Immunophenotypic Analysis of Melanoma Metastases

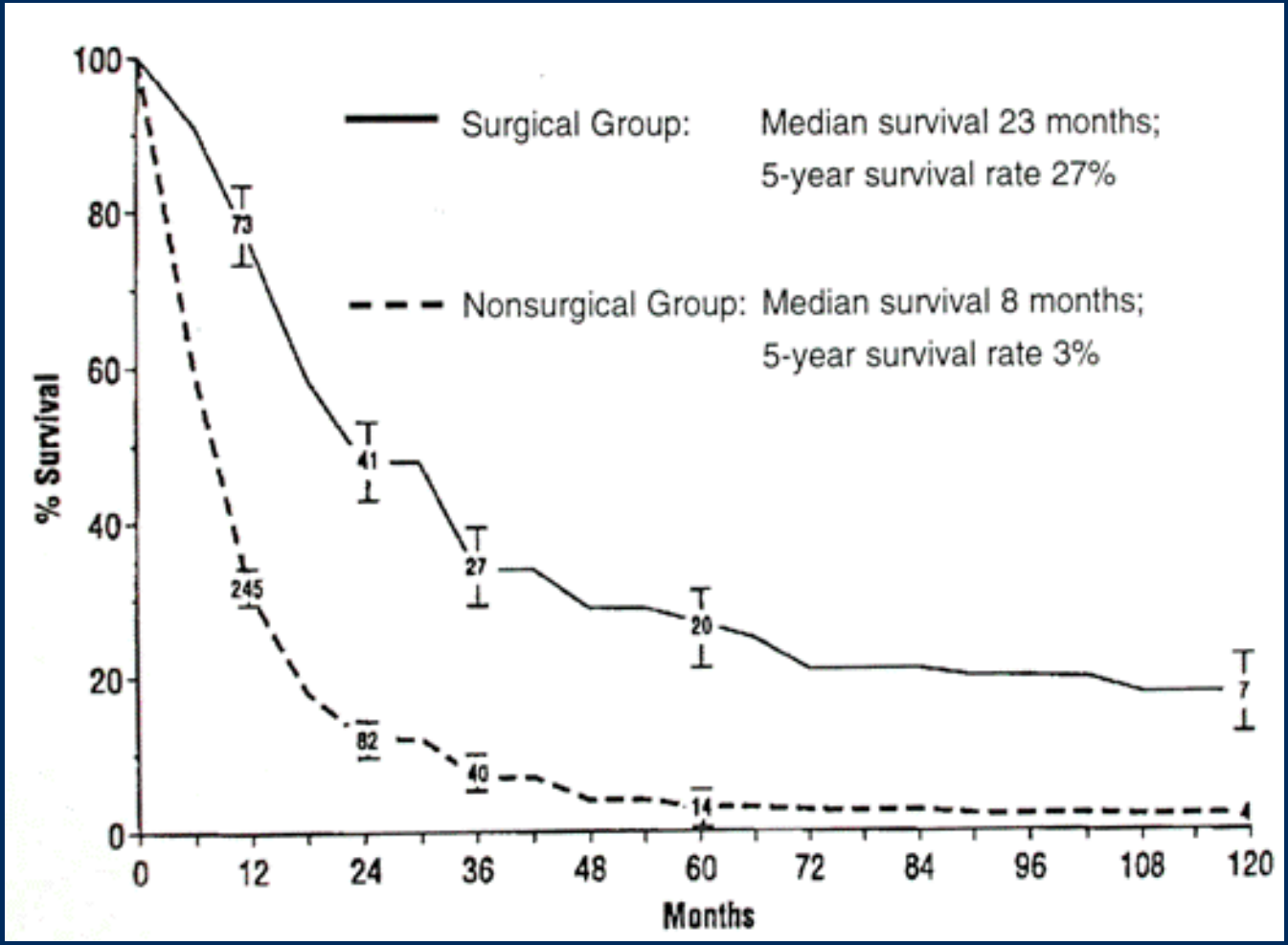
Alicia M. Terando, David Elashoff, Donald L. Morton, and Mark B. Faries

JOHN WAYNE
CANCER INSTITUTE
at Saint John's Health Center

Disclosures

The authors have no conflicts of interest to disclose.

Surgical Resection of Melanoma Metastatic to the Lung (Stage IV M1b)



Tafra L, et al. *J Thorac Cardiovasc Surg.* 1995;110:119-128.

Survival After Resection of Stage IV M1b Melanoma

Study	N	Median Survival (mos)	5-Yr OS (%)
Gorenstein[1]	56	18	25
Meyer[2]	10	28	50
Karakousis[3]	39	14	14
Harpole[4]	84	19	20
Tafra[5]	106	18	27
Wong[6]	38	24	31
La Hei[7]	83	19	22
Leo[8]	282	19	22

1. Gorenstein LA, et al. *Ann Thorac Surg*. 1991;52:204.
2. Meyer T, et al. *Cancer*. 2000;89:1983.
3. Karakousis CP, et al. *Surgery*. 1994;115:295.
4. Harpole DH Jr, et al. *J Thorac Cardiovasc Surg*. 1992;103:743.
5. Tafra L, et al. *J Thorac Cardiovasc Surg*. 1995; 110:119.
6. Wong JH, et al. *Arch Surg*. 1988;123:1091.
7. La Hei ER, et al. *Asia Pacific Heart J*. 1996;5:111.
8. Leo F, et al. *Br J Cancer*. 2000;83:569.

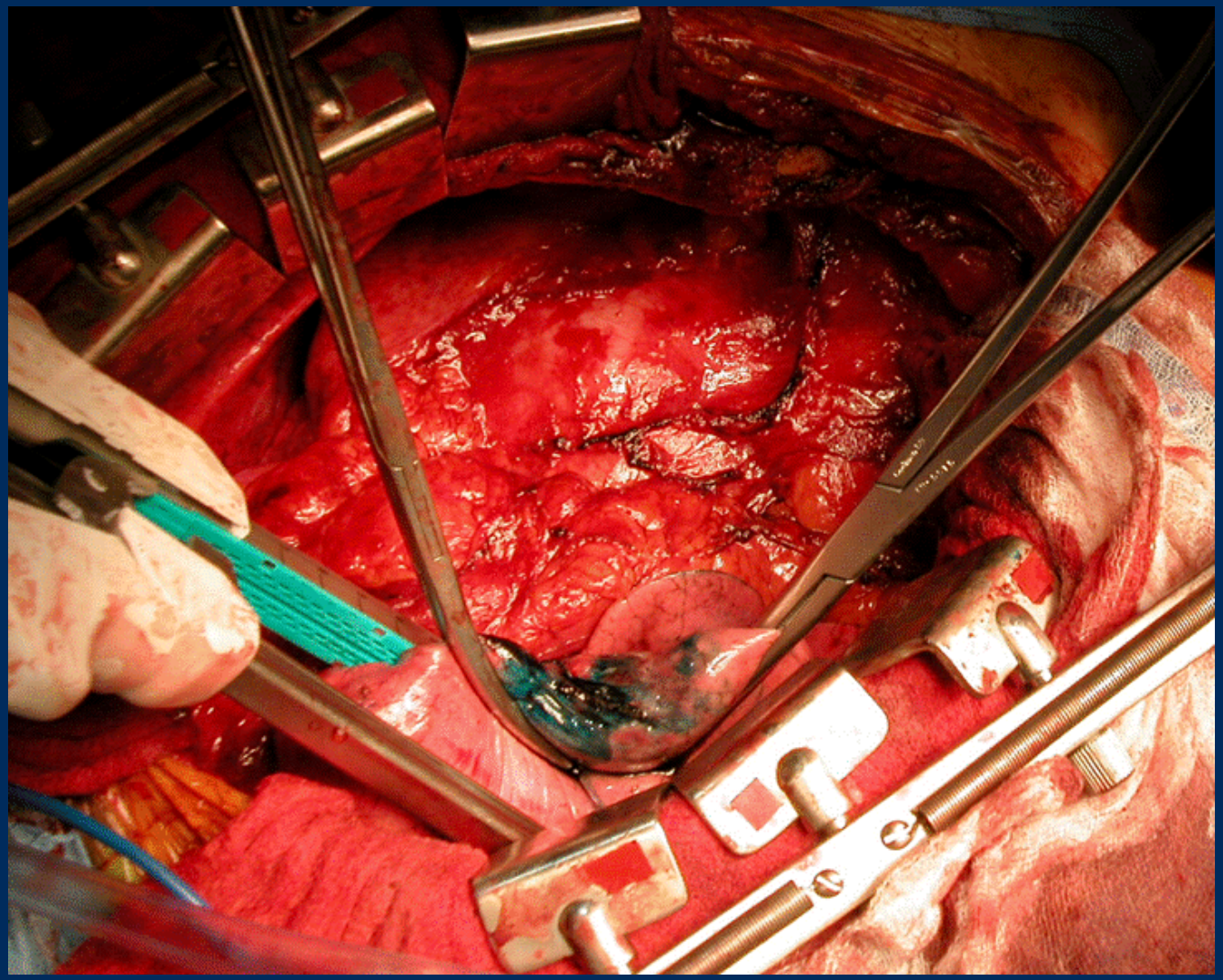
Clinical Significance of Intratumoral and Circulating Immune Profiles

- Tumor infiltrating lymphocytes associated with improved survival
- Improved outcome reported with increased NK cells and Dendritic Cells
- Circulating tumor-specific lymphocytes inconsistently correlated with clinical responses in immunotherapy trials

Hypothesis

- Intratumoral immune profiling may reliably predict patient outcome in metastatic melanoma

Pulmonary Resection for Metastatic Melanoma



Methods

- 32 specimens of pulmonary metastases were identified
- Thawed, washed and stained for surface and intracellular markers for flow cytometric analysis

Markers Used to Characterize the Immune Cell Infiltrate

Cell Type	Markers
Dendritic Cells	CD45 /CD11c+/HLA-DR+
Mature Dendritic Cells	CD45+/CD80&CD86+/HLADR+
Tumor Cells	CD45- / S100 +
Monocytes	CD14+
Granulocytes	CD15+
Macrophages	CD68+
Natural Killer Cells	CD56+
Lymphocytes	CD3+ and CD4+/CD8+ subsets
Memory Cells	CD3+/CD45RO
Regulatory T-cells	CD4+/CD25+/FOXP3+

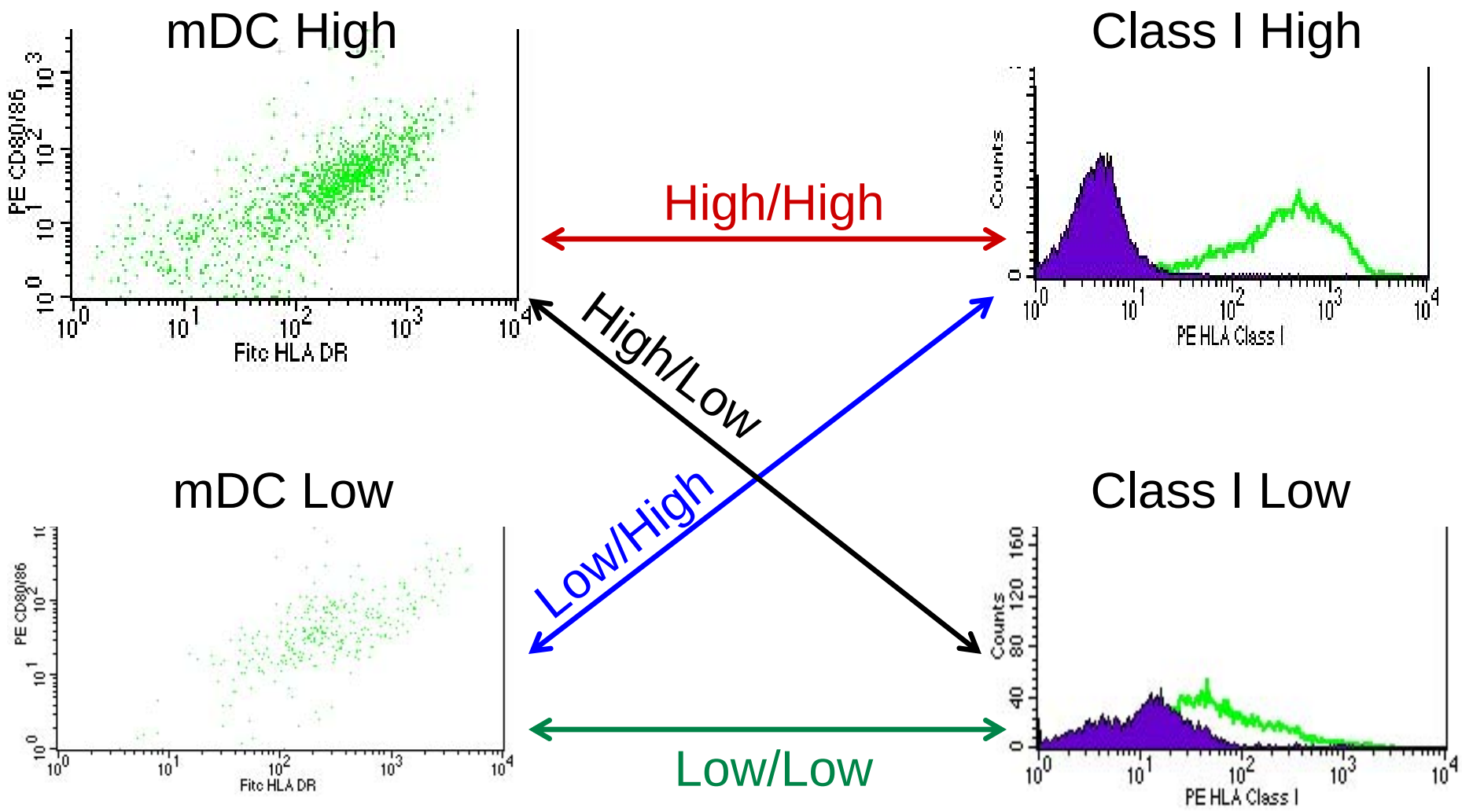
Data Analysis

- Determined the extent of infiltration of each of the various immune markers as a percentage of the total cell population
- MHC-Class I staining intensity of tumor cells = MFI of Class I /MFI Isotype control
- Data correlated with clinical outcome information

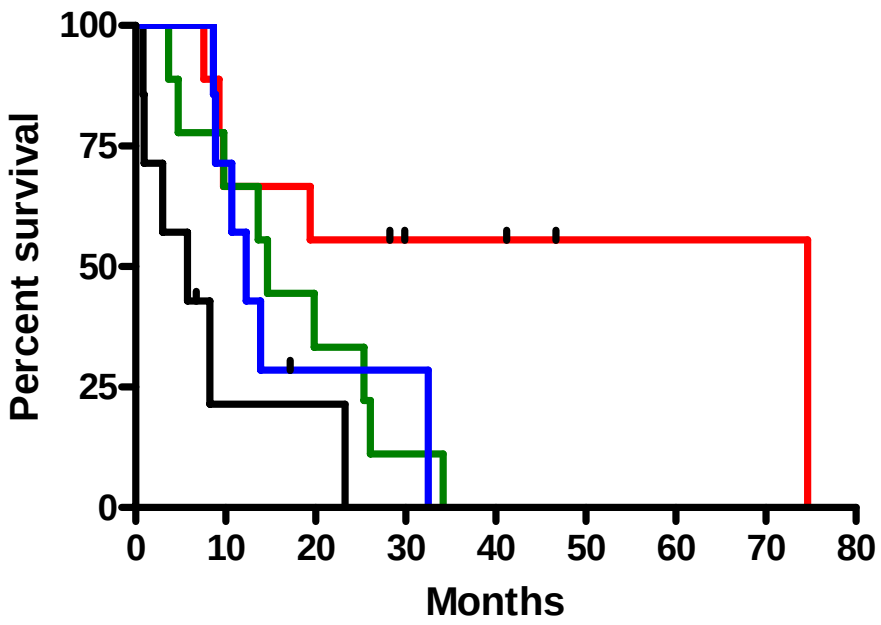
Univariate Analysis: Immune Infiltrate

	N	P-value	Hazard Ratio (95% CI)		
DC	32	0.3200	0.881	0.687	1.131
mDC	32	0.4730	0.870	0.595	1.272
Monocytes	32	0.4758	1.134	0.802	1.604
Lymphocytes	32	0.4938	0.982	0.934	1.034
CD4	32	0.4210	0.931	0.783	1.108
CD8	32	0.6488	0.983	0.914	1.057
CD3 & CD45RO	32	0.5083	0.981	0.928	1.038
NK	32	0.5204	1.232	0.652	2.327
Granulocytes	32	0.1302	1.121	0.967	1.301
Treg	32	0.5994	0.604	0.092	3.957
CD68	31	0.3515	0.926	0.789	1.088
CD4/Lymphocytes	32	0.3774	0.786	0.461	1.341
CD8/Lymphocytes	32	0.8932	1.062	0.440	2.562
Granulocytes/Lymphocytes	32	0.0582	1.223	0.993	1.507
Memory Cells/Lymphocytes	32	0.6920	0.842	0.361	1.968
NK/Lymphocytes	32	0.5442	1.112	0.789	1.566
Tregs/Lymphocytes	32	0.4478	1.253	0.700	2.242
ClassI MFI/Control MFI	32	0.0855	0.993	0.986	1.001

Analysis of Combined Effects of Immune Infiltrate and MHC-Class I Expression



Improved Prognosis with High Intratumoral Levels of Mature DC and Tumor-Specific MHC-Class I



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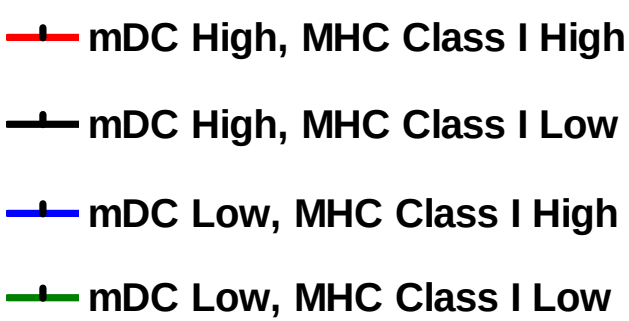
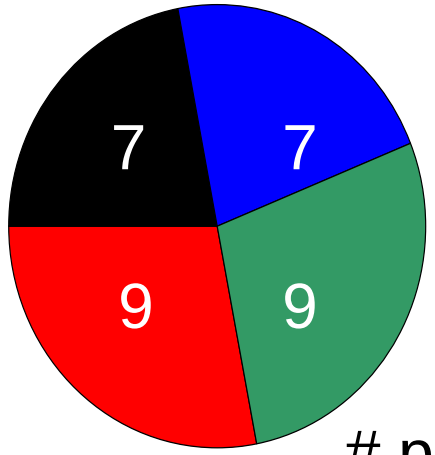
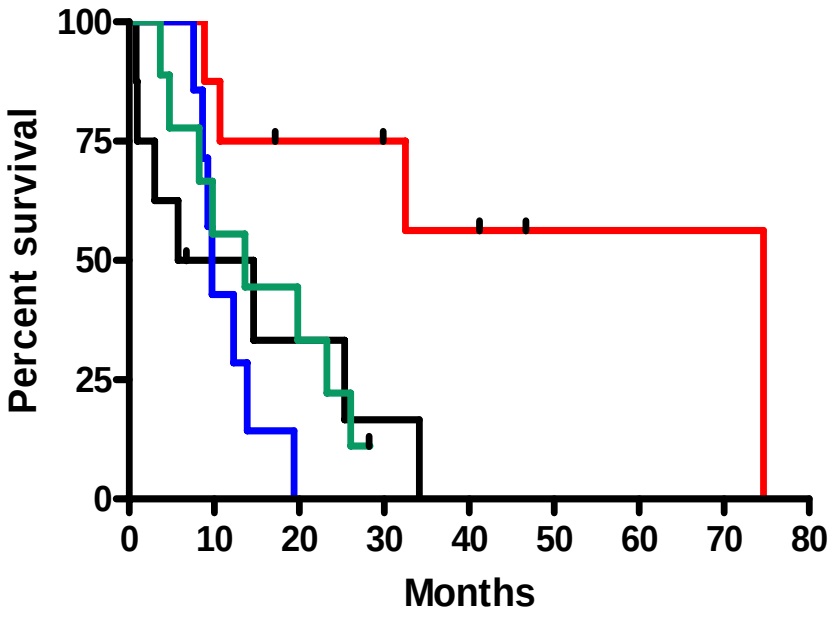




patients

Improved Prognosis with High Intratumoral Levels of NK Cells and Tumor-Specific MHC-Class I



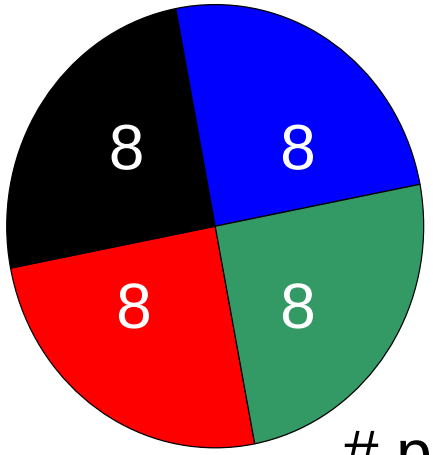
P < 0.05

NK High, MHC Class I High

NK High, MHC Class I Low

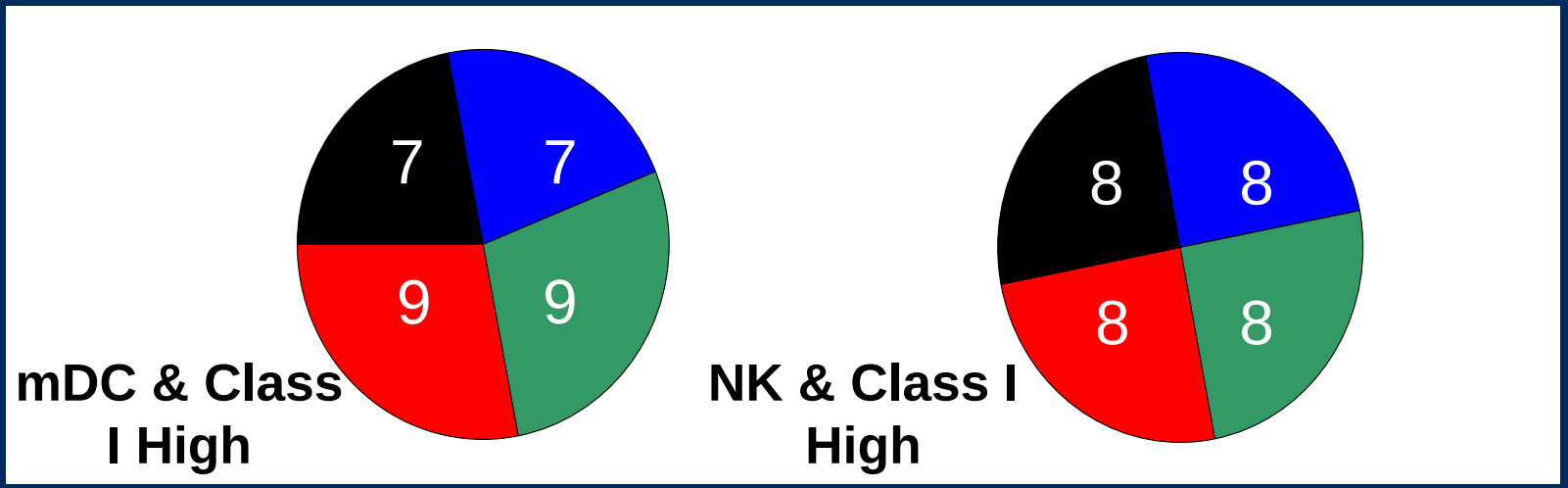
NK Low, MHC Class I High

NK Low, MHC Class I Low



patients

Subcategories of mDC and NK Infiltration Patterns



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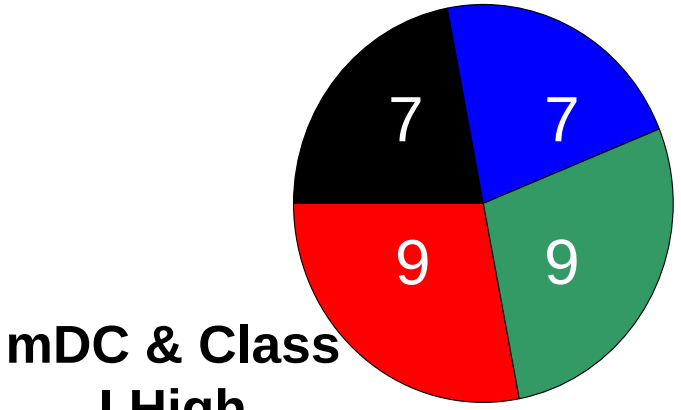
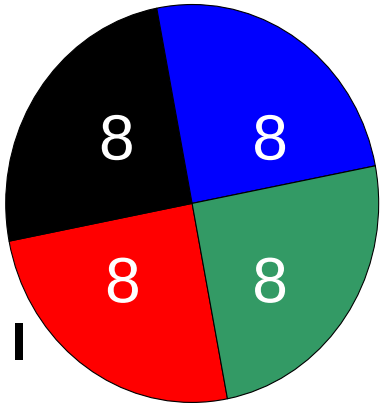
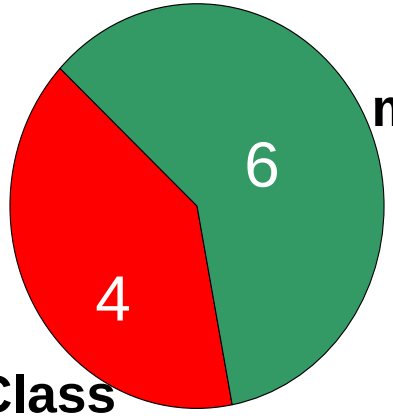


mDC & Class I High



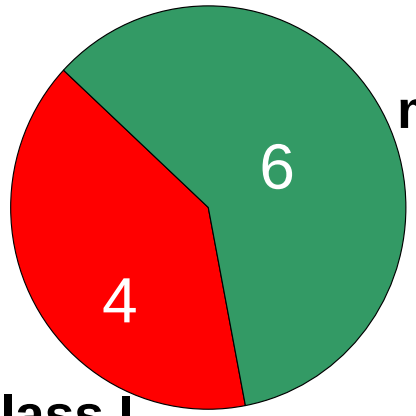
NK & Class I High



mDC, NK, Class I Low

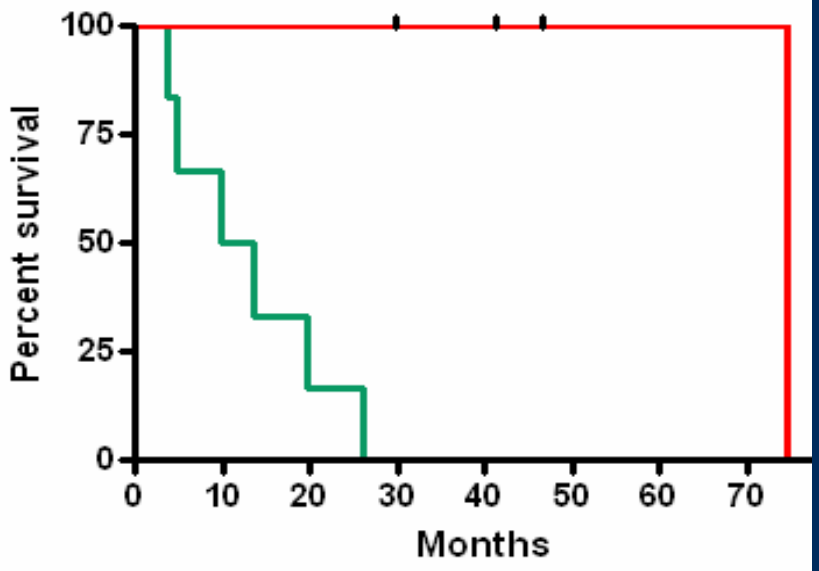
mDC, NK, Class I High

Improved Prognosis with Combined High mDC, NK, and Tumor-Specific Class I Expression



mDC,NK, Class I Low

mDC, NK, Class I High



Conclusions

- Improved outcomes noted when increased NK or mDC infiltrates corresponded with high tumor cell MHC-class I expression
- Demonstrates evidence of the combined actions of both the innate and adaptive immune responses in the control of advanced melanoma

Limitations

- Small sample sizes
- Retrospective
- Not controlled for differences in pre- or post-surgical treatments

Future Directions

- Analyze resected metastatic tumors in a prospective manner using fresh tissue
- Perform analyses on metastases to other organ sites
- Investigate the role of immunophenotyping in predicting response to immunotherapy in the context of a prospective, randomized clinical trial in patients with surgically resectable disease

Thank You

JOHN WAYNE
CANCER INSTITUTE
at Saint John's Health Center

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Mark B. Faries, M.D.
Donald L. Morton, M.D.
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David Elashoff, Ph.D.
Cynthia Ye