Monocyte response to SARS-CoV-2 protein ORF8 is associated with severe COVID-19 infection in patients with chronic lymphocytic leukemia

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Supplementary Figure Legends

Supplementary Figure 1: Multi-color flow cytometry gating strategy for intracellular cytokine staining on PBMCs from a patient with chronic lymphocytic leukemia. The gating is used to analyze cytokine expression on monocytes, while CD14-/CD16- cells are excluded. The lower right panel shows the increased level of IL-1β that is stimulated with open reading frame 8. This patient developed severe coronavirus disease 2019 (COVID-19). patient that required hospital admission due to COVID-19.

Supplementary Figure 2: Multi-color flow cytometry gating strategy for intracellular cytokine staining. This patient did not develop severe coronavirus disease 2019.

Supplementary Figure 3: Multi-color flow cytometry gating strategy for intracellular cytokine staining with Boolean gating strategy.

Supplementary Figure 4: Patterns of reactivity among ORF8-stimulated CLL sample

Supplementary Figure 5: Receiver operating curve analysis for adjusted MFI_{IL-6}, MFI_{IL-8}, and MFI_{CCL-2}

Supplemental Table 1. Comparisons of cytokine/chemokine expression levels between ORF8-stimulated monocytes from patients with chronic lymphocytic leukemia versus healthy controls.

	Normal Donors (n=26)	CLL (n=60)	p-value
ΔMFI			
IL-1β	230 (87-418)	378 (127-669)	0.11
IL-6	13 (5-27)	136 (111-247)	**<0.0001**
IL-8	80 (58-100)	238 (165-320)	**<0.0001**
CCL-2	269 (198-359)	339 (162-533)	0.05
% Monocytes staining positive			
IL-1β	31 (12-58)	27 (5-50)	0.46
IL-6	5 (1-21)	4 (2-6)	**0.02**
IL-8	30 (5-45)	30 (6-47)	0.56
CCL-2	48 (40-58)	25 (11-43)	**0.0003**
Adjusted MFI			
IL-1β	0.008 (0.004-0.01)	0.25 (0.06-1.38)	0.07
IL-6	0.001 (0.0006-0.003)	1.1 (0.2-5.9)	0.07
IL-8	0.004 (0.002-0.007)	0.28 (0.1-2.4)	**0.005**
CCL-2	0.005 (0.004-0.01)	0.42 (0.16-1.6)	**0.006**

Frequency	**% of Total Monocytes Median (25-75% IQR)**		
IL-1β+ cells (all)	18 (5-47)	27 (5-50)	0.89
IL-1β+/CCL2+/IL6+/IL8+	1 (0-4)	1 (0-3)	0.35
IL-1β+/CCL2+/Il6+/IL8-	0 (0-1)	0 (0-0.1)	0.3
IL-1β+/CCL2+/IL6-/IL8+	4 (1-15)	5 (0.3-16)	086
IL-1β+/CCL2+/IL6-/IL8-	**5 (2-9)**	**2 (0-4)**	**0.002**
IL-1β+/CCL2-/IL6+/IL8+	0.3 (0-1)	0.3 (0-2)	0.80
IL-1β+/CCL2-/IL6+/IL8-	0.1 (0-0.8)	0 (0-0.1)	0.20
IL-1β+/CCL2-/IL6-/IL8+	3 (0.4-7)	5 (0.3-14)	0.29
IL-1β+/CCL2-/IL6-/IL8-	4 (2-8)	3 (1-7)	0.51

MFI	**IL-1β (25-75% IQR)**		
IL-1β+ cells (all)	329 (198-512)	412 (153-700)	0.44
IL-β+/CCL2+/IL6+/IL8+	**1191 (670-1761)**	**2237 (1456-3024)**	**0.0002**
IL-1β+/CCL2+/Il6+/IL8-	223 (159-316)	307 (147-1273)	0.15
IL-1β+/CCL2+/IL6-/IL8+	429 (333-644)	484 (287-843)	0.35
IL-1β+/CCL2+/IL6-/IL8-	246 (182-342)	275 (154-479)	0.49
IL-1β+/CCL2-/IL6+/IL8+	**1103 (539-1825)**	**2382 (1300-3165)**	**0.0009**
IL-1β+/CCL2-/IL6+/IL8-	220 (152-290)	303 (146-710)	0.21
IL-1β+/CCL2-/IL6-/IL8+	421 (277-602)	532 (315-754)	0.28
IL-1β+/CCL2-/IL6-/IL8-	225 (176-293)	241 (125-395)	0.87

Supplemental Table 2. Previous CLL-directed therapy before COVID-19 diagnosis.

Treatment before COVID-19 diagnosis	N=60 (%)
Observation	30 (50)
BTK inhibitor (monotherapy)	17 (28)
Anti-CD20 (monotherapy)	3 (5)
BTK inhibitor and Anti-CD20	2 (3)
BTK inhibitor, anti-CD20, venetoclax	4 (7)
Chemotherapy, anti-CD20, BTK-inhibitor	1 (2)
Anti-CD20 and venetoclax	1 (2)
CAR-T	2 (3)

Supplemental Table 3. IL-1β$^+$ producing cell subsets among ORF8-stimulated monocytes from patients with chronic lymphocytic leukemia

Parameters	CLL (n=60)
Frequency	**% of Total Monocytes Median (25-75% IQR)**
IL-1β+ cells (all)	27 (5-50)
IL-1β+/CCL2+/IL6+/IL8+	1 (0-3)
IL-1β+/CCL2+/Il6+/IL8-	0 (0-0)
IL-1β+/CCL2+/IL6-/IL8+	5 (0.3-16)
IL-1β+/CCL2+/IL6-/IL8-	**2 (0-4)**
IL-1β+/CCL2-/IL6+/IL8+	0.3 (0-2)
IL-1β+/CCL2-/IL6+/IL8-	0 (0-0)
IL-1β+/CCL2-/IL6-/IL8+	5 (0.3-14)
IL-1β+/CCL2-/IL6-/IL8-	3 (1-7)
ΔMFI	**IL-1β (25-75% IQR)**
IL-1β+ cells (all)	412 (153-700)
IL-β+/CCL2+/IL6+/IL8+	**2237 (1456-3024)**
IL-1β+/CCL2+/Il6+/IL8-	307 (147-1273)
IL-1β+/CCL2+/IL6-/IL8+	484 (287-843)
IL-1β+/CCL2+/IL6-/IL8-	275 (154-479)
IL-1β+/CCL2-/IL6+/IL8+	**2382 (1300-3165)**
IL-1β+/CCL2-/IL6+/IL8-	303 (146-710)
IL-1β+/CCL2-/IL6-/IL8+	532 (315-754)
IL-1β+/CCL2-/IL6-/IL8-	241 (125-395)

CLL, chronic lymphocytic leukemia; ΔMFI, delta median fluorescence intensity; IQR, interquartile range

Supplemental Table 4. Univariate Analysis of IL-1β subsets and association with severe COVID-19 (Cox proportional hazards regression model)

Parameters	HR (95% CI)	p-value
Frequency		
IL-1β+/CCL2+/IL6+/IL8+	1.1 (0.97-1.2)	0.17
IL-1β+/CCL2+/Il6+/IL8-	1.2 (0.7-2.2)	0.55
IL-1β+/CCL2+/IL6-/IL8+	0.99 (0.95-1.04)	0.75
IL-1β+/CCL2+/IL6-/IL8-	0.96 (0.86-1.06)	0.42
IL-1β+/CCL2-/IL6+/IL8+	1.3 (0.99-1.6)	0.05
IL-1β+/CCL2-/IL6+/IL8-	2.2 (0.3-16.6)	0.43
IL-1β+/CCL2-/IL6-/IL8+	1.0 (0.97-1.1)	0.62
IL-1β+/CCL2-/IL6-/IL8-	0.95 (0.85-1.1)	0.35
ΔMFI		
IL-1β+/CCL2+/IL6+/IL8+	1.0 (0.99-1.0)	0.47
IL-1β+/CCL2+/Il6+/IL8-	1.00 (1.00-1.0007)	**0.01**
IL-1β+/CCL2+/IL6-/IL8+	1.0 (0.99-1.0)	0.31
IL-1β+/CCL2+/IL6-/IL8-	1.00 (1.00-1.003)	**0.03**
IL-1β+/CCL2-/IL6+/IL8+	1.0 (0.99-1.0)	0.47
IL-1β+/CCL2-/IL6+/IL8-	1.0006 (0.99-1.00)	0.08
IL-1β+/CCL2-/IL6-/IL8+	1.0004 (0.99-1.00)	0.55
IL-1β+/CCL2-/IL6-/IL8-	0.9997 (0.997-1.00)	0.81
IL-1β Adjusted MFI		
IL-1β+/CCL2+/IL6+/IL8+	0.99 (0.98-1.01)	0.86
IL-1β+/CCL2+/Il6+/IL8-	1.00 (0.97-1.04)	0.75
IL-1β+/CCL2+/IL6-/IL8+	0.99 (0.99-1.00)	0.24
IL-1β+/CCL2+/IL6-/IL8-	1.00 (0.99-1.01)	0.86
IL-1β+/CCL2-/IL6+/IL8+	1.00 (1.00-1.007)	0.08
IL-1β+/CCL2-/IL6+/IL8-	1.00 (1.001-1.01)	**0.02**
IL-1β+/CCL2-/IL6-/IL8+	1.00 (0.99-1.004)	0.31
IL-1β+/CCL2-/IL6-/IL8-	0.99 (0.98-1.1)	0.53