or cyclosporin alone.

Patient and AHSCT characteristics are summarized in Table 1.

^{18}F-FDG PET/CT Evaluation

All participants with clinically suspected Acute GIT-GVHD symptoms underwent a PET scan. Participants were asked to fast and refrain from vigorous activity for at least 6 hours prior to imaging. Administered ^{18}F-FDG activity was 3 MBq/kg to a maximum of 400 MBq. Molecular imaging was performed on a Philips Gemini PET/CT scanner (Philips Medical Systems, Cleveland, OH, USA) with scan range extending from the skull base to the proximal femora, 60-80 minutes after IV injection of 3 MBq/kg of ^{18}F-FDG. Low dose co-registered CT was used for anatomical localization and attenuation correction.

All images were interpreted independently by nuclear medicine specialists experienced in PET (RK, MC and TB) blinded to all investigation results including Endoscopy. Results of the PET scan were not made available to the patient's treating clinicians and did not influence subsequent clinical management of the patient.

Quantitative PET/CT Analysis

For quantitative PET analysis, the gastrointestinal tract (GIT) was divided into 8 segments: 4 upper GIT segments (oesophagus, stomach, duodenum and terminal ileum) and 4 lower GIT segments (ascending colon, transverse colon, descending colon and sigmoid/rectum) using the accompanying low dose CT for anatomical localization.

The highest intensity region within each of the 8 GIT segments was ascertained visually by two readers (RK & MC) and the maximum standardized uptake value (SUVmax) of this region measured and recorded independently with a standardized 2D planar region of interest (ROI) in the sagittal plane for the oesophagus, transaxial plane for the stomach, duodenum, terminal ileum, sigmoid/rectum and coronal plane for the ascending, transverse and descending colon. The size of the 2D planar region of interest used varied according to the GIT segment evaluated to ensure there was no overlap of other organs and only the SUVmax in the target GIT segment was measured. A 15mm 2D circular ROI was placed in the central lumen of the ascending aorta and mean standardized uptake value (SUVmean) recorded to establish background mediastinal blood pool ^{18}F-FDG uptake as a reference. The average SUVmax between both expert readers for each GIT segment was used for comparison with histology findings.

In order to evaluate overall ^{18}F-FDG activity in the entire colon, the parameters Min L4, Max L4 and Sum L4 were used. Min L4 and Max L4 described the lowest and highest SUVmax out of the Ascending, Transverse, Descending and Sigmoid/Rectal colon segments respectively. Sum L4 described the combined SUVmax of Ascending, Transverse, Descending and Sigmoid/Rectal colon segments.

Qualitative PET/CT Analysis

For qualitative PET analysis, the scan was considered positive for Acute GIT-GVHD if there was visually increased FDG uptake greater than 1.5x background liver uptake involving at least 50% of one or more upper GIT or colonic segments. In the event of disagreement between both expert readers (RK & MC), a third blinded expert reader (TB) was utilized to determine the final PET result.

PET findings were compared with histology, with patients considered positive for Acute GIT-GVHD if they had histological evidence of GVHD in at least one upper GIT or colonic segment.

Diagnosis of Acute Gastrointestinal GVHD

Gastroscopy and Colonoscopy were aimed to be performed within 7 days of the PET examination, utilizing a segmental unblinding method outlined as follows. Endoscopists were initially blinded to the results of the PET and were asked to macroscopically assess 4 upper GIT segments (oesophagus, stomach, duodenum and terminal ileum) and 4 colonic segments (ascending colon, transverse colon, descending colon and sigmoid/rectum) for active inflammation.

The results of the PET were then revealed to the endoscopists during endoscopy. Two biopsies were taken of each segment that appeared normal on both PET and macroscopically on endoscopy. Four biopsies were taken of each segment that was abnormal on either PET, macroscopic assessment or both. Hence, a total of 16-32 upper to lower GIT biopsies were obtained in each participant undergoing both Gastroscopy and Colonoscopy. All segments able to be endoscopically visualized were biopsied.

Histology

Each segmental GIT biopsy was deemed positive or negative for acute GVHD by a pathologist experienced in GVHD interpretation that was blinded to both the PET and endoscopy macroscopic findings. Bacterial, viral (including Cytomegalovirus), parasitic culture and Clostridioides Difficile Toxin and culture testing was also performed and documented to confirm or exclude other potential causes of non-GVHD inflammation.

Statistical Methods

All data was assessed for normality. Group comparisons of individual location data were performed using student t-tests and reported as means (standard deviation) while comparison of repeated measures data was performed using repeated measures analysis of variance with results reported as mean [95%CI]. To further explore the predictive capacity of colonic locations, summary

statistics (minimum, maximum, total) were calculated. For quantitative assessment of the relationship between Acute GIT-GVHD and SUVmax, sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) were determined from ROC curves derived from logistic regression. Qualitative assessment was determined by consensus expert visual interpretation of scans. Statistical analysis was performed using SAS version 9.4 (SAS Institute, Cary, NC, USA) and a two-sided p-value of 0.05 was used to indicate statistical significance.

RESULTS

Patients

51 patients were enrolled and underwent PET with a median of 47 days (range 12-166) post AHSCT. Median time from onset of clinical symptoms suspicious for Acute GIT-GVHD to PET examination was 6 days (range: 0-69). Four patients had diabetes and four had a history of steroid induced hyperglycaemia, however none were taking Metformin. 22 patients had clinical evidence of cutaneous GVHD, whilst 5 patients had elevated bilirubin suggestive of grade I-II hepatic GVHD (only 1 proven case of hepatic GVHD).

Endoscopic Findings

Participants underwent endoscopy within an average of 3 days (range: 0-13 days) of PET scanning. 2 patients were outside the target 7-day period post PET scan; one patient at 8 days was delayed due to severe illness and the other required urgent treatment for pericardial effusion receiving endoscopy 13 days post PET.

8/51 (16%) of patients did not have all 8 GIT segments biopsied due to logistical reasons or being too acutely unwell. Of these, 1 patient had a rectal biopsy only, 1 patient did not have gastroscopy, 4

patients did not have colonoscopy and 2 patients had no biopsy of the terminal ileum. Details of Endoscopic Pathology data in pre-specified GIT segments are presented in Supplementary Table 1.

Per Patient Histology Findings and Treatment

23/51 (45.1%) patients had biopsy confirmed Acute-GIT GVHD. 19/23 (83%) had upper GIT and 22/23 (96%) colonic involvement. 1/23 GVHD positive patient did not have colonic biopsies. 21/22 (95%) of patients with colonic GVHD had contiguous involvement of the entire colon. 14/51 (27%) patients had non-GVHD inflammation (6 Cytomegalovirus infection, 3 Clostridioides difficile infection, 5 non-specific oesophagitis and gastritis). 14/51 (27%) patients had normal upper GIT and colonic segments.

21/23 (91%) patients with histologically proven Acute GIT-GVHD required steroid treatment for clinical symptoms, 13/21 Intravenous Methylprednisolone and 8/21 oral Budesonide or Prednisolone.

Per GIT Segment Histology Findings

376 /408 (92%) intended GIT segments (191 upper GIT / 185 colonic) were biopsied in 51 patients. 131/376 (35%) were positive for GVHD (52 upper GIT / 79 colonic), 42/376 (11%) were positive for non-GVHD Inflammation (25 upper GIT/ 17 colonic), 199/376 (53%) (113 Upper GIT / 86 colonic) were normal and 4/376 (1.0 %) were equivocal for GVHD (1 upper GIT/3 colonic).

Relationship of PET SUVmax with Histology

No difference in SUVmax was demonstrated in normal Upper GIT segments 2.38 [2.24-2.52] or those with GVHD, 2.57 [2.36-2.77] or non-GVHD inflammation, 2.63 [2.34-2.91] (Figure 1A).

SUVmax was significantly increased in both colonic segments with GVHD, 4.06 [3.64-4.47] and Non-GVHD inflammation, 5.03 [4.13-5.93] compared to normal colonic segments, 2.29 [1.89-2.69] (Figure 1B.)

PET and Histology Images of Patient 49 are provided as an example of a positive case of Acute GVHD involving both the upper GIT and colon on histology with ^{18}F-FDG uptake only visibly increased in the colon on PET (Figure 2).

GIT segment histology and corresponding SUVmax values for all 51 patients are provided in Supplementary Table 2.

Quantitative PET SUVmax Analysis

Upper GIT and colonic segment PET SUVmax were compared between the 23 patients positive and 28 patients negative for Acute GIT-GVHD (Table 2). Patients positive for GVHD had significantly higher SUVmax in all colonic segments other than Ascending colon when compared with negative patients. The minimum SUVmax value in any of the 4 colonic segments (Min L4) was significantly higher in GVHD positive patients compared to GVHD negative patients. Similarly, the total SUVmax of all 4 colonic segments (Sum L4) was also significantly higher in GVHD positive patients compared to GVHD negative patients. No difference in SUVmax was demonstrated in any of the 4 upper GIT segments between GVHD positive and negative patients.

Area under receiver characteristic curve (AUROC) analysis demonstrated PET SUVmax of all colonic segments other than Ascending colon were independently predictive of Acute GIT-GVHD (Table 3). The Min L4 ROC curve was chosen to generate sensitivity, specificity, NPV and PPV values for GVHD as it had the highest AUROC and took into account all colonic segments.

From the Min L4 ROC curve, at Min L4 of 1.73 (uptake greater than mean background mediastinal blood pool activity) this resulted in Sensitivity 69%, Specificity 57%, NPV 73% and PPV 59% for detection of Acute GIT-GVHD. (Figure 3.)

Qualitative PET Analysis

PET scans of all 51 patients were qualitatively visually assessed for Acute GIT-GVHD in the upper GIT and colon. Both expert readers MC and RK were concordant in their appraisal of the presence or absence of GVHD on PET in 46/51 (90%) of cases. 5 cases required a third expert reader (TB) for final consensus determination of PET status. Qualitative visual assessment resulted in PET Sensitivity of 70%, Specificity 76%, NPV 76% and PPV 68% for the detection of Acute GIT-GVHD.

Of the 22 positive Acute GIT-GVHD patients who had colonic biopsies, 16/22 (73%) had at least 1 colonic segment, 15/22 (68%) at least 2 colonic segments, 13/22 (59%) at least 3 colonic segments and 9/22 (41%) all 4 colonic segments considered PET positive on qualitative visual assessment.

DISCUSSION

The main aim of our study was to determine the diagnostic utility of PET as a non-invasive test for Acute GIT-GVHD in patients with suggestive clinical symptoms post AHSCT. The few published studies(*9,10*) in this field have relied predominantly on qualitative assessment of PET for detection of Acute GIT-GVHD by consensus expert visual assessment which may be difficult to reliably reproduce across institutions.

In addition to qualitative visual PET assessment, we evaluated PET quantitatively using SUVmax. SUVmax is a widely accepted and validated parameter used both clinically and for research purposes to quantify and convey the degree/intensity of radiotracer uptake on PET scan(*11*). The higher the SUVmax value, the higher the degree of radiotracer uptake (inflammatory activity in this clinical scenario) on PET scan.

As SUVmax values are objective and generally reproducible across PET cameras and institutions it allows objective criteria and definitive thresholds to be defined when determining whether a PET scan

is considered positive or negative for Acute GIT-GVHD(*12*). This could provide a robust standardized technique for PET evaluation of Acute GIT-GHVD that is widely applicable across all institutions with PET.

One of the strengths of our study is the rigorous nature of data collection which included obtaining 376 biopsies out of a possible 408 upper GIT and colonic segments (92%) in 51 patients for direct correlation with PET scan findings which provided an extremely robust dataset which is highly novel and difficult to obtain in this patient population. 23/51 (45.1%) of patients in our cohort had biopsy confirmed Acute GIT-GVHD confirming the reasonably high prevalence of this condition when clinically suspected.

The involvement of the colon in all GVHD positive patients and in its entirety in 96% of positive patients is a significant finding as it suggests when Acute GIT-GVHD is suspected, sigmoidoscopy alone, a less invasive and resource consuming procedure may suffice for histological confirmation. Eliminating Gastroscopy +/- Colonoscopy as part of work up for Acute GIT-GVHD(*13*) would markedly reduce the number of endoscopic procedures and the associated risk of up to 1.8% mortality and morbidity in this vulnerable patient cohort(*6*).

We demonstrated quantitative PET assessment using SUVmax is only useful for assessing the presence of GVHD in the colon as no difference in SUVmax was demonstrated between GVHD positive and normal segments in the upper GIT. Stelljes et al. also reported similar findings and postulated higher lipopolysaccharide and microbial pro-inflammatory stimuli in the colon compared to the upper GIT(*9*) may account for this. Interestingly, Stelljes et al. found FDG uptake was invariably increased in the Ascending colon in patients positive for Acute GIT-GVHD. On the contrary, we found the Ascending colon was the only colonic segment not predictive for Acute GIT-GVHD on PET.

We demonstrated GVHD and non-GVHD causes of GIT inflammation in the colon are indistinguishable from each other and have similarly increased SUVmax. As such, further investigations including biopsy are required to determine the cause of inflammation when suggested on PET.

Our study yielded quantitative and qualitative PET Sensitivity of 69% and 70%, Specificity 57% and 76%, NPV 73% and 76% and PPV 59% and 68% respectively for detection of Acute GIT-GVHD. This suggests quantitative analysis using SUVmax is no better than qualitative visual analysis and qualitative analysis alone is sufficient.

Studies by Stelljes et al.(*9*) and Bodet-Milin et al.(*10*) which both only used qualitative visual PET assessment reported more favourable sensitivity of 82% and 81%, Specificity of 100% and 90% and NPV 81% and 96% respectively. They both provided limited details on how their images were standardized for review and did not have GIT segment histology datasets as comprehensive as our study. Interestingly, Stelljes et al. did provide quantitative SUVmax data as a figure which showed strikingly similar findings to our Figures 1A and 1B.

Non-invasive clinical algorithms based on patient symptoms, conventional imaging and serum biomarkers are not well established for Acute GIT-GVHD hence the low threshold for clinicians to proceed to more invasive procedures such as endoscopy(*14,15*).

The negative predictive value of 73% (quantitative) and 76% (qualitative) for PET detection of Acute GIT-GVHD in our study is reasonable and adds to the literature increasingly supporting the use of PET as a non-invasive diagnostic test for Acute GIT-GVHD. Our findings suggest PET fills a clinical need where endoscopy may not be readily accessible, the patient too unwell or risks of endoscopy too great.

A major factor which likely limits the sensitivity and specificity of ^{18}F-FDG PET for Acute GIT-GVHD is the marked variability in physiological ^{18}F-FDG uptake that can be seen in the gastro-intestinal

tract. It is not unusual to see very intense physiological ^{18}F-FDG uptake in the gastrointestinal tract which may relate to underlying peristaltic smooth muscle activity at the time of imaging.

The use of anti-spasmodic agents such as N-butylscopoolamine prior to scanning may decrease physiological gastrointestinal ^{18}F-FDG uptake and may improve the performance of PET in this cohort of patients(*16*). Metformin is also well known to significantly increase physiological gastrointestinal ^{18}F-FDG uptake and should be withheld for at least 48 hours when assessing the gastrointestinal tract on PET(*17*). Importantly, no patients in our study were taking Metformin prior to their PET scan.

Combining PET with other non-invasive markers such as serum inflammatory cytokines IL-17, IFN Gamma, Tumour Necrosis Factor and Granulocyte Macrophage colony stimulating factor which are known to be elevated in Acute GIT-GVHD (*18,19*) may also be an option to further improve non-invasive diagnostic test performance.

Novel PET radiotracers targeting cellular apoptosis(*20,21*), the histological hallmark of Acute GIT-GVHD may provide significantly improved sensitivity and specificity for detection of GVHD compared to ^{18}F-FDG PET and should be explored further.

Limitations of our study include no formal grading of severity of Acute GIT-GVHD on histology however given 21/23 (91%) of patients required steroid therapy (13 intravenous, 8 oral) suggests the majority of Acute GIT-GVHD cases were at least moderately severe.

CONCLUSION

^{18}F-FDG PET is a useful non-invasive diagnostic test for Acute GIT-GVHD particularly in the colon. Acute GIT-GVHD when present always involves the colon and usually in its entirety. This suggests only colonic biopsy is required for histological confirmation when Acute GIT-GVHD is suspected. Of note, ^{18}F-FDG PET cannot distinguish Acute GIT-GVHD from non-GVHD inflammatory changes in the colon.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

MH-C: designed the study, supervised research, collected, assembled and analyzed data and wrote the manuscript; RK, TB, KY, VK: analyzed data and helped write manuscript; SP: designed the study, recruited patients, helped write manuscript; SR, WK, AP: collected data and commented on the manuscript; SA: recruited patients and commented on manuscript; MB: performed statistical analysis and helped write manuscript.

KEY POINTS

QUESTION: How useful is ^{18}F-FDG PET/CT PET for non-invasive assessment of patients with clinically suspected Acute gastrointestinal tract Graft vs. host disease (acute GIT-GVHD) following allogeneic haemopoietic stem cell transplantation?

PERTINENT FINDINGS: In a prospective study evaluating ^{18}F-FDG PET/CT in 51 patients with clinically suspected acute GIT-GVHD with upper and lower gastrointestinal histology obtained from endoscopy, ^{18}F-FDG PET/CT was found to be a useful non-invasive test with sensitivity and specificity of 70% and 76% respectively for acute GIT-GVHD. The colon appears to always be involved in patients with acute GIT-GVHD and is the location of greatest increase in SUVmax on ^{18}F-FDG PET/CT.

IMPLICATIONS FOR PATIENT CARE: ^{18}F-FDG PET/CT is a useful adjunctive non-invasive diagnostic test for Acute GIT-GVHD when clinically suspected.

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TABLE 1. Patient Characteristics

Characteristics	Study Population n=51 (%)
Patient age, median (range)	46.5 [19.6-66.8]
Patient Gender	
Male	28 (54)
Female	23 (46)
CMV Status	
Seronegative donor-recipient pair	12 (24)
Underlying Diagnosis	
AML	21 (41)
ALL	9 (18)
MM	11 (22)
MDS	3 (6)
NHL	4 (8)
HL	1 (2)
Other (Adrenoleucodystrophy & BPD)	2 (4)
Stem Cell Source	
PBSC	46 (90)
DUCB	5 (10)
HLA Matching	
MRD	15 (29)
MUD	29 (57)
MISUD	7 (14)
Conditioning Regimen	
MAC	24 (47)
RIC	13 (25)
NMAC	14 (27)
ATG	
Yes	16 (31)
No	35 (69)
GVHD Prophylaxis	
CsA	10 (20)
CsA + MMF	22 (43)
CsA + MTX	19 (37)

Legend: ALL=Acute Lymphoblastic Leukaemia AML=Acute Myeloid Leukaemia, ATG=Anti-thymocyte globulin, BPD= Blastic plasmacytoid dendritic cell neoplasm, CsA= Cyclosporin A, DUBC=Double unit cord blood, HL=Hodgkin' Lymphoma, MAC=Myeloablative conditioning, MDS= Myelodysplastic Syndrome, MISUD=Mismatched Unrelated donor MM=Multiple Myeloma, MMF= mycophenolate mofetil, MRD=Matched related donor MTX=methotrexate, MUD=Matched unrelated donor, NHL=Non-Hodgkin' Lymphoma, NMAC=Non Myeloablative conditioning, PBSC=peripheral blood stem cell, RIC=Reduced intensity conditioning.

TABLE 2. Upper GIT and Colonic segment SUVmax - Positive vs. Negative GVHD patients

Variable	GVHD Positive n=23	GVHD Negative n=28	P value
Min L4	**2.96 (1.65)**	**1.88 (1.41)**	**0.02**
Transverse Colon SUVmax	**3.72 (2.25)**	**2.34 (1.95)**	**0.02**
Desc Colon SUVmax	**3.55 (2.16)**	**2.15 (1.93)**	**0.02**
Sigmoid/Rectum SUVmax	**3.81 (1.96)**	**2.77 (1.25)**	**0.03**
Sum L4	**15.00 (7.75)**	**10.60 (7.31)**	**0.04**
Terminal ileum SUVmax	2.51 (0.92)	2.12 (0.58)	0.07
Oesophagus SUVmax	2.22 (0.68)	2.52 (0.62)	0.11
Ascending Colon SUVmax	3.91 (1.89)	3.31 (2.48)	0.35
Max L4	4.50 (2.23)	3.61 (2.33)	0.18
Duodenum SUVmax	2.35 (0.62)	2.12 (0.49)	0.15
Stomach SUVmax	2.95 (0.69)	2.89 (0.76)	0.78

Legend: Min L4 / Max L4 = Lowest / Highest SUVmax out of Ascending, Transverse, Descending and Sigmoid/Rectal Colon. Sum L4= Combined SUVmax of Ascending, Transverse, Descending and Sigmoid/Rectal Colon
Variables with p value < 0.05 highlighted in **bold font.**

TABLE 3. AUROC Analysis – SUVmax as a predictor for GVHD

Variable	n	max	min	AUROC	P value
Min L4	**51**	**7.9**	**0.8**	**0.73**	**0.03**
Descending Colon SUVmax	**51**	**10.3**	**0.8**	**0.72**	**0.03**
Transverse Colon SUVmax	**51**	**10.7**	**1.0**	**0.73**	**0.04**
Sigmoid / Rectum SUVmax	**51**	**8.4**	**1.0**	**0.71**	**0.04**
Sum L4	51	40.2	5.2	0.69	0.06
Terminal Ileum SUVmax	51	1.2	4.7	0.62	0.08
Oesophagus SUVmax	51	4.0	1.1	0.64	0.12
Duodenum SUVmax	51	4.3	1.1	0.60	0.16
Max L4	51	11.3	1.7	0.63	0.18
Ascending Colon SUVmax	51	11.3	1.1	0.63	0.35
Stomach SUVmax	51	5.1	1.5	0.57	0.78

Legend: Min L4 / Max L4 = Lowest / Highest SUVmax out of Ascending, Transverse, Descending and Sigmoid/Rectal Colon. Sum L4= Combined SUVmax of Ascending, Transverse, Descending and Sigmoid/Rectal Colon.
Variables with p value < 0.05 highlighted in **bold font**

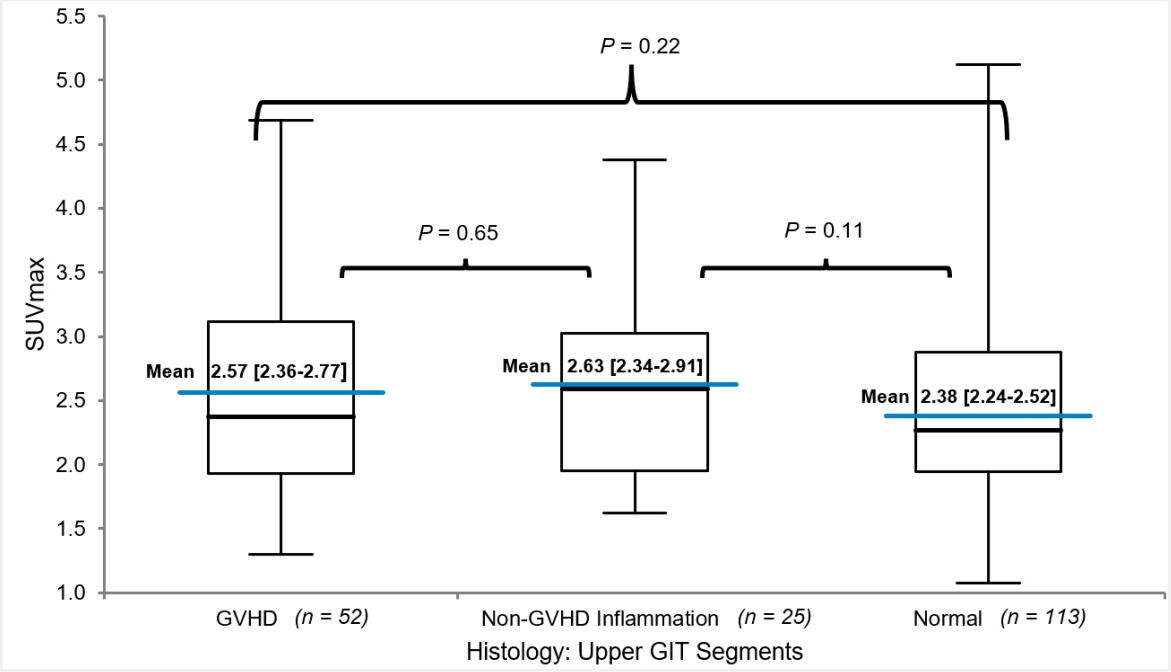


Figure 1A. Upper GIT Segments

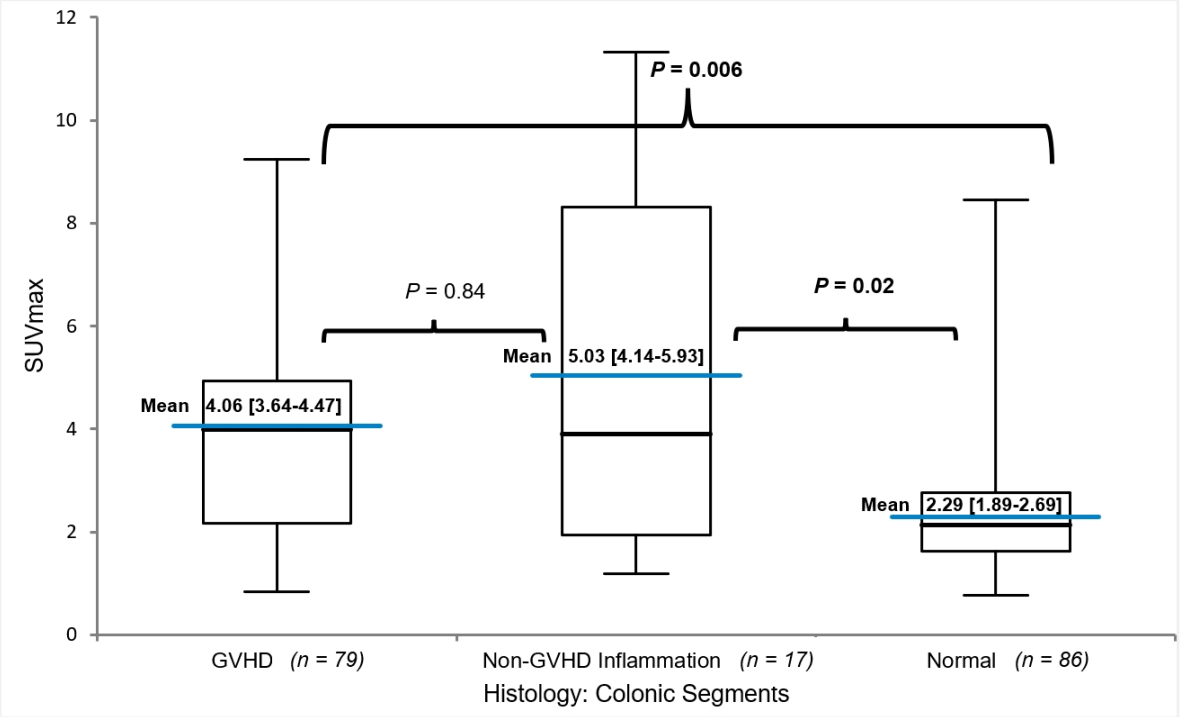


Figure 1B. Colonic Segments

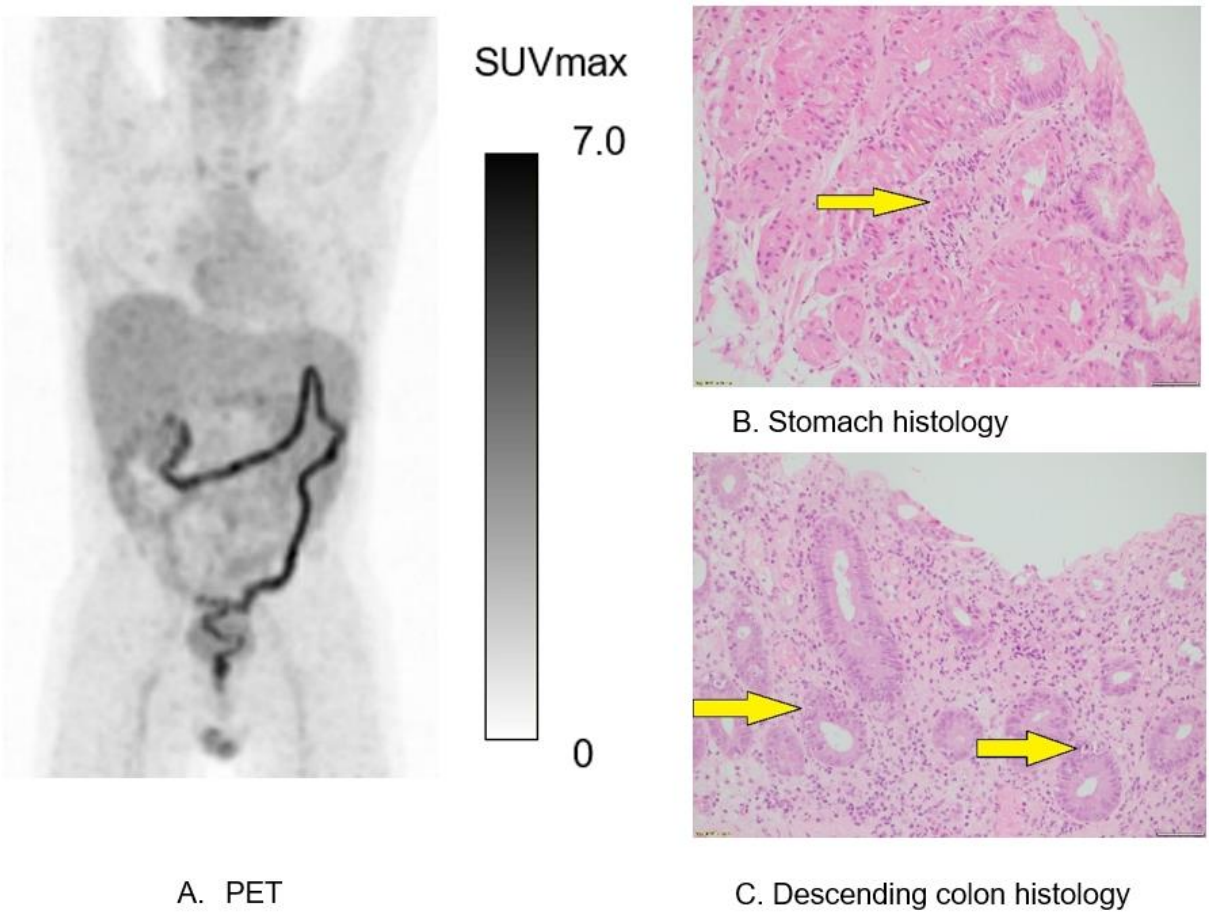


Figure 2. Patient 49. A.^{18}F-FDG uptake only increased in colon and not the upper gastrointestinal tract. B. Lymphocytic infiltration and necrosis (Arrow) of stomach crypt epithelium in keeping with Acute GIT-GVHD. C. Extensive colonic crypt destruction with frequent apoptotic bodies (Arrows), the histological hallmark of Acute GIT-GVHD.

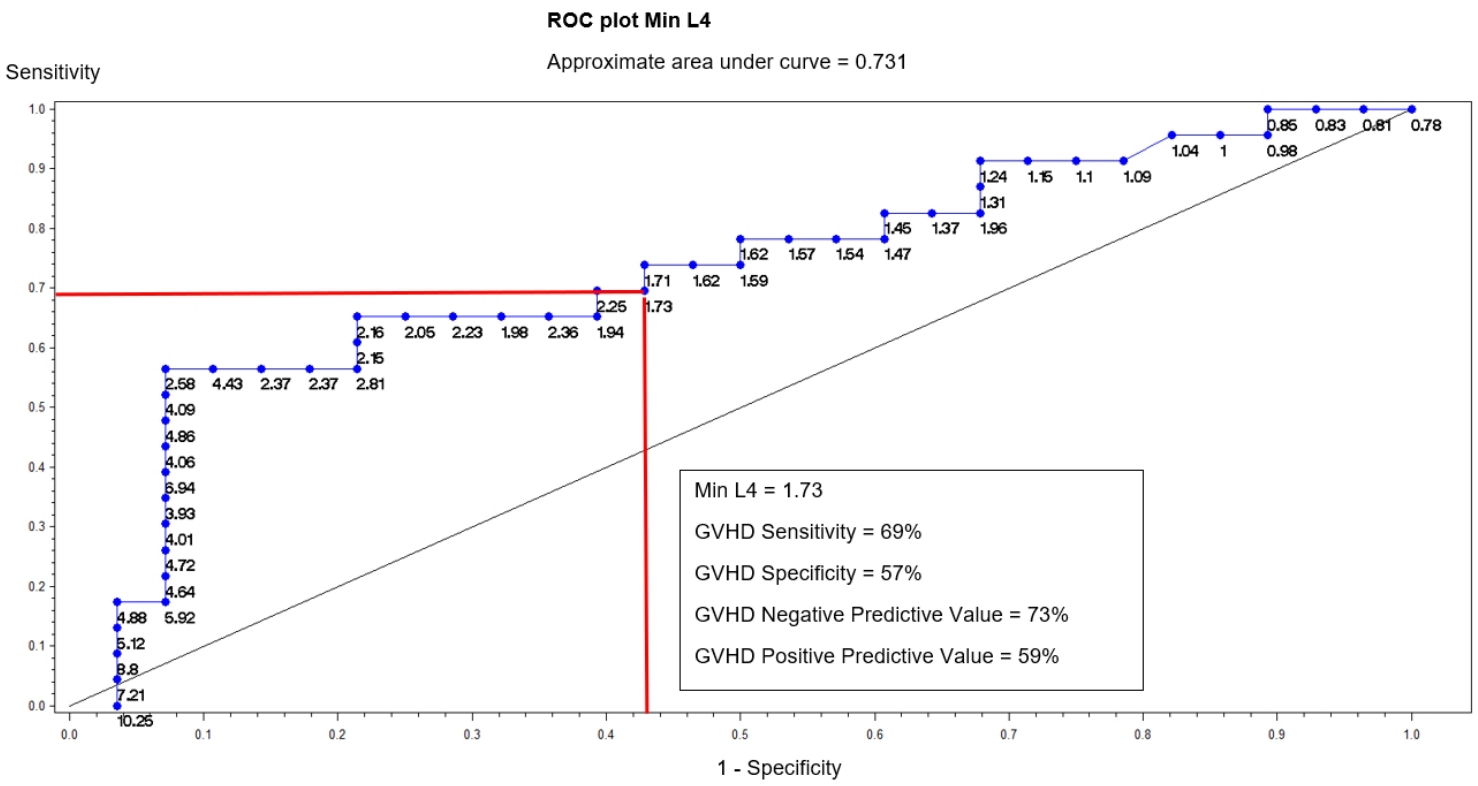
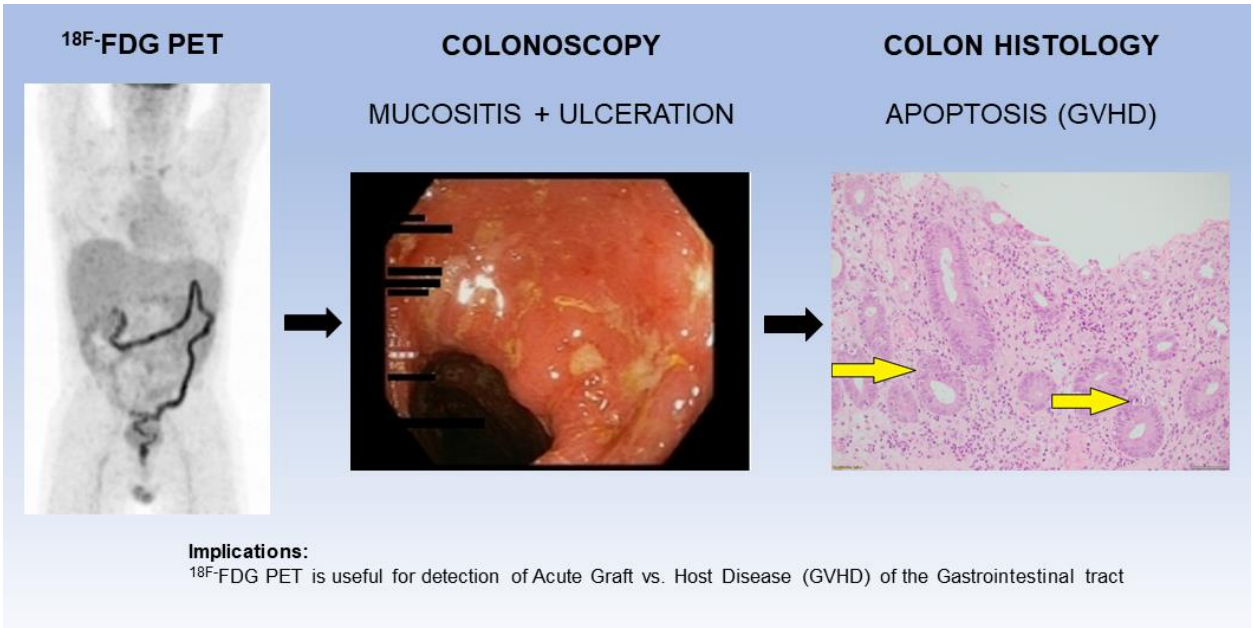


Figure 3. Min L4 Receiver Operator Characteristic Curve (ROC)

Graphical Abstract



Supplementary Table 1. Endoscopic Biopsy Histology

Pt No.	Biopsy post PET (Days)	Oesoph	Stomach	Duoden	Term Ileum	Asc Colon	Trvse Colon	Desc Colon	Sig/Rect	GVHD
1	1	-	I	-	-	-	-	-	-	N
2	1	+	+	+	+	+	+	+	+	Y
3	0	I	E	-	-	I	I	I	I	N
4	5	-	I	-	-	-	-	-	-	N
5	4	-	I	I	NB	+	+	+	+	Y
6	3	-	I	-	NB	NB	NB	NB	NB	N
7	1	-	-	-	-	-	-	-	-	N
8	2	-	-	-	I	I	I	I	I	N
9	4	I	-	-	-	-	-	-	-	N
10	3	-	I	-	-	-	-	-	-	N
11	0	-	-	+	+	+	+	+	+	Y
12	3	-	-	-	-	-	-	-	-	N
13	6	-	-	-	-	-	-	-	-	N
14	6	-	-	-	-	-	-	-	-	N
15	3	-	-	-	-	-	-	-	-	N
16	5	+	+	+	+	+	+	+	+	Y
17	5	-	I	I	-	I	I	I	I	N
18	4	-	-	-	-	-	-	-	-	N
19	0	-	-	+	+	+	+	+	+	Y
20	2	-	-	I	+	+	I	I	I	Y
21	8	-	-	-	-	I	-	-	-	N
22	1	-	-	-	-	-	-	-	-	N
23	1	-	-	-	-	-	-	-	-	N
24	1	+	+	+	+	+	+	+	+	Y
25	2	-	-	-	+	+	+	+	+	Y
26	0	-	-	+	+	+	+	+	+	Y
27	3	-	-	-	NB	NB	NB	NB	NB	N
28	5	I	I	-	NB	NB	NB	NB	NB	N
29	1	-	-	+	+	+	+	+	+	Y
30	3	I	-	+	NB	NB	NB	NB	NB	Y
31	1	I	-	-	-	-	-	-	-	N
32	0	NB	NB	NB	NB	NB	NB	NB	+	Y
33	0	-	-	-	-	-	-	-	-	N
34	1	NB	NB	NB	-	-	-	-	-	N
35	2	-	-	-	-	-	-	-	-	N
36	3	-	+	I	+	+	+	+	+	Y
37	2	-	-	-	-	-	-	-	-	N
38	0	-	I	+	+	+	+	+	+	Y
39	2	+	+	+	+	+	+	+	+	Y
40	2	I	I	+	+	+	+	+	+	Y
41	6	-	+	+	+	+	+	+	+	Y
42	7	-	I	I	I	-	-	-	-	N
43	5	-	-	+	+	+	+	+	+	Y
44	1	-	-	-	-	-	-	-	-	N
45	5	-	-	+	+	-	-	-	+	Y
46	5	-	+	+	+	+	+	+	+	Y
47	1	-	+	+	+	+	+	+	+	Y
48	4	I	I	I	-	E	E	-	E	N
49	13	-	+	+	+	+	+	+	+	Y
50	2	+	+	+	NB	+	+	+	+	Y
51	7	-	I	-	-	-	-	-	-	N
										N=23

Legend (+) GVHD positive, (-) GVHD Negative, (I) Non-GVHD Inflammation, (E) Equivocal (NB) Not Biopsied

Supplementary Table 2. GIT Segment PET SUVmax + Histology

Pt	SUV Mean BP	Oes SUV Max	Oeso Histo	Stom SUV Max	Stom Histo	Duod SUV Max	Duod Histo	Terminal ileum SUVMax	Terminal Ileum Histo	Asc Col SUVMax	Asc Col Histo	Tvse Col SUVMax	Tvse Col Histo	Desc Col SUVMax	Desc Col Histo	Sig/Rect SUVMax	Sig/Rect Histo
1	1.80	1.95	-	2.78	I	2.07	-	2.35	-	5.09	-	2.30	-	1.62	-	2.17	-
2	1.76	3.36	+	4.13	+	2.94	+	2.00	+	3.99	+	3.29	+	2.15	+	4.93	+
3	1.32	2.50	I	2.59	E	2.38	-	1.37	-	3.91	I	2.04	I	1.73	I	2.88	I
4	1.01	3.12	-	1.85	I	1.62	-	1.82	-	8.45	-	4.39	-	4.43	-	2.37	-
5	1.64	1.66	-	2.62	I	2.84	I	1.99	NB	2.56	+	2.12	+	2.16	+	2.26	+
6	1.39	1.67	-	2.59	I	1.75	-	1.77	NB	2.23	NB	1.62	NB	1.57	NB	1.79	NB
7	1.35	2.33	-	2.22	-	2.26	-	2.29	-	2.13	-	1.02	-	1.00	-	1.20	-
8	2.01	2.31	-	3.05	-	2.12	-	2.56	I	11.33	I	10.71	I	10.25	I	7.92	I
9	1.80	2.78	I	2.93	-	2.10	-	2.20	-	3.79	-	1.79	-	1.10	-	2.61	-
10	2.39	4.04	-	4.38	I	2.89	-	3.22	-	3.07	-	2.61	-	2.37	-	3.70	-
11	1.27	1.85	-	1.97	-	1.87	+	2.50	+	4.79	+	3.64	+	3.93	+	3.74	+
12	1.48	3.01	-	3.14	-	1.90	-	2.42	-	2.77	-	2.14	-	1.98	-	2.79	-
13	1.95	2.66	-	3.73	-	2.18	-	2.18	-	3.28	-	2.36	-	2.37	-	3.58	-
14	1.81	3.35	-	2.80	-	2.05	-	1.72	-	2.55	-	1.96	-	2.36	-	3.09	-
15	2.13	2.07	-	3.51	-	2.33	-	2.89	-	1.79	-	1.62	-	1.59	-	2.17	-
16	1.24	1.56	+	2.36	+	1.52	+	2.05	+	1.34	+	1.87	+	1.23	+	1.04	+
17	1.59	1.94	-	3.73	I	3.51	I	3.08	-	9.12	I	6.30	I	5.92	I	4.69	I
18	1.50	2.66	-	3.13	-	1.54	-	1.94	-	2.01	-	1.33	-	1.96	-	2.77	-
19	1.49	2.10	-	1.98	-	1.75	+	1.65	+	3.40	+	1.76	+	1.71	+	1.83	+
20	1.74	2.88	-	2.94	-	1.96	I	1.99	+	1.57	+	1.73	I	1.62	I	2.23	I
21	1.65	2.00	-	2.36	-	2.58	-	2.27	-	2.06	I	1.19	-	0.98	-	2.37	-
22	2.18	2.93	-	3.68	-	2.20	-	3.04	-	5.18	-	2.21	-	2.81	-	3.22	-
23	1.98	2.38	-	2.72	-	2.19	-	3.04	-	2.48	-	1.71	-	1.47	-	2.80	-
24	1.15	1.36	+	3.32	+	3.06	+	3.17	+	4.94	+	4.30	+	4.01	+	3.68	+
25	1.73	2.39	-	2.96	-	1.93	-	3.50	+	5.30	+	2.85	+	4.06	+	3.94	+
26	1.44	1.08	-	1.45	-	1.77	+	1.77	+	4.79	+	4.77	+	4.88	+	5.28	+
27	1.44	2.09	-	1.96	-	1.61	-	1.50	NB	1.24	NB	1.49	NB	1.09	NB	1.67	NB
28	1.22	1.94	I	2.55	I	2.43	-	1.70	NB	1.40	NB	1.19	NB	0.83	NB	2.01	NB
29	1.83	3.77	-	2.23	-	1.69	+	1.92	+	1.85	+	1.67	+	1.45	+	1.77	+
30	1.82	1.84	I	3.54	-	2.86	+	1.44	NB	2.09	NB	1.33	NB	1.31	NB	1.43	NB
31	2.17	3.21	I	1.95	-	2.23	-	1.49	-	2.79	-	2.34	-	2.05	-	2.76	-
32	1.73	1.94	NB	3.08	NB	2.57	NB	3.37	NB	3.86	NB	1.80	NB	2.25	NB	3.88	+
33	1.41	1.84	-	2.35	-	1.56	-	1.21	-	1.09	-	1.19	-	0.81	-	2.07	-
34	2.08	2.83	NB	2.93	NB	2.60	NB	2.54	-	1.80	-	1.63	-	0.78	-	2.01	-
35	0.97	1.41	-	1.65	-	1.14	-	1.49	-	1.93	-	1.26	-	1.04	-	1.38	-
36	1.30	1.82	-	3.21	+	2.54	I	2.67	+	4.68	+	4.95	+	4.09	+	2.54	+
37	1.98	2.52	-	5.13	-	2.42	-	2.18	-	2.27	-	2.00	-	2.23	-	3.20	-
38	1.93	1.97	-	3.71	I	2.17	+	2.25	+	4.62	+	4.23	+	4.72	+	4.51	+
39	1.67	2.71	+	3.52	+	2.08	+	1.95	+	2.48	+	3.68	+	2.58	+	3.43	+

40	2.08	2.98	I	3.13	I	2.39	+	4.69	+	7.63	+	9.24	+	5.12	+	6.80	+
41	1.43	2.25	-	2.31	+	1.98	+	2.17	+	1.94	+	1.59	+	0.85	+	1.97	+
42	1.42	2.28	-	2.71	I	1.62	I	1.63	I	1.85	-	1.55	-	1.54	-	2.84	-
43	1.59	2.01	-	2.61	-	2.15	+	2.45	+	6.63	+	6.99	+	7.21	+	8.36	+
44	2.89	3.34	-	3.01	-	2.65	-	2.36	-	3.07	-	2.04	-	1.94	-	2.87	-
45	1.49	2.00	-	3.66	-	2.51	+	1.30	+	1.68	-	1.70	-	1.24	-	1.67	+
46	1.88	2.35	-	2.91	+	2.71	+	4.66	+	3.57	+	2.81	+	4.86	+	5.32	+
47	1.71	3.42	-	4.11	+	4.30	+	3.60	+	8.16	+	7.98	+	8.80	+	6.26	+
48	1.39	1.98	I	2.68	I	1.67	I	1.42	0	1.58	E	1.74	E	1.37	0	2.47	E
49	2.04	2.05	-	2.85	+	2.79	+	2.19	+	3.45	+	6.89	+	6.94	+	6.27	+
50	1.38	1.88	+	3.18	+	1.76	+	2.57	NB	4.54	+	4.47	+	4.64	+	4.42	+
51	1.96	3.46	-	2.78	I	1.96	-	1.84	-	2.60	-	1.81	-	1.15	-	2.21	-

Legend (+) GVHD positive, (-) GVHD Negative, (I) Non-GVHD Inflammation, (E) Equivocal (NB) Not Biopsied