

Multiplex immunohistochemistry elucidates increased distance between cytotoxic T cells and plasma cells in relapsed myeloma, and identifies Lag-3 as the most common checkpoint receptor on cytotoxic T cells of myeloma patients

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Supplementary Table 1: Plasma cell percentage as reported by the diagnostic pathology laboratory by morphology [aspirate], trephine anti-CD138 chromogenic IHC estimation and as assessed by mIHC in this study.

	Aspirate	Trephine CD138 IHC	mIHC CD138	P value *
MGUS (n=32)	3.8 ± 2.7	6.5 ± 2.6	11.6 ± 7.8	<0.0001 1 vs 2 = 0.0014 1 vs 3 < 0.0001 2 vs 3 = 0.3125
NDMM (n=65)	23.3 ± 18.6	34.6 ± 25.2	36.1 ± 24.8	<0.0001 1 vs 2 = 0.0026 1 vs 3 < 0.0001 2 vs 3 = 0.5220
RRMM (n=59)	25.2 ± 30.1	33.4 ± 31.2	30.1 ± 26.2	0.0003 1 vs 2 = 0.0004 1 vs 3 = 0.0150 2 vs 3 = 0.9337
Combined (n=156)	20.0 ± 23.5	28.4 ± 27.4	28.8 ± 24.6	<0.0001 1 vs 2 < 0.0001 1 vs 3 < 0.0001 2 vs 3 = 0.9654

Values represent plasma cell percentage mean ± SD unless otherwise specified. *Friedman test one-way ANOVA for matched-measures, non-parametric data with corresponding Dunn's multiple comparisons tests shown where 1 = Aspirate, 2 = Trephine CD138, 3 = mIHC CD138 cohort.

Supplementary Table 2: Summary of mIHC sub-group analyses.

Characteristic	MGUS - Risk group comparison Low/Inter. (n=21) vs. Inter.-High/High Risk (n=11)			NDMM 2 - Response to 1 st line Rx Response (n=41) vs. SOR & EP (n=18)			NDMM 2 - Extremes of response < PR (n=12) vs. > CR (n=9)			RRMM - Number of prior lines (PL) 1 PL (n=22) vs. > 3 PL (n=15)			RRMM - Prior AutoSCT No (n=25) vs. Yes (n=34)		
	Low/Inter.	Inter.-High/High	p-value	Response	SOR & EP	p-value	< PR	> CR	P-value	1 PL	> 3 PL	p-value	No	Yes	p-value
BM cellularity, %	46.8 ± 11.4	47.4 ± 12.8	0.8976	50.3 ± 17.0	58.3 ± 16.1	0.0965	60.6 ± 14.3	47.8 ± 19.3	0.0970	49.9 ± 13.8	54.0 ± 17.7	0.4394	55.3 ± 14.2	52.1 ± 15.8	0.4376
Fat space, %	36.0 ± 9.4	35.1 ± 16.4	0.8468	36.4 ± 17.7	36.2 ± 16.0	0.9749	34.9 ± 15.4	39.2 ± 20.4	0.5897	34.9 ± 11.3	31.9 ± 15.4	0.4963	34.6 ± 12.6	32.7 ± 13.3	0.5850
Total number of nucleated cells assessed	32870 ± 21220	29026 ± 19038	0.6184	36839 ± 27075	48178 ± 29971	0.1571	51505 ± 31534	60880 ± 30162	0.5006	31794 ± 20601	31962 ± 29816	0.4720	23877 ± 19831	40640 ± 28688	0.0149
Plasma cells (PC), %	10.9 ± 8.0	13.0 ± 7.4	0.4694	35.3 ± 26.1	39.2 ± 20.9	0.5845	60.0 ± 21.7	80.1 ± 14.5	0.0216	28.7 ± 22.7	35.2 ± 35.2	> 0.9999	32.5 ± 26.2	28.4 ± 26.5	0.4692
Non-Plasma cells, %	89.1 ± 8.0	87.0 ± 7.4	0.4694	64.7 ± 26.1	60.8 ± 20.9	0.5845	39.9 ± 21.7	19.0 ± 14.5	0.0216	71.3 ± 22.7	64.8 ± 35.2	> 0.9999	68.5 ± 26.2	71.6 ± 26.5	0.4692
CD3 and CD8 expression															
CD3+ T-cells (As % of all nucleated cells)	11.8 ± 4.9	17.8 ± 14.4	0.0909	11.5 ± 6.4	10.1 ± 5.3	0.4466	8.95 ± 5.3	13.3 ± 8.4	0.1614	13.4 ± 8.4	11.5 ± 10.3	0.1807	11.7 ± 8.9	11.1 ± 7.6	0.9818
CD3+ CD8+ T-cells (As % of all nucleated cells)	3.9 ± 2.7	5.0 ± 4.4	0.3708	3.3 ± 2.5	4.5 ± 4.0	0.1883	3.82 ± 4.5	4.23 ± 2.6	0.3100	4.1 ± 2.8	4.2 ± 5.5	0.4720	3.4 ± 2.7	4.1 ± 3.9	0.1860
Tim-3 expression															
Tim-3+ cell, % (As % of all nucleated cells)	2.57 ± 2.6	1.8 ± 2.2	0.4113	3.78 ± 4.8	2.10 ± 2.5	0.1674	2.12 ± 2.9	2.14 ± 1.7	0.8078	2.63 ± 3.8	5.14 ± 7.9	0.4395	2.38 ± 3.5	3.87 ± 6.3	0.3953
Tim-3+ CD3+CD8+ T-cell (As % of all nucleated cells)	0.08 ± 0.08	0.07 ± 0.05	0.6794	0.14 ± 0.29	0.09 ± 0.11	0.4981	0.25 ± 0.6	0.24 ± 0.3	0.4522	0.14 ± 0.22	0.15 ± 0.33	0.6407	0.08 ± 0.20	0.15 ± 0.24	0.0581
Tim-3+ CD3+CD8- T-cell (As % of all nucleated cells)	0.34 ± 0.38	0.27 ± 0.30	0.5971	0.57 ± 0.78	0.24 ± 0.28	0.5665	0.19 ± 0.27	0.45 ± 0.73	0.2469	0.59 ± 1.3	0.50 ± 0.70	0.7483	0.47 ± 1.21	0.49 ± 0.77	0.9415
Lag-3 expression															
Lag-3+ cell, % (As % of all nucleated cells)	5.8 ± 4.5	4.9 ± 3.1	0.5376	6.28 ± 8.4	5.09 ± 6.8	0.8148	5.34 ± 7.8	4.35 ± 5.2	0.8078	5.11 ± 7.8	4.65 ± 5.7	0.7025	6.14 ± 7.7	3.52 ± 4.2	0.0468
Lag-3+ CD3+CD8+ T-cell (As % of all nucleated cells)	0.29 ± 0.40	0.45 ± 0.47	0.3148	0.22 ± 0.25	0.33 ± 0.62	0.3167	0.25 ± 0.6	0.24 ± 0.3	0.4522	0.41 ± 0.43	0.13 ± 0.14	0.0250	0.37 ± 0.43	0.20 ± 0.26	0.1344
Lag-3+ CD3+CD8- T-cell (As % of all nucleated cells)	0.57 ± 0.90	1.11 ± 1.61	0.2186	0.85 ± 1.48	0.67 ± 1.41	0.6782	0.87 ± 1.7	0.79 ± 1.0	0.4750	0.73 ± 0.90	0.45 ± 0.77	0.2794	0.54 ± 0.82	0.53 ± 0.86	0.9738
PD-1 expression															
PD-1+ cell, % (As % of all nucleated cells)	3.4 ± 4.8	3.6 ± 2.8	0.9279	5.17 ± 6.6	5.73 ± 11.9	0.8148	8.08 ± 14.2	4.52 ± 4.4	0.9170	3.30 ± 3.0	3.49 ± 6.5	0.3996	3.18 ± 3.8	3.39 ± 4.9	0.7929
PD-1+ CD3+CD8+ T-cell (As % of all nucleated cells)	0.12 ± 0.25	0.23 ± 0.33	0.2896	0.16 ± 0.32	0.11 ± 0.23	0.5083	0.15 ± 0.27	0.16 ± 0.17	0.4743	0.16 ± 0.28	0.14 ± 0.29	0.7767	0.11 ± 0.18	0.15 ± 0.28	0.9848
PD-1+ CD3+CD8- T-cell (As % of all nucleated cells)	0.76 ± 0.72	1.42 ± 1.72	0.1258	0.92 ± 1.4	0.85 ± 1.8	0.8796	0.86 ± 2.2	0.81 ± 1.2	0.6646	1.40 ± 2.1	0.85 ± 2.1	0.0380	1.30 ± 2.1	0.77 ± 1.4	0.1200
Absolute number of CD3+CD8+ T-cells within a specified distance from plasma cells															
Within 25µm	649 ± 586	1408 ± 2668	0.2270	978 ± 1175	1968 ± 3270	0.0916	2064 ± 3912	1682 ± 1594	0.5538	1010 ± 1181	837 ± 1570	0.1886	507 ± 575	1192 ± 1492	0.0443
Within 50µm	922 ± 739	1822 ± 3306	0.2485	1142 ± 1382	2131 ± 3315	0.1090	2198 ± 3957	2164 ± 1935	0.4221	1152 ± 1267	1343 ± 3297	0.0935	583 ± 597	1543 ± 2447	0.0242

Within 100µm	1102 ± 823	2014 ± 3468	0.2672	1233 ± 1520	2216 ± 3358	0.1237	2238 ± 3959	2472 ± 2218	0.3824	1247 ± 1293	1847 ± 5083	0.0417	628 ± 595	1862 ± 3539	0.0150
Proportion of CD3+CD8+ T-cells within a specified distance from plasma cells															
Within 25µm	54.0 ± 21.2	56.0 ± 22.4	0.8188	76.7 ± 24.7	86.3 ± 16.8	0.1400	88.6 ± 10.2	64.7 ± 17.9	0.0010	69.2 ± 27.3	66.4 ± 36.5	0.8855	70.7 ± 26.4	66.2 ± 30.8	0.8062
Within 50µm	77.4 ± 18.1	78.0 ± 23.1	0.9314	88.8 ± 17.8	94.6 ± 12.7	0.2143	97.0 ± 2.79	85.1 ± 8.4	0.0024	83.6 ± 21.3	76.7 ± 33.7	0.9681	84.9 ± 19.5	79.0 ± 26.7	0.6825
Within 100µm	93.6 ± 18.4	91.2 ± 18.4	0.6414	96.3 ± 9.8	97.5 ± 9.5	0.6735	99.7 ± 0.54	97.4 ± 2.2	0.0028	94.8 ± 10.4	85.7 ± 29.3	0.8364	95.8 ± 8.1	89.6 ± 20.8	0.3697
Average distance to a PC from each CD3+CD8+ T-cell	36.7 ± 20.7	40.9 ± 41.2	0.7035	25.4 ± 35.8	20.4 ± 28.9	0.6024	14.3 ± 6.4	26.9 ± 9.1	0.0043	29.0 ± 26.2	149 ± 442.3	0.9108	27.0 ± 21.4	82.3 ± 288.6	0.8152
Average distance to a CD3+CD8+ T-cell from each plasma cell	51.2 ± 29.6	50.1 ± 22.7	0.9139	79.2 ± 67.6	67.2 ± 71.9	0.5399	82.1 ± 84.7	44.7 ± 26.1	0.1694	107.2 ± 248	53.6 ± 22.4	0.6888	61.6 ± 38.5	122 ± 283	0.3472
Average number of CD3+CD8+ T-cells within 100µm of a PC	1.76 ± 0.75	1.78 ± 0.41	0.9618	1.36 ± 0.42	1.29 ± 0.34	0.5578	1.27 ± 0.28	1.54 ± 0.37	0.0491	1.51 ± 0.78	2.07 ± 2.5	0.4703	1.37 ± 0.33	1.82 ± 1.72	0.5125
Proportion of unique plasma cells, %	25.3 ± 16.2	26.1 ± 15.7	0.9027	12.3 ± 12.7	10.5 ± 9.8	0.6067	8.26 ± 0.79	16.1 ± 8.2	0.0118	16.8 ± 17.4	23.3 ± 33.3	0.4703	12.5 ± 11.3	19.5 ± 25.3	0.6401
Proportion of unique CD3+CD8+ T-cells	59.9 ± 20.6	55.1 ± 20.8	0.5530	79.5 ± 21.2	82.9 ± 19.4	0.5681	84.5 ± 15.3	67.6 ± 18.3	0.0409	73.6 ± 22.8	68.5 ± 37.0	0.5748	77.8 ± 19.7	69.0 ± 30.1	0.5887

Supplementary Table 3: Summary of mIHC results comparing MGUS cohort by <10% plasma cells with ≥ 10% by mIHC.

Characteristic	MGUS Reclassified by mIHC PC% <10% PCs by mIHC (n=16) vs. ≥10% PCs by mIHC (n=16)		
	<10% PCs by mIHC	≥10% PCs by mIHC	p-value
BM cellularity, %	48.6 ± 10.8	45.4 ± 12.8	0.451
Fat space, %	33.8 ± 9.8	37.6 ± 13.9	0.838
Total number of nucleated cells assessed	35,401 ± 22,705	27,697 ± 17,377	0.149
Plasma cells (PC), %	4.9 ± 2.7	18.3 ± 4.6	<0.0001 *
Non-Plasma cells, %	95.1 ± 2.7	81.7 ± 4.6	<0.0001 *
CD3 and CD8 expression			
CD3+ T-cells (As % of all nucleated cells)	10.0 ± 3.8	17.8 ± 11.9	0.0022 *
CD3+ CD8+ T-cells(As % of all nucleated cells)	3.16 ± 2.0	5.4 ± 4.1	0.061
Tim-3 expression			
Tim-3+ cell, % (As % of all nucleated cells)	2.6 ± 2.6	2.0 ± 2.3	0.809
Tim-3+ CD3+CD8+ T-cell (As % of all nucleated cells)	0.07 ± 0.07	0.09 ± 0.08	0.428
Tim-3+ CD3+CD8- T-cell (As % of all nucleated cells)	0.30 ± 0.35	0.33 ± 0.37	0.423
Lag-3 expression			
Lag-3+ cell, % (As % of all nucleated cells)	4.6 ± 3.6	6.4 ± 4.4	0.231
Lag-3+ CD3+CD8+ T-cell (As % of all nucleated cells)	0.19 ± 0.16	0.51 ± 0.54	0.075
Lag-3+ CD3+CD8- T-cell (As % of all nucleated cells)	0.44 ± 0.64	1.10 ± 1.51	0.058
PD-1 expression			
PD-1+ cell, % (As % of all nucleated cells)	2.7 ± 4.0	4.3 ± 4.4	0.270
PD-1+ CD3+CD8+ T-cell (As % of all nucleated cells)	0.07 ± 0.12	0.24 ± 0.36	0.121
PD-1+ CD3+CD8- T-cell (As % of all nucleated cells)	0.54 ± 0.56	1.43 ± 1.43	0.0136 *
Absolute number of CD3+CD8+ T-cells within a specified distance from plasma cells			
Within 25µm	365.5 ± 335.7	1438 ± 2142	0.004 *
Within 50µm	634.7 ± 513.9	1809 ± 2674	0.037 *
Within 100µm	874.8 ± 669.6	1938 ± 2827	0.149
Proportion of CD3+CD8+ T-cells within a specified distance from plasma cells			
Within 25µm	37.7 ± 15.5	71.6 ± 8.8	<0.0001 *
Within 50µm	64.5 ± 19.5	90.7 ± 6.0	<0.0001 *
Within 100µm	86.7 ± 15.9	98.8 ± 1.4	<0.0001 *

Average distance to a PC from each CD3+CD8+ T-cell	54.0 ± 32.9	22.1 ± 5.5	<0.0001 *
Average distance to a CD3+CD8+ T-cell from each plasma cell	56.2 ± 28.5	45.6 ± 25.5	0.174
Average number of CD3+CD8+ T-cells within 100µm of a PC	2.03 ± 0.8	1.51 ± 0.3	0.013 *
Proportion of unique plasma cells, %	32.6 ± 17.8	18.5 ± 9.6	0.017 *
Proportion of unique CD3+CD8+ T-cells	47.1 ± 18.9	69.5 ± 15.5	0.001 *