**CD8 T cells compensate for impaired humoral immunity in COVID-19 patients with hematologic cancer**

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47  **Abstract**

48  Cancer patients have increased morbidity and mortality from Coronavirus Disease 2019

49  (COVID-19), but the underlying immune mechanisms are unknown. In a cohort of 100 cancer

50  patients hospitalized for COVID-19 at the University of Pennsylvania Health System, we found

51  that patients with hematologic cancers had a significantly higher mortality relative to patients

52  with solid cancers after accounting for confounders including ECOG performance status and

53  active cancer status. We performed flow cytometric and serologic analyses of 106 cancer

54  patients and 113 non-cancer controls from two additional cohorts at Penn and Memorial Sloan

55  Kettering Cancer Center. Patients with solid cancers exhibited an immune phenotype similar to

56  non-cancer patients during acute COVID-19 whereas patients with hematologic cancers had

57  significant impairment of B cells and SARS-CoV-2-specific antibody responses. High

58  dimensional analysis of flow cytometric data revealed 5 distinct immune phenotypes. An

59  immune phenotype characterized by CD8 T cell depletion was associated with a high viral load

60  and the highest mortality of 71%, among all cancer patients. In contrast, despite impaired B cell

61  responses, patients with hematologic cancers and preserved CD8 T cells had a lower viral load

62  and mortality. These data highlight the importance of CD8 T cells in acute COVID-19,

63  particularly in the setting of impaired humoral immunity. Further, depletion of B cells with anti-

64  CD20 therapy resulted in almost complete abrogation of SARS-CoV-2-specific IgG and IgM

65  antibodies, but was not associated with increased mortality compared to other hematologic

66  cancers, when adequate CD8 T cells were present. Finally, higher CD8 T cell counts were

67  associated with improved overall survival in patients with hematologic cancers. Thus, CD8 T

68  cells likely compensate for deficient humoral immunity and influence clinical recovery of COVID-

69  19.  These observations have important implications for cancer and COVID-19-directed

70  treatments, immunosuppressive therapies, and for understanding the role of B and T cells in

71  acute COVID-19.

**Main Text**

Severe illness affects up to 20% of those hospitalized with Coronavirus Disease 2019 (COVID-19)[1] and is manifested by acute respiratory distress syndrome (ARDS), multi-organ failure, and/or death[2]. Severe disease has been linked to immune dysregulation, including deficiency in the production of type I and type III interferons[3–5], marked lymphopenia[6–10], and a paradoxical increase in pro-inflammatory cytokines, such as TNFα, IL-1β, and IL-6[3, 6, 11–15]. In addition, alteration of the lymphocyte compartments has been reported during COVID-19 with increases in activated CD4 and CD8 T cells[15–18], skewing of CD8 T cells towards effector[16, 17] and exhausted phenotypes[18], and increased differentiation of CD4 T cells towards the Th17 lineage[17, 19]. Despite these marked alterations in their T cell compartment, COVID-19 patients have robust plasmablast responses[15, 20], and the majority of patients generate IgM and IgG antibodies to SARS-CoV-2 over the course of disease[20–22]. More recently, integrated and multi-omic analyses have highlighted the tremendous heterogeneity of the human immune response to SARS-CoV-2, with distinct immunophenotypes that are associated with COVID-19 disease severity and disease trajectory[5, 11, 12, 15, 16]. Understanding how clinical features, particularly patient comorbidity, impact host immune responses to SARS-CoV-2 will elucidate determinants of immunotype and disease severity.

Cancer patients have an increased risk of severe illness from COVID-19[23–26] with an estimated case fatality rate of 25%[27] compared to 2.7% in the general population[28]. Importantly, cancer is a heterogeneous disease with even higher mortality rates reported for patients with particular subtypes of cancer. For example, several cohort and registry studies have demonstrated particularly poor outcomes among patients with hematologic cancers, with mortality rates as high as 55%[23, 26, 29–37]. However, it remains unknown whether the increased mortality by cancer subtype is independent of the confounding effects of other prognostic factors such as Eastern Cooperative Oncology Group (ECOG) performance status, active cancer

97    status, and cancer therapy. Further, data is limited on the immune landscape of cancer patients;

98    whether components of cellular and humoral immunity are compromised, the impact of immune-

99    modulating therapies such as B cell depleting therapy, and how these factors influence mortality

100   in the setting of COVID-19 is also not known. To address these questions, we studied three

101   cohorts of cancer patients with acute COVID-19 across two hospital systems to understand the

102   immunologic determinants of COVID-19 mortality in cancer.


103   **Hematologic cancer is an independent risk factor of COVID-19 mortality**

104   To understand the clinical impact of COVID-19 on cancer patients, we first conducted a

105   prospective multi-center observational cohort study of cancer patients hospitalized with COVID-

106   19 (COVID-19 Outcomes in Patients with Cancer, COPE). Between April 28 and September 15

107   2020, 114 patients with history of hematologic or solid tumor malignancy, and laboratory-

108   confirmed SARS-CoV-2 infection or presumed COVID-19 diagnosis, were enrolled across 4

109   hospitals in the University of Pennsylvania Health System. 14 patients were excluded from the

110   analyses due to either low suspicion for COVID-19 infection, or benign tumor diagnosis. The

111   median age of this cohort was 68 years, 52% were male, 54% Black, and 57% were current or

112   former smokers (**Table 1**), reflecting the demographics of severe COVID-19[38, 39]. In terms of

113   cancer-specific factors, 78% of patients had solid cancers, with prostate and breast cancers

114   most prevalent; 46% had active cancer, defined as diagnosis or treatment within 6 months; and

115   49% had a recorded ECOG performance status of 2 or higher (**Table 1**). During follow up, 48%

116   of subjects required ICU level care, and 38% of patients died within 30 days of admission

117   (**Table 2**), consistent with previously reported rates for severe COVID-19 in this population[30, 34,

118   37].

119          To understand key determinants of COVID-19 disease severity, we performed univariate

120   analysis to identify factors associated with all-cause mortality within 30 days of discharge. We

121   included relevant covariates, including patient factors such as age, race, gender, and smoking

122   history (ever versus never)[2, 38–40]; cancer-specific factors including ECOG performance status[35],

123   status of cancer (e.g., active versus remission)[36, 36]; cancer type (e.g., heme versus solid

124   cancer)[29, 34, 36, 41, 42]; and cancer treatment[26, 37].  Increased mortality was significantly associated

125   with prior or current smoking (p = 0.028), poor ECOG performance (ECOG 3-4, p=0.001), and

126   active cancer status (p=0.024) (**Fig. 1**). In addition, patients with hematologic cancers (mostly

127   lymphoma and leukemia), appeared to have an increased risk of mortality relative to solid

128   cancers (54% versus 33% respectively, p=0.075) (**Table 3**). This is consistent with recent data

129   showing increased disease severity and mortality in patients with hematologic malignancies[23, 29,

130   34–36, 41]. Notably, cancer treatment, including cytotoxic chemotherapy, was not significantly

131   associated with COVID-19 mortality, also consistent with published literature in patients with

132   cancer[29, 30, 34, 36, 41].

133         To determine whether the increased mortality observed in patients with hematologic

134   malignancy was independent of potential confounding effects from smoking history, poor ECOG

135   performance, and active cancer, which were not corrected for in the prior studies, we performed

136   multivariable logistic regression. Patients with hematologic cancers tended to be younger, male,

137   less likely to have coexisting comorbidities, and more likely to have received recent cytotoxic

138   chemotherapy (**Supplemental Table 1**). In this fully adjusted analysis, hematologic malignancy

139   was strongly associated with mortality, in comparison to solid cancer (OR 3.3, 95% CI 1.01-

140   10.8, p=0.048) (**Table 3**). Similar results were observed in time-to-event analyses using Kaplan

141   Meyer methods (**Fig. 2a**, median overall survival (mOS) not reached for patients with solid

142   cancers vs 47 days for patients with heme cancers, p-value=0.030) and Cox regression models

143   (**Table 3,** HR 2.56, 95% CI 1.19-5.54, p=0.017). Moreover, patients with hematologic cancers

144   had higher levels of many inflammatory markers on admission laboratory testing, including

145   ferritin, IL-6, and LDH (**Fig. 2b**). There were no significant differences in CRP, fibrinogen, D-

146   dimer, lymphocyte counts, and neutrophil counts, while ESR was higher in patients with solid

147   cancer (**Extended Data Fig. 1 a,b**). Thus, hematologic malignancy was an independent risk

148   factor of death, with signs of a dysregulated inflammatory response.


149   **Hematologic cancer patients have an impaired SARS-CoV2-specific antibody response.**

150   To understand the immune landscape in cancer patients, as compared to patients without

151   cancer, we leveraged an observational study of hospitalized COVID-19 patients at the

152   University of Pennsylvania Health System where blood was collected (MESSI-COVID)[15]. This

153   analysis included 130 subjects with flow cytometric and/or serologic analysis. In particular, we

154   focused on 22 subjects with active cancer (**Supplemental Tables 2, 3**), including patients

155   undergoing cancer-directed therapies such as chemotherapy, immunotherapy, or B cell directed

156   therapies (**Supplemental Table 4**). Age, gender, and race were similarly distributed in COVID-

157   19 patients with active cancer and those without, and both groups had a similar timeframe of

158   symptom onset and disease severity (**Fig. 3a**, **Supplemental Table 2**). However, cancer

159   patients had a higher all-cause mortality (36.4% versus 11.1%, **Fig. 3a**), consistent with our

160   COPE clinical cohort, and what has been reported in other cohorts of COVID-19 patients[23, 26, 29,]

161   [30].

162          As humoral immunity is critical for protective immunity against SARS-CoV-2, we

163   hypothesized that a defect in SARS-CoV-2-specific antibodies may be associated with the

164   increase in mortality seen in patients with active cancer. We assessed the levels of IgM and IgG

165   antibodies that recognized the SARS-CoV-2 receptor binding domain (RBD), using an enzyme-

166   linked immunosorbent assay (ELISA) based approach[43, 44]. Cancer patients had significantly

167   decreased SARS-CoV-2-specific IgG and IgM responses compared to non-cancer patients

168   (**Extended Data Fig. 2a**). These differences were not due to the timing of SARS-CoV-2

169   infection as time from symptom onset was similar (**Supplemental Table 2**). As hematologic

170   malignancies directly involve the lymphoid and myeloid immune compartments, we suspected

171   that hematologic cancers may have an impaired humoral immunity against SARS-CoV-2.

172    Indeed, the vast majority of hematologic cancer patients (6/7) had IgM and IgG levels below the

173    cutoff of positivity of 0.48 arbitrary units (**Fig. 3b, Extended Data Fig. 2b**). In contrast, those

174    with solid cancers had IgG and IgM antibody responses that were more comparable to patients

175    without cancer (**Fig. 3b**).


176    **A T cell-depleted immune phenotype is associated with COVID-19 mortality.**

177    Protective antibody responses require effective T cell and B cell responses. We therefore

178    examined whether cancer patients had an altered cellular response to SARS-CoV-2. We first

179    performed exploratory high-dimensional analysis on the lymphocyte compartment of 45 patients

180    with COVID-19 including 37 non-cancer, 6 solid cancer, and 2 hematologic cancer patients.

181    UMAP (Uniform Manifold Approximation and Projection) representation of 27-parameter flow

182    cytometry data highlighted discrete islands of CD4 and CD8 T cells, and CD19+ B cells

183    (**Extended Data Fig. 3a and Fig. 3c**). To understand whether there were major global

184    differences in lymphocytes between solid, hematologic, and non-cancer patients, we used the

185    Earth Mover's Distance (EMD) metric[45] to calculate the distance between the UMAP projections

186    for every pair of patients. Clustering on EMD values identified 5 clusters of patients with similar

187    lymphocyte profiles (**Fig. 3c**). Differences between these clusters of patients were driven by

188    both the distribution (**Fig. 3d)** and phenotype (**Extended Data Fig. 3b and Fig. 3e**) of CD4,

189    CD8, and B cells. EMD cluster 1 was defined by depleted CD4 and B cells, increased CD8 T

190    cells, and increased activation and effector markers, including PD-1, CX3CR1, Ki67, and HLA-

191    DR (**Extended Data Fig. 3b and Fig. 3 d,e**). EMD cluster 3 had decreased T cell and B cells,

192    with an inactivated immune profile, and EMD Cluster 5 was depleted of both CD4 and CD8 T

193    cells, but had preserved B cells. In contrast, EMD cluster 4 was defined by robust

194    CCR7+CD27+ memory CD4 T cell responses and heterogenous B cell responses; EMD cluster

195    2 had the most balanced responses, with CD4, CD8, and B cells represented (**Fig. 3d,e and**

196    **Extended Data Fig. 3b**). We then correlated these 5 patterns of immune responses with clinical

197    and serological variables. EMD cluster 5 patients with depleted T cells had the highest mortality

198    and disease severity, despite generating SARS-CoV-2-specific IgM and IgG antibodies (**Fig. 3f,**

199    **Extended Data Fig. 5d).** In contrast, EMD clusters 2 and 4, with robust CD4 and/or CD8 T cell

200    responses, had the lowest mortality and a low disease severity (**Fig. 3f, Extended Data Fig.**

201    **5d).** These findings suggest a key role for T-cell immunity in facilitating viral clearance, even in

202    the presence of intact humoral immunity.


203    **Distinct immune landscape in hematologic cancer compared to solid cancer or no**

204    **cancer.**

205    To further understand the immune response of patients with cancer and COVID-19, we explored

206    the role of cancer subtype (solid tumor versus hematologic) on immune phenotype. Four out of

207    the 6 solid cancer patients were in EMD cluster 2, with a balanced immune phenotype (**Fig. 3e**).

208    In contrast, both hematologic cancer patients were in EMD cluster 1, which had marked

209    depletion of CD4 and B cells. Indeed, UMAP projections showed that while solid cancer patients

210    had an immune landscape similar to non-cancer patients, the two hematologic cancer patients

211    demonstrated loss of islands associated with CD4 and B cells (**Fig. 3g**). We then extended this

212    analysis by measuring the frequency and phenotype of key lymphocyte populations in the entire

213    MESSI-COVID cohort and healthy donor controls. COVID-19 patients with hematologic cancers

214    had a significantly lower frequency of CD4 and B cells compared to solid cancer patients, non-

215    cancer patients, and healthy donors without COVID-19 (**Fig. 3h**). As T follicular helper cells

216    (Tfh) and plasmablasts are critical in the generation of effective antibody responses, we

217    assessed circulating Tfh and plasmablast responses. Although limited by sample size, patients

218    with hematologic cancers had low circulating Tfh (PD1+ CXCR5+) and plasmablast responses

219    (CD19+CD27$^{hi}$CD38$^{hi}$), and decreased CD138 expression (**Extended Data Fig. 4a**). Thus,

220    patients with hematologic malignancy appear to have quantitative defects in CD4 and B cells

221    that may be required for effective SARS-CoV-2-specific antibody responses.

222       Patients with hematologic cancers had a preserved frequency of CD8 T cells. Therefore,

223    we wanted to determine whether there were qualitative changes within the CD8 T cell

224    compartment. We performed FlowSOM clustering analysis on non-naïve CD8 T cells from 118

225    COVID-19 patients and 30 healthy donors and visualized the clusters using UMAP. UMAP

226    clearly separated CX3CR1 and Tbet expressing effector cells from memory CD8 T cells

227    expressing CD27 and TCF-1 (**Extended Data Fig. 4b and Fig. 3i**). The effector island was

228    composed of CD45RA$^{lo}$CD27$^{lo}$ effector memory cells (clusters 2 and 3) and CD45RA+ TEMRA

229    cells (cluster 1). The memory island was composed of CCR7$^{lo}$ transitional memory (cluster 5),

230    and effector memory cells (clusters 7 and 8), and CCR7$^{hi}$ central memory cells (cluster 9).

231    Activated cells, characterized by high HLA-DR, CD38, and Ki67 expression, were identified in

232    clusters 3, 4, and 5 (**Extended Data Fig. 4c**). Stem cell memory cells (cluster 10) and

233    exhausted phenotype CD8 T cells (cluster 6) were present, but at low frequencies of below

234    0.5% (**Data not shown**).

235       We then compared the landscape of CD8 T cells in patients with and without cancer.

236    CD8 T cell subsets including central memory, effector memory, transitional memory and EMRA,

237    were similar between patients with and without cancer (**Extended Data Fig. 4d)**. However,

238    UMAP representation of non-naïve CD8 data demonstrated preferential enrichment of cells

239    expressing HLA-DR and CD38 in cancer patients compared to non-cancer patients (**Fig. 3j**).

240    Indeed, cancer patients had higher frequencies of activated HLA-DR, CD38, and Ki67-

241    expressing FlowSOM clusters (clusters 3, 4, and 5) compared to non-cancer patients and

242    healthy donors (**Extended Data Fig. 4e and Fig. 3k**). When stratified by cancer type, the

243    increased HLA-DR and CD38 expression was restricted to the patients with hematologic

244    cancers; patients with solid cancers and those without cancer had comparable levels of

245    activation (**Fig. 3l**). Altogether, solid cancer patients with COVID-19 had an immune landscape

246    similar to non-cancer COVID-19 patients. In contrast, patients with hematologic malignancies

247    had defects in CD4, B cells, and humoral immunity but preserved and highly activated CD8 T

248  cells, suggesting that CD8 T cells might at least partially compensate for blunted humoral

249  immune responses in patients with hematologic malignancies.


250  **CD8 T cell adequacy increases survival in the setting of impaired B cell and humoral**

251  **immunity in hematologic cancer.**

252  Patients with hematologic cancer had significantly impaired humoral immunity and a mortality

253  rate of 55% (**Table 2**). We hypothesized that CD8 T cells partially compensated for defective

254  humoral immunity and influenced survival in acute COVID-19. We tested this hypothesis in a

255  cohort of cancer patients hospitalized with COVID-19 at the Memorial Sloan Kettering Cancer

256  Center (MSKCC), which included a larger number of hematologic malignancies patients,

257  including those treated with B cell depleting therapy. This cohort included 39 solid cancer

258  patients and 45 hematologic cancer patients. The median age was 65 years, and in contrast to

259  the MESSI cohort at Penn, 81% of the cohort was white (**Fig. 4a**, **Supplementary Table 5,6**). A

260  significant portion of patients were treated with remdesevir and convalescent plasma – 21.4%,

261  and 46.4%, respectively (**Supplementary Table 5**). Consistent with the Penn COPE and

262  MESSI cohorts, patients with hematologic cancers did poorly, with a mortality rate of 44.4%

263  (**Fig. 4a, Supplementary Table 5**). Clinical grade 12-parameter flow cytometry and serologic

264  testing for SARS-CoV-2-specific antibodies were performed. In the MSKCC cohort, both CD4

265  and CD8 T cells were significantly decreased in patients with active solid and hematologic

266  cancers, compared with patients in clinical remission (**Extended Data Fig. 5a**). Moreover,

267  despite the fact that a substantial number of patients with hematologic cancers from the MSKCC

268  cohort received convalescent plasma, they had a significant defect in SARS-CoV-2-specific IgG

269  and IgM responses as compared to solid cancers (**Extended Data Fig. 5b**). This was

270  independent of disease severity and viral load, as assessed by RT-PCR cycle threshold.

271  (**Extended Data Fig. 5c,d**).

272       We performed high dimensional analyses on flow cytometry data that included

273    information on CD4 T cells, CD8 T cells, and B cells. EMD and clustering of 20 solid cancer, 31

274    hematologic cancer, and 6 remission patients identified 4 immune phenotypes (**Extended Data**

275    **Fig. 6a,b and Fig. 4b,c**) that corresponded to the immune phenotypes 1,2,4, and 5 identified in

276    the Penn-MESSI cohort (**Fig. 3c,d**).  The Penn phenotype 3, the only cluster that did not have

277    cancer patients, was not identified in the MSKCC cancer cohort. Consistent with the Penn data,

278    MSKCC EMD cluster 5, with depleted of CD4 and CD8 T cells and preserved B cells, had the

279    highest mortality of 71%, and was associated with a high disease severity and viral load (**Fig**

280    **4d**).

281          Intriguingly, the clinical outcomes of patients with immune phenotype 4 was the greatest

282    contributor to the overall mortality difference between patients with solid and liquid cancers;

283    hematologic cancer patients with phenotype 4 had a mortality of 61% versus 9% in patients with

284    solid cancers (**Extended Data Fig. 7a**), with a corresponding higher viral load as assessed by

285    RT-PCR threshold cycle (**Extended Data Fig. 7b**). Immune phenotype 4 was characterized by

286    robust CD4 responses and decreased, but still intact, CD8 responses (**Extended Data Fig 6b)**.

287    Within immune phenotype 4, patients with solid and hematologic cancers had similar CD4 and

288    CD8 T cell counts (**Extended Data Figure 7c**). However, patients with hematologic cancers had

289    near-complete abrogation of B cells (phenotype 4A), that corresponded with a mortality rate of

290    61% (**Extended Fig 7a and d**). In contrast, patients with solid cancers had intact B cells counts

291    (phenotype 4B, **Extended Data Fig 7a and d**), with a mortality of 9%. Thus, in a setting with

292    similar CD4 and CD8 T cell numbers, B cell depletion was associated with higher mortality; B

293    cells, therefore, likely play an important role in acute COVID-19.

294          Anti-CD20 therapy (αCD20) with rituximab or obinutuzumab-containing regimens

295    depleted B cells with near-complete abrogation of SARS-CoV-2-specific IgG and IgM responses

296    (**Fig. 4e**). Notably, hematologic cancer patients on chemotherapy and solid cancer patients on

297    immune checkpoint blockade also had significant depletion of B cells (**Extended Data Fig. 8a**).

298   αCD20 therapy was not associated with quantitative changes in CD4 and CD8 T cells. However,

299   patients treated with anti-CD20 therapy displayed dramatic reduction in CD4 and CD8 naïve

300   and memory T cells, instead skewing towards effector differentiation and an activated HLA-

301   DR+CD38+ phenotype (**Extended Data Fig. 8b,c**). Importantly, despite the loss of B cells and

302   humoral immunity, αCD20 therapy was not associated with increased mortality, disease

303   severity, or viral load when compared to chemotherapy or observation (**Fig. 4f**).

304        We sought to understand why αCD20 therapy was not associated with greater mortality

305   in these patients. Patients treated with αCD20 therapy were restricted to immune phenotypes 1

306   and 4, characterized by depleted B cells (**Fig 4g**). However, phenotype 1, characterized by

307   preserved CD8 T cells, was associated with a lower mortality (**Fig 4h**). Indeed, αCD20 treated

308   patients who survived their COVID-19 hospitalization had higher CD8 T cell counts (**Fig 4i**), and

309   lower viral load (**Extended Data Fig. 9a**). We extended these analyses to other patients with

310   hematologic cancers, including those on chemotherapy who also had quantitative (**Extended**

311   **Data Fig. 8a**), and possibly qualitative B cell defects. Hematologic cancer patients who survived

312   had higher CD8 T cell count (**Fig. 4j**), which was not seen in solid cancer patients (**Extended**

313   **Data Fig. 9b**). Conversely, CD4 T cell counts were not associated with mortality, and higher B

314   cell counts were associated with increased mortality (**Extended Data Fig. 9b, Fig 4j**). Thus,

315   patients with hematologic cancers, in the setting of defective humoral immunity, were more

316   highly dependent on adequate CD8 T cell counts than patients with solid cancers. Finally,

317   Classification and Regression Tree Analysis (CART) identified a CD8 T cell level that was

318   predictive of survival after COVID-19 in patients with hematologic cancers (**Fig. 4k**). Taken

319   together, these findings suggest that CD8 T cells are critical for anti-viral immunity in

320   hematologic malignancy patients and may at least partially mitigate the negative impact of B-cell

321   depletion on COVID outcomes.

322

**Discussion**

324    A notable feature of the COVID-19 pandemic has been the dramatic heterogeneity in clinical

325    presentations and outcomes, yet mechanistic explanations for the wide variance in disease

326    severity have remained elusive. Early on, acute phase reactants and systemic cytokines were

327    implicated in patient outcomes[46] and hospital stay and mortality were decreased by

328    dexamethasone[47], suggesting that an excessive host immune response might contribute to

329    COVID-19 mortality. However, there were also indications that inadequate host immunity might

330    contribute to adverse COVID-19 outcomes, including the association of lymphopenia with

331    mortality as well as the potentially inferior outcomes of patients on chronic immunosuppression,

332    such as patients with autoimmune diseases or organ transplant recipients[48–51]. Recent studies

333    defined immune signatures associated with severe COVID-19, including activated CD4, CD8 T

334    cells, plasmablasts, and robust antibody responses[15, 16, 20, 52]. Nevertheless, the individual roles

335    of these cell types in acute COVID-19 remained unclear. We speculated that investigating both

336    the clinical outcomes and immunologic profile of cancer patients might shed valuable insight into

337    how arms of the immune system contribute to viral control and mortality during COVID-19.

338    Immune investigation in hematologic malignancies is especially relevant because the disease

339    directly impacts the lymphoid and myeloid immune cells, and is commonly treated with

340    myelosuppressive and B cell-depleting therapies including CD20 targeting antibodies.

341    Our investigation reveals several novel findings. First, we establish in a prospective

342    clinical cohort that hematologic malignancy is an independent predictor of COVID-19 mortality

343    after adjusting for ECOG performance and disease status. We observed a higher mortality rate

344    in patients with hematologic (53%) versus solid cancers (34%), which were substantially higher

345    than in the general population (2.7%)[28]. The high mortality rates for hematologic cancer in this

346    study were consistent with a recent meta-analysis of 2,361 hospitalized patients with

347    hematologic cancer (40%)[53]. This finding highlights the importance of transmission mitigation

348    efforts for this vulnerable population[54]. Furthermore, we demonstrate that excess mortality

349    observed with hematologic cancers persisted (HR 2.5) after adjustment for independent

350    predictors of cancer mortality, including age, smoking history, poor performance status, and

351    active or advanced disease. Adjustment for these factors was necessary to determine that the

352    increased mortality difference seen in hematologic cancer was in fact, driven by cancer subtype,

353    rather than differences in patient characteristics. These data can better inform hospitalized

354    patients with hematologic cancers of their expected outcomes, irrespective of performance

355    status or active cancer status, thereby improving decision-making between best supportive care

356    or aggressive interventions. The disease-specific increased risk of COVID-19 associated

357    mortality in hematologic cancer patients may also influence the prioritization and distribution of

358    vaccinations to this very high-risk population.

359         Second, using high dimensional analyses, we define immune phenotypes associated

360    with mortality during COVID-19. In particular, we identify the immune phenotype that drives the

361    mortality difference between solid and liquid malignancy. A balanced immunity that included

362    CD4, CD8, and B cells responses (phenotypes 2 and 4b) was associated with low mortality. In

363    contrast, an immune signature with robust B cell and humoral responses, but absent T cell

364    responses (phenotype 5), was associated with the highest mortality (>60%). A high mortality for

365    patients with immune phenotype 5 was consistent in both the Penn and MSKCC cohorts, and in

366    patients with solid cancer, hematologic cancer, and infected patients without cancer. Thus,

367    humoral immunity alone is often not sufficient in acute COVID-19. In fact, greater B cell

368    responses was associated with higher mortality in both solid and liquid cancer. B cell responses

369    may be a marker of disease severity, as seen with plasmablasts[15, 20] and neutrophils[20, 55, 56] in

370    severe COVID-19. Alternatively, some components of the B cell and humoral responses may be

371    aberrant and pathogenic, as may be the case with autoantibodies targeting type I interferons in

372    severe COVID-19[57].

373         Consistent with recent data[58], patients with solid cancers had a similar cellular immune

374     landscape and SARS-CoV-2-specific IgG responses as compared to patients without cancer.

375     Patients with hematologic cancers, however, had substantial defects in B cells and humoral

376     immunity. These defects were associated with a high mortality or 45%, as compared to 25% in

377     solid cancers. This difference in survival was driven by immune phenotype 4, which was

378     characterized by robust CD4 T cell responses in conjunction with a diminished, but not absent

379     CD8 T response. This phenotype (phenotype 4B), in the setting of preserved B cells seen in

380     solid cancer patients, was associated with a low mortality of 9%. However, this phenotype in the

381     setting of depleted B cells (phenotype 4A) seen in liquid cancer patients, was associated with a

382     mortality of 61%. This highlights the fact that CD8 T cell responses that are normally sufficient

383     may no longer be adequate in the setting of compromised humoral immunity. Thus, CD4 or B

384     cells responses, in the absence of an intact CD8 T cell response, may not be sufficient to

385     control an acute SARS-CoV-2 infection. This is reminiscent of published data demonstrating

386     that uncoordinated immune responses in the elderly was associated with severe disease and

387     poor outcomes[59].

388           Finally, by leveraging a population of COVID-19 patients in the setting of B cell depletion

389     (anti-CD20), we uncovered a critical protective role for CD8 T-cell responses. CD8 T cells are

390     known to be critical for viral clearance, particularly in response to higher viral inocula[60]. Recent

391     data from transgenic mouse models show that both CD4 and CD8 T cells are necessary for

392     optimal viral clearance of SARS-CoV-2[61]. In patients treated with anti-CD20, absolute CD8 T

393     cell count, but not CD4 counts, was associated with survival from COVID-19 and lower viral

394     load. Although conclusions are limited by sample size, these data suggest that CD8 T cells play

395     a key role in limiting SARS-CoV-2, even in the absence of humoral immunity. Indeed, SARS-

396     CoV-2-specific CD8 T cell responses have been identified in acute and convalescent

397     individuals[59, 62–65]. Further, in our cohort, absolute CD8 counts were predictive of outcomes in

398     the broader cohort of patients with hematologic malignancy. The compensatory role of CD8 T

399     cells was restricted to patients with hematologic, but not solid, malignancies. Thus, CD8 T cells

400    likely play an important role in the setting of quantitative and qualitative B cell dysfunction in

401    patients with lymphoma, multiple myeloma, and leukemia, undergoing anti-CD20,

402    chemotherapy, or Bruton tyrosine kinase (BTK) inhibition. CD8 T cell counts may inform on the

403    need for closer monitoring and a lower threshold for hospitalization in COVID-19 patients with

404    hematologic malignancies. Furthermore, the clinical benefit of dexamethasone, which

405    demonstrated an overall mortality benefit in hospitalized COVID-19+ patients but is known to

406    suppress CD8 T cell responses[66], should be investigated further in patients who recently

407    received anti-CD20 therapy.

408        Recent analysis demonstrated that patients treated with B-cell depleting agents had the

409    highest mortality rate, although this analysis did not account for whether the risk was modulated

410    by CD8 count. Our findings do not exclude the possibility that B-cell depleting therapies may be

411    associated with adverse outcomes in this population but rather extend these findings to suggest

412    that an adequate CD8-dependent T cell response is essential for patients in whom humoral

413    immunity is compromised. We did, however, observe a profound depletion of both naive CD4

414    and CD8 T cells in patients receiving B-cell depleting agents. Naive T-cells, and particularly

415    naive CD4 T cells, are known to require tonic TCR signaling driven by APC-presented self-

416    antigens for persistence[67, 68]. We speculate that depletion of functional B cells, particularly in the

417    context of B cell depleting therapy, might lead to concomitant naive T cell depletion and a

418    corresponding increase in the effector and activated CD8 T cells. Although the clinical relevance

419    of naive T cell depletion in the setting of anti-CD20 is still unclear - it is notable that depletion of

420    naive T cells in the elderly was associated with increased disease severity and poor

421    outcomes[59].

422        Importantly, both B-cell depleting therapies and cytotoxic chemotherapy agents which

423    can compromise the T-cell compartment are mainstays of lymphoma therapy. Both are

424    administered, often in combination, with curative intent for patients with aggressive lymphomas,

425    but also for debulking or palliation in patients with indolent lymphomas. Based on our data, we

426    would suggest that oncologists and patients considering treatment regimens that combine B cell

427    depletion with cytotoxic agents carefully weigh the associated increased risk of immune

428    dysregulation against the benefit of disease control when making an educated decision on

429    whether to initiate such treatments, particularly in non-curative settings.

430         Finally, our finding that CD8 T cell immunity is critical for survival in hematologic

431    malignancy patients with COVID-19 has profound implications for the vaccination of these

432    patients.  Both the Pfizer and Moderna vaccines, as well as the Johnson and Johnson vaccine

433    currently under investigation, induce robust CD8 T cell responses in addition to humoral

434    responses[69–71]. Our findings suggest that vaccination of hematologic patients might enhance the

435    protective capacity of CD8 T-cells despite the likely absence of a humoral response. We are

436    conducting ongoing studies to monitor the immune profile of patients undergoing vaccination

437    prospectively to determine if this is the case.  Ultimately, understanding how the immune

438    response relates to disease severity, cancer type, and cancer treatment will provide important

439    insight into the pathogenesis of and protective immunity from SARS-CoV-2, which may have

440    implications for the development and prioritization of therapeutics and vaccines in cancer

441    subpopulations.

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**Methods:**

**COVID-19 Outcomes in Patients with Cancer, COPE**

General Design/Patient Selection

We conducted a prospective cohort study of patients with cancer hospitalized with COVID-19 (UPCC 06920). Informed consent was obtained from all patients. Adult patients with a current or prior diagnosis of cancer and hospitalized with a probable or confirmed diagnosis of COVID-19, as defined by the WHO criteria[72], within the University of Pennsylvania Health System (UPHS) between April 28, 2020 and September 15, 2020 were approached for consent. Participating hospitals included the Hospital of the University of Pennsylvania, Presbyterian Hospital, Pennsylvania Hospital, and Lancaster General Hospital. The index date was defined as the first date of hospitalization within the health system for probable or confirmed COVID-19. Repeat hospitalizations within 7 days of discharge were considered within the index admission. Patients who died prior to being approached for consent were retrospectively enrolled. Patients were followed from the index date to 30-days following their discharge or until death by any cause. This study was approved by the institutional review boards of all participating sites.

Data Collection

Baseline characteristics including patient (age, gender, race/ethnicity, comorbidities, smoking history, body mass index) and cancer (tumor type, most recent treatment, ECOG performance status, active cancer status) factors as well as COVID-19 related clinical factors including change in levels of care, complications, treatments such as need for mechanical ventilation, laboratory values (complete blood counts with differentials and inflammatory markers including LDH, CRP, ferritin, and IL-6), and final disposition were extracted by trained research personnel using standardized abstraction protocols. Active cancer status was defined by diagnosis or treatment within 6 months of admission date. Cancer treatment status was determined by the most recent treatment within 3 months prior to admission date.

475    The primary study endpoint was all-cause mortality within 30-days of hospital discharge.

476    Disease severity was categorized using the NIH ordinal scale including all post-hospitalization

477    categories: 1,hospitalized, not requiring supplemental oxygen but requiring ongoing medical

478    care; 2, hospitalized requiring any supplemental oxygen; 3, hospitalized requiring noninvasive

479    mechanical ventilation or use of high-flow oxygen devices; 4, hospitalized receiving invasive

480    mechanical ventilation or extracorporeal membrane oxygenation (ECMO); 5, death[73] , and was

481    assessed every 7 days throughout a patients admission.

482    Statistical Analysis

483    Cohort characteristics were compared using standard descriptive statistics. One-time

484    imputation of missing values for ECOG was done using the predicted mean value from an

485    ordinal logistic model (proportional odds) of complete data. The ordinal model was fitted with

486    forward stepwise selection, with entry at p=0.1 and removal at 0.2, using clinical variables

487    expected to be correlated with ECOG performance status.  Those variables included several

488    items in the Charlson and severity score, and other clinical variables.

489    Univariate analyses examined demographic and clinical variables and cancer subtype

490    (hematologic versus solid cancer) as predictors of death within 30 days of discharge and of ICU

491    admission.  Odds ratios and 95% CIs were used to generate the forest plot illustration. Baseline

492    laboratory tests were compared by cancer type using Mann Whitney tests and available RT-

493    PCR data was used to determine length of RT-PCR positivity by cancer type.

494    Rates of ICU admission and death were calculated for the overall cohort and

495    stratified by cancer subtype. A multivariate logistic model was used to examine the

496    adjusted effect of solid versus hematologic designation.  Covariates included

497    demographic variables of age and sex (race was omitted for missing data).  Covariates

498    also included clinical variables that attained a p-value of 0.1 in the univariate analyses.

499    The final model included age, sex, smoking status, active disease status, and ECOG

500    performance status. A cox proportional hazards regression model was also performed

501    to determine the association between cancer type and mortality and identically adjusted

502    for age, sex, smoking status, active cancer status, and ECOG performance

503    status.  Overall survival (OS) was measured from date of hospitalization to last follow up

504    or death and the median OS was estimated using Kaplan-Meier method and differences

505    by cancer subtype compared using log-rank test.

506

507    **Immune profiling of patients hospitalized for COVID-19, MESSI**

508    Information on clinical cohort, sample processing, and flow cytometry is described in Mathew et

509    al, Science 2020. Briefly, Patients admitted to the Hospital of the University of Pennsylvania with

510    a positive SARS-CoV-2 PCR test were screened and approached for informed consent within 3

511    days of hospitalization. Peripheral blood was collected from all subjects and clinical data were

512    abstracted from the electronic medical record into standardized case report forms. All

513    participants or their surrogates provided informed consent in accordance with protocols

514    approved by the regional ethical research boards and the Declaration of Helsinki. Methods for

515    PBMC processing, flow cytometry, and antibodies used were previously described[15].

516

517    **Serologic enzyme-linked immunosorbent assay (ELISA)**

518         ELISAs were completed using plates coated with the receptor binding domain (RBD) of

519    the SARS-CoV-2 spike protein as previously described[44]. Briefly, Prior to testing, plasma and

520    serum samples were heat-inactivated at 56°C for 1 hour. Plates were read at an optical density

521    (OD) of 450nm using the SpectraMax 190 microplate reader (Molecular Devices). Background

522    OD values from the plates coated with PBS were subtracted from the OD values from plates

523    coated with recombinant protein. Each plate included serial dilutions of the IgG monoclonal

524    antibody CR3022, which is reactive to the SARS-CoV-2 spike protein, as a positive control to

525   adjust for inter assay variability. Plasma and serum antibody concentrations were reported as

526   arbitrary units relative to the CR3022 monoclonal antibody. A cutoff of 0.48 arbitrary units was

527   established from a 2019 cohort of pre-pandemic individuals and used for defining seropositivity.

528

529   **Flow Cytometry and statistical analysis**

530   Samples were acquired on a 5 laser BD FACS Symphony A5. Standardized SPHERO

531   rainbow beads (Spherotech, Cat#RFP-30-5A) were used to track and adjust PMTs over time.

532   UltraComp eBeads (ThermoFisher, Cat#01-2222-42) were used for compensation. Up to 2 ×

533   $10^6$ live PBMC were acquired per each sample. During the early sample acquisition period,

534   three antibodies in the flow panel were changed. Three cancer patients and twelve non-cancer

535   patients were stained using this earlier flow panel. Flow features of these patients were visually

536   assessed for batch variations against data from the later flow panel. The three cancer patients

537   were included with the rest of the cohort when batch effects were determined to have little

538   impact on confidence in gated populations. These three cancer patients were excluded in

539   analysis of cell populations defined by proteins associated with the three changed antibodies.

540   Due to the heterogeneity of clinical and flow cytometric data, non-parametric tests of

541   association were preferentially used throughout the study. Tests of association between mixed

542   continuous variables versus non-ordered categorical variables (n=2) were performed by Mann-

543   Whitney test. Tests of association between binary variables versus non-ordered categorical

544   variables (n=2) were performed using Pearson Chi Square test. All tests were performed using

545   a nominal significance threshold of $P<0.05$ with Prism version 9 (GraphPad Software) and Excel

546   (Microsoft Office Suite). Classification and Regression Tree analysis (CART) was performed

547   using R package 'rpart'.

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550   **High dimensional data analysis of flow cytometry data**

551    UMAP analyses were conducted using R package *uwot*. FlowSOM analyses were

552    performed on Cytobank (https://cytobank.org). Lymphocytes and non-naive CD8 T cells were

553    analyzed separately. An artifact due to monocyte contamination was removed from the FCS as

554    defined by high CD16 and side scatter area (SSC-A). UMAP analysis was performed using

555    equal down sampling of 10000 cells from each FCS file in lymphocytes and 1500 cells in non-

556    naive CD8 T cells, with a nearest neighbors of 15, minimum distance of 0.01, number of

557    components of 2, and a euclidean metric. The FCS files were then fed into the FlowSOM

558    clustering algorithm. A new self-organizing map (SOM) was generated for both lymphocytes and

559    non-naive CD8 using hierarchical consensus clustering. For each SOM, 225 clusters and 10

560    metaclusters were identified. For lymphocytes, the following markers were used in the UMAP

561    and FlowSOM analysis: CD45RA, PD-1, IgD, CXCR5, CD8, CD19, CD3, CD16, CD138,

562    Eomes, TCF-1, CD38, CD95, CCR7, CD21, Ki-67, CD27, CD4, CX3CR1, CD39, T-bet, HLA-

563    DR, and CD20. For non-naive CD8 T cells, the following markers were used: CD45RA, PD-1,

564    CXCR5, CD16, Eomes, TCF-1, CD38, CCR7, Ki-67, CD27, CX3CR1, CD39, T-bet, and HLA-

565    DR. For FlowSOM analysis of non-naive CD8 T cells, two patients at day seven without data

566    from day zero were included. Heatmaps were visualized using R function *pheatmap*.

567    To group individuals based on lymphocyte landscape, pairwise Earth Mover's Distance

568    (EMD) value was calculated on the lymphocyte UMAP axes using the *emdist* package in R.

569    Resulting scores were hierarchically clustered using the *hclust* package in R.

570

571    **Immune profiling of patients hospitalized for COVID-19, MSKCC**

572    Patients admitted to Memorial Sloan Kettering Cancer Center with a positive SARS-CoV-2 PCR

573    test were eligible for inclusion.  For inpatients, clinical data were abstracted from the electronic

574    medical record into standardized case report forms. Clinical laboratory data were abstracted

575    from the date closest to research blood collection. Peripheral blood was collected into BD

576    Horizon Dri tubes (BD, Cat#625642). Immunophenotyping of peripheral blood mononuclear

577 cells via flow cytometry was performed in the MSKCC clinical laboratory. The lymphocyte panel

578 included CD45 FITC (BD, 340664, clone 2D1), CD56+16 PE (BD 340705, clone B73.1; BD

579 340724, clone NCAM 16.2), CD4 PerCP-Cy5.5 (BD 341653, clone SK3), CD45RA PC7 (BD

580 649457, clone L48), CD19 APC (BD 340722, clone SJ25C1), CD8 APC-H7 (BD 641409, clone

581 SK1), and CD3 BV 421 (BD 562426, clone UCHT1). The naive/effector T panel included CD45

582 FITC (BD 340664, clone 2D1), CCR7 PE (BD 560765, clone 150503), CD4 PerCP-Cy5.5 (BD

583 341653, clone SK3), CD38 APC (BioLegend, 303510, clone HIT2), HLA-DR V500 (BD 561224,

584 clone G46-6), CD45RA PC7 (BD 649457, clone L48), CD8 APC-H7 (BD 641409, clone SK1),

585 and CD3 BV 421 (BD 562426, clone UCHT1). The immune phenotypes were based on NIH

586 vaccine consensus panels and the Human Immunology Project[74]. Samples were acquired on a

587 BD Facs Canto using FACSDiva software.

588

589 **Data Availability Statement:** Flow Cytometry data collected in this study was deposited to the
590 Human Pancreas Analysis Program (HPAP-RRID:SCR_016202) Database and Cytobank62
591

**Author contributions**
ACH, RM, SV, and EMB conceived the project; ACH, SV, NAH designed all experiments. ACH, RM, EMB, AMD, IPM conceived the PENN COPE cohort. EMB, JR, FP, OO, KN, CZ, MG, ARW, CAGI, EMK, CR, KRB, ST, and CW enrolled patients and collected data for COPE. EMB, RM, AMD, and ACH designed data and statistical analysis for COPE. PW performed statistical analysis for COPE. NJM conceived the PENN MESSI-COVID clinical cohort, ARW, CAGI, OO, RSA, TGD, TJ, HMG, JPR, and NJM enrolled patients and collected data for MESSI-COVID. NAH, AEB, and JYK performed downstream flow cytometry analysis for MESSI-COVID. SG, MEW, CMM, SEH analyzed COVID-19 patient plasma and provided antibody data. NAH, JRG, ARG, CA, DAO performed computational and statistical analyses. CA compiled and JRG, DO, and CA analyzed clinical metadata for MESSI-COVID.
SV and JW conceived the MSKCC cohort. SV, AK, AW, SD, and SB provided clinical samples from MSKCC Cohort. PM performed downstream flow cytometry analysis. NEB performed quantitative PCR experiments for COVID viral load. NAH and ACH compiled figures. KNM, LS, RHV, JDW, EJW, provided intellectual input. EMB, SV, RM, and ACH wrote the manuscript; all authors review the manuscript.

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828

**Table 1 | COPE: Patient demographics and clinical characteristics.**

| | Total (N=100) |
|---|---|
| **Age, median (IQR)** | 68 (57.5-77.5) |
| **Gender, female** | 48 (48%) |
| **Race** | |
| Black | 54 (54%) |
| White | 33 (33%) |
| Asian | 4 (4%) |
| Hispanic | 3 (3%) |
| Unknown | 6 (6%) |
| **Smoking History, Ever[+]** | 57 (57%) |
| **Comorbidities** | |
| Cardiac | 78 (78%) |
| Pulmonary | 41 (41%) |
| **Use of immunosuppressive drugs[++]** | 30 (30%) |
| **BMI, median (IQR)** | 26.84 (23.2-31.5) |
| **Cancer Type** | |
| Solid malignancy | 78 (78%) |
| Genitourinary | 19 (19%) |
| Breast | 14 (14%) |
| Gastrointestinal | 14 (14%) |
| Thoracic | 9 (9%) |
| Other[+++] | 8 (8%) |
| Gynecologic | 7 (7%) |
| Head and Neck | 4 (4%) |
| Sarcoma | 3 (3%) |
| Heme malignancy | 22 (22%) |
| Lymphoma | 10 (10%) |
| Leukemia | 7 (7%) |
| Myeloma | 3 (3%) |
| MDS/MPN | 2 (2%) |
| **Cancer Status, Active[#]** | 46 (46%) |
| **Cancer treatment in last 3 months** | |
| Active surveillance/surgery | 53 (53%) |
| Cytotoxic Chemotherapy | 24 (24%) |
| Hormone therapy | 15 (15%) |
| Other* | 8 (8%) |
| **ECOG Performance Status** | N=73 |
| 0-1 | 37 (50.7%) |
| 2 | 13 (17.8%) |
| 3-4 | 23 (31.5%) |

[+]Current or prior smoker
[++]Exposure to immunosuppressive medications not including cancer treatment
[+++]Tumor types with less than 2 subjects: CNS-2, Thyroid-2, Thymus-1, Neuroendocrine-1
[#]Diagnosis or treatment within 6 months
*Single agent immunotherapy, targeted therapy, monoclonal antibodies

**Table 2 | COPE: COVID-19 related treatment and outcomes.**

| | Total (N=100) | Solid (N=78) | Heme (N=22) |
|---|---|---|---|
| **COVID-19 Disease Severity** | | | |
| At Presentation | | | |
| No Supplemental Oxygen | 35 (35.0%) | 28 (35.9%) | 7 (31.85) |
| Supplemental Oxygen | 44 (44.0%) | 32 (41.0%) | 12 (54.6%) |
| Non-invasive ventilation | 9 (9.00%) | 7 (8.97%) | 2 (9.09%) |
| Invasive ventilation | 12 (12.0%) | 11 (14.1%) | 1 (4.55%) |
| Maximum throughout hospitalization | | | |
| No supplemental Oxygen | 28 (28.0%) | 24 (30.8%) | 4 (18.2%) |
| Supplemental Oxygen | 24 (24.0%) | 19 (24.4%) | 4 (18.2%) |
| Non-invasive ventilation | 11 (11.0%) | 8 (10.3%) | 3 (13.6%) |
| Invasive ventilation | 9 (9.00%) | 9 (11.5%) | 0 (0.00%) |
| Death | 28 (28.0%) | 18 (23.1%) | 12 (54.5%) |
| **COVID-19 Directed Treatment** | | | |
| Steroids | 51 (51.0%) | 39 (50.0%) | 12 (54.6%) |
| Remdesivir | 18 (18.0%) | 13 (16.7%) | 5 (22.7%) |
| Convalescent Plasma | 10 (10.0%) | 6 (7.69%) | 4 (18.2%) |
| **COVID-19 Outcomes** | | | |
| Thrombosis | 11 (11.0%) | 7 (9.09%) | 4 (18.2%) |
| Intubation | 28 (28.0%) | 21 (26.9%) | 7 (31.8%) |
| ICU admission | 48 (48.0%) | 37 (47.4%) | 11 (50.0%) |
| Death | 38 (38.0%) | 26 (33.3%) | 12 (54.6%) |
| Hospital Length of stay, median (IQR) | 8 (4-19) | 8 (4-20) | 8 (4-18) |

**Table 3 | COPE: Event rates and point estimates of outcomes by cancer type.**

| | Heme | Solid |
|---|---|---|
| **Death within 30 days of discharge** | | |
| Event rate (%) | 12 (54.6%) | 26 (33.3%) |
| Unadjusted OR (95% CI) | 2.4 (0.82-7.06) | ref |
| Adjusted OR (95% CI)[+] | 3.3 (1.01-10.8) | ref |
| Adjusted HR (95% CI)[+] | 2.6 (1.19-5.54) | ref |

[+]Logistic regression computed odds ratio (OR) and Cox regression computed hazard ratio (HR), respectively. Adjusted for age, gender, smoking status, active cancer status, and ECOG performance status.

**Fig. 1**
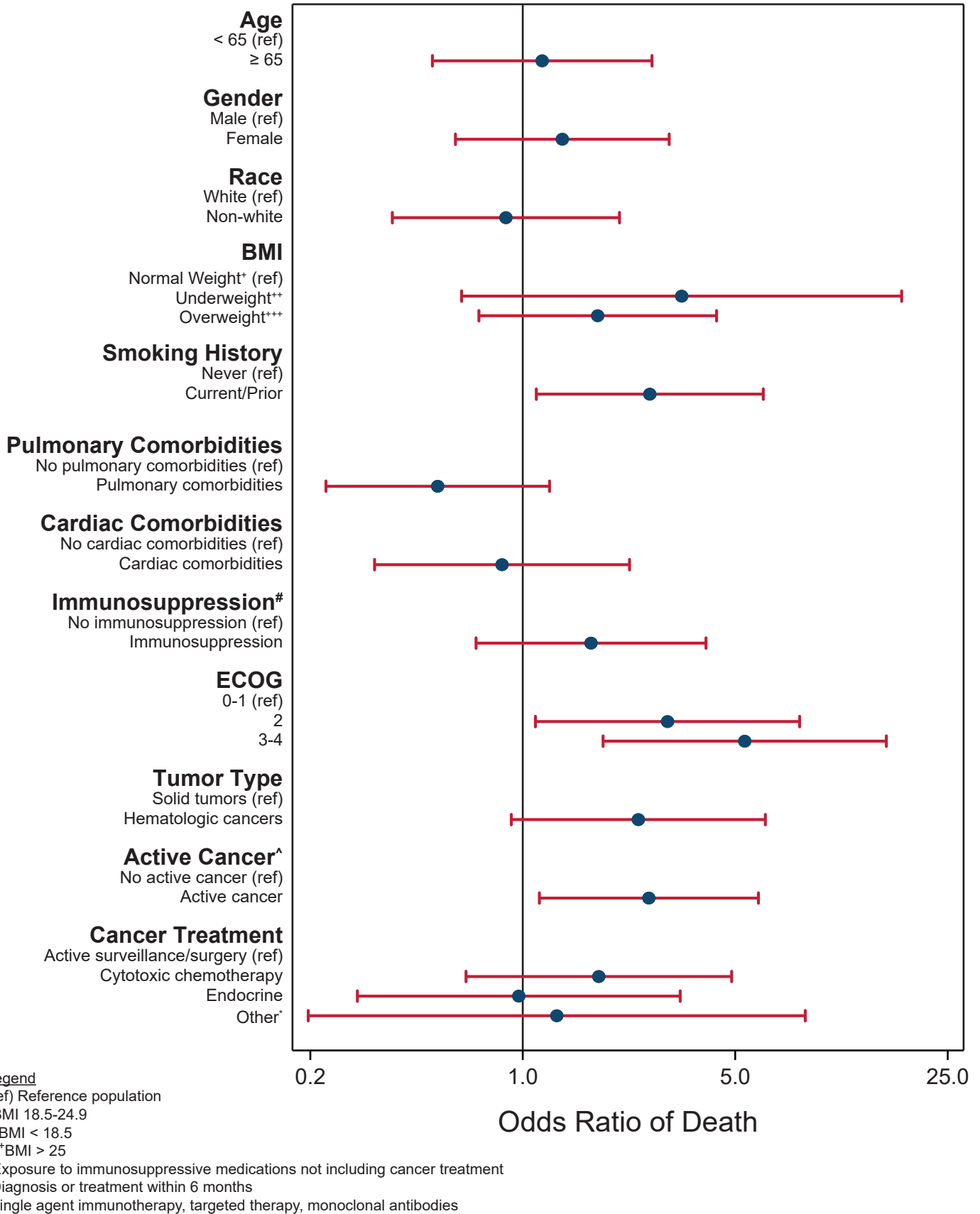


Fig. 1 | Univariate analysis of potential risk factors in COVID-19 mortality.

# Fig. 2
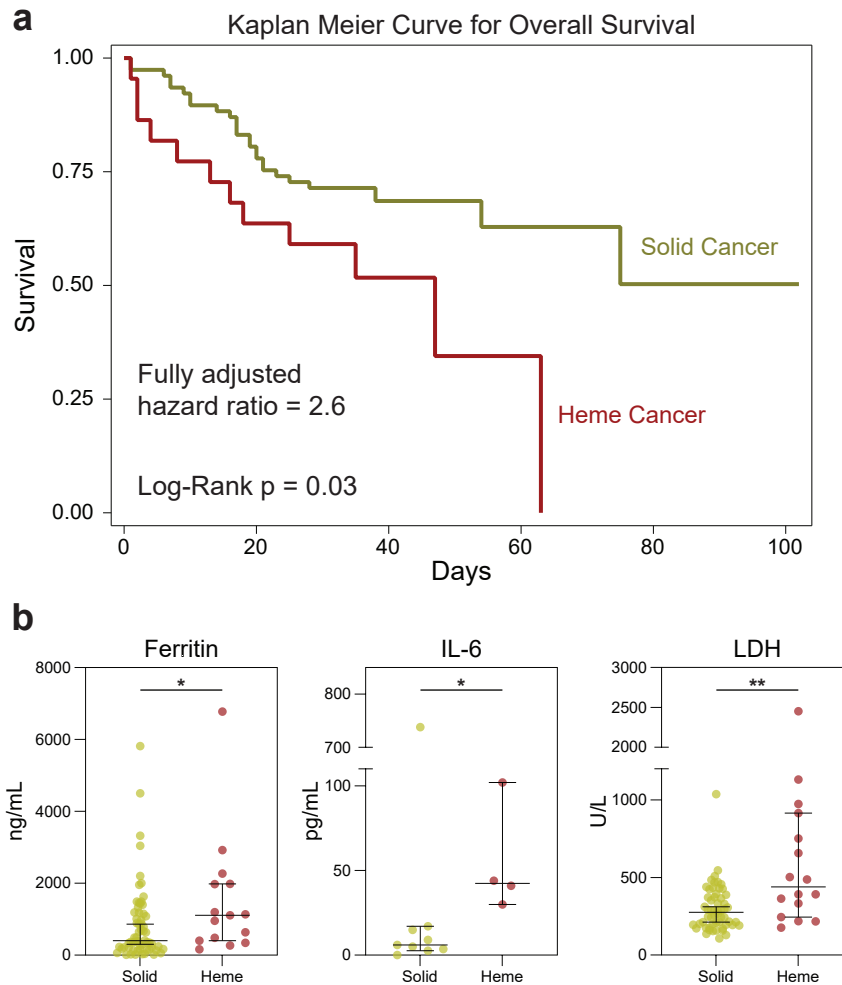


**Fig. 2 | Hematologic cancer is an independent risk factor for COVID-19 related mortality.** (**a**) Kaplan Meier curve for COVID-19 survival of patients with solid (n=77) and hematologic (n=22) cancer. Cox regression-computed hazard ratio for mortality in hematologic vs solid cancer, adjusted for age, gender, smoking status, active cancer status, and ECOG performance status. (**b**) Ferritin, IL-6, and LDH in solid (n=62) and hematologic (n=15) cancer hospitalized for COVID-19. (All) Significance determined by Mann Whitney test: *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. Median and 95% CI shown.

# Fig. 3



**Fig. 3 | High dimensional analyses reveal immune phenotypes associated with mortality and distinct phenotypes between solid and hematologic cancers.** (**a**) Demographic and mortality data for MESSI cohort at Penn. (**b**) Relative levels of SARS-CoV-2 IgG and IgM of solid (n=14) and hematologic (n=7) cancer patients and non-cancer patients (n=108). (**c**) (Left) Global UMAP projection of lymphocyte populations for all 45 patients pooled. (Right) Hierarchical clustering of Earth Mover's Distance (EMD) using Pearson correlation, calculated pairwise for lymphocyte populations. (**d**) UMAP projection of concatenated lymphocyte populations for each EMD cluster. (Yellow: High Density; Black; Low Density) (**e**) Heatmap showing expression patterns of various markers, stratified by EMD cluster. Heat scale calculated as column z-score of MFI. (**f**) Mortality, disease severity, and SARS-CoV-2 antibody data, stratified by EMD cluster (Cluster 5 n=5; Cluster 1,2,3,4 n=40). Mortality significance determined by Pearson Chi Square test. Severity assessed with NIH ordinal scale for COVID-19 clinical severity (1: Death; 8: Normal Activity)[15]. (**g**) UMAP projections of concatenated lymphocyte populations for solid cancer, hematologic cancer, and non-cancer patients. (**h**) CD8 and CD4 T cell and B cell frequencies in healthy donors (HD) (n=33), non-cancer (n=108), solid cancer (n=7), and heme cancer (n=4). (**i**) UMAP projection of non-naïve CD8 T cell clusters identified by FlowSOM. (**j**) (Top) UMAP projections of non-naïve CD8 T cells for non-cancer and cancer patients. (Bottom) UMAP projections indicating HLA-DR and CD38 protein expression on non-naive CD8 T cells for all patients pooled. (**k**) Frequency of activated FlowSOM clusters in HD (n=30), non-cancer (n=110), and cancer patients (n=8). (**l**) Representative flow plots and frequency of HLA-DR and CD38 co-expression in HD (n=30), non-cancer (n=110), solid cancer (n=7), and hematologic cancer (n=3) patients. (All) Significance determined by Mann Whitney test: *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. Median and 95% CI shown.

# Fig. 4



**Fig. 4 | CD8 T cell counts associated with survival in hematologic cancer patients with COVID-19.** (**a**) Demographic and mortality data of MSKCC cohort. (**b**) (Left) Hierarchical clustering of Earth Mover's Distance (EMD) using Pearson correlation, calculated pairwise for lymphocyte populations. (Right) Global UMAP projection of lymphocyte populations pooled. (**c**) UMAP projection of concatenated lymphocyte populations for each EMD cluster. (Yellow: High Density; Black: Low Density) (**d**) Mortality (Cluster 5 n=7; Cluster 1,2,4 n=50), severity, and RT-PCR cycle threshold (Cluster 1 n=14; Cluster 2 n=5; Cluster 4 n=24; Cluster 5 n=6) (Lower Ct: Higher viral load) stratified by EMD cluster. Mortality significance determined by Pearson Chi Square test. (**e**) Relative levels of SARS-CoV-2 IgG and IgM of patients with recent cancer treatments (solid tx n=9; heme αCD20 n=7; heme other tx n=5). (**f**) Mortality, severity, and RT-PCR cycle threshold stratified by cancer treatment (remission n=9; solid obs n=6; solid tx n=19; heme obs n=5; heme chemo n=4; heme αCD20 n=10). Severity assessed with NIH ordinal scale for COVID-19 clinical severity. (**g**) Recent cancer treatment of patients in each EMD cluster. (**h**) Mortality of patients treated with B cell depleting therapy in EMD cluster 1 (red) and EMD cluster 4 (blue). (**i**) Absolute CD8 and CD4 T cell counts in patients treated with B cell depleting therapy (alive n=7; dead n=4). (**j**) Absolute CD8 and CD4 T cell counts and B cell counts in hematologic cancer patients (alive n=17; dead n=18). (**k**) Kaplan-Meier curve for survival in hematologic cancer patients stratified by CD8 T cell counts (threshold = 55.9; log-rank hazard ratio) (>=55.9 n=28; <55.9 n=13). CD8 count threshold determined by Classification and Regression Tree (CART) analysis. (All) Significance determined by Mann Whitney test: *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. Median and 95% CI shown.

# Extended Data Fig. 1

**a**



**b**
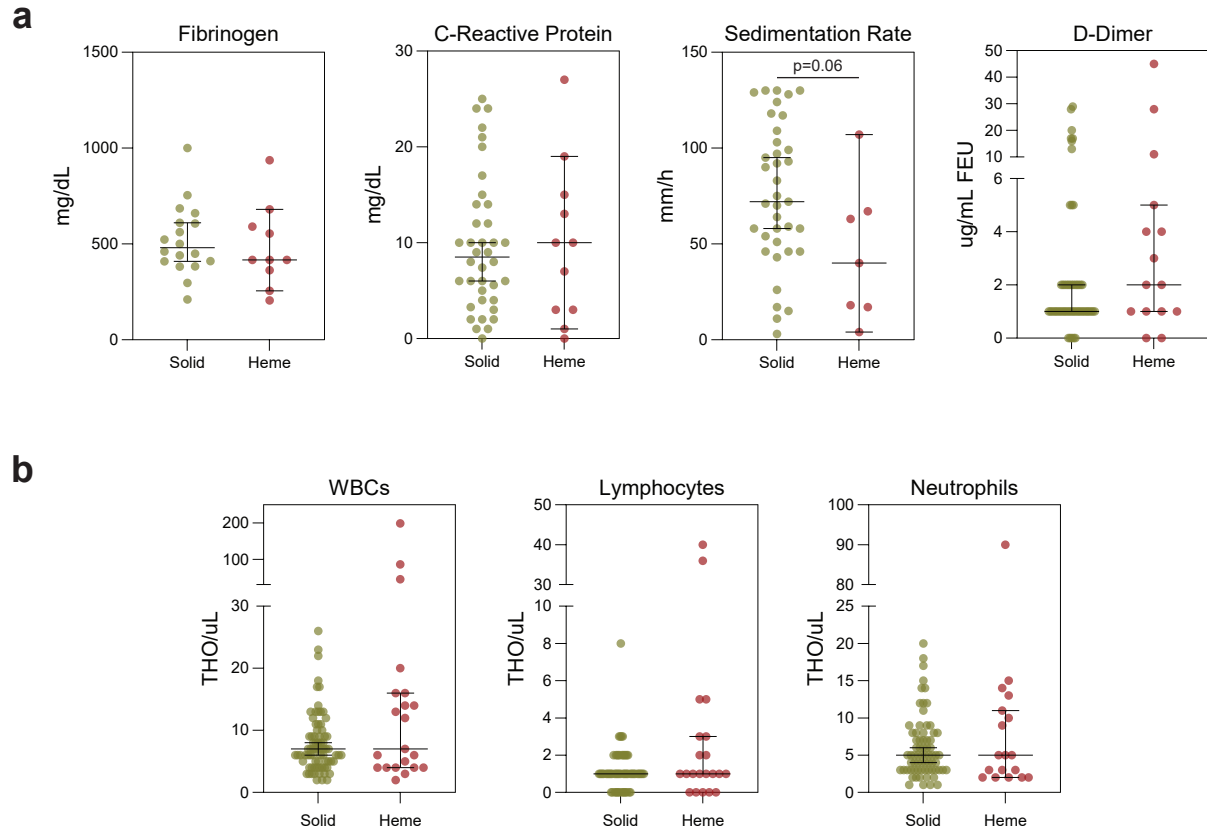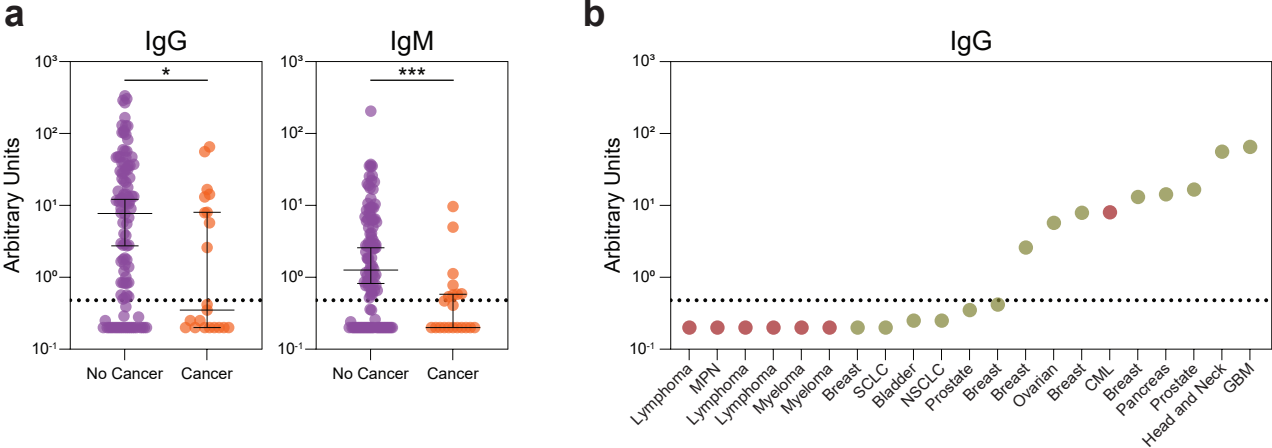


**Extended Data Fig. 1 | Inflammatory markers and blood cell counts in cancer patients with COVID-19.** Clinical laboratory values for (**a**) inflammatory markers and (**b**) cell counts in solid (n=62) and hematologic (n=21) cancer patients. (All) Significance determined by Mann Whitney test: *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. Median and 95% CI shown.

# Extended Data Fig. 2



**Extended Data Fig. 2 | SARS-CoV-2 antibody levels.** (**a**) Relative levels of SARS-CoV-2 IgG and IgM in non-cancer (n=108) and cancer (n=21) patients. (**b**) Relative IgG levels in cancer patients. Each dot represents a cancer patient (Heme: Red; Solid: Yellow). (All) Significance determined by Mann Whitney test: *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. Median and 95% CI shown.

# Extended Data Fig. 3

**a**



**b**
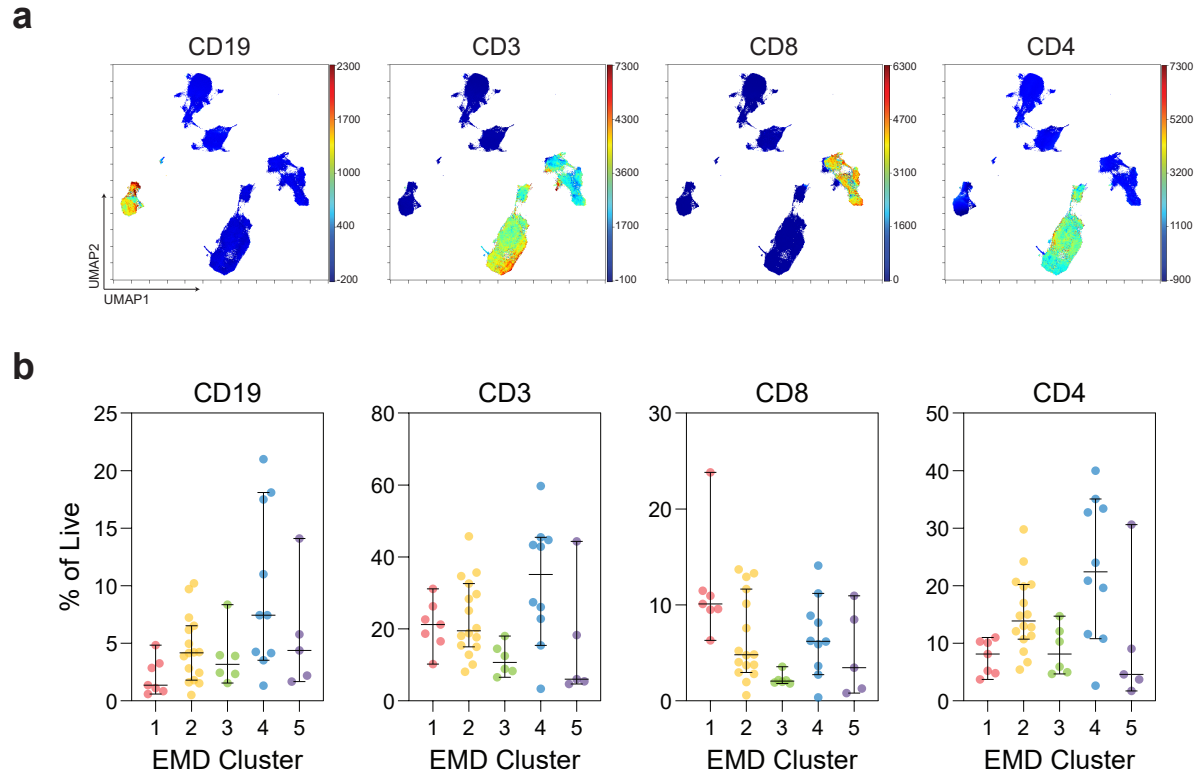


**Extended Data Fig. 3 | Dimensionality reduction and EMD clustering of MESSI cohort.** (**a**) UMAP projections of lymphocytes with indicated protein expression. (**b**) Frequencies of CD19+, CD3+, CD3+CD8+, and CD3+CD4+ cells of patients in each EMD cluster (Cluster 1 n=7; Cluster 2 n=16; Cluster 3 n=6; Cluster 4 n=10; Cluster 5 n=5). (All) Median and 95% CI shown.
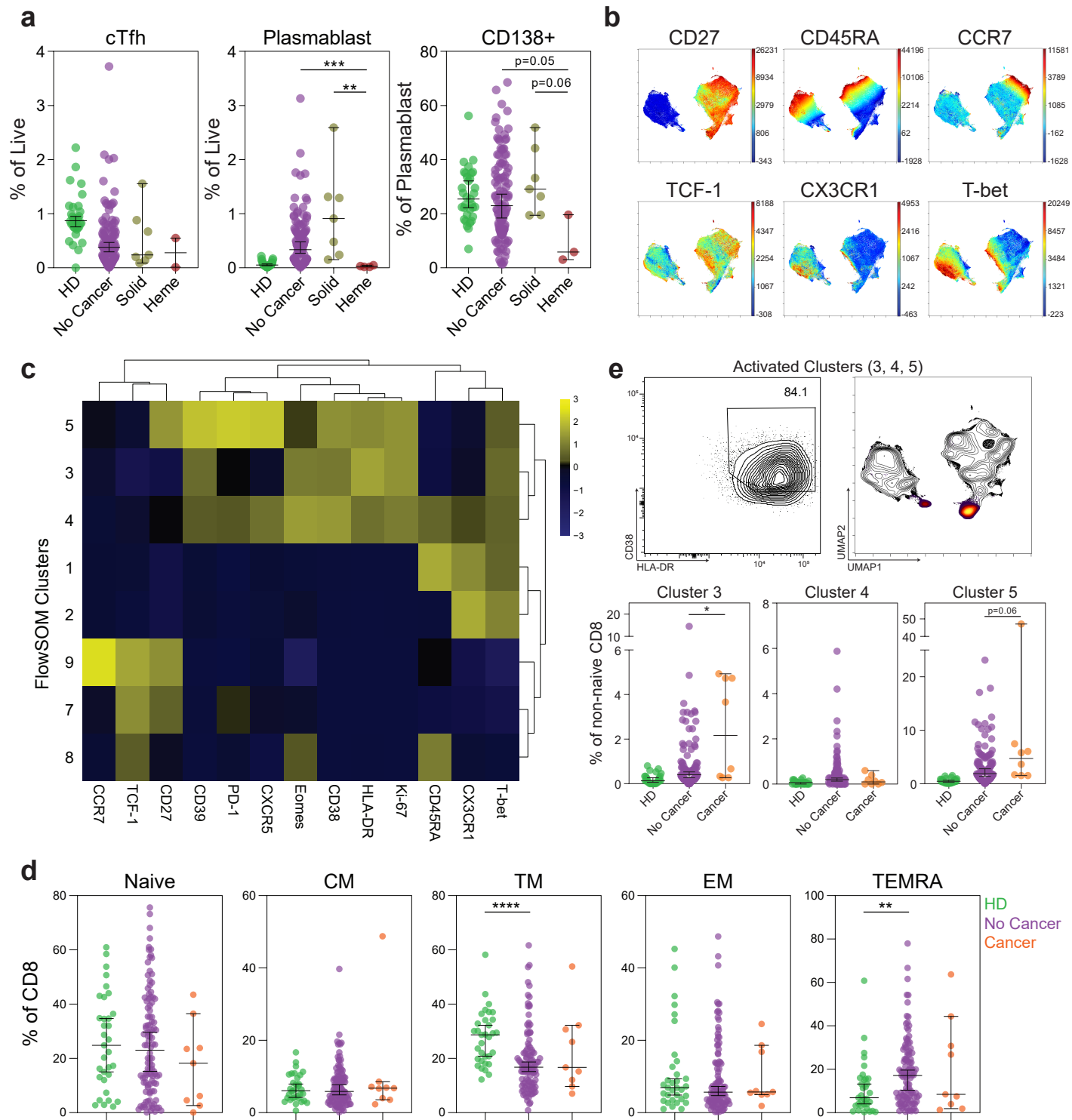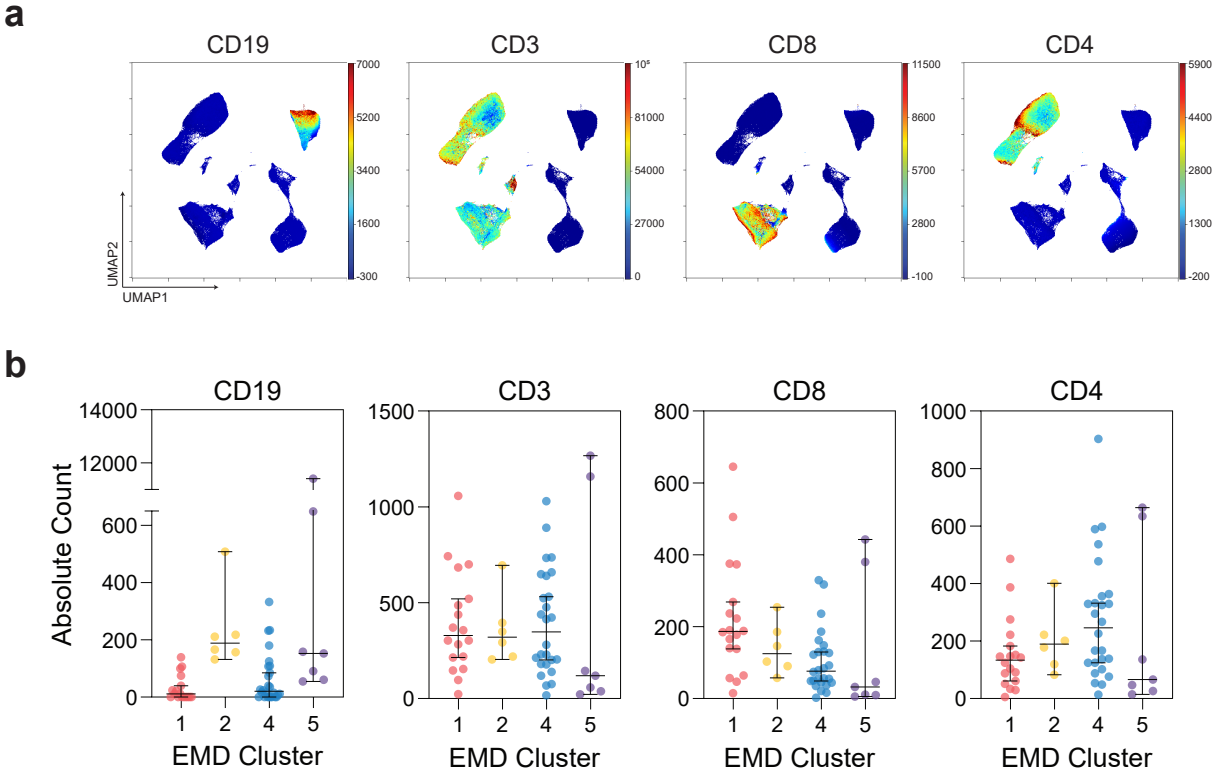
# Extended Data Fig. 4



**Extended Data Fig. 4 | Cellular phenotyping of COVID-19 patients with cancer.** (**a**) Frequencies of circulating T follicular helper cells (cTfh), plasmablasts, and CD138 expression on plasmablasts (HD n=33; non-cancer n=108; solid cancer n=7; heme cancer n=3). (**b**) UMAP projection of non-naïve CD8 T cells with indicated protein expression. (**c**) Heatmap showing expression patterns of various markers, stratified by FlowSOM clusters. Heat scale calculated as column z-score of MFI. (**d**) Frequencies of CD8 subsets: naive (CD45RA+CD27+CCR7+), central memory (CD45RA-CD27+CCR7+), transition memory (CD45RA-CD27+CCR7-), effector memory (CD45RA-CD27-CCR7-), and TEMRA (CD45RA+CD27-CCR7-) (HD n=33; non-cancer n=108; cancer n=9). (**e**) (Top) HLA-DR and CD38 coexpression in concatenated activated clusters (3, 4, and 5) and associated UMAP localization. (Bottom) Frequency of activated clusters (3, 4, and 5) in each patient (HD n=30; non-cancer n=110; solid-cancer n=8). (All) Significance determined by Mann Whitney test: *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. Median and 95% CI shown.
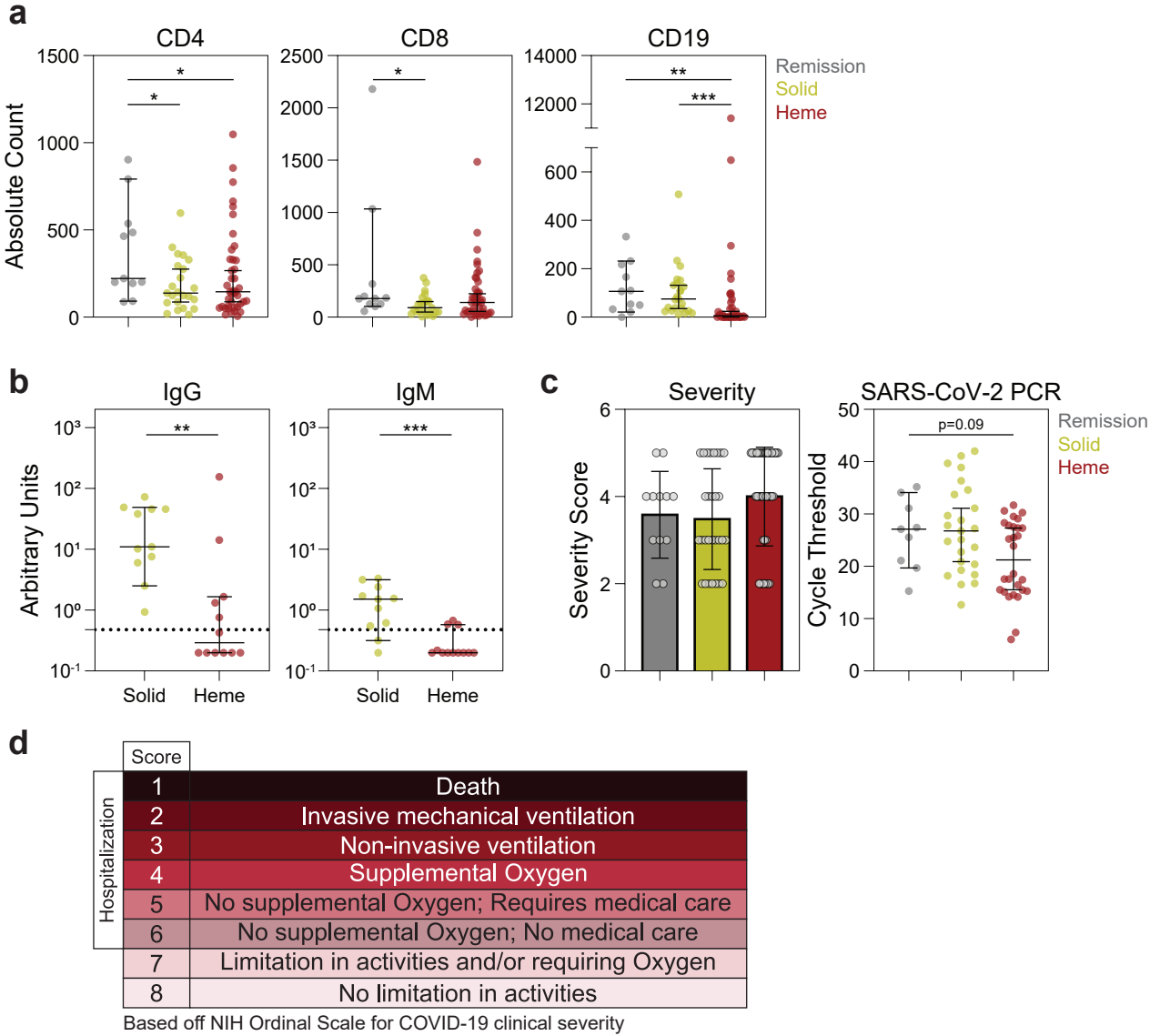
# Extended Data Fig. 5



**Extended Data Fig. 5 | Cellular, serologic, and clinical features in solid and hematologic cancer patients with COVID-19.** (**a**) Absolute counts of CD4, CD8, and CD19 expression in remission (n=11), solid cancer (n=23), and hematologic cancer (n=41) patients. (**b**) Relative levels of SARS-CoV-2 IgG and IgM in solid (n=11) and hematologic cancer (n=14) patients. (**c**) Severity (NIH ordinal scale for COVID-19 clinical severity) and RT-PCR cycle threshold (remission n=9; solid n=25; heme n=28) (Lower Ct: Higher viral load). (d) NIH ordinal scale for COVID-19 clinical severity. (All) Significance determined by Mann Whitney test: *$p<0.05$, **$p<0.01$, ***$p<0.001$, and ****$p<0.0001$. Median and 95% CI shown.

# Extended Data Fig. 6

**a**



**b**



**Extended Data Fig. 6 | Dimensionality reduction and EMD clustering of MSKCC cohort.** (**a**) UMAP projections of lymphocytes with indicated protein expression. (**b**) Absolute counts of CD19+, CD3+, CD3+CD8+, and CD3+CD4+ cells of patients in each EMD cluster (Cluster 1 n=18; Cluster 2 n=6; Cluster 3 n=26; Cluster 4 n=7). (All) Median and 95% CI shown.

# Extended Data Fig. 7



**Extended Data Fig. 7 | EMD Cluster 4 drives differences in mortality between hematologic and solid cancer patients.** (**a**) (Left) Number of patients with hematologic, solid, and remission cancer statuses within each EMD cluster. (Right) Mortality of patients within each EMD cluster for hematologic and solid cancers. (**b**) RT-PCR cycle threshold of solid and heme cancer patients in EMD cluster 4 (solid n=11; heme n=11). (**c**) Absolute CD8 and CD4 T cell counts for subjects in EMD cluster 4 stratified by solid (n=11) and heme (n=13) cancer. (**d**) Global UMAP projections of lymphocytes for subjects in EMD cluster 4: (Left) Hematologic cancer; (Middle) Solid cancer. (Right) Absolute B cell counts for subjects in EMD cluster 4 stratified by solid (n=11) and heme (n=13) cancer. (All) Significance determined by Mann Whitney test: *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. Median and 95% CI shown.
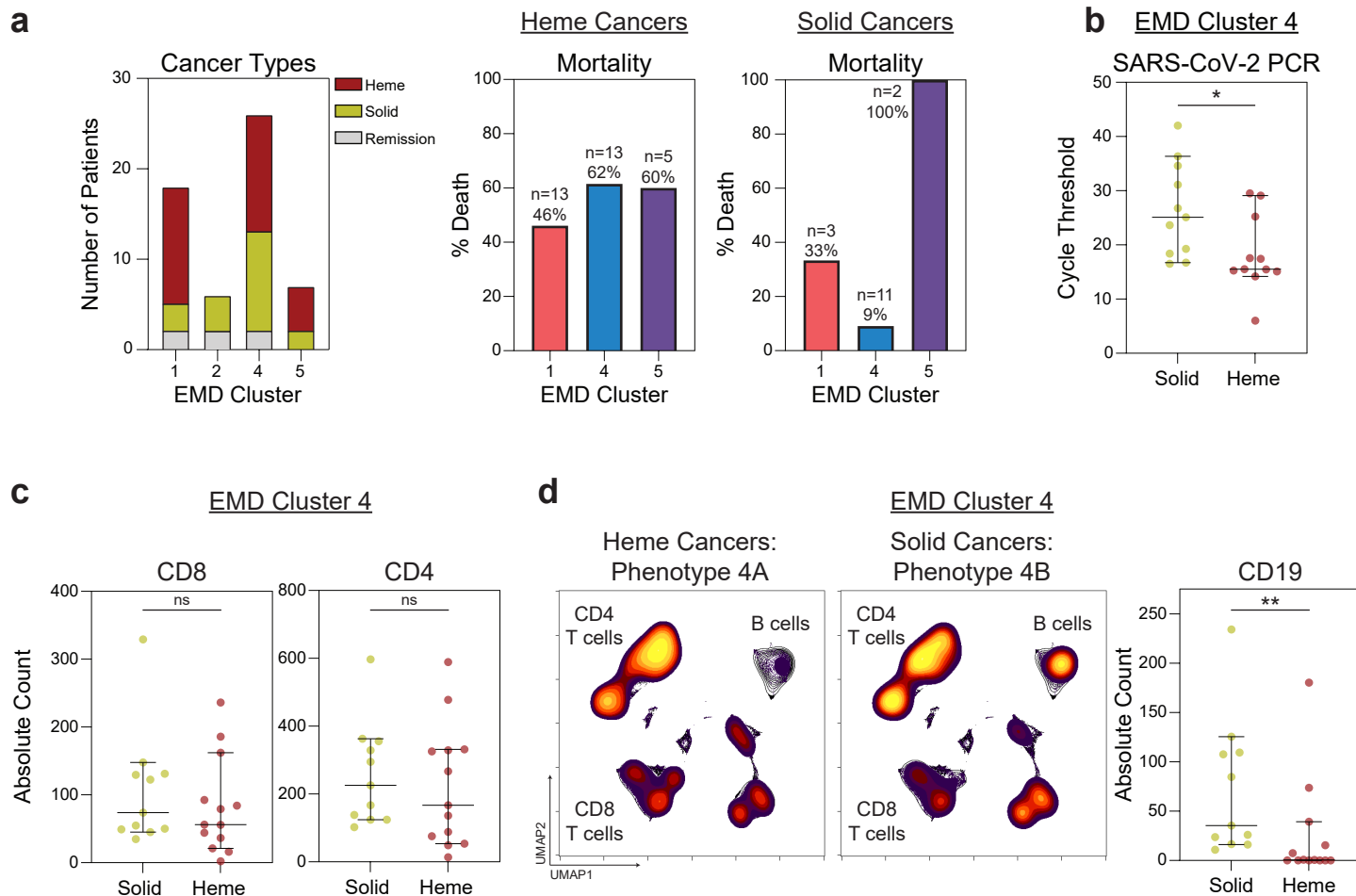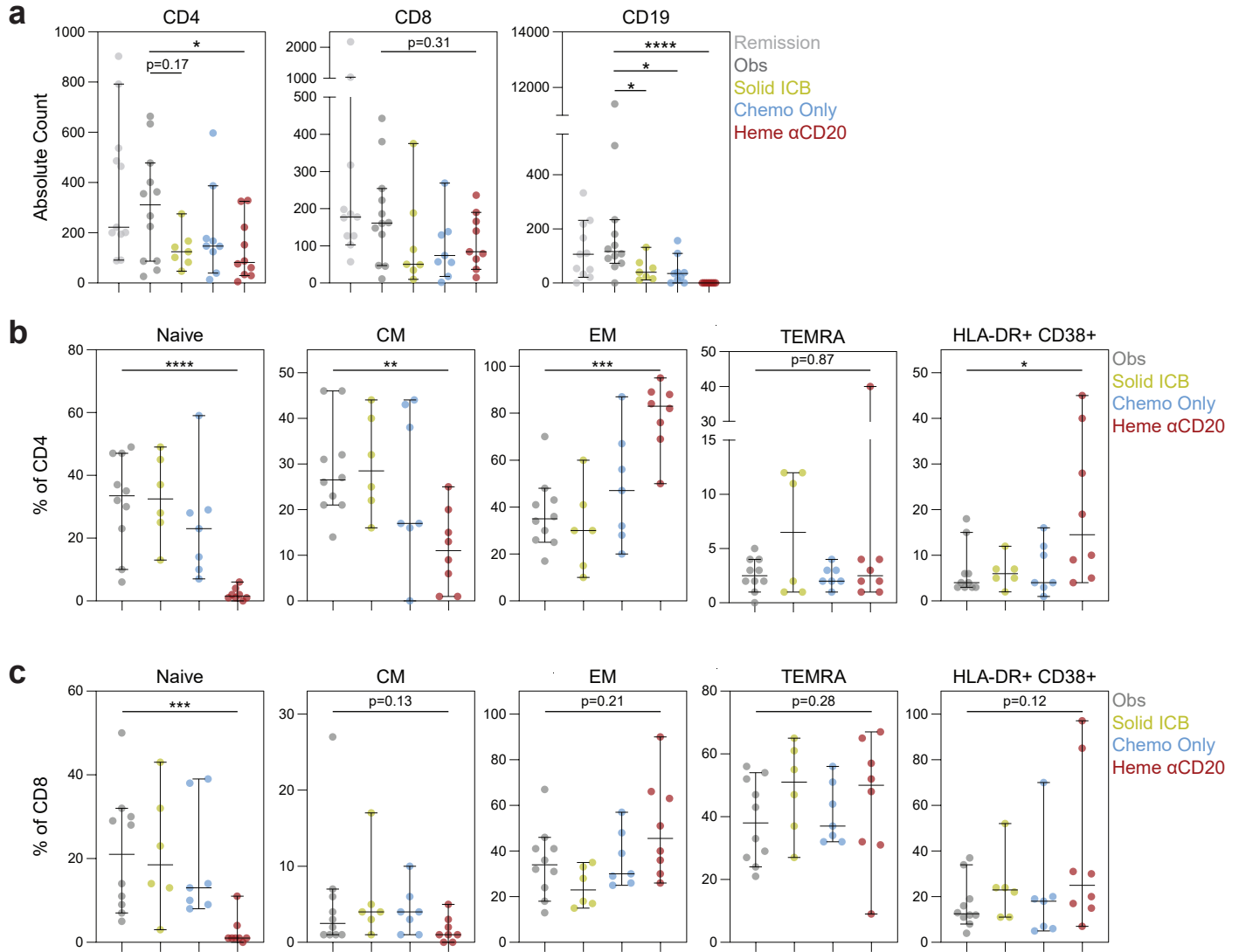
# Extended Data Fig. 8



**Extended Data Fig. 8 | Effect of cancer treatment on T cell differentiation in cancer patients with COVID-19.** (**a**) Absolute counts of CD4, CD8, and CD19 expressing cells. Frequencies of (**b**) CD4 and (**c**) CD8 T cell subsets in cancer patients treated with immune checkpoint blockade therapies, chemotherapies, and B cell depleting therapies. Naive (CD45RA+CCR7+), CM (CD45RA-CCR7+), EM (CD45RA-CCR7-), TEMRA (CD45RA+C-CR7-). (All) Remission n=11, obs n=12, chemo only n=9, solid ICB n=7, and heme αCD20 n=10. Significance determined by Mann Whitney test: *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. Median and 95% CI shown.

# Extended Data Fig. 9



**Extended Data Fig. 9 | Association of mortality with cell counts and viral load.** (**a**) RT-PCR cycle threshold of patients treated with αCD20 therapy (alive n=7; dead n=3). (**b**) Absolute counts of CD8+, CD4+, and CD19+ cells in solid cancer patients (alive n=16; dead n=7). (All) Significance determined by Mann Whitney test: *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. Median and 95% CI shown.
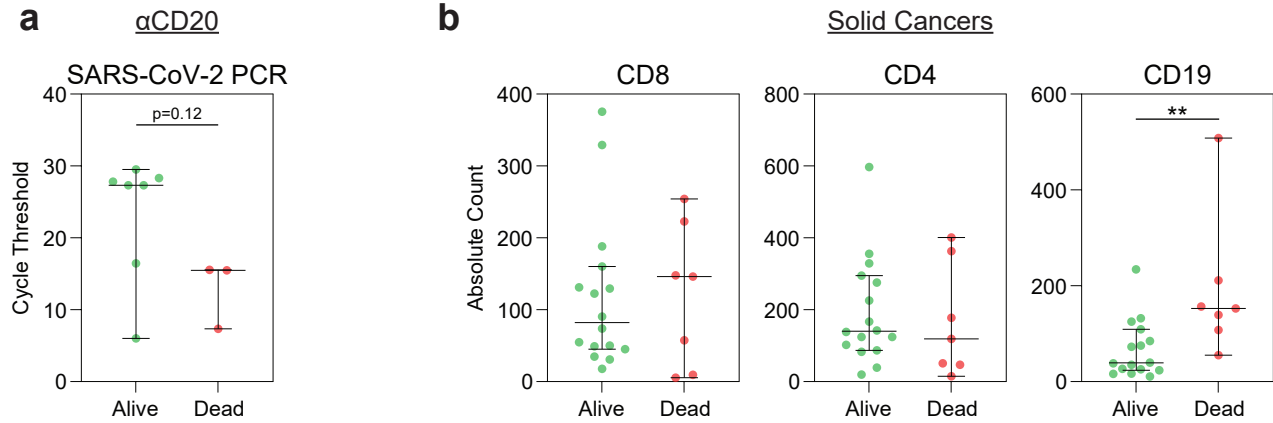
**Supplementary Table 1 | COPE: Patient demographics and clinical characteristics by tumor type.**

| | Solid (N=78) | Heme (N=22) |
|---|---|---|
| Age, median (IQR) | 70.5 (57-78) | 64.5 (60-77) |
| Gender, female | 39 (50.0%) | 9 (40.9%) |
| Race | | |
|     White | 24 (30.8%) | 9 (40.9%) |
|     Non-white | 49 (62.8%) | 12 (54.6%) |
|     Unknown | 5 (6.41%) | 1 (4.55%) |
| Smoking History, Current/Prior | 44 (56.4%) | 13 (59.1%) |
| Comorbidities | | |
|     Cardiac | 63 (80.8%) | 15 (68.2%) |
|     Pulmonary | 38 (48.7%) | 3 (13.6%) |
| Use of immunosuppressive drugs[+] | 23 (29.5%) | 7 (31.8%) |
| BMI, median (IQR) | 26.6 (23.2-30.9) | 28.7 (24.0-33.4) |
| Cancer Status, Active[++] | 32 (41.0%) | 14 (63.6%) |
| Cancer Treatment | | |
|     Treatment in last 3 months | | |
|         Cytotoxic Chemotherapy | 16 (20.5%) | 8 (36.4%) |
|         Hormone therapy | 15 (19.2%) | 0 (0.00%) |
|         Active Surveillance/surgery | 43 (55.1%) | 10 (45.5%) |
|         Other* | 4 (5.13%) | 4 (18.2%) |
| ECOG Performance Status | N=58 | N=15 |
|     0-1 | 28 (48.3%) | 9 (60.0%) |
|     2 | 9 (15.5%) | 4 (26.7%) |
|     3-4 | 21 (36.2%) | 2 (13.3%) |

*Single agent immunotherapy, targeted therapy, monoclonal antibodies
[+]Exposure to immunosuppressive medications not including cancer treatment
[++]Diagnosis or treatment within 6 months

**Supplementary Table 2 | MESSI: Patient demographics and clinical characteristics by cancer status.**

| | Non-Cancer (N=108) | Cancer (N=22) | Overall (N=130) |
|---|---|---|---|
| Gender | | | |
| Female | 49 (45.4%) | 14 (63.6%) | 63 (48.5%) |
| Male | 59 (54.6%) | 8 (36.4%) | 67 (51.5%) |
| Age (median) | 60 | 66 | 60.5 |
| Race | | | |
| Asian | 5 (4.6%) | 1 (4.5%) | 6 (4.6%) |
| Black | 74 (68.5%) | 16 (72.7%) | 90 (69.2%) |
| White | 28 (25.9%) | 5 (22.7%) | 33 (25.4%) |
| Pacific Islander | 1 (0.9%) | 0 (0%) | 1 (0.8%) |
| Symptoms (Days before hospitalization) (median) | 9 | 8 | 9 |
| Severity (At hospitalization) (median) | 3.5 | 3 | 3 |
| Mortality (28 days) | 12 (11.1%) | 8 (36.4%) | 20 (15.4%) |

**Supplementary Table 3 | MESSI: Patient demographics and clinical characteristics by cancer type.**

| | Heme (N=7) | Solid (N=15) | Overall (N=22) |
|---|---|---|---|
| Gender | | | |
|     Female | 4 (57.1%) | 10 (66.7%) | 14 (63.6%) |
|     Male | 3 (42.9%) | 5 (33.3%) | 8 (36.4%) |
| Age (median) | 67 | 65 | 66 |
| Race | | | |
|     Asian | 0 (0%) | 1 (6.7%) | 1 (4.5%) |
|     Black | 5 (71.4%) | 11 (73.3%) | 16 (72.7%) |
|     White | 2 (28.6%) | 3 (20.0%) | 5 (22.7%) |
| Symptoms (Days before hospitalization) (median) | 9 | 7.5 | 8 |
| Severity (At hospitalization) (median) | 3 | 4 | 3 |
| Mortality (28 days) | 2 (28.6%) | 6 (40.0%) | 8 (36.4%) |
| COVID Treatments | | | |
|     Remdesivir | 1 (14.3%) | 2 (13.3%) | 3 (13.6%) |
|     Convalescent Plasma | 1 (14.3%) | 3 (20.0%) | 4 (18.2%) |
|     Early Steroids | 4 (57.1%) | 5 (33.3%) | 9 (40.9%) |

**Supplementary Table 4 | MESSI: Cancer type and cancer treatment.**

|  | Heme (N=7) | Solid (N=15) | Overall (N=22) |
|---|---|---|---|
| Cancer Type |  |  |  |
| CML | 1 (14.3%) | 0 (0%) | 1 (4.5%) |
| CTCL | 1 (14.3%) | 0 (0%) | 1 (4.5%) |
| Lymphoma | 1 (14.3%) | 0 (0%) | 1 (4.5%) |
| Mantle Cell Lymphoma | 1 (14.3%) | 0 (0%) | 1 (4.5%) |
| MM | 1 (14.3%) | 0 (0%) | 1 (4.5%) |
| MPN | 1 (14.3%) | 0 (0%) | 1 (4.5%) |
| Myeloma | 1 (14.3%) | 0 (0%) | 1 (4.5%) |
| Bladder | 0 (0%) | 1 (6.7%) | 1 (4.5%) |
| Breast | 0 (0%) | 6 (40.0%) | 6 (27.3%) |
| GBM | 0 (0%) | 1 (6.7%) | 1 (4.5%) |
| Head and Neck | 0 (0%) | 1 (6.7%) | 1 (4.5%) |
| NSCLC | 0 (0%) | 1 (6.7%) | 1 (4.5%) |
| Ovarian | 0 (0%) | 1 (6.7%) | 1 (4.5%) |
| Pancreas | 0 (0%) | 1 (6.7%) | 1 (4.5%) |
| Prostate | 0 (0%) | 2 (13.3%) | 2 (9.1%) |
| SCLC | 0 (0%) | 1 (6.7%) | 1 (4.5%) |
| Cancer Treatment |  |  |  |
| αCD20 + Chemotherapy | 1 (14.3%) | 0 (0%) | 1 (4.5%) |
| Chemo | 5 (71.4%) | 4 (26.7%) | 9 (40.9%) |
| None | 1 (14.3%) | 1 (6.7%) | 2 (9.1%) |
| Chemotherapy + Radiation | 0 (0%) | 1 (6.7%) | 1 (4.5%) |
| Hormonal | 0 (0%) | 4 (26.7%) | 4 (18.2%) |
| Hormonal + CDK Inhibitor | 0 (0%) | 1 (6.7%) | 1 (4.5%) |
| Hormonal + Radiation | 0 (0%) | 1 (6.7%) | 1 (4.5%) |
| ICB | 0 (0%) | 3 (20.0%) | 3 (13.6%) |

**Supplementary Table 5 | MSKCC: Patient demographics and clinical characteristics by cancer type.**

| | Heme (N=45) | Solid (N=39) | Overall (N=84) |
|---|---|---|---|
| Gender | | | |
|     Female | 21 (46.7%) | 20 (51.3%) | 41 (48.8%) |
|     Male | 24 (53.3%) | 19 (48.7%) | 43 (51.2%) |
| Age (median) | 64 | 66 | 65 |
| Race | | | |
|     Asian | 2 (4.4%) | 3 (7.7%) | 5 (6.0%) |
|     Black | 5 (11.1%) | 4 (10.3%) | 9 (10.7%) |
|     White | 38 (84.4%) | 30 (76.9%) | 68 (81.0%) |
| Disease Severity (median) | 4 | 3 | 4 |
| Mortality (In hospital) | 20 (44.4%) | 8 (20.5%) | 28 (33.3%) |
| COVID Treatments | | | |
|     Remdesivir | 12 (26.7%) | 6 (15.4%) | 18 (21.4%) |
|     Convalescent Plasma | 25 (55.6%) | 14 (35.9%) | 39 (46.4%) |
|     Early Steroids | 17 (37.8%) | 21 (53.8%) | 38 (45.2%) |

**Supplementary Table 6 | MSKCC: Cancer type and cancer treatment.**

| | Heme (N=45) | Solid (N=39) | Overall (N=84) |
|---|---|---|---|
| Cancer Type | | | |
| ALL | 4 (8.9%) | 0 (0%) | 4 (4.8%) |
| AML | 6 (13.3%) | 0 (0%) | 6 (7.1%) |
| CLL | 4 (8.9%) | 0 (0%) | 4 (4.8%) |
| Lymphoma | 23 (51.1%) | 0 (0%) | 23 (27.4%) |
| MDS/Myelofibrosis | 3 (6.7%) | 0 (0%) | 3 (3.6%) |
| Myeloma | 5 (11.1%) | 0 (0%) | 5 (6.0%) |
| Bladder | 0 (0%) | 2 (5.1%) | 2 (2.4%) |
| Breast | 0 (0%) | 8 (20.5%) | 8 (9.5%) |
| CNS | 0 (0%) | 3 (7.7%) | 3 (3.6%) |
| Colorectal | 0 (0%) | 5 (12.8%) | 5 (6.0%) |
| GYN | 0 (0%) | 3 (7.7%) | 3 (3.6%) |
| Head and Neck | 0 (0%) | 1 (2.6%) | 1 (1.2%) |
| Kidney | 0 (0%) | 1 (2.6%) | 1 (1.2%) |
| Liver | 0 (0%) | 1 (2.6%) | 1 (1.2%) |
| Lung | 0 (0%) | 5 (12.8%) | 5 (6.0%) |
| Melanoma | 0 (0%) | 2 (5.1%) | 2 (2.4%) |
| Prostate | 0 (0%) | 3 (7.7%) | 3 (3.6%) |
| Renal | 0 (0%) | 1 (2.6%) | 1 (1.2%) |
| Sarcoma | 0 (0%) | 2 (5.1%) | 2 (2.4%) |
| Thymoma | 0 (0%) | 1 (2.6%) | 1 (1.2%) |
| Thyroid | 0 (0%) | 1 (2.6%) | 1 (1.2%) |
| Cancer Treatment | | | |
| αCD20 | 9 (20.0%) | 0 (0%) | 9 (10.7%) |
| αCD20 + chemo | 9 (20.0%) | 0 (0%) | 9 (10.7%) |
| αnti-CD30 | 1 (2.2%) | 0 (0%) | 1 (1.2%) |
| αHER2 | 0 (0%) | 2 (5.1%) | 2 (2.4%) |
| AXL inhibitor | 1 (2.2%) | 0 (0%) | 1 (1.2%) |
| Bispecific | 1 (2.2%) | 0 (0%) | 1 (1.2%) |
| BTK inhibitor | 4 (8.9%) | 0 (0%) | 4 (4.8%) |
| CAR-T | 1 (2.2%) | 0 (0%) | 1 (1.2%) |
| Chemotherapy | 8 (17.8%) | 15 (38.5%) | 23 (27.4%) |
| EZH inhibitor | 1 (2.2%) | 0 (0%) | 1 (1.2%) |
| PI3K Inhibitor | 1 (2.2%) | 0 (0%) | 1 (1.2%) |
| Proteasome inhibitor | 3 (6.7%) | 0 (0%) | 3 (3.6%) |
| Radiation | 1 (2.2%) | 0 (0%) | 1 (1.2%) |
| Tyrosine kinase inhibitor | 1 (2.2%) | 0 (0%) | 1 (1.2%) |
| Hormonal | 0 (0%) | 5 (12.8%) | 5 (6.0%) |
| Immune checkpoint blockade | 0 (0%) | 7 (17.9%) | 7 (8.3%) |
| VEGF inhibitor | 0 (0%) | 1 (2.6%) | 1 (1.2%) |
| None | 4 (8.9%) | 9 (23.1%) | 13 (15.5%) |