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1 **Monocyte reconstitution and gut microbiota composition after hematopoietic stem cell**
2 **transplantation**

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30

31 **Background:**

32 Monocytes are an essential cellular component of the innate immune system that support the
33 host's effectiveness to combat a range of infectious pathogens. Hemopoietic cell transplantation (HCT)
34 results in transient monocyte depletion, but the factors that regulate recovery of monocyte populations
35 are not fully understood. In this study, we investigated whether the composition of the gastrointestinal
36 microbiota is associated with the recovery of monocyte homeostasis after HCT.

37

38 **Methods:**

39 We performed a single-center, prospective, pilot study of 18 recipients of either autologous or
40 allogeneic HCT. Serial blood and stool samples were collected from each patient during their HCT
41 hospitalization. Analysis of the gut microbiota was done using 16S rRNA gene sequencing and flow
42 cytometric analysis was used to characterize the phenotypic composition of monocyte populations.

43

44 **Results:**

45 Dynamic fluctuations in monocyte reconstitution occurred after HCT and large differences were
46 observed in monocyte frequency among patients over time. Recovery of absolute monocyte counts and
47 monocyte subsets showed significant variability across the heterogeneous transplant types and
48 conditioning intensities; no relationship to the microbiota composition was observed in this small
49 cohort.

50

51 **Conclusion:**

52 A relationship between the microbiota composition and monocyte homeostasis could not be
53 firmly established in this pilot study.

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54

55 **Keywords: microbiota; hematopoietic cell transplantation; monocytes; systems biology; immune**

56 **reconstitution**

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78 **BACKGROUND**

79

80 Hematopoietic cell transplantation (HCT) is a potentially curative procedure for patients with
81 hematologic malignancies but its success has been limited by the morbidity and mortality of post-
82 transplant infections and relapse. Impaired immune reconstitution post-HCT increases the risk of both of
83 these complications [1-4]. Multiple factors influence the vulnerability of patients to these complications
84 including: time since transplantation, graft source (i.e., autologous (auto-HCT) or allogeneic (allo-HCT)),
85 and persisting myelosuppression (humoral and cell-mediated) post-HCT [2, 3]. Alterations in the
86 microbiome have been associated with clinical outcomes of patients who have undergone HCT,
87 including their survival. However, the mechanisms by which the gut microbiota exerts its effects, both
88 beneficial and detrimental, have not been fully elucidated [5, 6].
89 In humans there are three main circulating monocyte subsets with diverse functions, classified
90 based on their expression of CD14 and CD16 surface proteins and cytokine production. These monocyte
91 subsets are named "classical", "intermediate", and "non-classical monocytes" [7-9] and typically
92 comprise 85%, 10%, and 5% respectively of the circulating monocyte pool in a healthy individual under
93 homeostatic conditions [10, 11]. Classical monocytes specialize in phagocytosis and produce the
94 cytokine IL-10 [12], while intermediate monocytes have elevated surface expression of MHC class II,
95 suggesting they have an important role in antigen presentation [13]. In contrast, non-classical
96 monocytes secrete substantial levels of inflammatory cytokines (TNF and interleukin [IL]-1β) and are
97 known to exert endothelial surveillance by endovascular slow patrolling when tissue damage is present
98 [14]. These roles make monocytes a critical cell line for host defense against common post-HCT
99 infections including *Aspergillus,* and for mitigating the risk of developing GVHD [15-22].
100 While it has been shown that monocyte reconstitution in the first 100 days post-HCT is
101 associated with improved survival [23, 24], a better understanding of the mechanisms that regulate

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102 monocyte reconstitution in this setting is needed. Given the known immunomodulating properties of
103 the microbiota [25-29], we investigated whether the composition of the intestinal microbiota is
104 associated with monocyte recovery. Monocyte migration out of the bone marrow and into the blood
105 circulation is driven by low level lipopolysacharide (LPS)-mediated signaling [30]. Further, basal
106 circulating LPS levels derived from the intestinal microbiota have been detected in
107 immunocompromised hosts [31, 32]. Therefore, we hypothesized that microbial communities of gram-
108 negative bacteria through the production of LPS [33, 34] and commensal obligate anaerobes [35]
109 influence monocyte maturation post-HCT.
110
111 **METHODS**
112
113 **Study Patients, Specimen Collection, and Patient Tracking**
114 We followed 18 adult recipients of auto- or allo-HCT at Memorial Sloan Kettering Cancer Center
115 (MSKCC) from July 2015 to January 2016. There were 7 female and 11 male patients; their ages ranged
116 from 40 to 75. Fecal samples were collected longitudinally from each patient during their transplant
117 hospitalization using a prospective institutional fecal biospecimen collection protocol (described
118 previously) [36]. For the majority of patients, daily collection began at the start of pre-transplant
119 conditioning (7-10 days before hematopoietic cell infusion) and continued until discharge. The median
120 average length of stay for patients in this cohort was 27 days. Clinical metadata for all patients, including
121 medication administrations (i.e. chemotherapy agents, antibiotics, etc.), absolute white blood cell values
122 (obtained from routine daily complete blood counts), and other patient characteristics, were retrieved
123 from the electronic health record. The study protocol was approved by the institutional review board.
124 Informed consent was obtained from all subjects prior to specimen collection.
125

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126 **Transplantation Practices**

127 Antimicrobial prophylaxis was given routinely to patients undergoing HCT. Subjects undergoing
128 either auto- or allo-HCT were given ciprofloxacin two days prior to hematopoietic cell infusion as
129 prophylaxis against gram-negative bacterial infections. Allo-HCT recipients were given intravenous (IV)
130 vancomycin for prophylaxis against viridans-group streptococci [28]. Antibiotic prophylaxis against
131 *Pneumocystis jiroveci* pneumonia was generally administered using either trimethoprim-
132 sulfamethoxazole, aerosolized pentamidine, or atovaquone; the time at which prophylaxis was initiated
133 (during conditioning or after engraftment, defined as an absolute neutrophil count ≥ 500
134 neutrophils/mm3 for three consecutive days) varied. In the event of a new fever during times of
135 neutropenia, patients were usually started on empiric antibiotics, such as piperacillin-tazobactam,
136 cefepime, or meropenem. Recipients of an autograft received pegfiltastrim on day +1 and recipients of
137 an allograft received daily filgastrim starting on day+7 until engraftment to accelerate recovery from
138 neutropenia [37].

139

140 **Sample Analysis and Defining Microbial Predictors**

141 *Sample Analysis-*

142 Stool DNA was extracted and purified, and the V4-V5 region of the 16S rRNA gene was amplified
143 by polymerase chain reaction using modified universal bacterial primers [38]. Sequencing was
144 performed using the Illumina Miseq platform[39] to obtain paired-end reads. These reads were
145 assembled, processed, filtered for quality, and grouped into operational taxonomic units of 97%
146 similarity using a previously described UPARSE pipeline [25]. Taxonomic assignment to species level was
147 performed using nucleotide BLAST (Basic Local Alignment Search Too) [40] with the National Center for
148 Biotechnology Information RefSeq (refseq_rna) as the reference database [41]. Alpha diversity was
149 calculated using the inverse Simpson index at the OTU level [42].

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150

151 *Microbial Predictors-*

152 We analyzed obligate anaerobic bacteria by major anaerobic groups defined at various
153 taxonomic levels for their importance in maintaining 'healthy' immunity [35, 43-46]: *Clostridia* (class),
154 *Bacterioidetes* (phylum), *Negativicutes* (class) and *Fusobacteria* (genus). Percent anaerobes in a given
155 stool sample was calculated by adding the percent 16S rRNA gene sequences of these obligate
156 anaerobic bacteria.

157 **Monocyte subsets, analysis of blood samples, monocyte isolation and flow cytometry**

158 A median of 7 blood samples were obtained from each patient during transplant days -10 to
159 +30. 5-10 cc of blood in heparinized tubes were processed within four hours of collection. The first
160 blood sample was collected within two days of hospital admission (prior to any perturbations to white
161 blood cells from chemotherapy or radiation) and subsequent blood samples were collected at the start
162 of white blood cell reconstitution through engraftment [47, 48].

163 Peripheral blood mononuclear cells were isolated by density gradient centrifugation
164 (Histopaque 1119; Sigma). Single-cell suspensions were stained for surface antigens with fluorescently
165 conjugated antibodies and samples were acquired with LSR II (Becton Dickinson). All flow cytometry
166 data were analyzed using FlowJo software. For flow cytometry staining, the following antibodies were
167 used: CD14-PE (clone M5E2; BD Biosciences) , CD16-FITC (clone 3G8;BD Biosciences), CCR2-APC (clone
168 K036C2; BioLegend), CD45-Alexa Fluor 700 (clone Hl30; BioLegend), CD11b (APC-Cy7; clone lCRF44;
169 BioLegend), HLA-DR-PE-Texas Red (clone L243; BioLegend), CD86-PE-Cy7 (clone lT2.2; BioLegend), CD15-
170 Pacific Blue (clone Hl98; eBioscience), CD20-PerCP-Cy5.5 (clone 2H7; BioLegend), CD3- PerCP-Cy5.5
171 (clone OKT3; BioLegend), CD19- PerCP-Cy5.5 (clone SJ25C1; BioLegend), CD56- PerCP-Cy5.5 (clone
172 5.1H11; BioLegend), and CD5- PerCP-Cy5.5 (clone UCHT2; BioLegend). Fluorescent minus one controls
173 (FMO) were used to determine positive staining gate [49]. The monocyte gating strategy employed to

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174 define monocyte subsets (classical, intermediate, and non-classical) [10, 11, 50] is shown with CD14 on
175 the x-axis and CD16 on the y-axis. A dump gate excluded B cells, T cells, and NK cells using CD19/CD20,
176 CD3, and CD56, respectively **(Supplementary Figure 2)**.
177
178 **Analytical Approach:**
179 We analyzed absolute monocyte count recovery as a function of time at the start of immune
180 reconstitution defined as: engraftment day minus 2 days ("reconstitution day") until hospital discharge,
181 using conditioning intensity and transplant type as stratification variables. Linear mixed models were fit
182 using patient as a random effect (for intercept), absolute monocyte count as the dependent variable,
183 and reconstitution day as a fixed effect. Fixed effect sizes and 95% confidence intervals are shown.
184 Descriptive statistics (box plots) were used to visualize the proportions of classical
185 ($CD14^{hi}CD16^{neg}$), intermediate ($CD14^{hi}CD16^{Int}$), and non-classical ($CD14^{Int}CD16^{hi}$) monocytes in the last
186 blood sample collected from each patient, to assess whether the subpopulations of circulating
187 monocytes reached their estimated targets: ~85% classical monocytes; ~10% non-classical monocytes;
188 ~5% intermediate monocytes. These thresholds were determined based on previous studies [10, 11, 51].
189 We calculated the Pearson correlation coefficient to measure the strength of the relationship
190 between the proportion of anaerobic commensal gut microbes and microbial diversity to monocyte
191 subset recovery. Next, we trained linear regression models while controlling for false discovery rates
192 [52] to assess whether or not different clinical predictors correlated with monocyte recovery in the last
193 blood sample tested (using the above mentioned 'target' values). The following clinical variables were
194 used in the regression: 1. conditioning type (RIC versus MAC) 2. transplant type (T-cell depleted versus
195 unmodified versus auto-HCT) 3. GCSF administration within 7 days of blood collection 4. A bloodstream
196 infection (excluding positive blood cultures considered to be a "contaminant") within 7 days of blood
197 collection and 5. percent gram-negative bacteria (in the phyla, *Bacteroidetes* and *Proteobacteria*, major

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198 gram-negative taxa) in the stool sample collected within 3 days of the last blood sample collected.
199 Statistical analyses were performed using R (v. 3.3.1).
200
201 **RESULTS**
202
203 **Description of study population and biospecimens**
204 Our cohort consisted of 18 patients who underwent auto- or allo-HCT at MSKCC between July
205 2015 to January 2016. Patients underwent different types of HCT for different hematologic
206 malignancies. Four patients received an auto-HCT after myeloablative conditioning (MAC) with
207 Carmustine (BCNU), etoposide, cytarabine, and melphalan (BEAM). Four patients received an allo-HCT
208 after MAC conditioning consisted of the following regimens: total body irradiation (TBI), thiotepa, and
209 cyclophosphamide or busulfan, melphalan, and fludarabine. Ten patients were recipients of an allo-HCT
210 after reduced intensity conditioning (RIC) regimens. Clinical characteristics for each patient are shown in
211 Table 1.
212 The duration of transplant hospitalization ranged from 20 to 38 days, during which antibiotics
213 were given for both prophylactic and treatment purposes. Throughout this period, we sought to collect
214 fecal samples on a daily basis. Out of the total 352 hospital days for these 18 patients, 318 stool samples
215 were collected (90% of total hospital days). Of those samples, 236 (74%) yielded 16S amplicons that
216 could be sequenced. Each blood sample was paired with a stool sample collected within 3 days. A
217 median of 15 stool samples and 7 blood samples were collected per patient during their HCT
218 hospitalization **(Table 1)**.
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222 **Monocyte Recovery**

223 Monocyte counts were considered during an analysis window from two days prior to the date of
224 neutrophil engraftment ("reconstitution day") until hospital discharge. Significant differences in
225 reconstitution trajectories were found among groups stratified by conditioning regimens and transplant
226 type. Patients receiving reduced intensity conditioning and unmodified transplants demonstrated the
227 most variability ($CI_{95\%}$ = [0.196±0.062] and $CI_{95\%}$ =[0.194±0.060], respectively). All 18 patients reached a
228 minimum normal absolute monocyte count value of 0.26 x 10^9/L defined elsewhere, [53-55] and
229 patients who received RIC and an unmodified transplant were more likely to overshoot the upper
230 reference limit of 1.3 x 10^9 (a value determined by the MSKCC laboratory) **(Figure 1A, Figure 1B)**.

231 Three representative flow cytometry plots collected longitudinally from 3 patients who received
232 different transplant types (Auto-HCT; Allo-HCT (RIC); Allo-HCT (MAC) show significant monocyte
233 heterogeneity. That is, the commonly observed distribution of monocytes ('banana shape') within a
234 patient [56, 57] changes over time **(Figure 2A)**. All other patients (n = 15) are shown in **Supplementary**
235 **Figure 1.** The gating strategy used to identify the monocyte subsets of interest is shown in
236 **Supplementary Figure 2**.

237 Some patterns in monocyte reconstitution were present; classical monocytes had an initial
238 robust recovery consistent with previous findings [58], followed by restoration of intermediate and non-
239 classical monocytes at later timepoints **(Figure 2A)**. This observation was especially true with respect to
240 the recovery of non-classical monocytes. On average, patients who were recipients of either an auto-
241 HCT or allo-HCT with RIC were more likely than allo-HCT patients with MAC to recover classical
242 monocytes to target threshold (~85%). The threshold of ~5% intermediate monocytes was met in 72%
243 (13/18) of patients, while those that received an allo-HCT with MAC conditioning were more likely to be
244 discharged with levels of circulating non-classical monocytes that met target (~10%) **(Figure 2B)**.

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245 We next compared the frequency of circulating monocytes subsets with the composition of the
246 intestinal microbiota**.** Diversity of intestinal microbial communities, as measured by inverse Simpson
247 index, were not associated with monocyte recovery **(Figure 3A)**. Additionally, the proportion of obligate
248 anaerobes associated with a healthy flora (*Negativacutes*, *Clostridia*, *Bacteroidiales*, and *Fusobacteria*)
249 within the microbiota did not correlate with monocyte recovery **(Figure 3B)**.
250 While the proportion of monocyte subset recovery did not correlate with the microbiota, we
251 next sought to investigate whether immune activation status of monocytes subsets was altered by the
252 microbiota composition. Monocyte immune activation phenotype as measured by the expression of cell
253 surface markers (CD86, HLA-DR, and CCR-2 **(Supplementary Table 1)**). was assessed in each three
254 subsets of monocytes. No correlation was observed between expression of these markers and
255 microbiota diversity **(Supplementary Figure 3)** or percent of obligate anaerobic bacteria in the
256 microbiota **(Supplementary Figure 4)**.
257 We used a simple linear regression model to test whether different compositional
258 characteristics of the microbiota and different clinical variables are related to monocyte recovery. In a
259 linear regression model, microbiota diversity and the proportion of gram-negative Proteobacteria? in
260 the gut were not observed to be associated with monocyte recovery of each subset. Exposure to GCSF
261 within 7 days of blood sample collection/processing was associated with successful reconstitution of
262 classical and intermediate monocytes ($p = 0.037$ and $p =0.002$, respectively). Conversely, a T-cell
263 depleted transplant was negatively associated with these monocyte subsets ($p = 0.041$ and $p = 0.008$,
264 respectively) **Table 2**. Non-classical monocytes were not associated with any of the clinical predictors we
265 defined.
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269 **DISCUSSION**

270

271 HCT involves the administration of intense chemotherapy with or without radiation and
272 antibiotic regimens, resulting in large shifts in leukocyte and microbiota compartments, often leading to
273 complete compositional changes from one day to the next. The myeloablation associated with HCT
274 provides a unique platform for exploring the nascent, re-development of immune reconstitution. Prior
275 studies have supported the symbiotic relationship between the gut microbiota and the systemic
276 immune system [59, 60], but most prior work has focused on the role the microbiota has on shaping
277 different populations of T cells [30-32]; little is known about the impact intestinal commensalism has on
278 monocyte maturation. We followed a systematic strategy to identify three subsets of monocytes
279 (classical, intermediate, and non-classical monocytes) [10] during immune reconstitution post-HCT to
280 assess whether constituents of the microbiota affect monocyte recovery.

281 This pilot study involved high-frequency collection of stool and blood samples from 18 patients
282 to assess whether a link between the microbiota and monocyte recovery could be found. We found no
283 correlation between the microbiota composition and differences in monocyte recovery when analyzing
284 a higher-diversity microbiota or a microbiota composed primarily of either commensal anaerobes or
285 Gram-negative organisms. We also found no association between microbiota composition and the
286 expression of co-stimulatory markers, including CD86, HLA-DR and the chemokine receptor, CCR2.

287 Analysis of factors associated with immune reconstitution revealed that any link between the
288 microbiota and immune reconstitution is weak relative to other clinical variables. As expected, GCSF
289 exposure correlated positively with classical and intermediate monocyte reconstitution, while T cell
290 depletion by means of CD34 positive selection was negatively associated with classical and intermediate
291 monocyte subset reconstitution. We also found significant variability in the reconstitution of absolute

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292 monocyte counts and monocyte subsets across patients. The biological significance that the varying rate
293 of monocyte recovery has on patient outcomes needs to be further explored.

294 Discerning the effects of many patient variables in the setting of HCT, including medications
295 delivered and clinical complications that ensue (bacteremia, fever, GVHD, etc.) make forming a direct
296 relationship between the microbiota and monocytes in HCT patients challenging [61]. Medications given
297 to HCT patients that have immunomodulating properties (i.e., antibiotics and steroids), could serve as
298 confounders in our analysis. For example, 16/18 patients received ciprofloxacin and 7/18 patients
299 received steroids. Ciprofloxacin can dampen the effects of LPS [62, 63], a potent stimulator of monocyte
300 mobilization out of the bone marrow [30, 64], while steroids can reduce blood levels of monocytes [30,
301 65]. Further, testing for intestinal LPS permeability in human serum or blood is technically challenging
302 [61].

303 While we were not able to discern an association between microbiota composition and
304 monocyte recovery in this heterogeneous pilot cohort, this study demonstrates the feasibility of high-
305 resolution sampling of blood and stool samples and opens the door to future studies where sampling
306 many more patients with daily frequency may help distinguish which microbial and clinical factors drive
307 HCT patient outcomes.

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316

317 **Figure Legends**

318 **Figure 1. Recovery of absolute monocyte counts (AMC) is variable among patients receiving different**
319 **conditioning regimens and transplant types.** Graph showing absolute monocyte counts obtained for all
320 patients when there was any sign of white blood cell recovery defined as "reconstitution day"
321 (engraftment day – 2). Patient groups were divided by **(A)** conditioning, reduced intensity conditioning
322 (RIC) and myeloablative conditioning (MAC) and **(B)** transplant type. Each color represents a different
323 patient and a dashed line in each panel marks the upper limit of a "normal" AMC, defined by the MSKCC
324 laboratory to be 1.3 K/mcL. In the upper righthand corner is the coefficient (i.e. slope/effect size)
325 multiplied by 1.96 (+/- 95% confidence interval).

326

327 **Figure 2 Variable recoveries of monocyte populations (classical, intermediate, and non-classical)**. **(A)**
328 Time series for 3 representative patients using transplant day: Auto-HCT (top panel), Allo-HCT (reduced
329 intensity conditioning (RIC; middle panel), and Allo-HCT myeloablative conditioning (MAC; bottom
330 panel). The first figure (from left-to-right for each panel) is a cartoon illustration depicting each
331 monocyte subtype, classical (C), intermediate (I), and non-classical (NC) (clockwise from left to right)
332 that corresponds to successive multicolor flow cytometry plots that follow. The numbers in each gate
333 are percentages of each monocyte population. The gating strategy is detailed in Supplementary Figure 1.
334 **(B)** A boxplot showing the distribution of monocyte subsets determined from a patient's last blood
335 sample collected. Colored dashed lines indicate the upper limit of "normal" for the percent values for
336 each monocyte subset: 85% classical monocytes (blue), 10% non-classical monocytes (yellow), 5%
337 intermediate monocytes (green). Data points (dots) indicate the monocyte subset type using the same
338 color scheme.

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340 **Figure 3: Monocyte subset frequency does not correlate with microbiota diversity or proportion of**
341 **obligate anaerobes. (A)** Relationship between the proportion of each monocyte subset and microbiota
342 diversity measured by Inverse Simpson and **(B)** the proportion of obligate anaerobes (percent 16S rRNA
343 gene sequences of *Negativacutes + Clostridia + Bacteroidiales +Fusobacteria*) in the stool microbiota for
344 each matched stool and blood collection. Pearson correlation coefficient values are shown in the upper
345 righthand corner of each panel.
346
347 **Supplementary Figure 1: Circulating monocyte subsets in transplant patients following immune**
348 **reconstitution.** Time series of blood samples collected for 15 representative patients with transplant day
349 used to show when samples were collected: Auto-HCT patients (top panel), Allo-HCT patients (RIC)
350 (middle panel), and Allo-HCT patients (MAC) (bottom panel). A cartoon illustration in the upper
351 righthand corner of each panel is again shown depicting the relative location of each monocyte subtype,
352 classical (C), intermediate (I), and non-classical (NC) (clockwise from left to right).
353
354 **Supplementary Figure 2: Monocyte gating strategy**. **(A)** Cells are first visualized on Live/Dead vs. FSC
355 and a gate is drawn around the live cells. **(B)** A gate is drawn around lymphocytes and monocytes (SSC
356 vs. FSC). **(C & D)** Doublets are then discriminated in two steps (FSC-A VS. FSC-W and SSC-A and SSC-W).
357 **(E)** CD45+ (hematopoietic cells), lineage negative (Non-T, Non-B, Non-NK) cells were gated for. **(F)**
358 Gating to further discriminate monocytes from granulocytes (CD15+, CD16+). **(G)**
359 (CD14+CD16+) monocytes subsets (classical, intermediate, non-classical) are shown (plot f). Numbers
360 are percentages of each population within each gate.
361
362 **Supplementary Figure 3: Monocyte expression of immune activation markers does not correlate with**
363 **microbiota diversity.** Relationship between compositional diversity of the gut microbiota, measured by

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364 Inverse Simpson and the proportion of surface marker expression of each monocyte subsets (classical,
365 intermediate, and nonclassical): CCR2 (top panel), CD86 (middle panel) and HLA-DR (bottom panel).
366 Pearson correlation coefficient values are shown in the upper righthand corner of each panel.
367
368 **Supplementary Figure 4: Monocyte expression of immune activation markers does not correlate with**
369 **proportion of obligate anaerobes in the microbiota.**
370 Relationship between the proportion of commensal anaerobes that make up the gut microbiota and the
371 level of surface marker expression of each monocyte subsets (classical, intermediate, and nonclassical):
372 CCR2 (top panel), CD86 (middle panel) and HLA-DR (bottom panel). Pearson correlation coefficient
373 values are shown in the upper righthand corner of each panel.
374
375 **Table 1: Clinical characteristics of all 18 HCT patients including the number of blood samples and**
376 **stools samples collected from each patient.** Abbreviations: Pt, patient; Auto, autologous; Allo,
377 allogeneic; RIC, reduced intensity conditioning; MAC, myeloablative conditioning; DLBCL, diffuse large B
378 cell lymphoma; MDS, myelodysplastic syndrome; CLL, chronic lymphocytic leukemia; ALL, acute
379 lymphoblastic leukemia; AML, acute myeloid leukemia; cy, cyclophosphamide.
380
381 **Table 2: Parameters assessed for association with monocyte subset recovery**
382 Abbreviations: Auto, autologous; Allo, allogeneic; RIC, reduced intensity conditioning; MAC,
383 myeloablative conditioning; GCSF, granulocyte colony-stimulating factor.
384
385 **Supplemental Table 1:**
386 Fluorochromes used to label antibodies used and their function
387

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388 **Footnotes**

389 **Conflict of interest:** Dr. Perales reports honoraria from Abbvie, Bellicum, Bristol-Myers Squibb, Incyte,
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555

A

95% CI = −0.0136+/−0.0065

MAC

95% CI = 0.196+/−0.0616

RIC

Absolute monocyte count (K/mcL)

Reconstitution day

B

95% CI = 0.0511+/−0.0202

auto

95% CI = −0.0664+/−0.111

T−cell depleted

95% CI = 0.194+/−0.0596

unmodified

Absolute monocyte count (K/mcL)

Reconstitution day

Fig 1

Figure 2A

Allo-HCT; MAC

NC
I
C
Day +12
Day +14
Day +17
Day +19
CD 16
CD 14

Auto-HCT; MAC

NC
I
C
Day +11
Day +14
Day +17
Day +18
CD 16
CD 14

Allo-HCT; RIC

NC
I
C
Day +16
Day +17
Day +18
Day +19
CD 16
CD 14

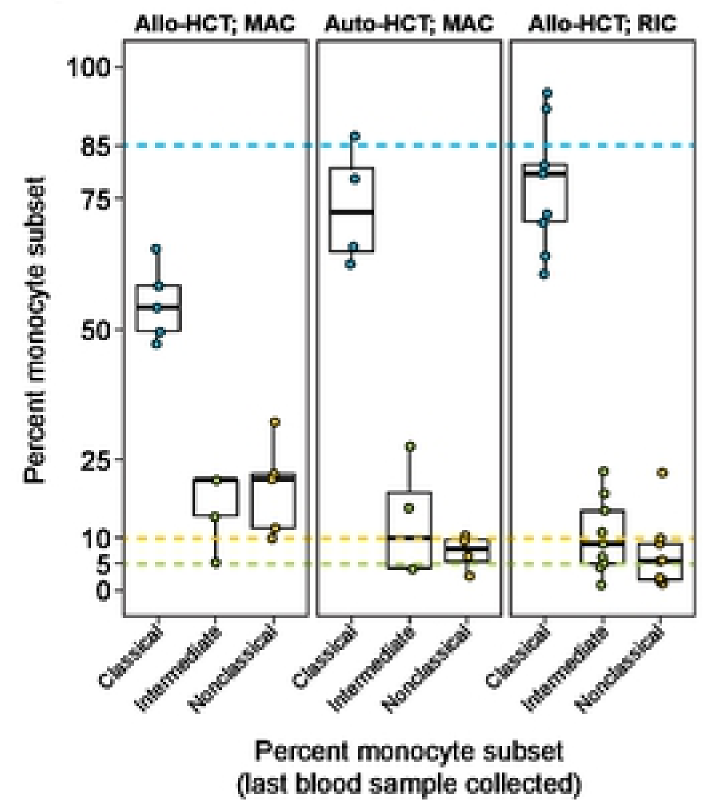

Fig 2

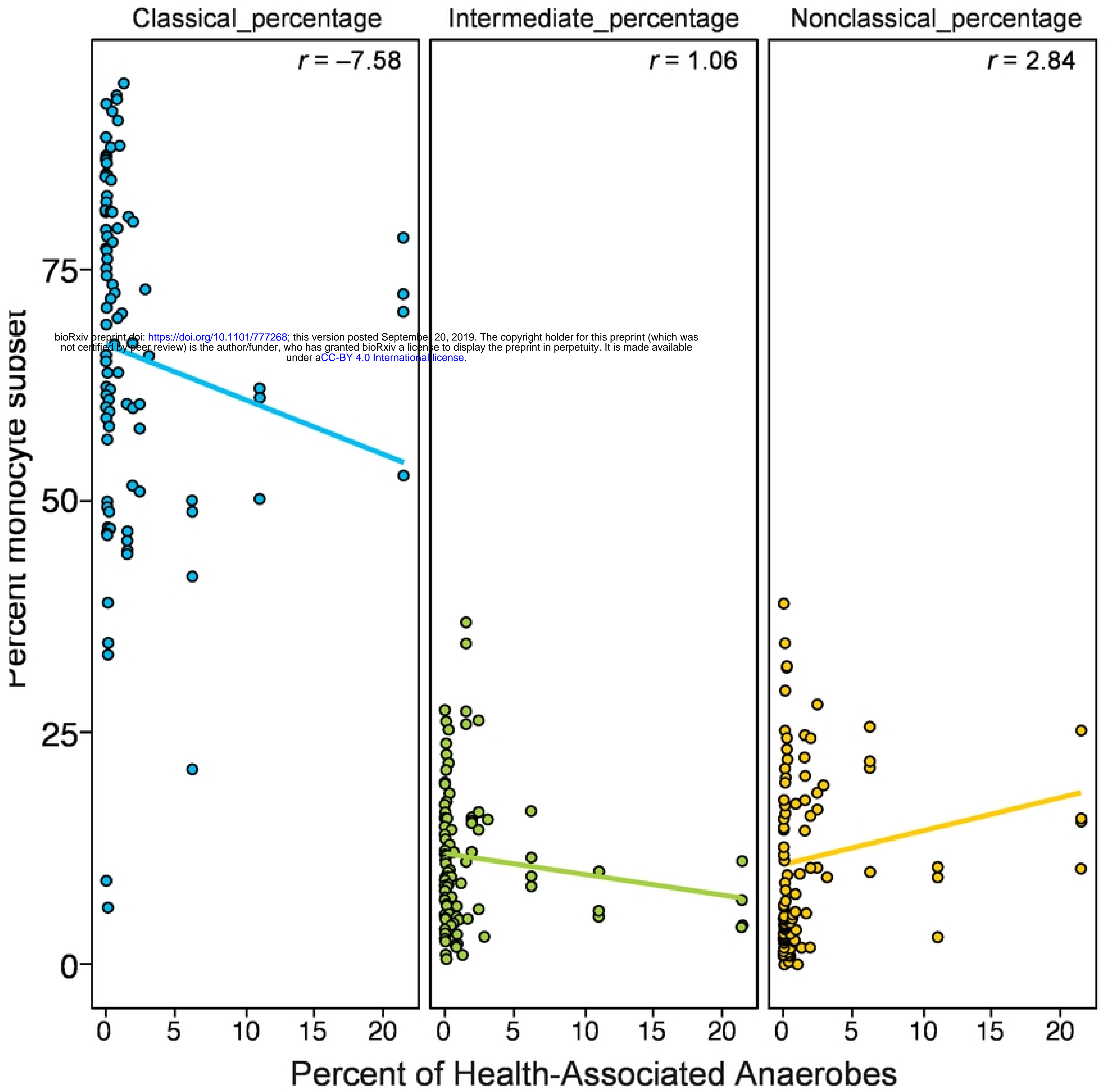

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Fig 3