SUPPLEMENTARY CONTENT

Appendix 1: Institutional Review Boards and Ethics Committees Approving Trial (NCT00351533)

Human Subjects Division
University of Washington Medical Center – Coordinating Center (IRB #45721)
Seattle, Washington, United States

Human Subjects Division
Harborview Medical Center
Seattle, Washington, United States

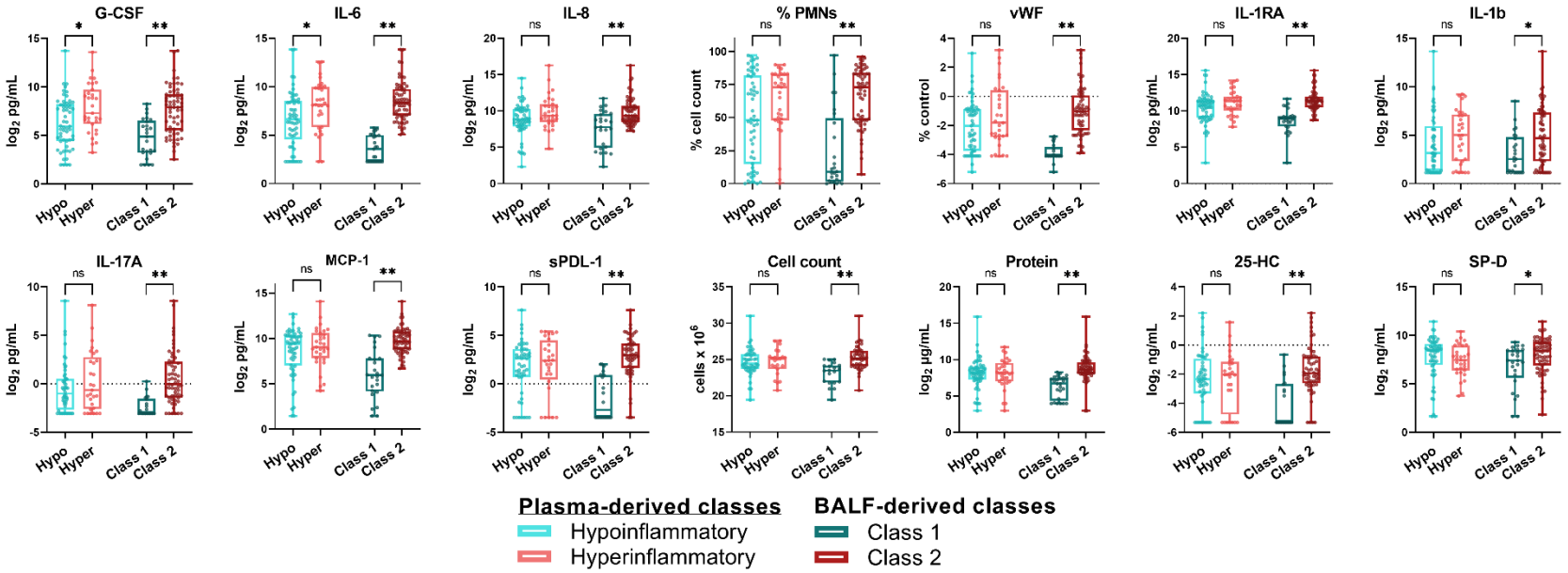
Research Ethics Board
St. Michael's Hospital
Toronto, Ontario, Canada

Institutional Review Board
St. Alphonsus Medical Center
Boise, Idaho, United States

Research Protections Office
Fletcher Allen Health Care
Burlington, Vermont, United States

Data Safety Monitoring Board (DSMB)
Appointed by the National Heart, Lung, and Blood Institute (NHLBI)

Supplemental Figure 1. Alveolar biomarkers by plasma-derived and BALF-derived classes. Each dot represents an individual measurement. Boxes indicate median and interquartile range; whiskers indicate minimum and maximum. Biomarkers are sorted by magnitude of difference between plasma-derived classes. ns = not significant, *Nominal P < 0.05, and **Bonferroni corrected P < 0.05 for difference between plasma-derived classes or BALF-derived classes using Mann Whitney U tests. Abbreviations: BALF = bronchoalveolar lavage fluid, G-CSF = granulocyte colony stimulating factor, IL-6 = interleukin-6, IL-8 = interleukin-8, % PMNs = % neutrophils of total leukocyte count, vWF = von Willebrand factor, IL-1RA = interleukin-1 receptor antagonist, IL-1b = interleukin-1 beta, IL-17A = interleukin-17A, MCP-1 = monocyte chemoattractant protein-1, sPD-L1 = soluble programmed cell death ligand 1, cell count = total leukocyte count, protein = total protein, 25-HC = 25-hydroxycholesterol, SP-D = surfactant protein D.



Supplemental Table 1: BALF measurements excluded from analysis

Biomarkers	Reason for exclusion
% lymphocytes	correlated with % neutrophils
% macrophages	correlated with % neutrophils
Tumor necrosis factor (TNF)	>25% at lower limit of detection
Interleukin-10 (IL-10)	>25% at lower limit of detection
Soluble programmed cell death ligand 2 (sPD-L2)	correlated with sPDL-1
Soluble programmed cell death protein 1 (sPD-1)	correlated with sPDL-1
Matrix metallopeptidase-28 (MMP-28)	>25% at lower limit of detection

Supplemental Table 2: Alveolar biomarkers by plasma-derived and BALF-derived classes

	Plasma Classes				BALF Classes			
	Hypoinflammatory N=57	**Hyperinflammatory** N=31	**P**	**P adjusted**	**Class 1** N=25	**Class 2** N=63	**P**	**P adjusted**
IL-8, pg/mL	449 (235-1227)	656 (350-1969)	0.069	0.966	218 (32-723)	640 (350-1629)	<0.001	0.006
IL-6, pg/mL	100 (30-368)	282 (57-1009)	0.014	0.196	12 (5-31)	326 (131-868)	<0.001	<0.001
G-CSF, pg/mL	61 (22-295)	157 (72-846)	0.008	0.112	29 (9-86)	239 (46-603)	<0.001	<0.001
MCP-1, pg/mL	672 (133-1284)	530 (224-1526)	0.56	>0.99	62 (19-222)	814 (454-1749)	<0.001	<0.001
IL-17A, pg/mL	0.50 (0.17-1.45)	0.64 (0.18-5.19)	0.34	>0.99	0.12 (0.12-0.33)	0.98 (0.37-4.92)	<0.001	<0.001
sPD-L1, pg/mL	6 (2-12)	5 (2-21)	0.79	>0.99	0 (0-2)	8 (3-18)	<0.001	<0.001
IL-1RA, pg/mL	1729 (537-2834)	2560 (1065-4080)	0.12	>0.99	492 (242-602)	2623 (1448-4080)	<0.001	<0.001
SP-D, ng/mL	347 (127-509)	176 (84-497)	0.16	>0.99	169 (61-351)	333 (117-635)	0.020	0.280
vWF, % control	0.25 (0.07-0.54)	0.28 (0.14-1.35)	0.16	>0.99	0.06 (0.06-0.08)	0.48 (0.20-1.05)	<0.001	<0.001
IL-1b, pg/mL	9 (3-49)	32 (5-140)	0.13	>0.99	6 (2-25)	25 (5-166)	0.008	0.109
25-HC, ng/mL	0.20 (0.10-0.49)	0.25 (0.07-0.44)	0.84	>0.99	0.03 (0.03-0.15)	0.26 (0.16-0.58)	<0.001	<0.001
Total leukocyte count x 10^6	22 (14-56)	36 (14-46)	0.69	>0.99	12 (4-19)	36 (19-77)	<0.001	<0.001
% PMN	48 (15-82)	73 (48-83)	0.10	>0.99	9 (2-46)	73 (48-83)	<0.001	<0.001
Total protein, µg/mL	294 (158-505)	290 (128-750)	0.95	>0.99	104 (24-175)	392 (268-790)	<0.001	<0.001

Median (interquartile range) listed for all variables. P values for Mann Whitney U tests, both nominal and Bonferroni-adjusted. Abbreviations: BALF = bronchoalveolar lavage fluid, IL-8 = interleukin-8, IL-6 = interleukin-6, G-CSF = granulocyte colony stimulating factor, MCP-1 = monocyte chemoattractant protein -1, IL-17A = interleukin-17A, sPDL-1 = soluble programmed cell death-ligand 1, IL-1RA = interleukin-1 receptor antagonist, SP-D = surfactant protein D, vWF = von Willebrand factor, IL-1b = interleukin-1 beta, 25-HC = 25-hydroxycholesterol, % PMN = % neutrophils of total leukocyte count, sTNFR-1 = soluble tumor necrosis factor receptor-1.

Supplemental Table 3: Plasma biomarkers by plasma-derived and BALF-derived classes

	Plasma Classes			BALF Classes		
	Hypoinflammatory N=57	**Hyperinflammatory** N=31	**P**	**Class 1** N=25	**Class 2** N=63	**P**
Bicarbonate, mmol/L	25 (23-29)	21 (17-24)	<0.001	24 (22-26)	25 (21-27)	>0.99
IL-8, pg/mL	20 (20-21)	35 (23-97)	<0.001	20 (20-24)	21 (20-36)	0.28
sTNFR-1, pg/mL	1853 (1353-2615)	4286 (2600-6524)	<0.001	2441 (1660-3713)	2237 (1471-4018)	0.78
IL-6, pg/mL	58 (32-116)	151 (94-436)	<0.001	52 (21-75)	121 (54-201)	<0.001
G-CSF, pg/mL	37 (21-83)	110 (16-431)	0.036	37 (16-58)	71 (22-161)	0.038
MCP-1, pg/mL	165 (104-318)	259 (139-777)	0.033	144 (88-294)	229 (120-412)	0.078
IL-17A, pg/mL	2.66 (1.31-6.49)	6.56 (2.69-22.18)	0.002	2.26 (1.02-3.66)	4.92 (2.29-10.89)	0.010
sPDL-1, pg/mL	80 (69-115)	149 (94-177)	<0.001	82 (71-132)	106 (72-143)	0.34
IL-1RA, pg/mL	1976 (1154-3081)	4501 (2404-11282)	<0.001	2201 (1227-3081)	2447 (1325-5137)	0.26
SP-D, ng/mL	122 (65-179)	123 (47-253)	0.79	61 (33-173)	128 (72-223)	0.031
vWF, % control	211 (151-536)	292 (184-629)	0.24	205 (141-349)	297 (162-586)	0.13

Median (interquartile range) listed for all variables. P values for Mann Whitney U tests, uncorrected for multiple hypothesis testing. Abbreviations: BALF = bronchoalveolar lavage fluid, IL-8 = interleukin-8, IL-6 = interleukin-6, G-CSF = granulocyte colony stimulating factor, MCP-1 = monocyte chemoattractant protein -1, IL-17A = interleukin-17A, sPDL-1 = soluble programmed cell death-ligand 1, IL-1RA = interleukin-1 receptor antagonist, SP-D = surfactant protein D, vWF = von Willebrand factor, IL-1b = interleukin-1 beta, 25-HC = 25-hydroxycholesterol, % PMN = % neutrophils of total leukocyte count, sTNFR-1 = soluble tumor necrosis factor receptor-1.

Supplemental Table 4: Alveolar biomarkers by PaO_2 to FIO_2 ratio > 150 and ≤ 150

	PaO_2:FIO_2 > 150 N=51	**PaO_2:FIO_2 ≤150** N=37	**P**
IL-8, pg/mL	529 (187-1542)	486 (350-1345)	0.52
IL-6, pg/mL	111 (28-368)	282 (78-815)	0.026
G-CSF, pg/mL	122 (31-448)	84 (23-295)	0.59
MCP-1, pg/mL	471 (90-1227)	814 (328-1526)	0.075
IL-17A, pg/mL	0.42 (0.12-1.62)	0.75 (0.33-3.31)	0.080
sPDL-1, pg/mL	4 (1-8)	9 (2-20)	0.034
IL-1RA, pg/mL	1451 (464-3225)	2046 (1437-3203)	0.11
SP-D, ng/mL	260 (91-509)	233 (98-491)	0.95
vWF, %	0.15 (0.07-0.48)	0.51 (0.17-1.13)	0.006
IL-1b, pg/mL	21 (3-176)	9 (4-71)	0.34
25-OH cholesterol, ng/mL	0.16 (0.03-0.27)	0.40 (0.16-0.61)	0.001*
Cell count x 10^6	31 (12-54)	22 (18-49)	0.55
% PMN	51 (15-83)	69 (46-82)	0.15
Total protein, µg/ml	268 (103-376)	426 (233-898)	0.001*

Median (interquartile range) listed for all variables. P values for Mann Whitney U tests, uncorrected for multiple hypothesis testing. *Bonferroni corrected $P < 0.05$

Supplemental Table 5: BALF-to-plasma ratios of biomarkers, stratified by ARDS etiology

	Total N=87	Indirect N=39	Direct N=48	P
Ratios of BALF-to-plasma biomarker concentrations				
Ratio IL-6	1.28 (0.45-5.07)	1.11 (0.47-4.89)	1.80 (0.43-5.12)	0.44
Ratio IL-8	19.07 (9.08-49.74)	14.24 (9.14-47.49)	23.32 (8.70-64.52)	0.49
Ratio MCP-1	2.92 (0.74-6.31)	2.61 (0.58-6.17)	3.32 (0.82-7.46)	0.51
Ratio G-CSF	1.54 (0.61-4.97)	1.88 (0.66-4.97)	1.35 (0.58-4.89)	0.46
Ratio IL-1RA	0.64 (0.30-1.80)	0.45 (0.22-0.89)	0.87 (0.43-2.55)	0.005
Ratio IL-17a	0.17 (0.08-0.55)	0.12 (0.07-0.32)	0.29 (0.10-0.80)	0.046
Ratio sPDL-1	0.05 (0.01-0.13)	0.04 (0.01-0.11)	0.06 (0.01-0.17)	0.13
Ratio SP-D	2.50 (0.75-5.33)	2.67 (1.22-6.75)	1.94 (0.33-4.53)	0.12
BALF biomarker concentrations				
BALF IL-6, pg/mL	150 (36-489)	141 (36-400)	164 (40-747)	0.57
BALF IL-8, pg/mL	498 (254-1399)	471 (292-1212)	640 (235-1640)	0.40
BALF MCP-1, pg/mL	672 (205-1344)	672 (205-1360)	605 (197-1260)	0.77
BALF G-CSF, pg/mL	110 (27-448)	123 (37-344)	78 (22-627)	0.87
BALF IL-1RA, pg/mL	1904 (602-3225)	1368 (537-2372)	2591 (1344-3557)	0.006
BALF IL-17a, pg/mL	0.56 (0.18-2.08)	0.33 (0.12-1.06)	0.92 (0.37-4.92)	0.009
BALF sPDL-1, pg/mL	5 (2-13)	3 (2-8)	7 (2-21)	0.13
BALF SP-D, ng/mL	260 (98-500)	333 (163-446)	239 (77-514)	0.49
Plasma biomarker concentrations				
Plasma IL-6, pg/mL	84 (44-173)	86 (52-184)	80 (30-162)	0.32
Plasma IL-8, pg/mL	20 (20-36)	21 (20-40)	20 (20-31)	0.67
Plasma MCP-1, pg/mL	201 (113-342)	284 (120-452)	189 (89-310)	0.083
Plasma G-CSF, pg/mL	46 (20-124)	79 (24-121)	37 (19-141)	0.38
Plasma IL-1RA, pg/mL	2355 (1302-4670)	2380 (1278-4389)	2298 (1313-5159)	0.97
Plasma IL-17a, pg/mL	3.58 (1.59-9.61)	2.69 (1.31-6.49)	4.89 (2.26-10.22)	0.15
Plasma sPDL-1, pg/mL	96 (71-141)	94 (71-137)	104 (71-143)	0.78
Plasma SP-D, ng/mL	122 (58-213)	105 (45-170)	139 (67-271)	0.13

Table includes both ratios as well as the constituent BALF and plasma biomarkers used to calculate ratios. Median (interquartile range) listed for all variables. P values for Mann Whitney U tests. 1 out of 88 patients in this manuscript did not have ARDS etiology ascertained and was excluded in this table. Abbreviations: BALF = bronchoalveolar lavage fluid, IL-6 = interleukin-6, IL-8 = interleukin-8, MCP-1 = monocyte chemoattractant protein -1, G-CSF = granulocyte colony stimulating factor, IL-1RA = interleukin-1 receptor antagonist, IL-17A = interleukin-17A, sPDL-1 = soluble programmed cell death-ligand 1, SP-D = surfactant protein D.