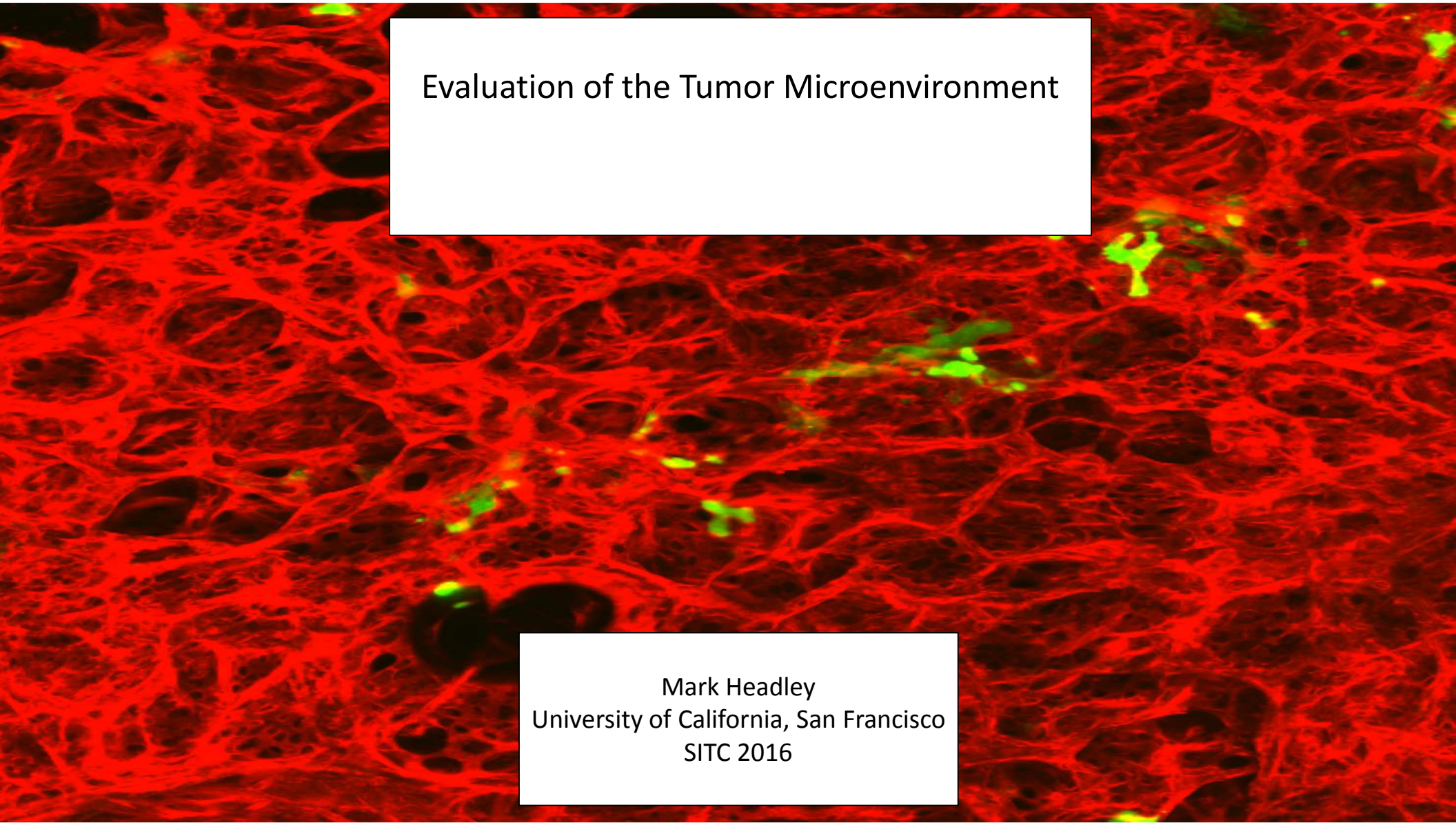


Evaluation of the Tumor Microenvironment

Mark Headley
University of California, San Francisco
SITC 2016

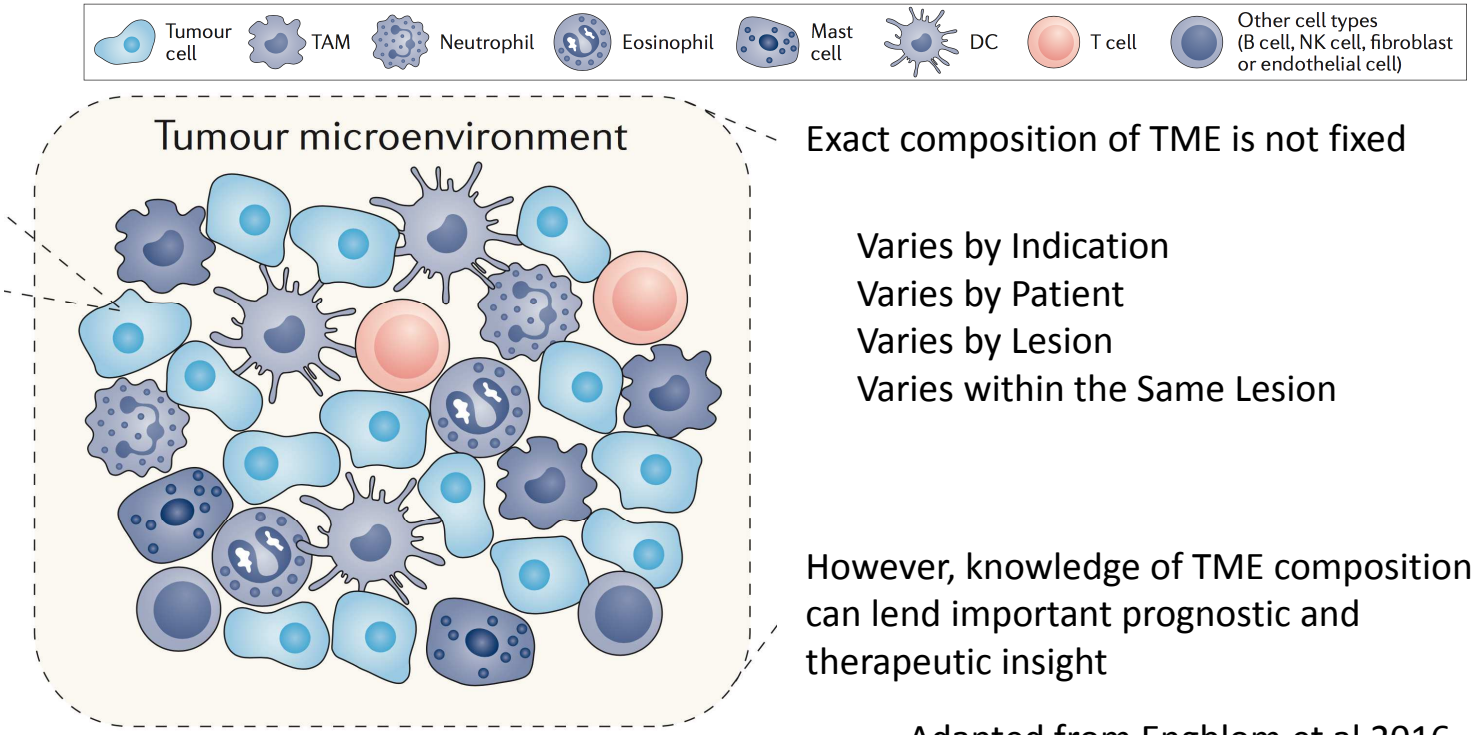
Presenter Disclosure Information

Mark B. Headley, PhD

In addition to appointment at UCSF
Joint position at Precision Immune, Inc. (as of Nov.1)

The Complexity of The Tumor Microenvironment

The Tumor Microenvironment is a Complex Network of Tumor Cells, Immune Cells, Fibroblasts, Endothelium, etc…



Exact composition of TME is not fixed

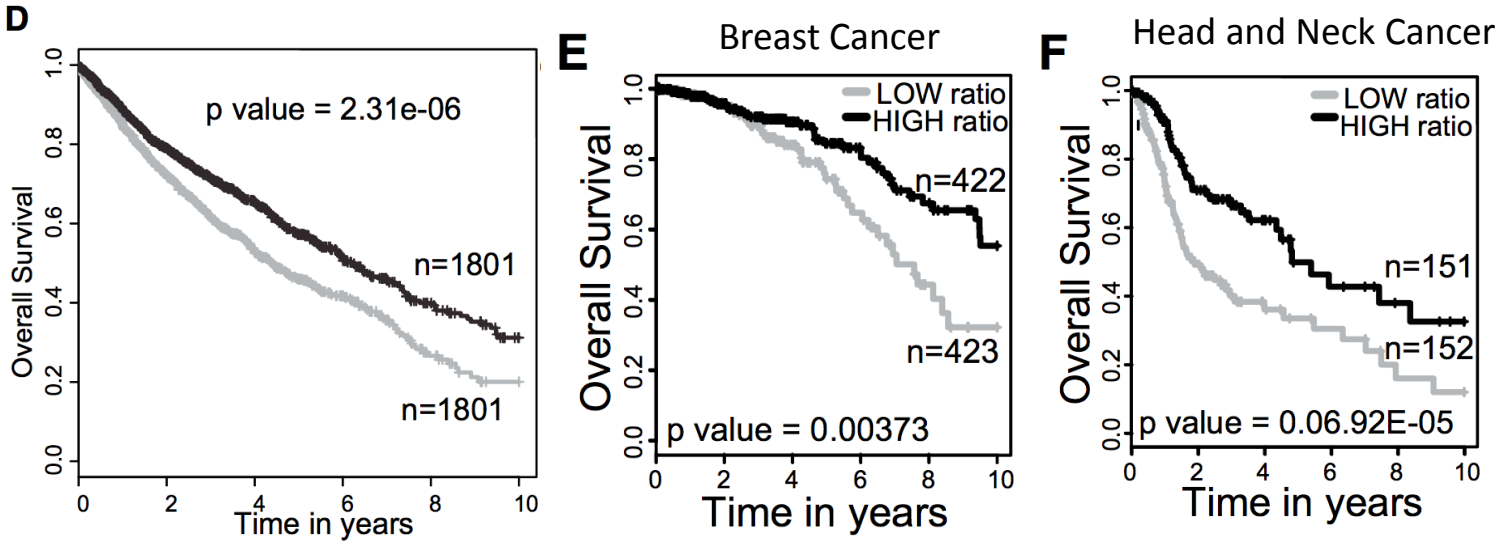
Varies by Indication
Varies by Patient
Varies by Lesion
Varies within the Same Lesion

However, knowledge of TME composition can lend important prognostic and therapeutic insight

Adapted from Engblom et al 2016

Elevated DC Gene Signature Predicts Increased Survival in multiple indications

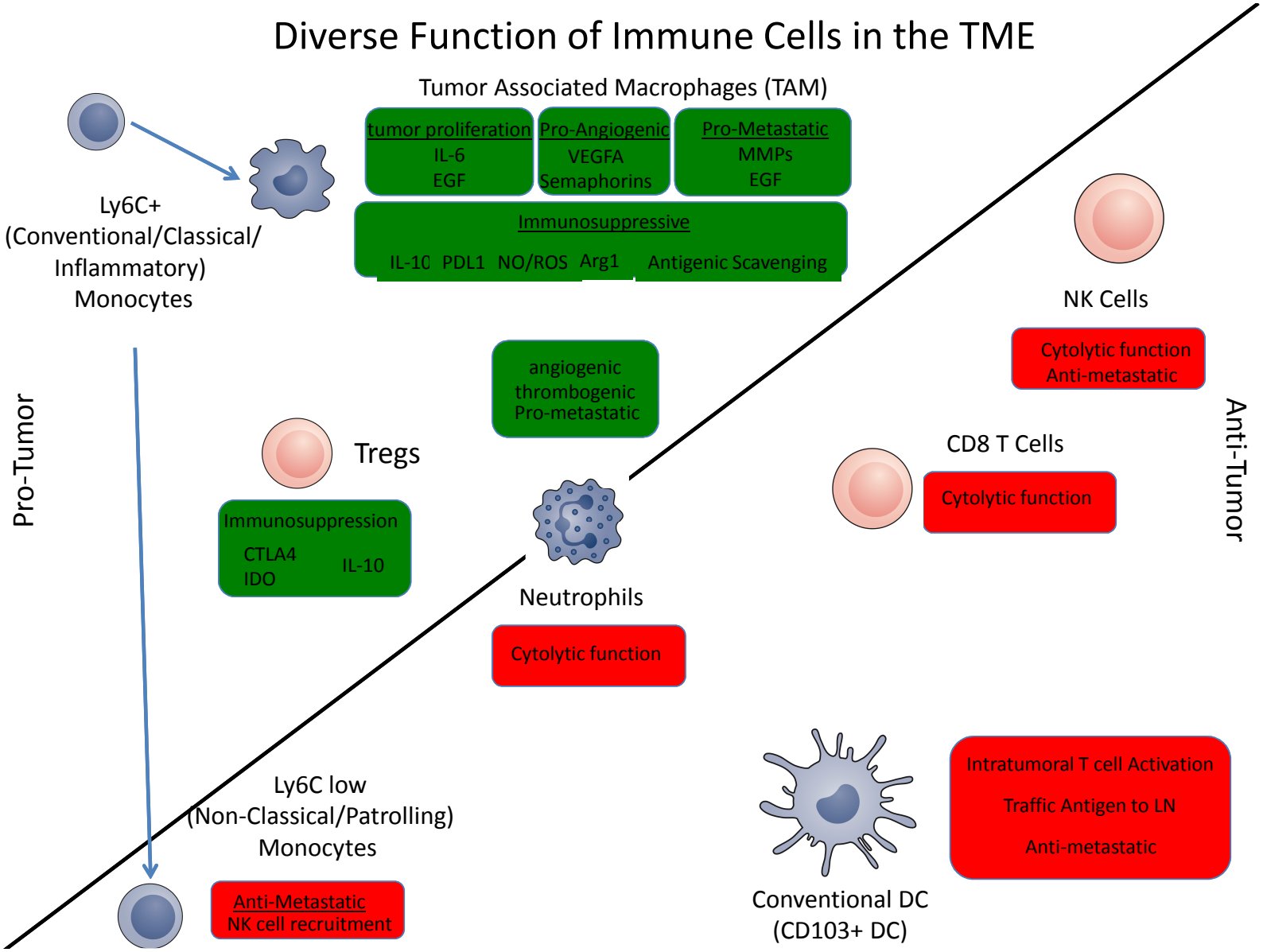
12 Cancer Indications from TCGA



Hi DC Signature

Low DC Signature

Broz et al. Cancer Cell 2014


Diverse Function of Immune Cells in the TME
Tumor Associated Macrophages (TAM)
tumor proliferation
IL-6
EGF
Pro-Angiogenic
VEGFA
Semaphorins
Pro-Metastatic
MMPs
EGF
Immunosuppressive
IL-10 PDL1 NO/ROS Arg1 Antigenic Scavenging
Ly6C+
(Conventional/Classical/
Inflammatory)
Monocytes
NK Cells
Cytolytic function
Anti-metastatic
Pro-Tumor
Anti-Tumor
angiogenic
thrombogenic
Pro-metastatic
Tregs
CD8 T Cells
Cytolytic function
Immunosuppression
CTLA4
IDO
IL-10
Neutrophils
Cytolytic function
Ly6C low
(Non-Classical/Patrolling)
Monocytes
Anti-Metastatic
NK cell recruitment
Intratumoral T cell Activation
Traffic Antigen to LN
Anti-metastatic
Conventional DC
(CD103+ DC)

Tools at our disposal For TME Study

Multiparameter Flow Cytometry/Mass Cytometry:

- Ever increasing capacity for more parameters theoretically >50 with recent technological advances.
- Effectively captures population identity and heterogeneity however gives no indication of spatial context
- Potential for High-throughput analysis

Multiparameter Immunofluorescence

- Ever increasing capacity for more parameters
- incorporates spatial localization
- Labor intensive and challenging to perform in a high throughput fashion

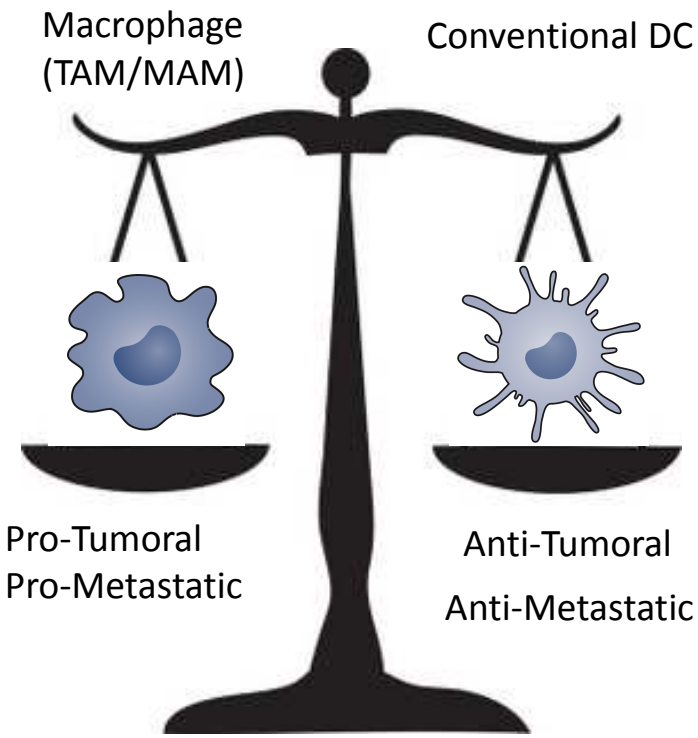
Transcriptomic Analysis

- Vast amounts of quantifiable molecular characterization data
- Bulk Population analysis – Can suggest but not define heterogeneity
- Single Cell analysis- incredible potential for defining heterogeneity but again relatively low throughput

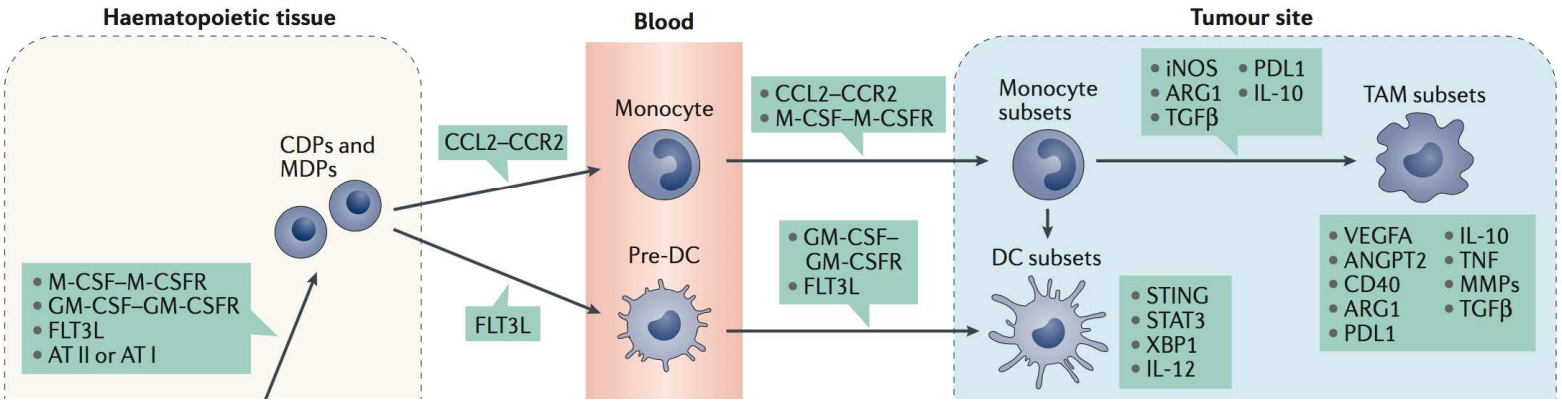
Intravital Microscopy

- Low potential for parallel analysis of multiple parameters
- Very low throughput
- The only method that gives insight into spatial and temporal context of TME interaction

The DC-Macrophage Dichotomy Within the TME



The Players



Engblom et al 2016

Function

TAM: Highly phagocytic – TME favors Macrophages with a “Wound Healing” phenotype

Dendritic Cells: Come in several flavors

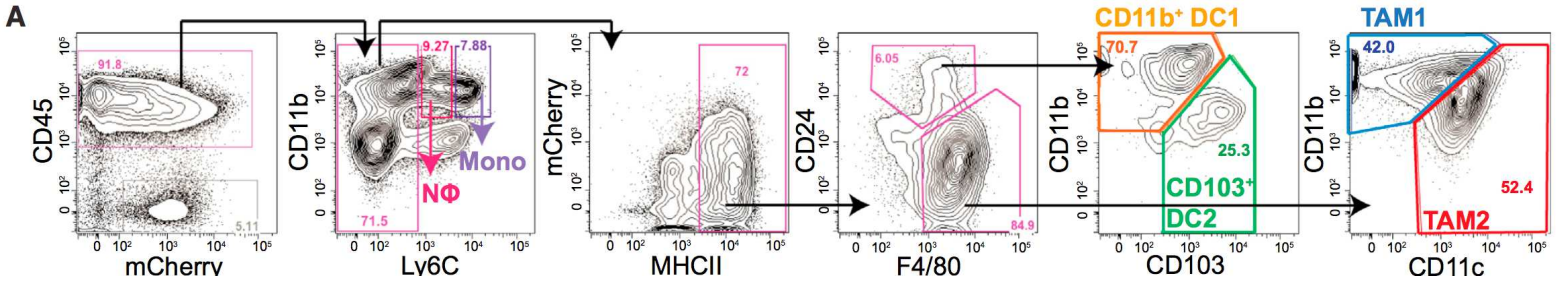
CD103+ DC/CD8+ DC/XCR1+DC: Phagocytic function diminishes with maturity, Professional APC, Cross-presentation

CD11b+ cDC: Postulated to be a primary source of CD4+ T cell activation

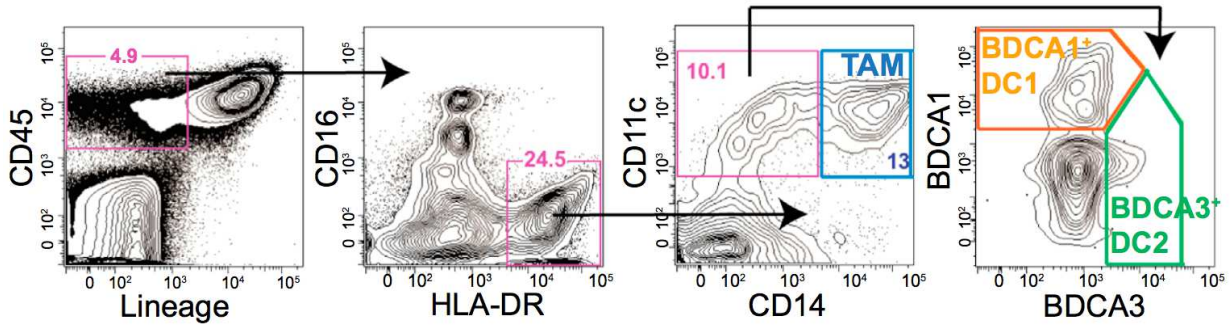
moDC: GM-CSF induced, function in TME largely unknown

Primary Tumors in Mice and Human Are Infiltrated with a Combination of Macrophage and DC Populations

Murine – Breast Tumor Model (PyMT-ChOVA)

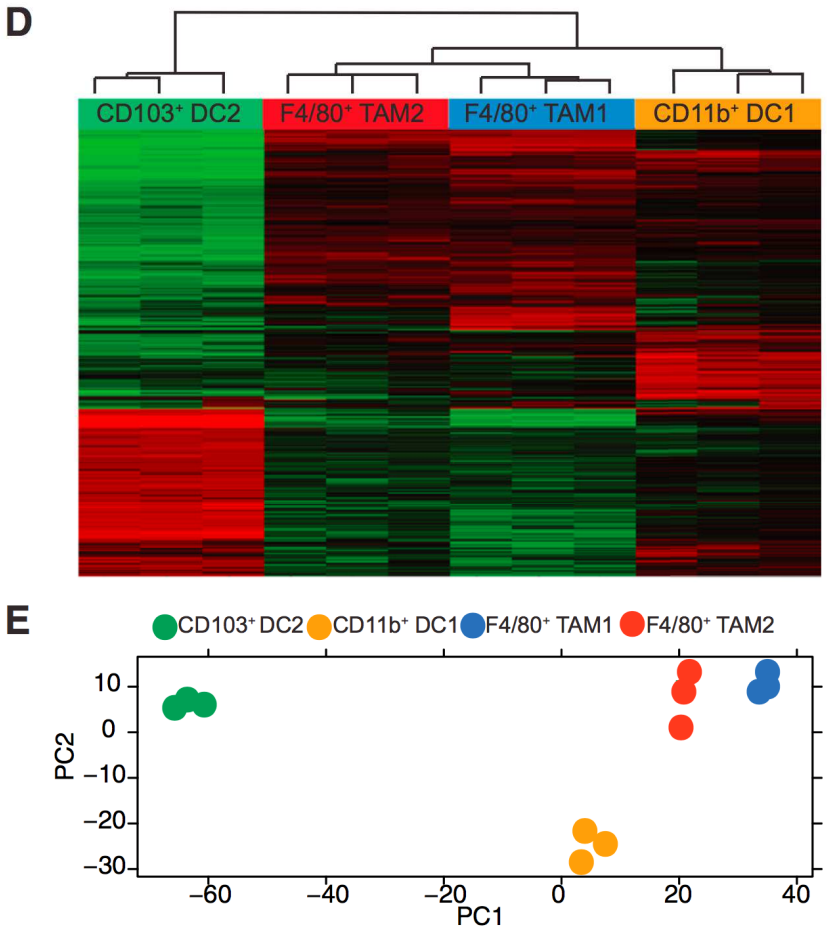


Human – Metastatic Melanoma Biopsy



Broz et al. Cancer Cell 2014

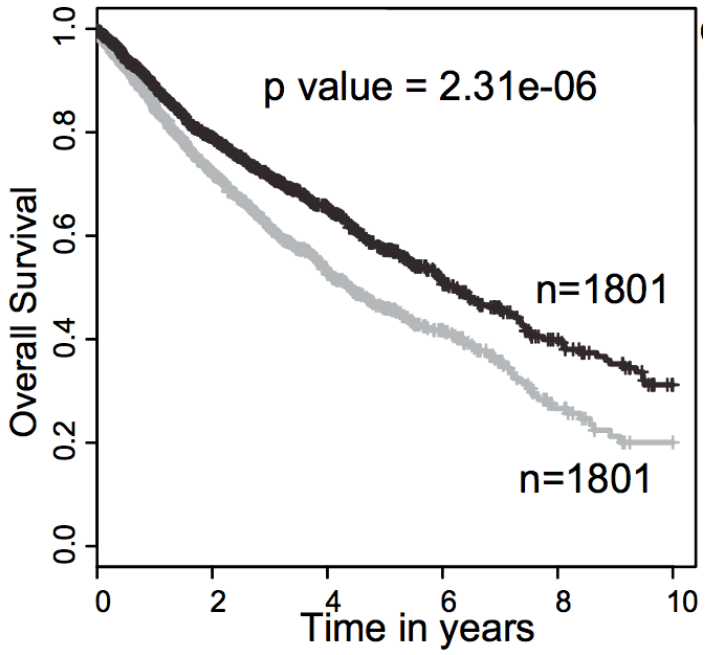
Tumor Macrophage and Dendritic Cell Populations within TME Represent Distinct Cell Lineages



Broz et al. Cancer Cell 2014

Murine RNAseq Profiles Yield Prognostic Value in Human Patient Data

D 12 Cancer Indications from TCGA

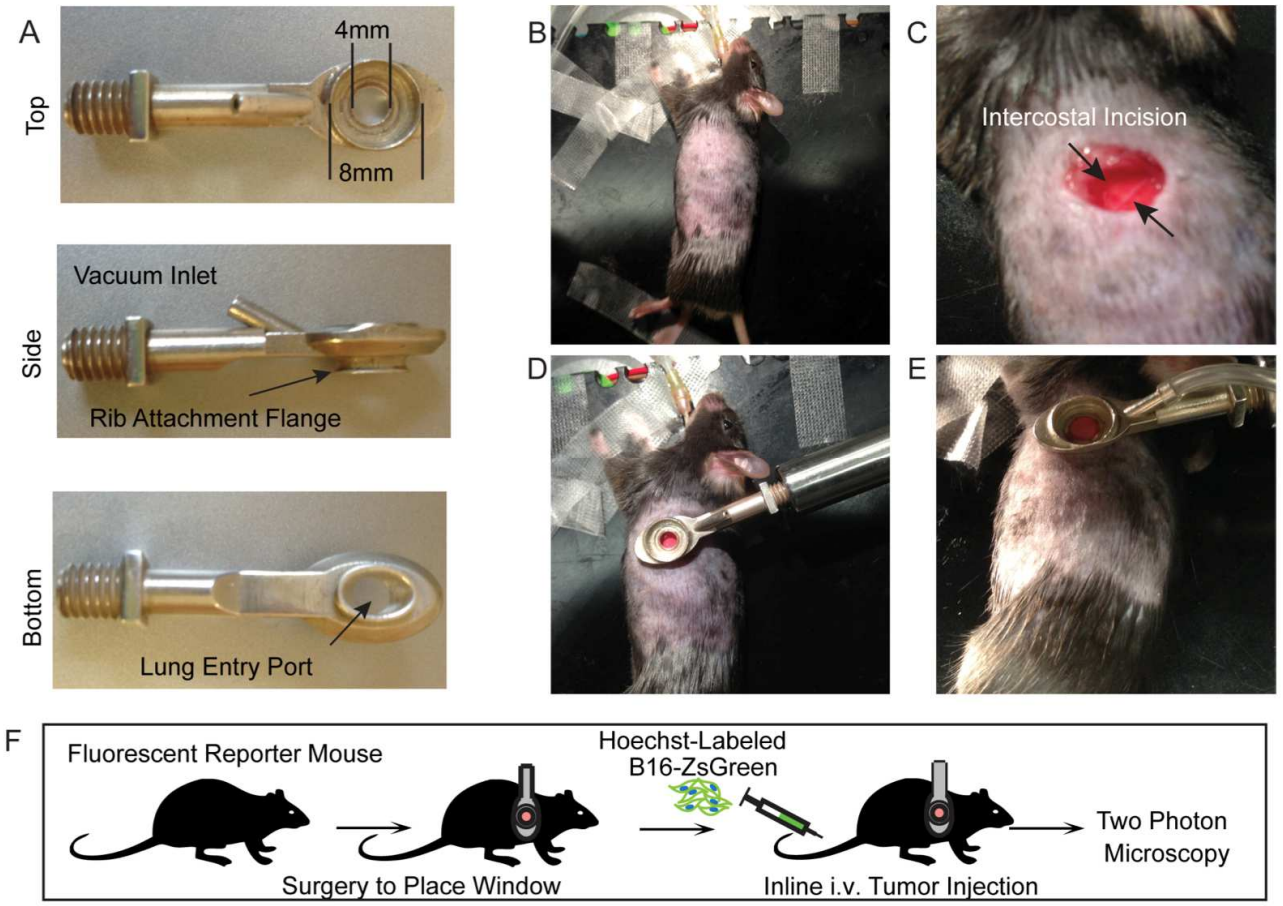


Hi DC Signature

Low DC Signature

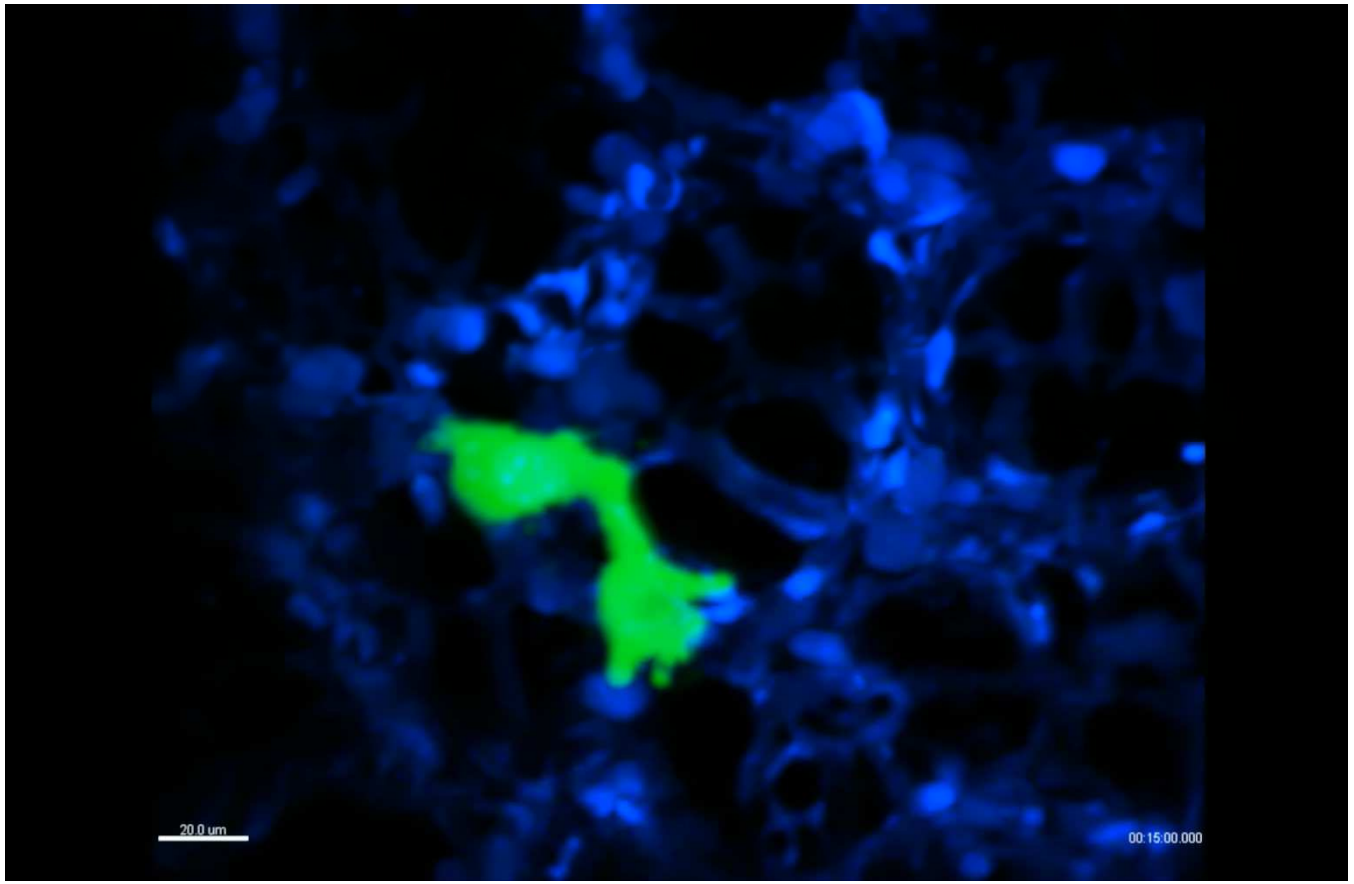
Broz et al. Cancer Cell 2014

A Metastatic Interlude: Intravital Imaging of the Metastatic Lung



Headley et al. Nature 2016

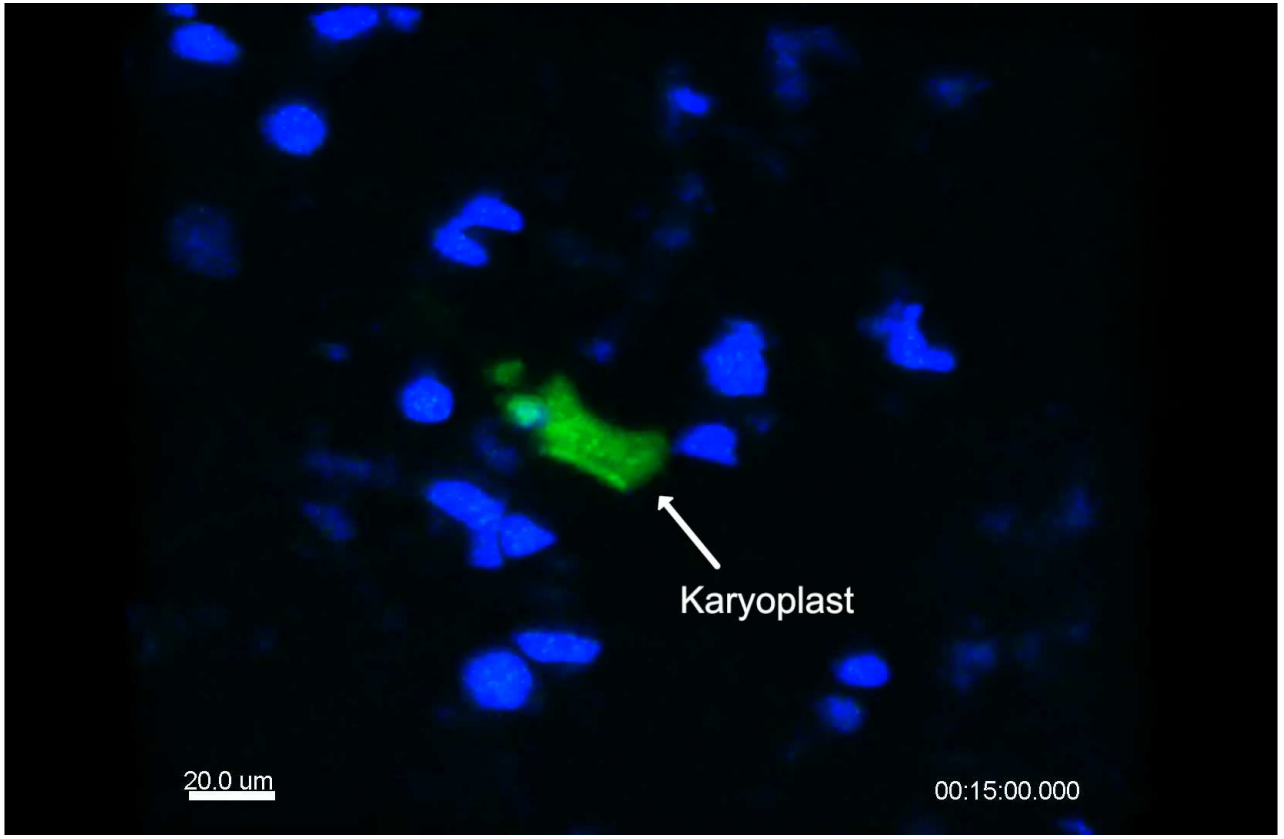
Prospective Metastatic Cells Shed Micron-Scale Cytoplasmic Blebs Rapidly During Metastasis



Actin CFP

B16-ZsGreen

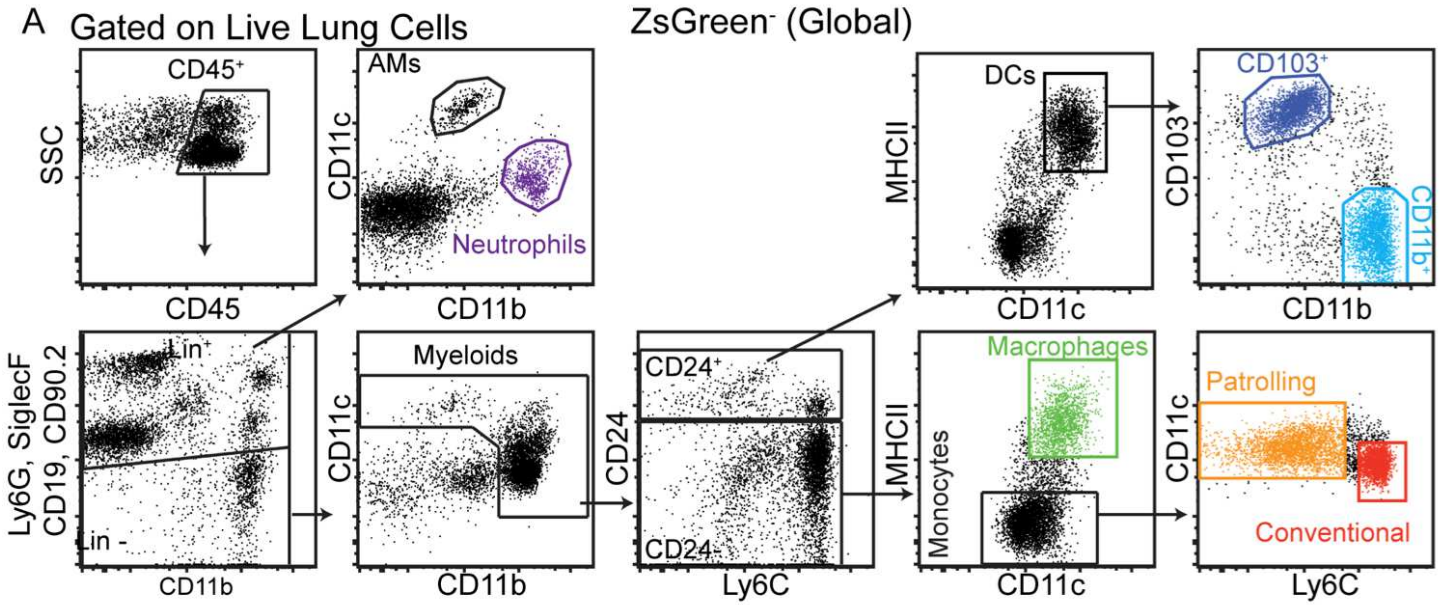
Detection and Uptake Of Cytoplast By The Lung Myeloid Phagocytic System



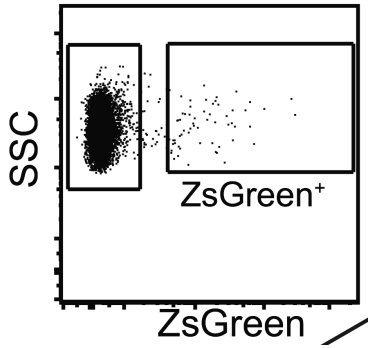
Monocytes/Macrophages

B16 Melanoma

Contrasting The Global Lung Myeloid Response To The Tumor Loaded Response

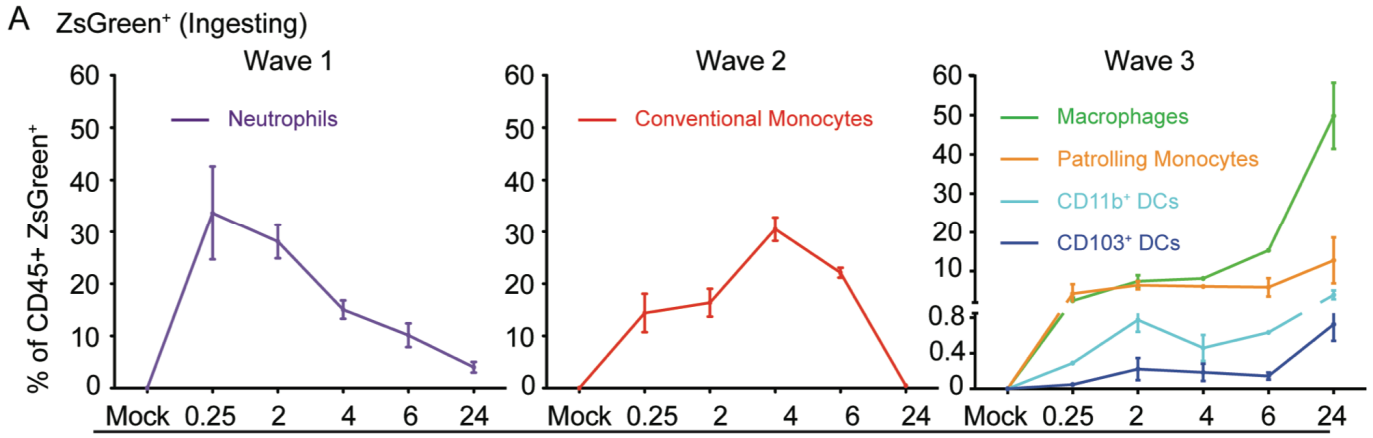


ZsGreen Allows Discrimination of Ingesting vs Non-Ingesting Myeloid Cells



Headley et al Nature 2016

Wave Structure of Myeloid Recruitment and Tumor Ingestion During Early Colonization

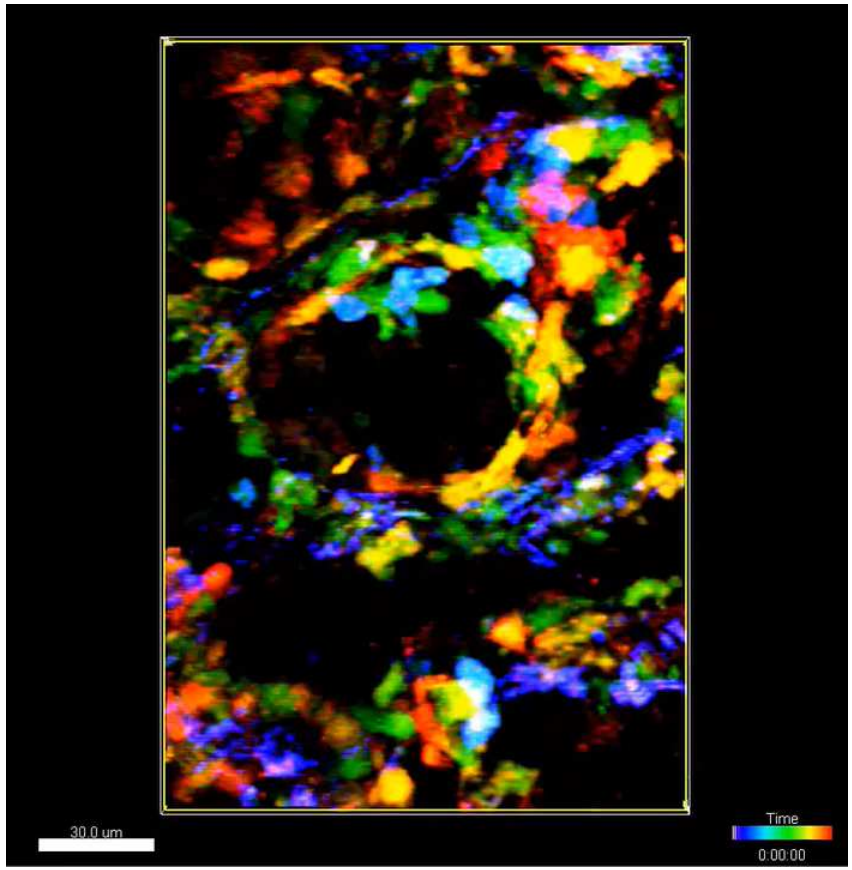


Qian et al. Nature 2010 – conventional monocytes and macrophages recruited to the metastatic niche within days of injection promote metastasis

Hanna et al. 2015 – Patrolling monocytes recruited to the metastatic niche within hours of injection oppose metastasis

Headley et al Nature 2016

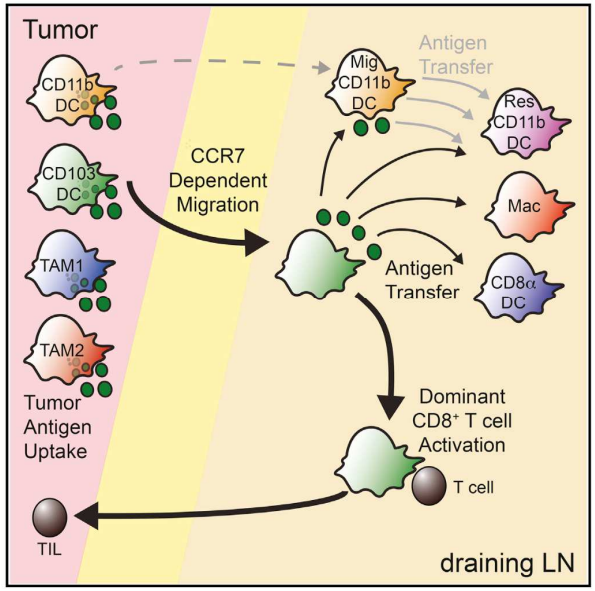
The Function Of cDC in the TME



Macrophage/Macrophage
Dendritic Cells
Antigen Specific CD8+ T cell

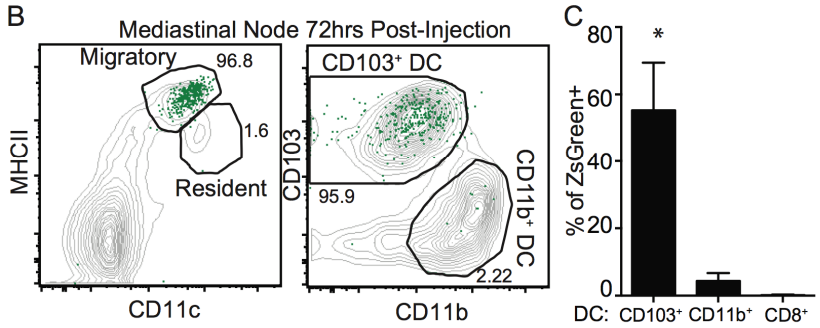
CD103+ DC migrate to LN bearing Tumor Antigen and Engage CD8 T cells

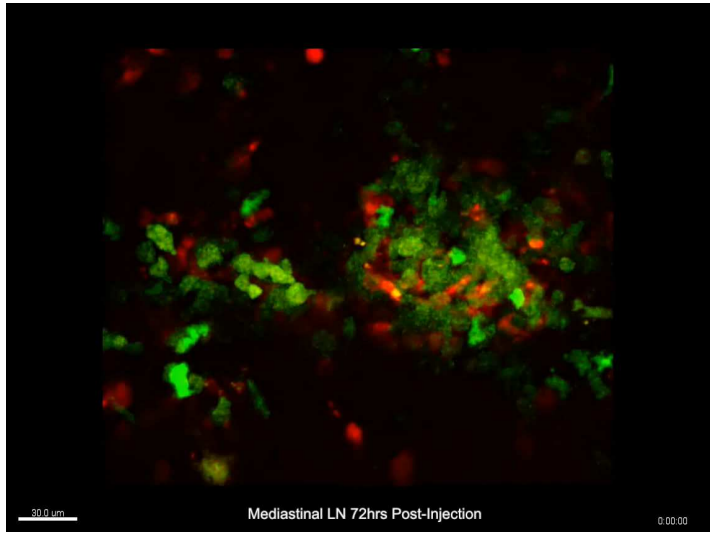
Primary Tumor DLN



Roberts et al. Cancer Cell 2016

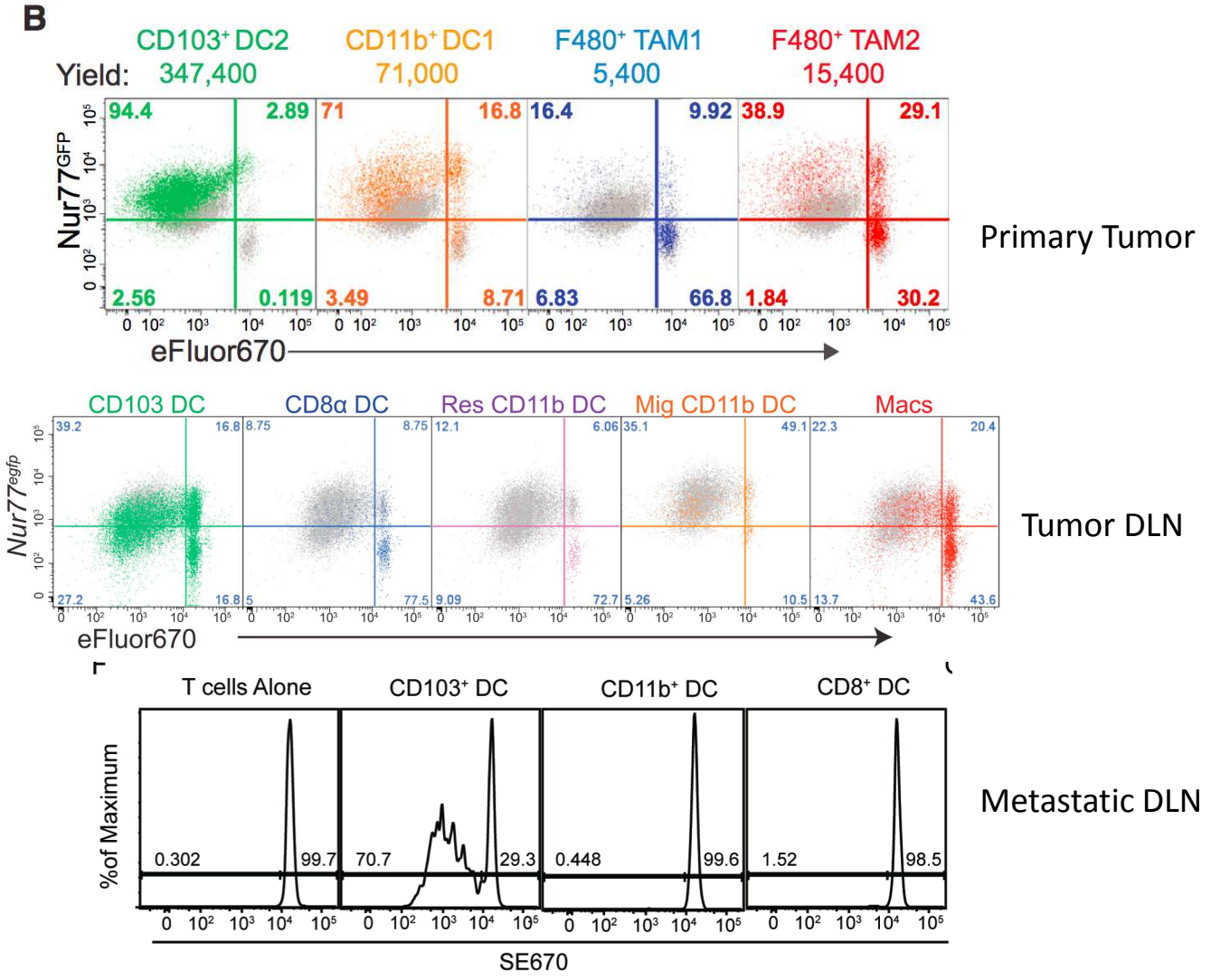
Metastatic DLN



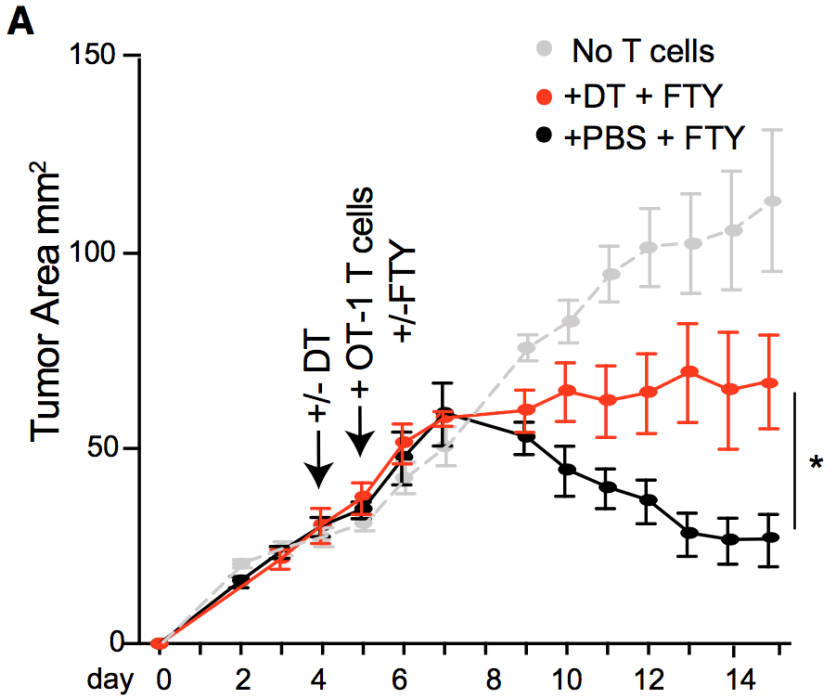


Headley et al Nature 2016

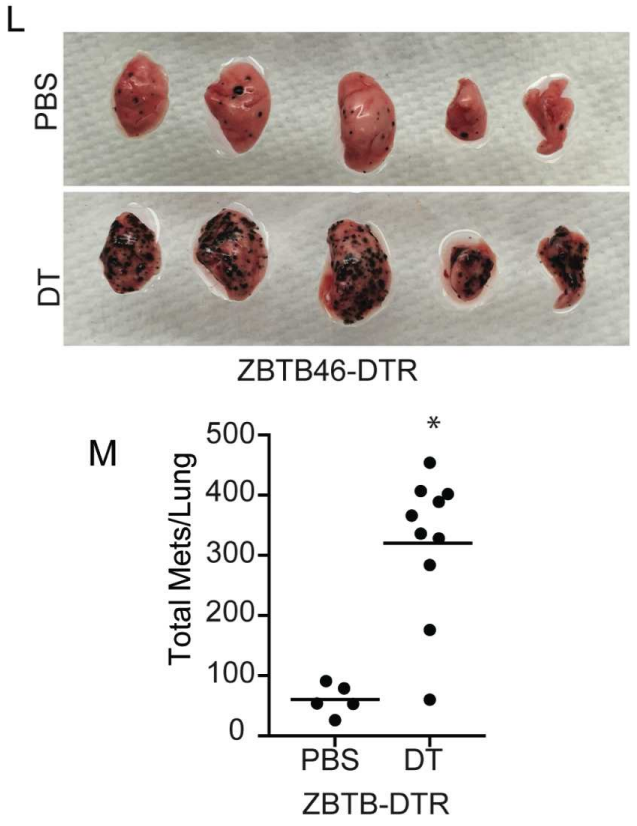
Conventional DC but Not TAMs Support Activation of CD8+ T cells



Conventional Dendritic Cells Restrict Primary and Metastatic Tumor Growth



Broz et al Cancer Cell 2014



Headley et al Nature 2016

Conclusions

Analysis of the Cells of the Tumor Microenvironment can yield critical knowledge into both the varied functions of these distinct populations as well as prognostic insight into human disease.

In both primary and metastatic tumors conventional DC (likely CD103+) set an equilibrium with macrophages restricting overall tumor success.

These DC in contrast to Macrophages support CD8 T cell activation

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