

Supplementary material:

Spatial landscape of malignant pleural and peritoneal mesothelioma tumor immune microenvironment

Xiaojun Ma¹, David Lembersky¹, Elena S Kim^{1,4}, Tullia C Bruno^{1,2}, Joseph R. Testa³, Hatice U Osmanbeyoglu^{1,4,5,6*}

¹UPMC Hillman Cancer Center, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

²Department of Immunology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

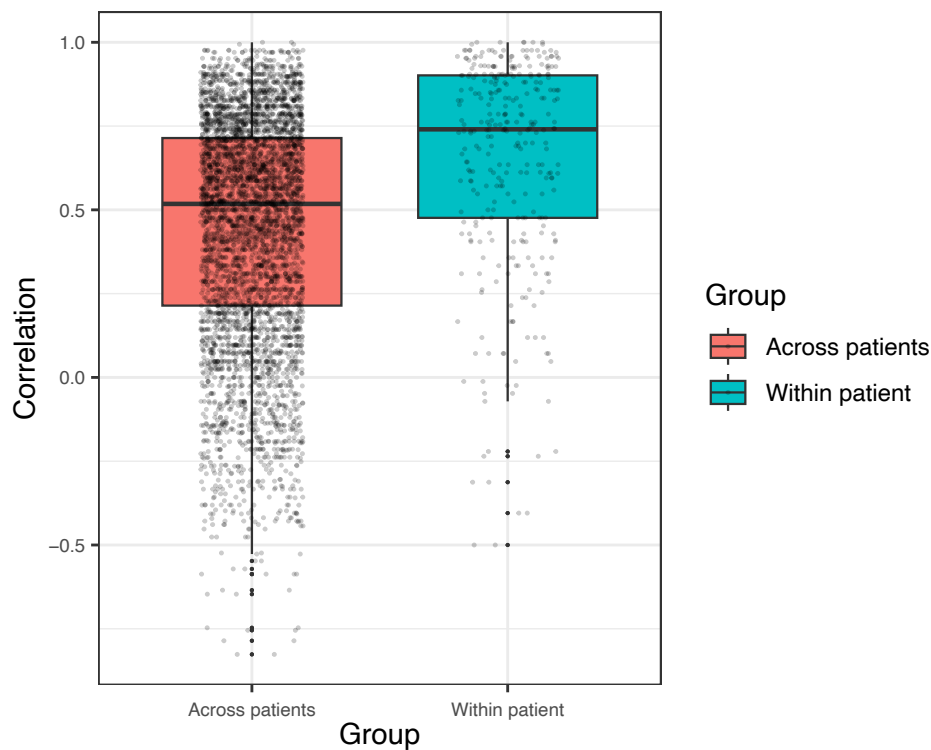
³Cancer Prevention and Control Program, Fox Chase Cancer Center, Philadelphia, PA, USA

⁴Department of Biomedical Informatics, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

⁵Department of Bioengineering, University of Pittsburgh School of Engineering, Pittsburgh, USA

⁶Department of Biostatistics, University of Pittsburgh School of Public Health, Pittsburgh, PA, USA

Supplementary Figures



Supplementary Figure 1: TMA core cell-type proportions are more similar within patients than across patients. Each value in the boxplot represents the Pearson correlation coefficients between cell-type proportions across patients and within patients.

Supplementary Tables

Supplementary Table 1: Comparison of cell-cell contact score differences between MPM and MPeM. Two-sided Kruskal Wallis test was used for statistical analysis with FDR-adjusted <0.15 considered significant.

Contact pair	P value	FDR-adjusted	Mean contact score difference (MPM – MpeM)
Tumor cells – tumor cells	0.0002	0.0036	0.091
CD8 ⁺ T cells – CD8 ⁺ T cells	0.0012	0.0125	-0.095
CD4 ⁺ T cells – tumor cells	0.0112	0.0784	0.058
Macrophages – tumor cells	0.0158	0.0830	0.035
Tregs – tumor cells	0.0300	0.1054	0.041
CD8 ⁺ T cells – tumor cells	0.0301	0.1054	0.043
B cells – Macrophages	0.0393	0.1118	-0.214
B cells – CD8 ⁺ T cells	0.0426	0.1118	-0.051
CD8 ⁺ T cells – Macrophages	0.0625	0.1458	-0.069
Macrophages – Macrophages	0.0714	0.1499	0.026
CD8 ⁺ T cells – FOXP3	0.1020	0.1947	-0.042
CD4 ⁺ T cells – CD8 ⁺ T cells	0.1180	0.2065	-0.040
B cells – B cells	0.1620	0.2460	-0.026
B cells – CD4 ⁺ T cells	0.1640	0.2460	-0.094
B cells – tumor cells	0.2280	0.3192	0.072
Tregs – Tregs	0.4020	0.5276	0.045
CD4 ⁺ T cells – Macrophages	0.4550	0.5621	-0.014
CD4 ⁺ T cells – Tregs	0.5730	0.6540	0.014
CD4 ⁺ T cells – CD4 ⁺ T cells	0.5930	0.6540	0.022
Tregs – Macrophages	0.6240	0.6540	0.009
B cells – Tregs	0.6540	0.6540	0.097

Supplementary Table 2: Distribution of immune/tumor cells across different histologies for MM cohort. Table listing P values of individual immune/tumor cell subsets. Two-sided Kruskal Wallis test was used for statistical analysis with $P < 0.05$ considered significant.

Cell Type	MPM			MPeM
	Epithelioid vs. Biphasic	Epithelioid vs Sarcomatoid	Biphasic vs Sarcomatoid	Epithelioid vs. Biphasic
B cells (CD20 ⁺)	0.044	0.095	0.416	0.217
CD4 ⁺ T cells	0.371	0.037	0.040	0.473
CD8 ⁺ T cells	0.053	0.475	0.077	0.420
Tregs (FOXP3 ⁺ CD4 ⁺)	0.163	0.256	0.447	0.469
Macrophages (CD68 ⁺)	0.366	0.282	0.337	0.101
DCs (CD11c ⁺)	0.410	0.677	0.637	0.230
NK (CD56 ⁺)	0.161	0.323	0.108	0.319
Pan-CK ⁺	0.491	0.001	0.001	0.420

Supplementary Table 3: Comparison of cell-type distributions between MPM and MPeM within same histologic subtypes*

Cell Type	MPM vs. MPeM	
	Epithelioid	Biphasic
B cells (CD20 ⁺)	0.234	0.122
CD4 ⁺ T cells	0.531	0.184
CD8 ⁺ T cells	0.065	0.479
Tregs (FOXP3 ⁺ CD4 ⁺)	0.206	0.077
Macrophages (CD68 ⁺)	0.625	0.093
DCs (CD11c ⁺)	0.689	0.246
NK (CD56 ⁺)	0.342	0.521
Pan-CK ⁺	0.072	0.184

*Table lists *P* values of differences for individual immune/tumor cell populations between MPM and MPeM tumors. One-sided Kruskal Wallis test was used for statistical analysis with *P* < 0.05 considered significant.

Supplementary Table 4: The association of immune cells proportion with overall survival in all MM as well as for MPM and MPeM separately

	MM	MPM	MPeM
CD4 ⁺ T cells	0.17	0.67	0.02
CD8 ⁺ T cells	0.63	0.46	0.97
Pan-CK ⁺	0.20	0.10	0.93
Tregs (FOXP3 ⁺ CD4 ⁺)	0.13	0.38	0.39
B cells (CD20 ⁺)	0.27	0.38	0.35
Macrophages (CD68 ⁺)	0.12	0.33	0.12
DCs (CD11c ⁺)	0.21	0.70	0.37
NK (CD56 ⁺)	0.36	0.72	0.36

*Table lists *P* values based on log-rank test. *P* < 0.05 considered significant.

Supplementary Table 5: Comparison of clinical parameters between MPM and MPeM for various cell types. FDR-corrected sided Wilcoxon test for sex, age, smoking status, asbestos exposure, stage

	CD4+ T cells	CD8+ T cells	Pan-CK+	B cells (CD20+)	Tregs (FOXP3+ CD4+)	Macrophages (CD68+)	DCs (CD11c+)	NK (CD56+)
High grade	0.680	0.061	0.054	0.897	0.696	0.827	0.266	0.725
Low/Median grade	NA	NA	NA	NA	NA	NA	NA	NA
Smoker	0.876	0.131	0.071	0.897	0.696	0.592	0.515	0.722
Non-smoker	0.876	0.131	0.204	0.897	0.696	0.827	0.847	0.725
Asbestos Exposure	0.065	0.123	0.136	0.897	0.696	0.827	0.818	0.722
No Asbestos Exposure	NA	NA	NA	NA	NA	NA	NA	NA
Young (<60)	0.876	0.091	0.122	0.897	0.721	0.592	0.815	0.725
Old (>60)	0.876	0.002	0.042	0.897	0.696	0.495	0.065	0.153
White	0.876	0.002	0.023	0.897	0.696	0.495	0.169	0.725
Not White	NA	NA	NA	NA	NA	NA	NA	NA
Male	0.680	0.011	0.071	0.897	0.696	0.964	0.818	0.510
Female	0.680	0.131	0.023	0.897	0.696	0.592	0.266	0.153

FDR < 0.15 were bolded.

Supplementary Table 6: Comparison of cell-cell contact score differences between MPM and MPeM*

Contact pair	MPM			MPeM		
	P value	FDR-adjusted	BAP1 ^{high} – BAP1 ^{low}	P value	FDR-adjusted	BAP1 ^{high} – BAP1 ^{low}
CD4 ⁺ T cells – tumor cells	0.074	0.120	-0.685	0.549	0.549	0.226
CD8 ⁺ T cells – tumor cells	0.003	0.013	-0.521	0.046	0.093	-0.630
Macrophages – tumor cells	0.090	0.120	-0.147	0.036	0.093	0.756
B cells – tumor cells	NA	NA	-0.147	NA	NA	0.756
Tregs – tumor cells	0.500	0.500	-0.460	0.500	0.549	-0.199

*Table lists P values and FDR values. Two-sided Kruskal Wallis test was used for statistical analysis with FDR-adjusted such that P < 0.15 was considered significant.

Supplementary Table 7: Antibody details

Antibody	Clone	Concentration	Supplier	Cat. #
FOXP3	D2W8E	1:200	Cell Signaling	98377S
CD8	C8/144B	1:150	Biocare Medical	ACI3160A
CD68	D4B96	1:800	Cell Signaling	76437S
Pan-cytokeratin	AE1/AE3	1:150	Santa Cruz Biotechnologies	sc-81714
CD20	L26	1:300	Leica Biosystems	NCL-L-CD20-L26
CD4	EP204	pre-dilute	Biocare Medical	API3209AA
LAG3	D2G4O™	1:250	Cell Signaling	45208S
CD11c	D3V1E	1:400	Cell Signaling	45581S
CD56	BC56C04	1:100	Biocare Medical	CM164A
BAP1	N/A	1:100	Biocare Medical	ACI3247A
MTAP	N/A	1:500	Abcam	Ab96231
NF2	N/A	1:300	Sigma	HPA003097