

Characterization and Differentiation of the Tumor Microenvironment (TME) of Orthotopic and Subcutaneously Grown Head and Neck Squamous Cell Carcinoma (HNSCC) in Immunocompetent Mice

Matthias Brand, Simon Laban, Marie-Nicole Theodoraki, Johannes Doescher, Thomas K. Hoffmann, Patrick J. Schuler and Cornelia Brunner

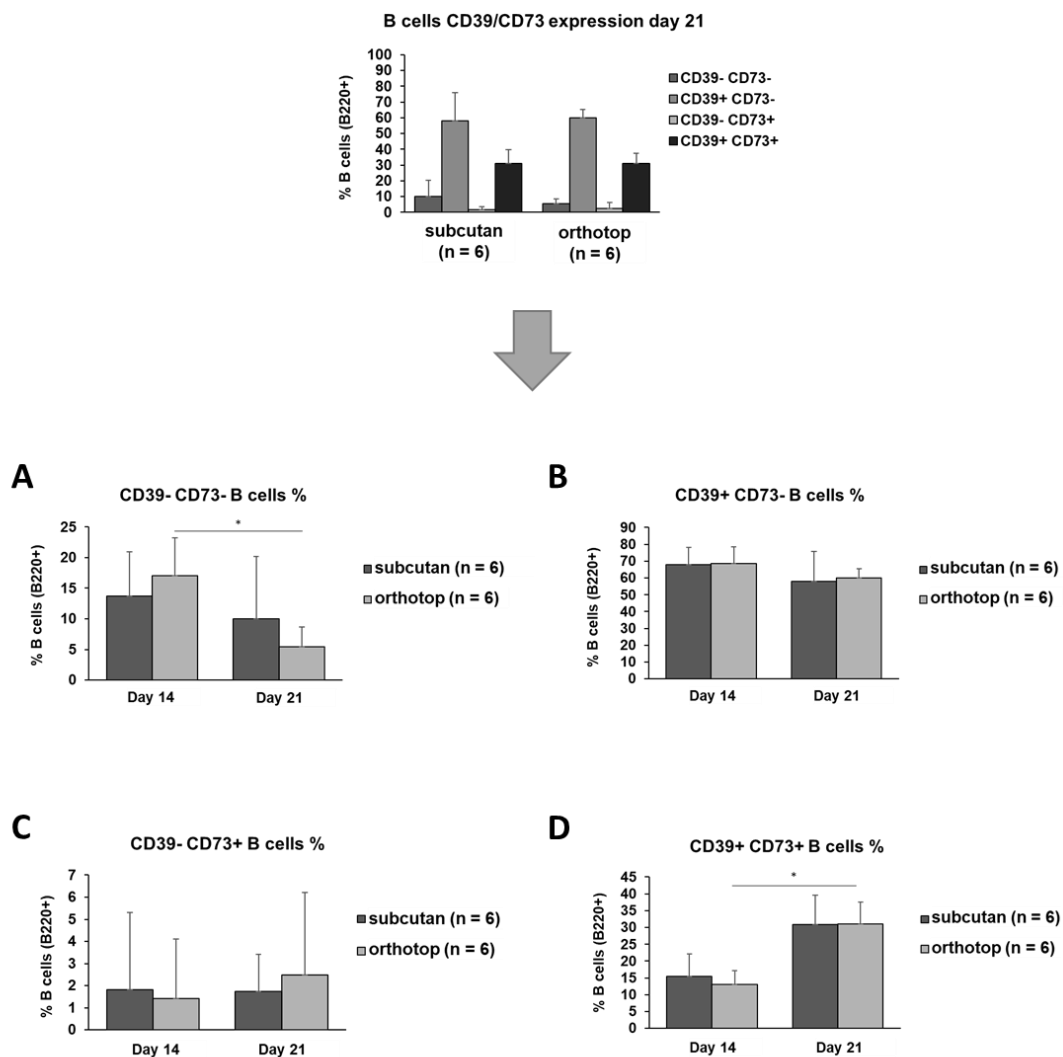


Figure S1. CD39/CD73 expression on B cells in the tumor of orthotopic and subcutaneous HNSCC bearing mice. 14 days and 21 days after tumor induction, tumors were harvested and populations of B cells with a different CD39/CD73 expression were analyzed using flow cytometry. On day 14 and day 21 the test group size for the subcutaneous group is $n = 6$, for the orthotopic group $n = 6$. p -values < 0.05 were considered to be significant with (*).

Table S1. Tumor-data summary of orthotopic and subcutaneous HNSCC bearing mice. Data is visualized in Figure 2–4. SD = standard deviation.

		Subcutan				Orthotop			
		Day 14		Day 21		Day 14		Day 21	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Tumorweight (g)		0.36	0.13	0.75	0.22	0.31	0.12	0.47	0.12
TIL ($\times 10^3$)		19,958.33	15,422.13	48,960.00	30,610.41	10,691.67	7617.71	36,800.00	9025.52
TIL/tumorweight ($\times 10^3/\text{g}$)		55,696.13	39,132.27	59,633.87	23,554.93	31,143.58	10,735.44	81,098.27	34366.33
% Myeloid cells	Macrophages			60.11	3.30			60.65	3.41
	Granulocytes			12.03	2.15			16.23	5.98
	Monocytes			3.62	0.74			2.59	0.87
	Dendritic cells			24.69	2.36			22.79	1.53
	Macrophages ($\times 10^3$)	1077.85	608.75	2595.01	1366.30	745.69	446.25	1731.31	769.28
Granulocytes ($\times 10^3$)		288.77	112.96	532.61	314.05	201.85	116.07	452.58	170.15
Monocytes ($\times 10^3$)		151.08	101.81	173.59	134.65	40.36	15.82	71.56	30.32
Dendritic cells ($\times 10^3$)		514.04	293.24	1064.87	596.93	308.72	173.29	647.32	274.00
B cells (%)		0.77	0.45	0.34	0.26	1.93	1.76	1.58	0.59
B cells ($\times 10^3$)		155.67	137.29	177.41	157.00	125.68	96.47	541.82	153.54
B cells/tumorweight ($\times 10^3/\text{g}$)		449.87	443.68	194.09	150.10	488.64	419.55	1206.85	503.18
Newly formed B cells ($\times 10^3$)		93.17	81.57	56.24	38.43	76.42	53.64	305.83	97.35
Follicular B cells ($\times 10^3$)		44.25	38.46	108.45	163.68	54.41	33.14	128.99	83.88
% B cells	IgM- IgD-			10.91	4.06			9.17	2.68
	IgM+ IgD-			1.21	1.01			0.98	0.35
	IgM+ IgD+			84.48	3.68			85.66	3.85
	IgM- IgD+			0.83	1.13			3.62	4.25
	CD39- CD73-			10.02	10.21			5.40	3.29
	CD39+ CD73-			58.02	17.84			59.97	5.41
	CD39- CD73+			1.75	1.67			2.47	3.75
	CD39+ CD73+			30.84	8.73			31.03	6.45
CD4+ T cells ($\times 10^3$)		292.24	211.43	299.84	121.40	275.63	101.02	540.94	72.96
CD8+ T cells ($\times 10^3$)		52.12	45.33	58.42	36.49	34.65	23.40	63.83	24.09
Regulatory T cells (% CD4+ T cells)		28.49	4.56	29.90	4.75	16.02	5.71	16.77	7.14
Regulatory T cells ($\times 10^3$)		94.79	64.88	61.88	33.02	28.44	15.52	52.48	17.84
% CD4+ T cells	CD39- CD73-			17.36	5.57			37.10	15.31
	CD39+ CD73-			17.03	6.87			12.36	7.46
	CD39- CD73+			17.34	6.65			19.56	4.43
	CD39+ CD73+			48.27	5.70			30.98	10.59
% CD8+ T cells	CD39- CD73-			71.63	8.09			70.76	5.97
	CD39+ CD73-			5.73	2.06			4.57	1.61
	CD39- CD73+			18.15	4.72			18.42	3.56
	CD39+ CD73+			4.50	2.37			6.25	1.78

Table S2. Data summary of peripheral blood of orthotopic and subcutaneous HNSCC bearing mice. Data is visualized in Figure 5. SD = standard deviation.

		Control								Subcutan								Orthotop							
		Day 0		Day 7		Day 14		Day 21		Day 7		Day 14		Day 21		Day 7		Day 14		Day 21					
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
WBC ($\times 10^3$)		8.59	0.90	10.27	0.03	7.64	0.23	10.38	0.38	9.81	0.35	11.15	1.76	14.38	1.40	10.24	0.79	11.57	0.62	13.56	1.39				
Lymphocytes ($\times 10^3$)		5.77	0.77	7.60	0.60	5.65	0.25	7.15	0.55	7.02	0.25	6.70	0.45	8.40	1.36	6.98	0.43	7.17	0.68	7.60	1.03				
B cells/ μ L		279.57	62.28	293.86	18.92	157.60	36.29	339.99	10.52	371.07	144.45	492.26	57.28	357.55	193.34	362.97	183.54	334.41	110.88	476.76	203.03				
T cells/ μ L		1729.36	602.02	2914.21	211.56	1629.85	76.59	2726.95	46.39	3141.56	575.74	2025.83	722.58	2418.24	867.25	2466.38	349.06	2628.27	425.25	2314.46	512.37				
CD4+ T cells/ μ L		1264.88	435.77	2076.40	159.14	1129.02	41.43	1966.65	48.17	1869.87	919.41	1454.37	528.37	1725.61	615.66	1513.10	625.34	1890.21	315.42	1690.68	375.58				
CD8+ T cells/ μ L		416.86	149.80	766.45	35.93	465.76	27.74	713.04	6.95	677.54	340.46	520.07	178.07	612.99	221.18	574.67	234.05	646.18	110.16	572.84	136.27				
% B cells	IgM- IgD-							3.51	0.25					3.19	1.32					3.83	0.79				
	IgM+ IgD-							27.46	2.87					22.63	5.07					14.00	2.70				
	IgM+ IgD+							56.55	2.30					55.06	5.79					47.73	5.34				
	IgM- IgD+							13.92	0.35					19.36	3.35					34.56	5.92				
	CD39- CD73-							8.34	1.64					11.99	5.79					10.65	2.18				
	CD39+ CD73-							88.90	2.26					85.93	5.56					86.87	1.80				
	CD39- CD73+							0.02	0.02					0.01	0.01					0.04	0.04				
	CD39+ CD73+							1.58	0.35					1.42	0.40					1.88	0.95				
% CD4+ T cells	CD39- CD73-							84.74	2.29					81.87	1.67					82.31	2.78				
	CD39+ CD73-							4.89	0.28					6.71	1.23					5.82	1.05				
	CD39- CD73+							6.67	1.55					7.08	1.07					7.58	1.04				
	CD39+ CD73+							3.71	0.47					4.34	0.66					4.30	1.14				
% CD8+ T cells	CD39- CD73-							82.78	0.29					81.10	2.11					79.39	2.47				
	CD39+ CD73-							4.96	1.82					3.31	1.00					3.02	0.94				
	CD39- CD73+							11.25	1.94					14.26	2.49					16.33	1.89				
	CD39+ CD73+							1.03	0.18					1.33	0.33					1.26	0.21				

Table S3. Data summary of the spleen of orthotopic and subcutaneous HNSCC bearing mice. Data is visualized in Figure 6. SD = standard deviation.

		Control						Subcutan						Orthotop					
		Day 7		Day 14		Day 21		Day 7		Day 14		Day 21		Day 7		Day 14		Day 21	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
B cells (×10 ³)		3622.8	1062.3	5575.0	543.24	4697.7	12.66	4243.6	2032.2	6166.9	2559.2	7375.9	1163.8	3416.9	715.36	5360.6	1016.0	7190.6	3522.2
		1	0	3		7		3	8	0	9	8	4	5		0	1	7	1
Newly formed B cells (×10 ³)		192.08	59.92	295.57	38.15	233.51	18.49	287.89	140.37	326.67	181.39	487.90	103.73	254.52	85.24	356.87	140.32	386.12	191.95
Follicular B cells (×10 ³)		2336.9	806.46	3106.5	666.32	3678.1	534.6	3441.8	1654.4	3382.7	1497.1	5502.2	1063.0	2789.7	725.97	3540.2	1297.8	4874.2	2067.8
		9		5		1	6	3	3	5	9	1	1	0		9	4	8	5
% B cells	IgM- IgD-					11.58	2.30					12.44	3.16					11.00	1.33
	IgM+ IgD-					13.34	3.89					34.40	5.17					25.25	4.88
	IgM+ IgD+					44.07	7.79					36.93	7.20					48.82	6.72
	IgM- IgD+					30.75	1.51					16.01	1.36					13.08	2.53
	CD39- CD73-					4.45	0.47					3.92	1.32					4.85	1.78
	CD39+ CD73-					88.05	0.23					88.50	2.91					89.33	4.04
	CD39- CD73+					0.00	0.00					0.01	0.01					0.01	0.01
	CD39+ CD73+					7.12	0.29					7.33	2.47					5.54	2.89
CD4+ T cells (×10 ³)		5976.4	1389.2	8087.2	3772.4	7797.6	431.9	6313.1	3210.1	7663.5	3021.9	6909.7	1534.1	4602.2	1517.7	7746.3	2863.0	7770.2	1831.9
		8	5	1	5	8	5	8	8	8	3	6	0	8	0	0	0	2	7
CD8+ T cells (×10 ³)		2877.3	684.99	3766.1	2222.8	4199.7	637.3	2960.3	1844.9	3911.2	1652.3	3748.4	1085.3	2505.4	863.03	4015.2	1724.6	3997.9	759.14
		3		7	6	2	9	1	4	5	2	5	0	1		6	2	1	
% CD4+ T cells	CD39- CD73-					57.77	11.51					54.80	3.43					56.66	6.38
	CD39+ CD73-					18.07	4.91					25.14	4.38					17.03	7.64
	CD39- CD73+					4.58	0.36					5.13	1.24					8.75	3.36
	CD39+ CD73+					19.57	6.96					14.93	3.80					16.99	3.07
% CD8+ T cells	CD39- CD73-					80.88	2.37					74.87	2.49					75.08	3.71
	CD39+ CD73-					4.24	0.79					6.80	1.11					5.10	3.02
	CD39- CD73+					10.63	0.13					11.62	1.27					16.04	4.09
	CD39+ CD73+					4.25	1.71					6.91	1.01					5.07	3.48

Table S4. Data summary of inguinal lymph nodes of orthotopic and subcutaneous HNSCC bearing mice. Data is visualized in Figure 7. SD = standard deviation.

	Control						Subcutan						Orthotop					
	Day 7		Day 14		Day 21		Day 7		Day 14		Day 21		Day 7		Day 14		Day 21	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
B cells (% total)	3.03	0.49	2.38	0.04	4.61	0.20	3.48	0.73	4.23	1.58	4.63	1.19	3.60	0.80	4.08	0.62	4.66	0.86
Newly formed B cells (% B cells)	11.79	0.47	10.51	3.87	9.16	2.09	10.07	1.94	8.91	2.82	8.94	1.51	8.00	1.95	10.33	1.82	8.16	1.49
Follicular B cells (% B cells)	81.52	0.03	80.84	4.20	85.90	2.47	84.08	3.86	86.86	4.00	86.89	1.43	86.82	2.59	84.51	3.33	89.25	2.37
% B cells	IgM- IgD-				7.05	0.66					10.76	1.24					9.03	0.83
	IgM+ IgD-				0.14	0.07					0.13	0.05					0.12	0.09
	IgM+ IgD+				82.57	2.80					79.21	2.70					81.40	1.92
	IgM- IgD+				10.35	2.00					9.73	2.94					8.81	2.10
	CD39- CD73-				6.26	0.63					9.59	4.27					12.22	11.30
	CD39+ CD73-				90.96	0.35					85.89	4.41					84.25	85.44
	CD39- CD73+				0.00	0.00					0.03	0.02					0.00	0.01
	CD39+ CD73+				2.90	0.37					4.35	1.29					3.26	2.83
CD4+ T cells (% T cells)	65.34	0.27	68.33	0.26	67.05	1.08	64.83	2.65	64.68	3.31	57.48	3.11	66.01	3.52	63.56	2.05	58.86	3.76
CD8+ T cells (% T cells)	26.34	0.18	26.20	0.30	24.20	0.91	26.30	1.07	23.76	1.52	22.95	1.72	25.49	1.38	24.37	1.69	21.62	1.57
% CD4+ T cells	CD39- CD73-				87.34	0.32					85.09	3.07					87.36	1.87
	CD39+ CD73-				4.89	0.63					5.61	2.18					4.51	0.84
	CD39- CD73+				4.17	0.27					4.49	0.86					4.51	0.71
	CD39+ CD73+				3.60	0.05					4.81	1.34					3.62	0.68
% CD8+ T cells	CD39- CD73-				91.55	0.56					88.73	1.56					88.30	2.28
	CD39+ CD73-				2.26	0.47					1.78	0.50					1.81	0.23
	CD39- CD73+				5.70	1.00					8.82	1.90					9.26	2.26
	CD39+ CD73+				0.49	0.04					0.67	0.11					0.65	0.16

Table S5. Data summary of the bone marrow of orthotopic and subcutaneous HNSCC bearing mice. Data is visualized in Figure 8. SD = standard deviation.

	Control						Subcutan						Orthotop					
	Day 7		Day 14		Day 21		Day 7		Day 14		Day 21		Day 7		Day 14		Day 21	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Leucocyte cells (% total)					34.93	0.09					42.35	4.58					40.75	4.95
Erythroid cells (% total)					61.07	1.07					54.06	4.09					56.39	4.79
B cells (×10 ³)	2982.90	667.16	3837.77	1088.69	4175.40	1215.01	3323.91	1873.42	4063.61	2917.45	3524.87	2790.37	3201.68	1165.76	3285.90	402.20	2996.99	1514.13
Pro-Pre-B cells (IgM(lo)) (% B cells IgD(lo))					61.58	2.04					69.58	3.17					75.28	3.89
Immature B cells (IgM(med)) (% B cells IgD(lo))					33.30	0.93					25.36	3.11					20.04	2.84
Transitional B cells (IgM(hi)) (% B cells IgD(lo))					4.24	0.74					4.90	0.31					4.39	1.22

Table S6. Data summary of the thymus of orthotopic and subcutaneous HNSCC bearing mice. Data is visualized in Figure 9. SD = standard deviation.

	Control						Subcutan						Orthotop					
	Day 7		Day 14		Day 21		Day 7		Day 14		Day 21		Day 7		Day 14		Day 21	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
T cells (×10 ³)	3156.56	942.76	2863.99	70.63	2218.02	641.61	2507.30	1262.51	2950.64	1300.66	2104.11	1089.10	2063.58	890.89	2764.92	461.89	2078.59	1262.38
CD4+ T cells (×10 ³)	1294.27	398.34	1131.29	93.32	1317.55	409.38	1072.96	559.91	1363.76	580.27	1194.01	633.98	826.60	359.12	1186.10	183.19	1072.92	638.63
CD8+ T cells (×10 ³)	1811.68	512.63	1660.82	145.14	868.90	229.19	1373.96	681.09	1534.16	691.97	872.78	447.01	1186.74	533.01	1504.12	273.96	963.29	598.14