

Corticosteroid modulated immune activation in the TB immune reconstitution inflammatory syndrome

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MATERIAL FOR ONLINE DATA SUPPLEMENT

Supplementary Tables 1, 2, 3 and 4

Supplementary Table 1: Log fold gene induction after 24 hrs restimulation of PBMC with heat-killed H37Rv *Mycobacterium tuberculosis* at weeks 0, 2 and 4 on clinical trial for placebo-treated and prednisone-treated participants

PLACEBO-TREATED						PREDNISONE-TREATED				
Cytokine/ chemokine	Week 0 N = 9	Week 2 N = 8	Week 4 N = 6	p-value (0 vs 2)	p-value (0 vs 4)	Week 0 N = 16	Week 2 N = 14	Week 4 N = 15	p-value (0 vs 2)	p-value (0 vs 4)
IL-1β	2.25 (0.80)	2.35 (0.41)	1.95 (0.76)	0.46	0.03 *	2.29 (1.11)	2.28 (0.94)	2.14 (0.74)	0.89	0.34
IL-2	0.97 (0.68)	0.99 (0.43)	0.97 (0.81)	0.57	0.76	0.78 (0.74)	0.97 (0.55)	0.68 (0.67)	0.45	0.61
IL-4	-0.13 (0.49)	-0.09 (0.34)	0.04 (0.30)	0.75	0.79	0.32 (0.42)	0.12 (0.52)	-0.26 (0.29)	0.43	0.002 *
IL-6	2.63 (1.37)	2.48 (1.10)	2.46 (1.15)	0.94	0.01 *	2.75 (1.56)	2.69 (1.19)	2.26 (1.11)	0.68	0.18
IL-8	1.29 (0.54)	1.15 (0.45)	1.14 (0.52)	0.91	0.26	1.52 (0.83)	1.33 (0.62)	1.12 (0.66)	0.34	0.09
IL-10	0.24 (0.84)	-0.04 (0.82)	0.41 (1.05)	0.23	0.93	0.38 (0.80)	0.13 (0.69)	-0.72 (1.08)	0.46	0.006 *
IL-12p40	2.02 (0.80)	2.24 (0.62)	2.47 (0.44)	0.41	0.62	2.40 (0.82)	2.04 (1.11)	2.21 (1.23)	0.45	0.84
IL-13	2.24 (1.06)	2.17 (0.96)	1.94 (0.96)	0.98	0.004 *	2.29 (0.86)	1.89 (0.90)	1.97 (0.75)	0.31	0.22
IL-15	0.43 (0.36)	0.24 (0.54)	0.63 (0.44)	0.17	0.38	0.67 (0.53)	0.73 (0.40)	0.50 (0.49)	0.51	0.33
IL-17A	1.53 (0.64)	1.46 (0.75)	1.52 (0.83)	0.79	0.27	1.28 (1.07)	1.11 (0.65)	1.10 (0.93)	0.63	0.65
IL-22	1.98 (0.89)	1.98 (0.66)	2.27 (0.80)	0.68	0.02 *	1.90 (1.11)	1.76 (0.77)	1.83 (0.82)	0.67	0.80

IL-27	1.42 (0.62)	1.28 (0.49)	2.00 (0.73)	0.95	0.37	1.39 (0.79)	1.52 (0.66)	1.34 (1.21)	0.82	0.72
TNFα	1.60 (0.30)	1.27 (0.80)	1.47 (0.42)	0.33	0.26	1.45 (0.65)	1.45 (0.32)	1.31 (0.64)	0.74	0.42
IFNγ	1.50 (0.78)	1.82 (0.53)	1.78 (0.42)	0.09	0.35	1.45 (0.88)	1.43 (0.70)	1.25 (0.71)	0.73	0.44
GM-CSF	2.55 (1.43)	2.62 (1.09)	2.00 (1.10)	0.88	0.049 *	3.01 (1.45)	2.74 (1.19)	2.48 (0.91)	0.32	0.15
CCL3	1.46 (0.51)	1.41 (0.30)	1.09 (0.68)	0.74	0.04 *	1.50 (0.73)	1.40 (0.54)	1.33 (0.58)	0.48	0.29
CCL4	1.11 (0.32)	0.93 (0.22)	0.82 (0.35)	0.31	0.09	1.17 (0.59)	1.02 (0.45)	0.95 (0.54)	0.41	0.26
CCL5	-0.09 (0.16)	-0.21 (0.26)	-0.11 (0.15)	0.21	0.73	-0.02 (0.27)	0.00 (0.30)	-0.17 (0.39)	0.61	0.24

Legend for Supplementary Table 1:

Mean log fold-induction (+/- standard deviation) in gene expression of 18 cytokines and chemokines after 24 hrs restimulation of PBMC with heat-killed H37Rv *Mycobacterium tuberculosis* are shown for placebo and prednisone-treated patients at weeks 0, 2 and 4 on the clinical trial. P-values were calculated for comparisons between 0 and 2 weeks values and 0 and 4 week values for each treatment group separately using Student's paired t-tests. Two patients in the placebo-treated arm switched to open label prednisone at week 2 and their week 4 samples were thus excluded from analysis.

Significant differences are indicated with *. After Bonferroni correction for multiple comparisons ($p_{corr} = p \times 18 - 1 = p \times 17$) only the change in IL-4 in prednisone-treated patients between week 0 and 4 remained significant ($p_{corr} = 0.03$).

Supplementary Table 2: Changes in concentrations of cytokines and chemokines in supernatants of cell cultures after restimulation of PBMC with heat-killed H37Rv *Mycobacterium tuberculosis* for 24 hrs assessed by Luminex multiplex assay

PLACEBO-TREATED						PREDNISONE-TREATED				
Cytokine/ chemokine	Week 0 N = 12	Week 2 N = 11	Week 4 N = 9	p-value (0 vs 2)	p-value (0 vs 4)	Week 0 N = 17	Week 2 N = 15	Week 4 N = 15	p-value (0 vs 2)	p-value (0 vs 4)
IL-1β	1556 298.1 - 5182	495 226.4 - 2915	184.6 65 - 1788	0.70	0.01 *	2375 112.2 - 3623	112.4 0 - 699.4	235.8 98 - 456.8	0.01 *	0.03 *
IL-2	94.6 9.1 - 491.3	101.8 51.7 - 295.5	215.9 69.8 - 529.8	0.28	0.82	55.1 9.9 - 291	64.8 13.7 - 206.3	65.2 5.1 - 126	0.45	0.71
IL-4	0 0 - 0	0 0 - 0	0 0 - 0	0.75	0.50	0 0 - 1.6	0 0 - 0	0 0 - 0	0.63	0.13
IL-5	0 0 - 0	0 0 - 0	0 0 - 0	0.50	1.0	0 0 - 0	0 0 - 0	0 0 - 0	1.0	1.0
IL-6	17957 5088 - 29776	13026 2954 - 24499	6884 1119 - 15462	0.64	0.01 *	17084 3805 - 33389	3213 868.2 - 16139	6496 3411 - 12239	0.02 *	0.04 *
IL-10	249.1 139.3 - 2082	465.9 130.5 - 1297	190.4 77.1 - 1617	1.0	0.02 *	751.1 160.9 - 1591	124.3 55.4 - 963.7	311.5 187 - 638.8	0.14	0.06
IL-12p40	41.3 27.2 - 207.4	54.2 1.0 - 103.3	29.0 4.3 - 59.4	0.83	0.73	7.4 0 - 327.7	7.1 0 - 76.4	3.2 0 - 85.1	0.08	0.91
IL-13	10.1 0.2 - 62	9.4 2.8 - 67.9	3.5 -5.1 - 13.8	0.03 *	0.20	3.8 2 - 14.3	5.4 2.1 - 7.1	5.3 0.6 - 14	0.49	0.98
IL-15	0 0 - 2.6	0 0 - 3.8	0 0 - 0.3	1.0	0.81	0 0 - 0.4	0 0 - 0	0 0 - 0	0.20	0.84
TNFα	3799 1010 - 16035	4749 947 - 13480	1372 875.6 - 11891	0.90	0.01 *	6375 1017 - 11679	2727 598.3 - 3712	2627 1448 - 5406	0.06	0.07
GM-CSF	214.7 76.5 - 2584	314.8 175 - 492.2	116.8 48.9 - 2747	1.0	0.02 *	486.9 152.5 - 1516	147.2 42 - 427.2	245.8 175 - 595.5	0.03 *	0.06

IFNγ	257.0	835.8	467.3	0.02 *	0.65	233.4	133.9	416.4	0.06	0.39
	37.6 - 5157	266.9 - 5830	219.6 - 983.8			28.5 - 2524	49.1 - 983	29 - 807.8		
IP-10	1430	11596	1201	0.17	0.43	1282	20133	9222	0.23	0.76
	157.4 - 36114	398.1 - 18355	315.6 - 25330			-30.8 - 17084	4289 - 36155	3964 - 18476		
CCL3	29972	29608	13635	0.52	0.03 *	27820	9218	20029	0.03 *	0.08
	8888 - 95230	12920 - 65686	3968 - 24054			5995 - 49179	4119 - 33611	10720 - 26486		
CCL4	14046	8279	5293	0.64	0.01 *	8037	6860	6993	0.45	0.25
	3428 - 20732	4556 - 11353	2165 - 8015			2947 - 16094	3128 - 11886	4756 - 11283		
CCL5	2179	3210	2580	0.28	0.20	4122	2288	4443	0.76	0.42
	486.2 - 7919	1719 - 7681	1486 - 3616			871.8 - 7449	636.5 - 14529	1981 - 16442		
IL-8 (Elisa) ng/ml	54	54.5	47	0.77	0.69	57	52	50	0.43	0.55
	13 - 63	0 - 64.3	0 - 59.8			39 - 65.5	34 - 62	39 - 62		

Legend for Supplementary Table 2:

Results shown are cytokine/chemokine concentrations assayed with Luminex multiplex subtracting concentration in unstimulated PBMC cultures from concentration in cultures of PBMC stimulated with heat-killed H37Rv for 24 hrs. Median and interquartile range values are presented and units are pg/ml. The exception was IL-8 which was assayed using ELISA and units are in ng/ml. P-values were calculated for comparisons between 0 and 2 weeks values and 0 and 4 week values for each treatment group separately using Wilcoxon matched pairs test. Two patients in the placebo-treated arm switched to open label prednisone at week 2 and thus their week 4 sample was excluded from analysis.

Significant differences are indicated with *. After Bonferroni correction for multiple comparisons ($p_{corr} = p \times 17 - 1 = p \times 16$) none of the differences remained significant.

Cytokines	Placebo (n=9)					Prednisone (n=16)				
	Week 0	Week 2	Week 4	p-value (Week 0 vs 2)	p-value (Week 0 vs 4)	Week 0	Week 2	Week 4	p-value (Week 0 vs 2)	p-value (Week 0 vs 4)
TNF α	8.65 (7.85-9.17)	8.99 (8.67-9.61)	8.40 (6.00-9.04)	0.05	1.00	8.63 (7.54-9.86)	8.82 (8.35-9.77)	8.80 (8.12-9.11)	0.36	0.56
IL-6	12.34 (10.55-15.88)	14.38 (12.48-15.29)	13.94 (8.58-17.09)	0.08	1.00	12.79 (11.81-14.55)	16.47 (13.30-17.37)	14.01 (12.81-18.04)	0.04	0.42
IL-12p40	17.07 (15.35-17.83)	17.26 (15.89-18.33)	17.03 (12.58-19.24)	0.58	0.44	17.22 (16.22-17.87)	17.49 (15.99-19.11)	17.60 (15.88-18.04)	0.63	0.93
IL-10	6.89 (6.58-7.62)	6.98 (6.64-7.75)	8.08 (7.53-11.47)	0.84	0.09	7.76 (6.37-9.65)	7.64 (7.09-9.17)	8.08 (6.56-11.16)	0.68	0.56

Supplementary Table 3: RT-PCR experiments showing delta Ct (Ct gene of interest – Ct β -actin) values of unstimulated samples after 24-hour cell culture. Statistical comparisons were made between week 0 and 2 and between week 0 and 4 values for placebo- and prednisone-treated participants separately.

Legend for Supplementary Table 3:

Values shown are median (IQR) of delta Ct values.

The paired Wilcoxon signed-rank test was used to compare medians.

Supplementary Table 4: Serum Luminex multiplex assay comparing the change (or delta) in concentrations of cytokines/chemokines between week 0 and week 2 for those participants who had symptom improvement over this period to those who had no improvement or deteriorated.

Cytokine/chemokine	Non-improvers (n= 20)	Improvers (n=35)	p-value
TNFα	-17.8 (-31.0; 0)	-15.8 (-29.5; -7.4)	0.69
IFNγ	-9.1 (-19.3; 0.7)	0 (-15.3; 6.0)	0.14
GM-CSF	0 (0; 0)	0 (0; 0)	0.72
IL-1β	0 (0; 0)	0 (0; 0)	0.73
IL-2	0 (0; 0)	0 (0; 0)	0.07
IL-6	-17.7 (-34.0; 0.8)	-15.7 (-23.4; -8.1)	0.90
IL-8	-7.6 (-58.9; 17.6)	-15.9 (-43.6; 3.6)	0.76
IL-10	-4.0 (-9.4; -0.2)	-3.7 (-8.1; 0)	0.89
IL-12	-12.4 (-25.3; 0)	-8.0 (-29.4; 0)	0.78
IP-10	-890.7 (-2480; -1.5)	-727.7 (-2478; 66.5)	0.84
CCL3	-1.4 (-33.5; 5.5)	0 (-11.3; 21.4)	0.10
CCL4	-9.2 (-30.0; 15.4)	-14.3 (-32.5; 10.2)	0.62

Legend for Supplementary Table 4:

Values shown are the median (IQR) of the delta (week 2 – week 0) for each of 12 serum cytokines/chemokines measured.

Statistical comparisons were performed using the Wilcoxon signed-rank test.