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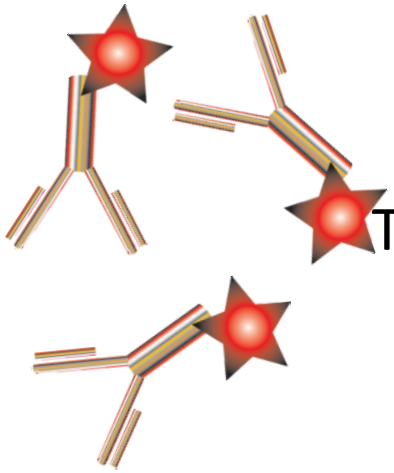
Cytokine Arrays Reveal "Black Ops" Tactics of Tumor-induced Immunosuppression



Jarad J Wilson, Ph.D.
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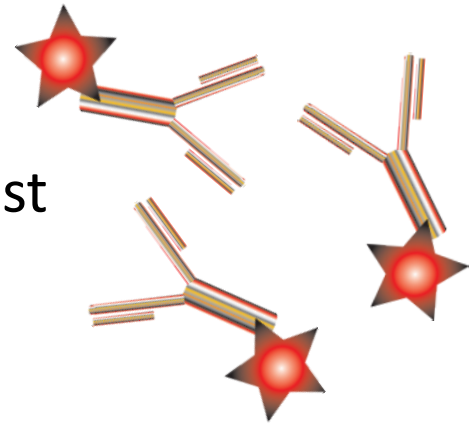
Founder and CEO



What are Antibody Arrays?

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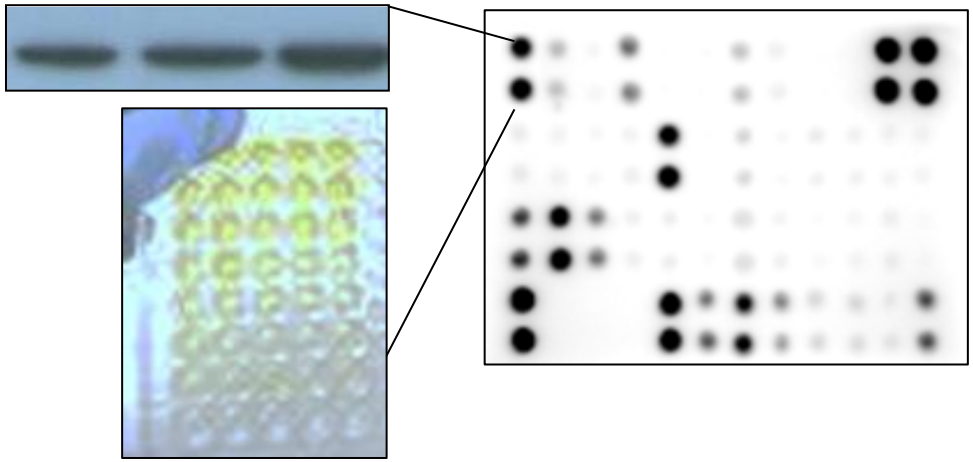

- Antibody arrays are merely multiplexed ELISA platforms
- Compresses multiple target detection from multiple Western's or ELISAs into a single experiment
- Utilize a specific capture antibody for the given cytokine/protein of interest, and detect the presence of this molecule in the sample via a direct labeled protein, or a secondary detection antibody
- Arrays are spotted in duplicate, providing and internal independent measurement of cytokines

Types of Antibody Arrays

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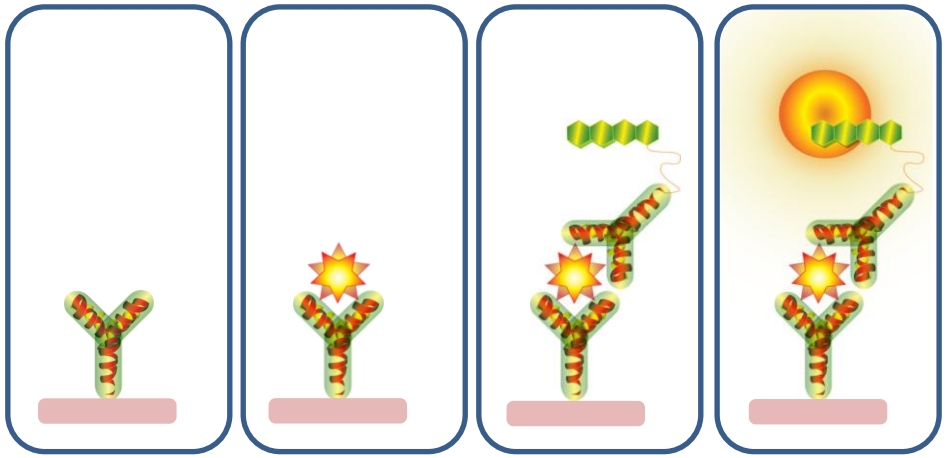
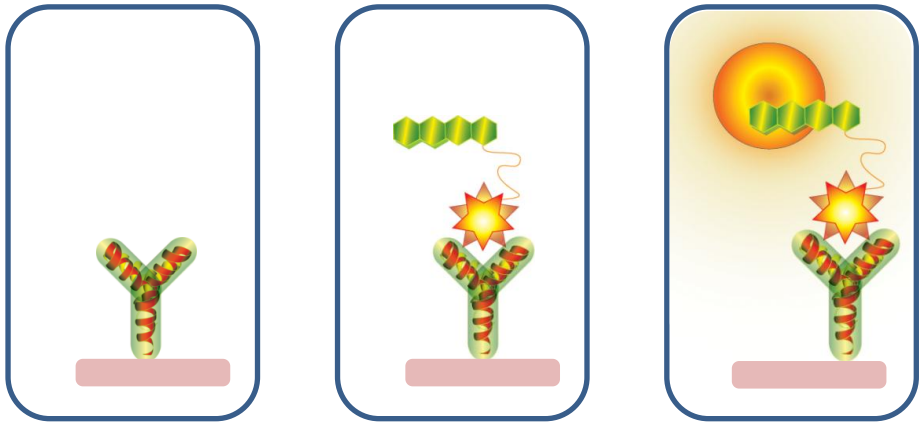
Sandwich Method (Antibody Pair)

- Capture antibody printed onto a solid surface (glass slide or membrane)
- Sample is added, incubated, and washed off
- Biotin labeled secondary antibody cocktail is added
- Streptavidin-HRP, or –Cy3 is added for signal detection



Labeling Method

- Capture antibody printed onto a solid surface (glass slide or membrane)
- Sample is directly biotinylated, the added, incubated, and washed
- Streptavidin-HRP, or –Cy3 is added for signal detection



How Tumors Promote Immunosuppression

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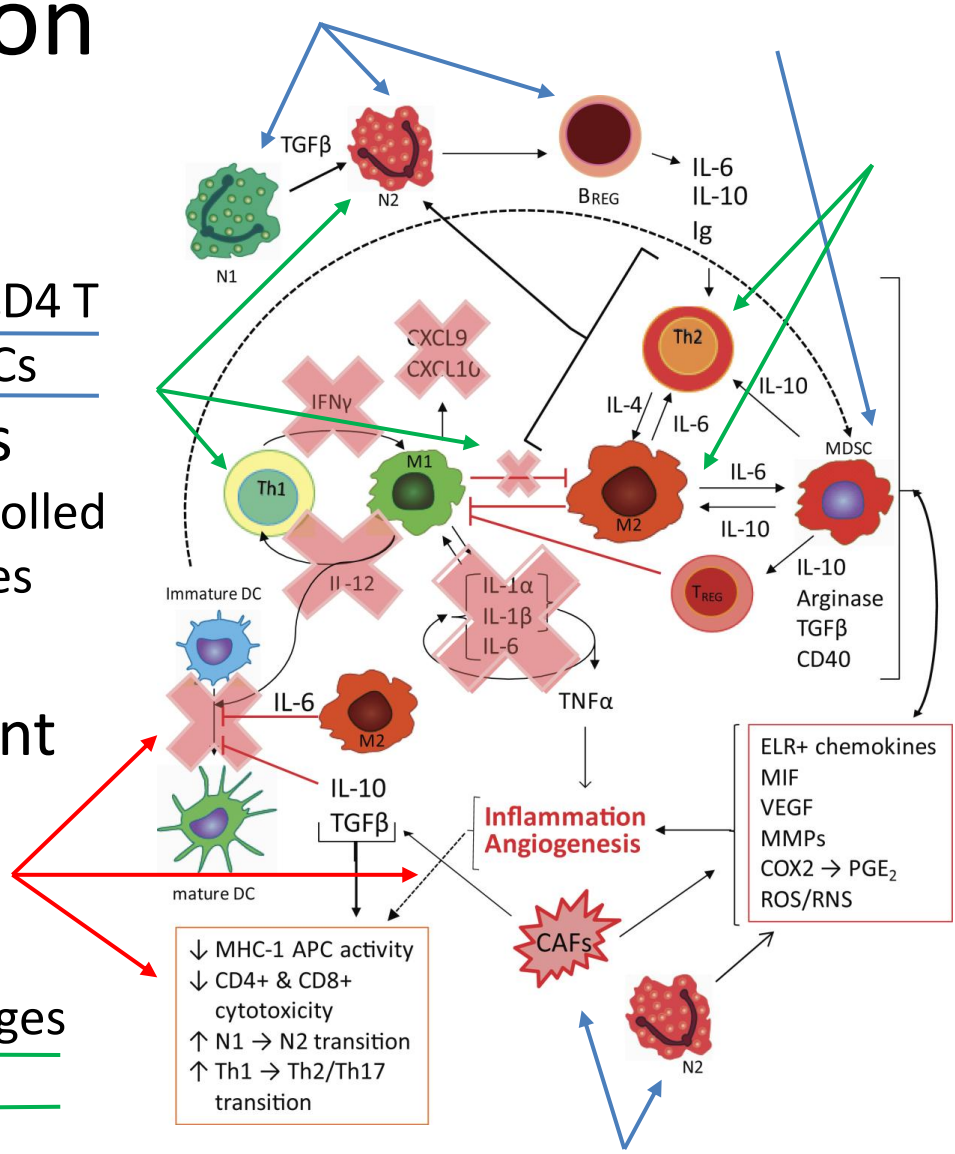
- Recruitment of Cellular Accomplices
  - Cancer-associated fibroblasts, T_H2 CD4 T cells, Regulatory B and T cells, MDSCs
- Subversion of normal pathways
  - Promotion of angiogenesis, uncontrolled cell growth, tumor invasion of tissues
- Promote tumor survival and pro-tumor cytokine environment
  - Decreased antigen presentation, cytotoxicity, and inflammation
- Conversion of Immune Cells
  - CD4's favor T_H2 over T_H1, macrophages and neutrophils polarized to Type 2



Seed to Soil Hypothesis

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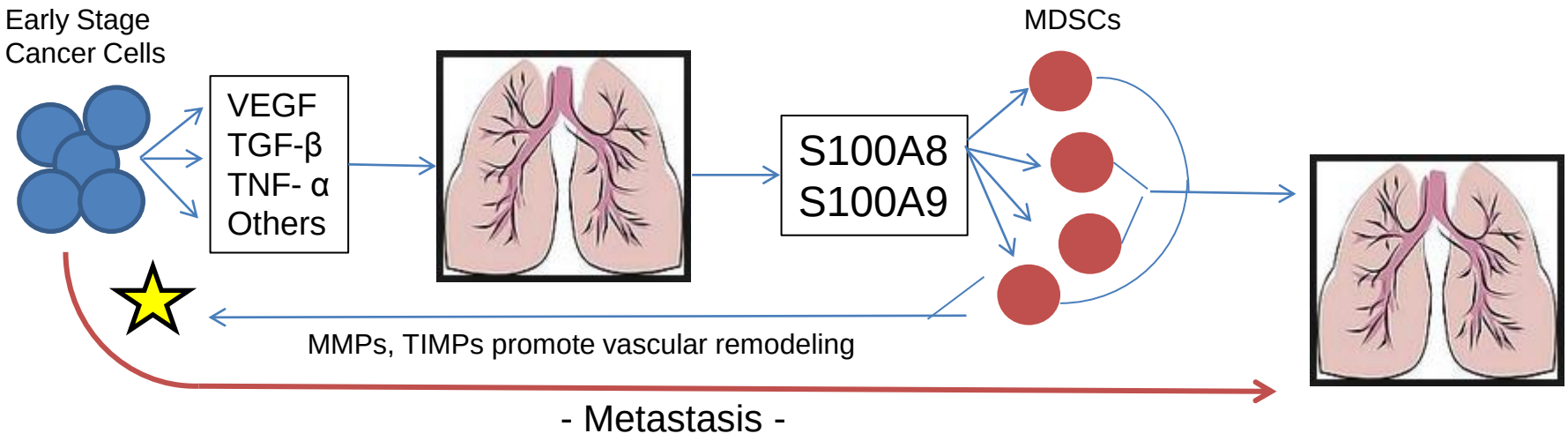

- Premetastatic niches is the development of areas of pro-tumor cells that facilitate tumor extravasation, tumor survival, and tumor metastasis.
- Myeloid Derived Suppressory Cells (MDSCs, Gr1+ CD11b+ in mice, and multiple phenotypes in Humans) are found at increased levels in almost all cancer patient
- MDSCs infiltrate tumors and promote angiogenesis, immunosuppression, and pro-tumor inflammation

MDSCs Arrive before Tumor Cells

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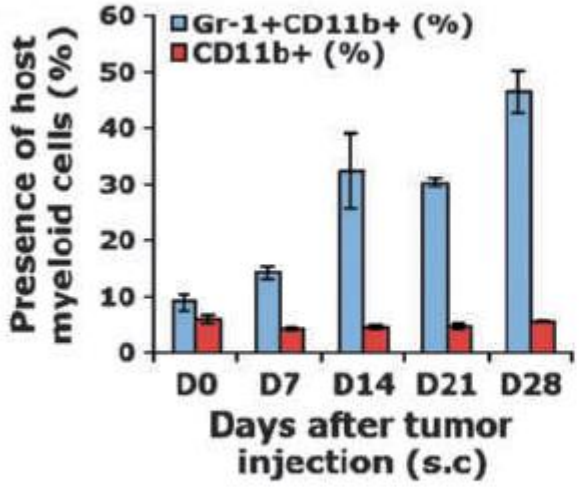
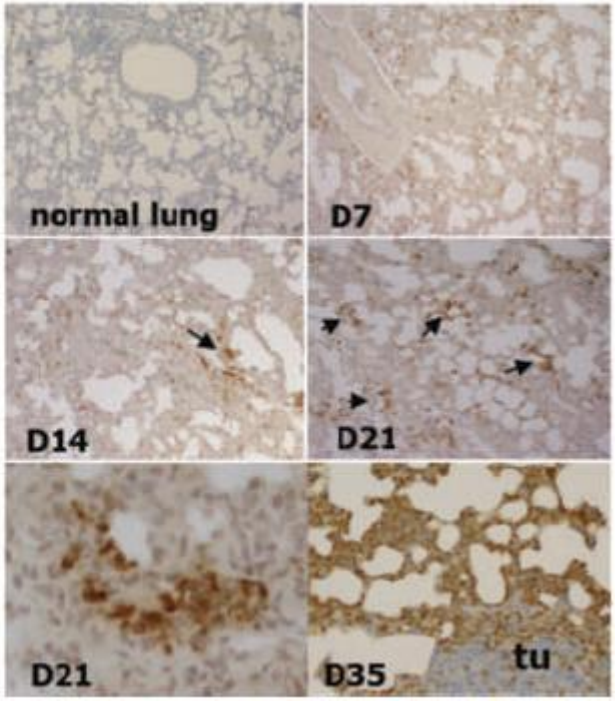
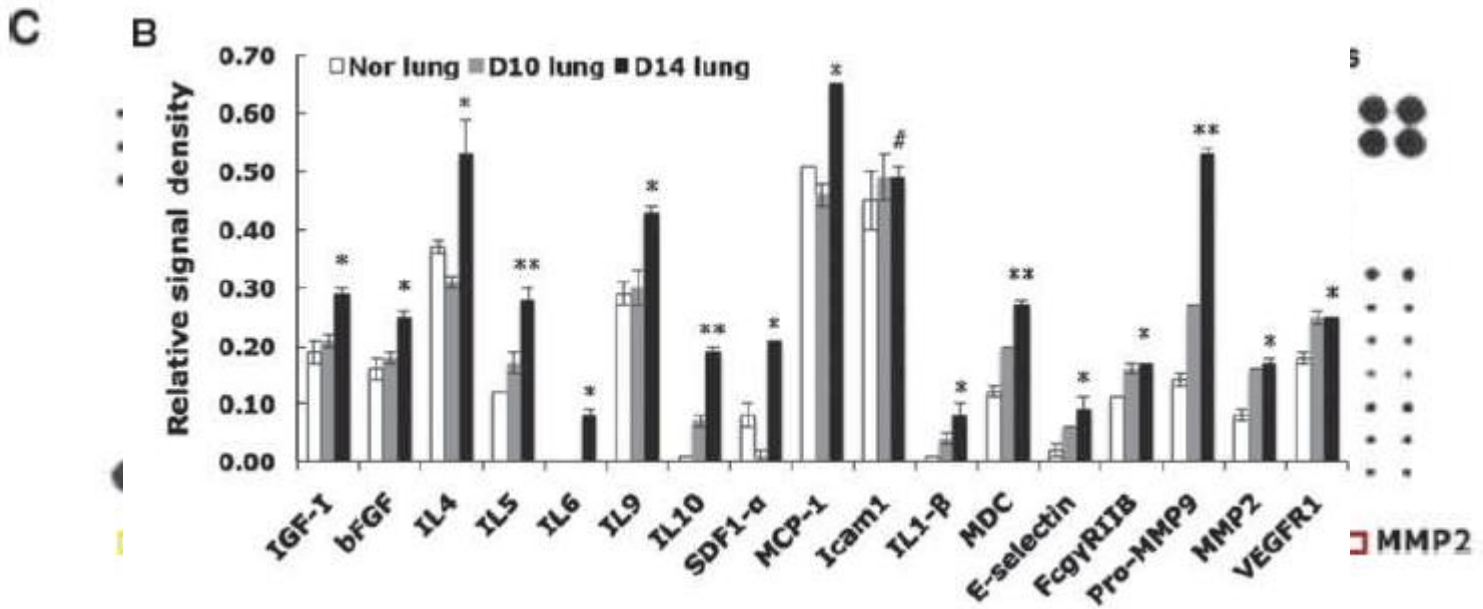




- Using 4T1 breast cancer cells, authors noted a large increase in lung cells prior to metastasis
- MDSCs began to arrive in large numbers as early as D7, 7-14 days prior to tumor cell arrival
- MDSC coculture reduced lung lymphocytes IFNg production, primary means of early M1 and T_H1 development.
- How are the MDSCs affecting the local lung environment once there?

Increased T_H2 Cytokines and MMP9



- Array Identified numerous cytokine changes in the premetastatic lung
- Notably large increase in MMP2 and MMP9 and important factor in remodeling and tumor escape.
- Follow-up experiments showed MDSCs linked to leaky lung vasculature, and MMP9 facilitated metastasis

Effects of MDSCs in Cancer

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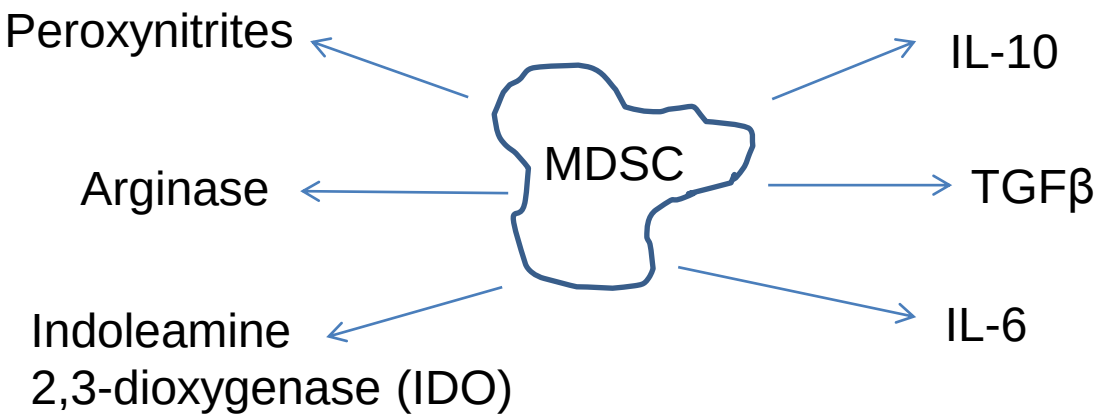

- MDSC immunosuppressive effects vary from cancer to cancer, but CD13+, CD33+ MDSCs in breast cancer express large amounts of IDO
- IDO in cancer is associated with significant decreases in IFNγ from T cells, and reduced proliferation.
- However, IFNγ is main driver of IDO, so what signal from MDSCs is functioning to drive IDO expression?

IDO production linked to STAT3

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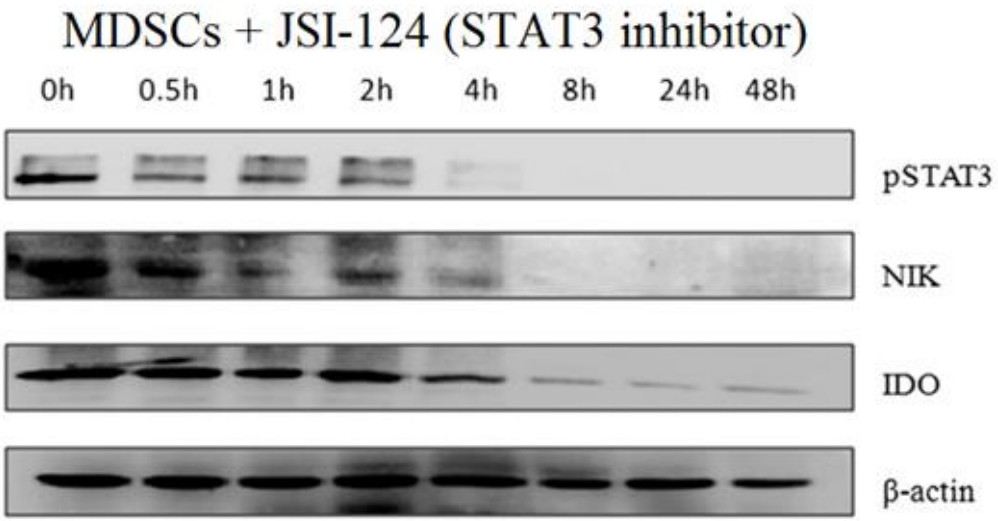

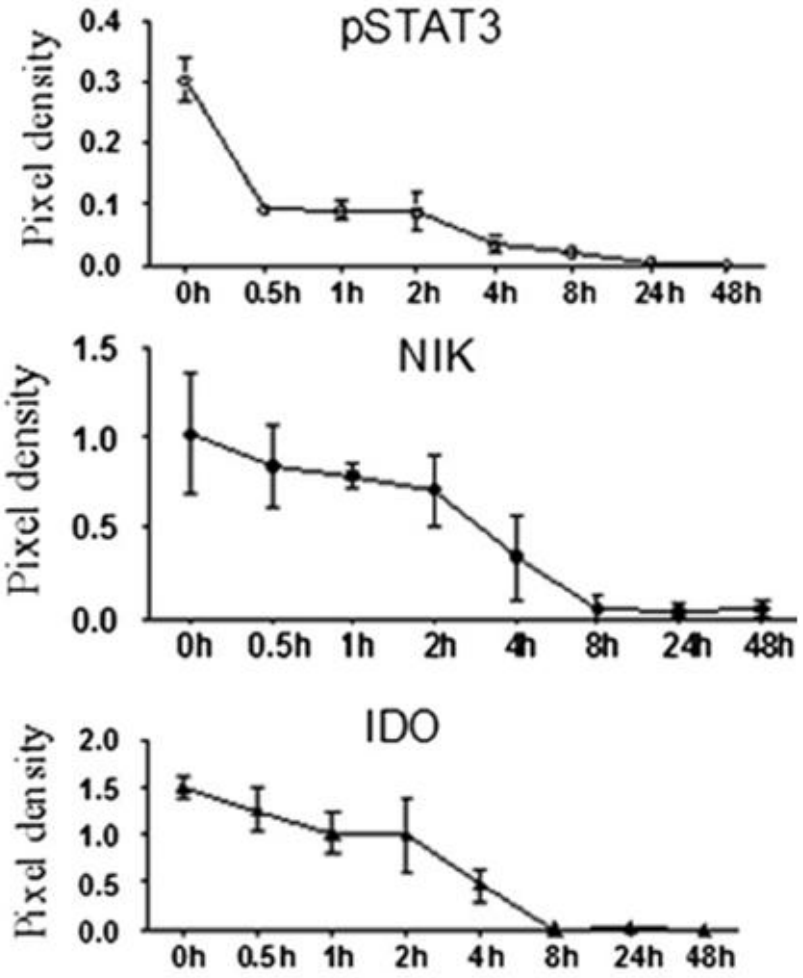

- Using derived MSDCs from MDA-MB-468 coculture.
- STAT3 inhibition with JSI-124 reduced IDO expression.
- However, STAT3 does not directly bind IDO promoter

Yu J, et al. The Journal of Immunology September 1, 2014 vol. 193 no. 5 2574-2586

STAT3 Driven by IL-6 from MDSCs

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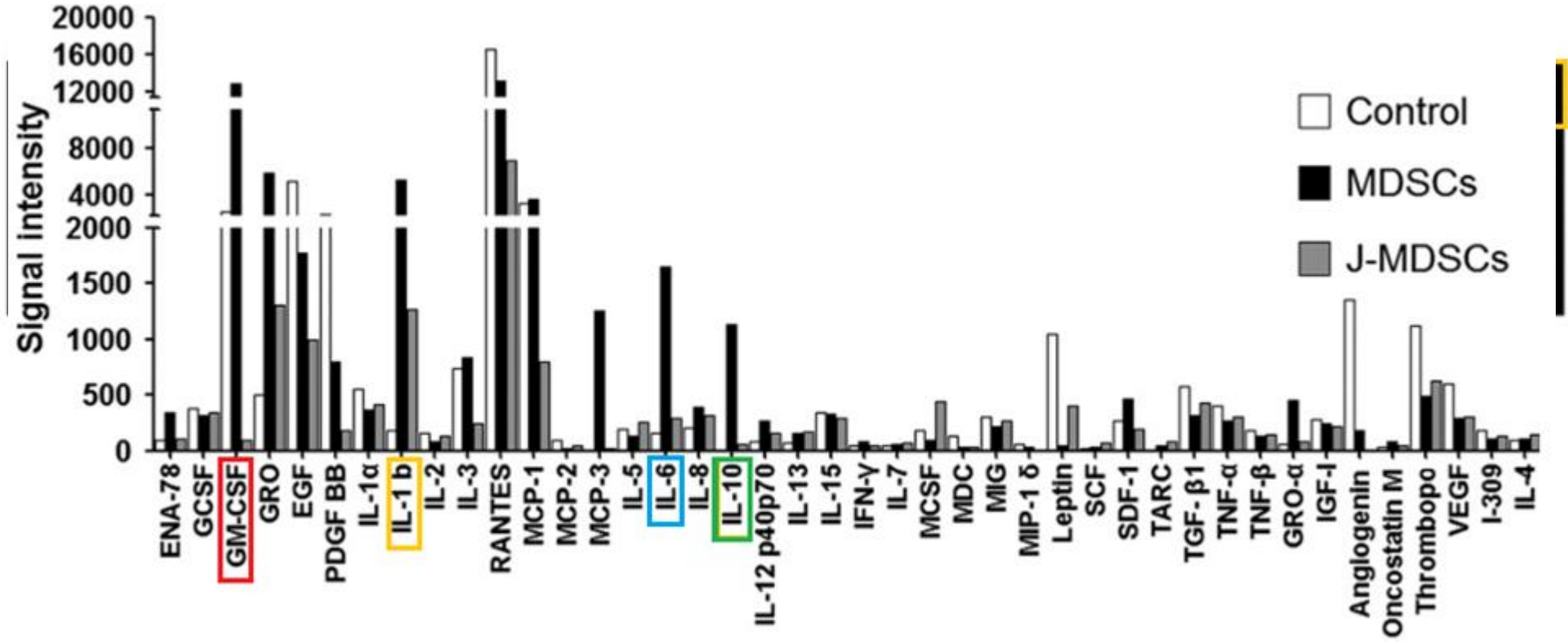

- Elevated GM-CSF, IL-1b, IL-6, and IL-10 all could drive a STAT3 signal
- Follow on treatments of MDSCs directly with IL-6 confirmed that IL-6 could promote IDO in a STAT3 dependent manner

Tumor DC Suppression

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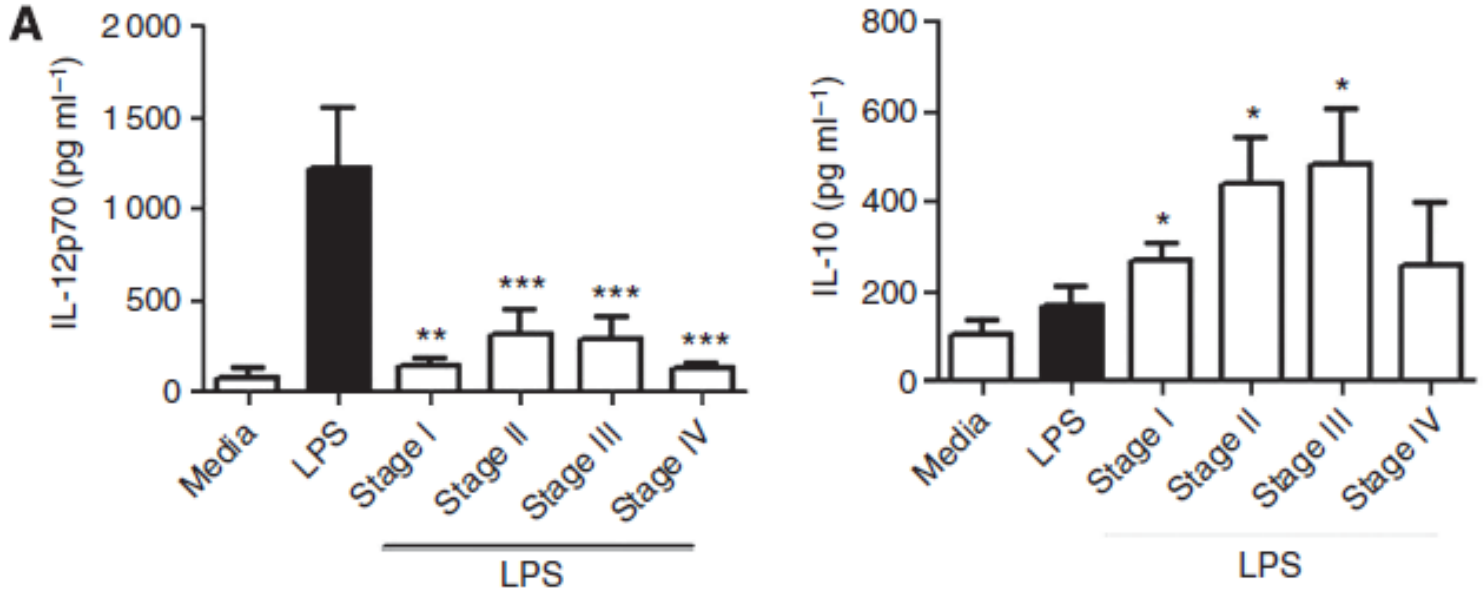
- Chronic Inflammation is critical to tumor progression in continued tumor growth, invasion, and angiogenesis
- The TME in late stage colorectal cancer involved continued suppression of IL-12p70 from monocyte-derived DCs, and promotion of IL-10 production
- Since DC based vacccines require IL-12p70 production for efficacy, how does the TME cytokine profile change?



Drive to Premetastatic Niches

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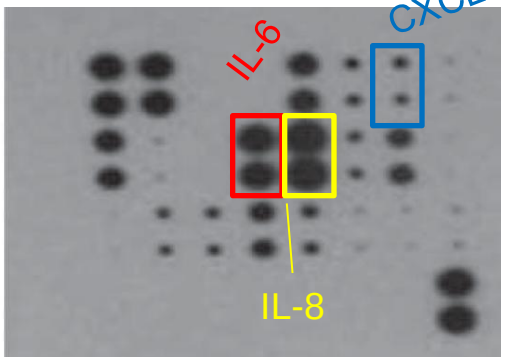
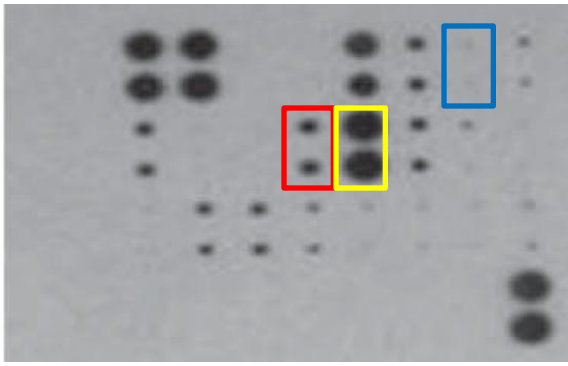
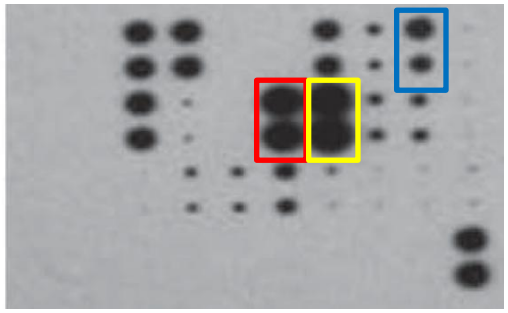
Stage I



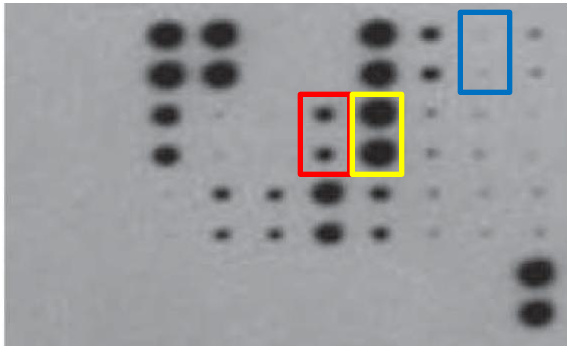
Stage II



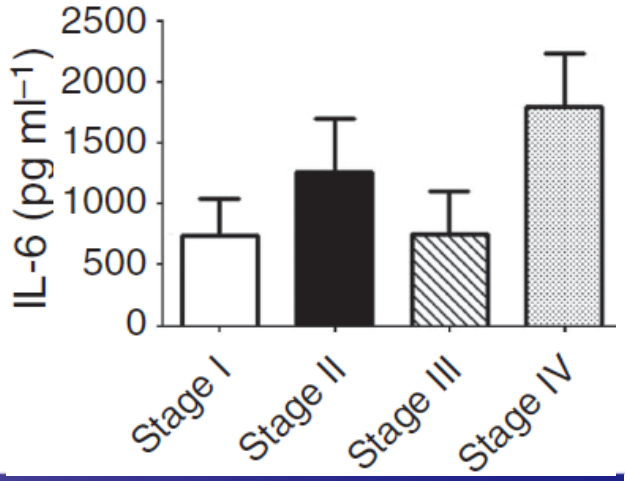
Stage III



Stage IV



- Some changes in IL-6 expression were found at different stages, but overall cytokines levels were similar
- Critical as most studies show IL-12p70 is required for DC based vaccines



Stromal Cell Involvement in MM

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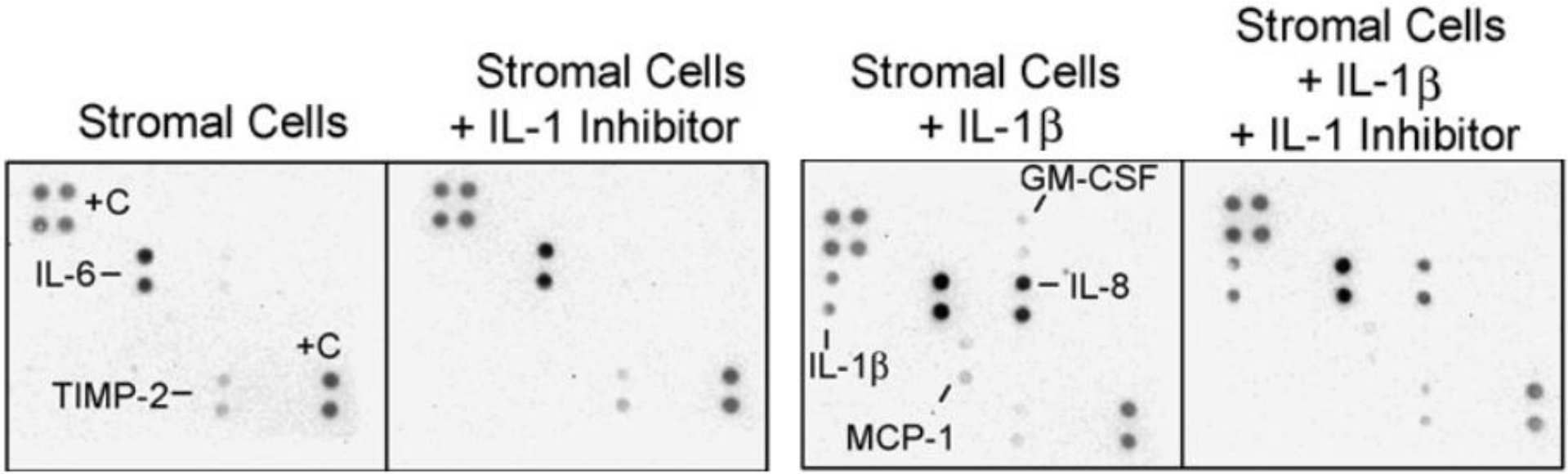
- Multiple Myeloma, and associated diseases (MGUS) are recruited to the Bone Marrow where the permissive environment promotes survival of plasma cells.
- BM Stromal cells support MM cells by secretion of IL-6 in response to MM produced VEGF, IL1 β, and TNF.
- What other factors are secreted by stromal cells?



- Kline M., et al. Leukemia Research Volume 31, Issue 5, May 2007, Pages 591–598

IL-6 and IL-8 in Active Myeloma

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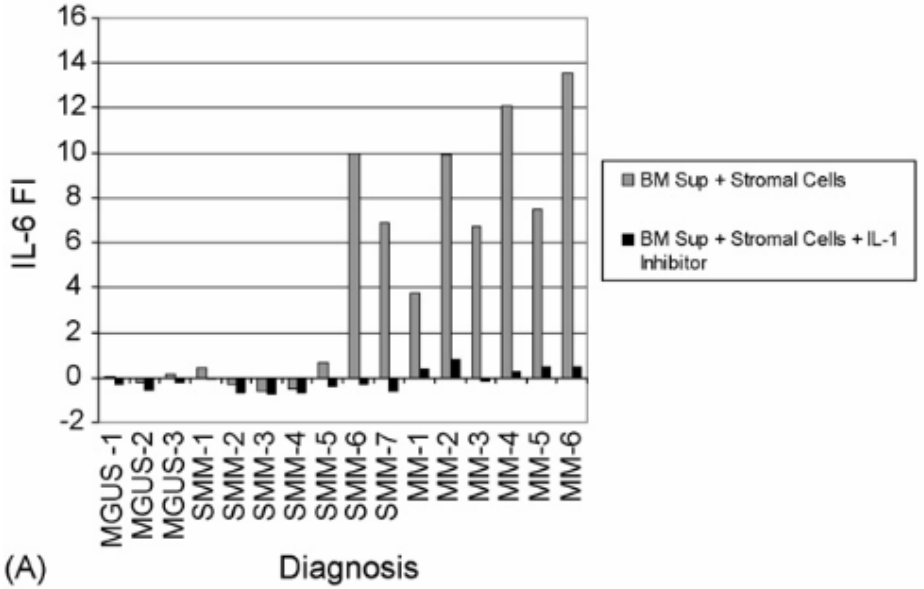
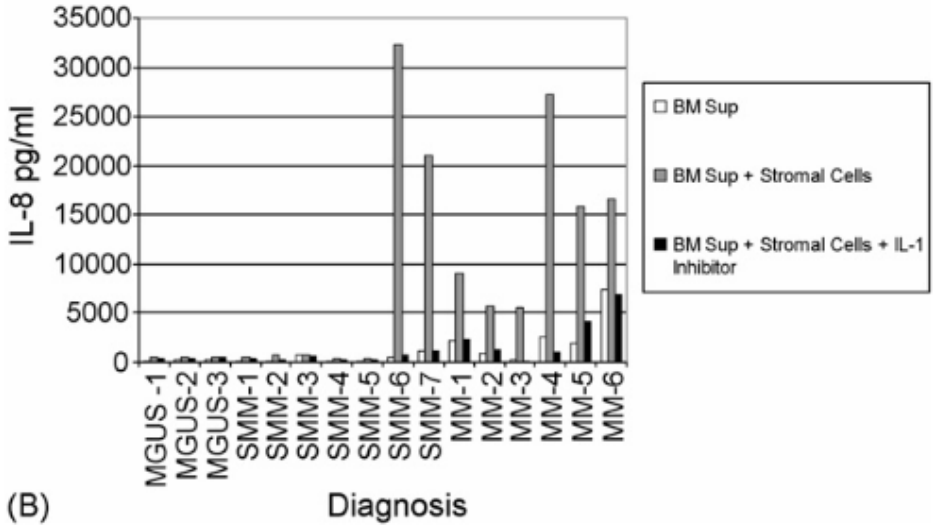
- IL-6 and IL-8 primarily produced in active myeloma cases
- MGUS and SMM patients show little production.
- Active myelome must initiate IL-1b production from plasma tumor cells to drive IL-6 and IL-8.
- Biomarker importance for disease, but also NFkB is a common chemotherapy target.





How do CAFs affect Neighbors?

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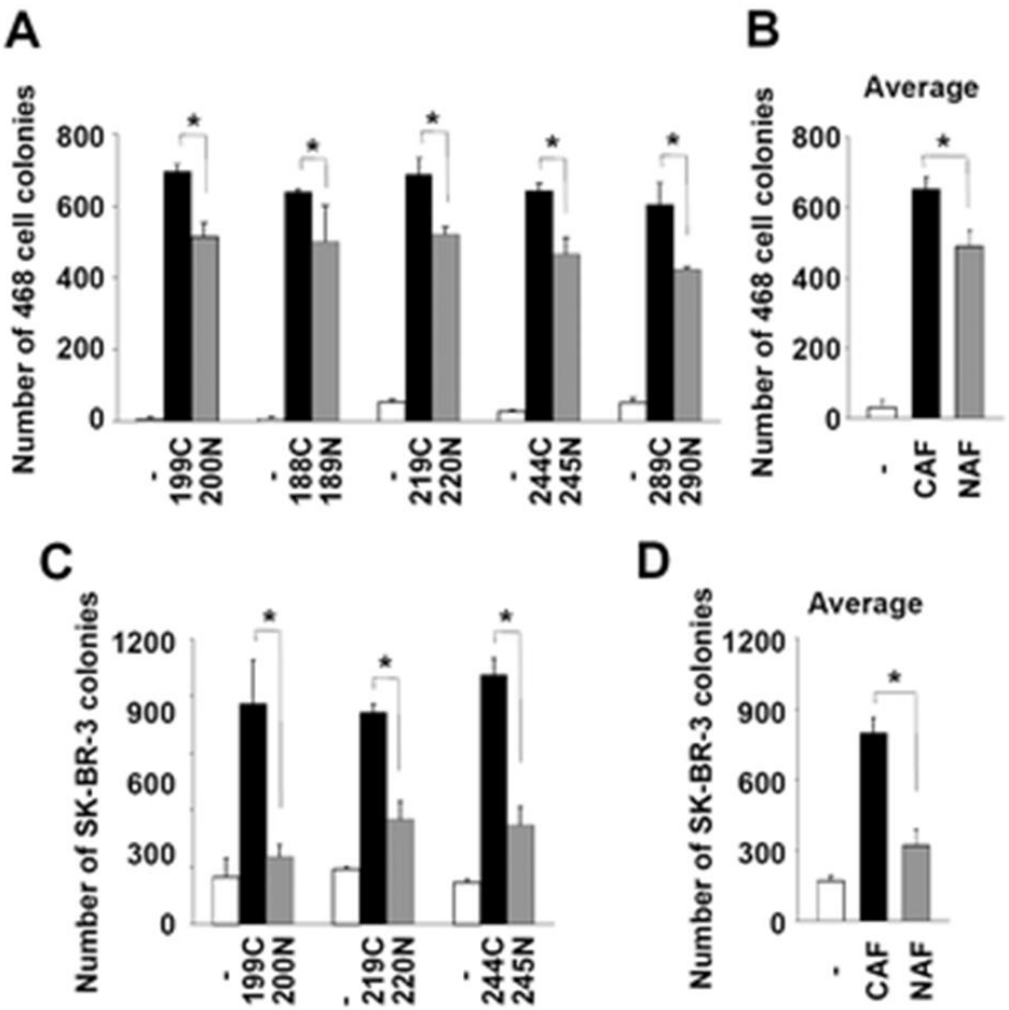
- Cancer Associated Fibroblasts (CAFs) facilitate tumor growth, angiogenesis, and metastasis
- How do CAFs effect in vitro grown breast cancer cells?
- CAFs, significantly expanded the number of tumor colonies developed compared to normal fibroblasts
- SDF-1 and Hepatocyte growth factor are known contributors to tumor growth
- How do CAFs affect other nearby fibroblasts?



CAFs Produce more HGF than NAFs

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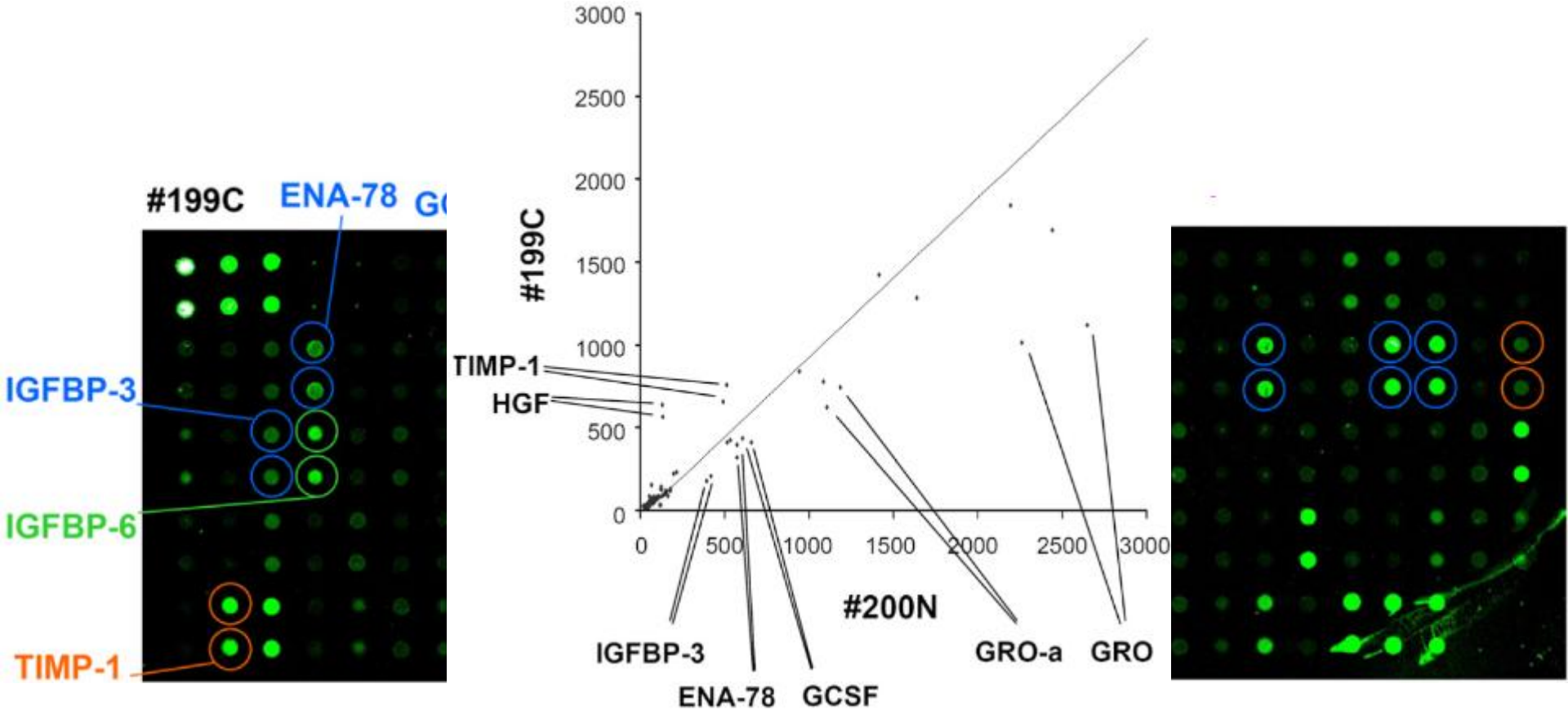
- CAFs express significantly more HGF than NAFs
- NAFs have a different expression profile of CXCL5, GRO, and GCSF, and IGFBP3



Tyan SW., et al. PLOS One. 2011.

Tumors Convert NAFs

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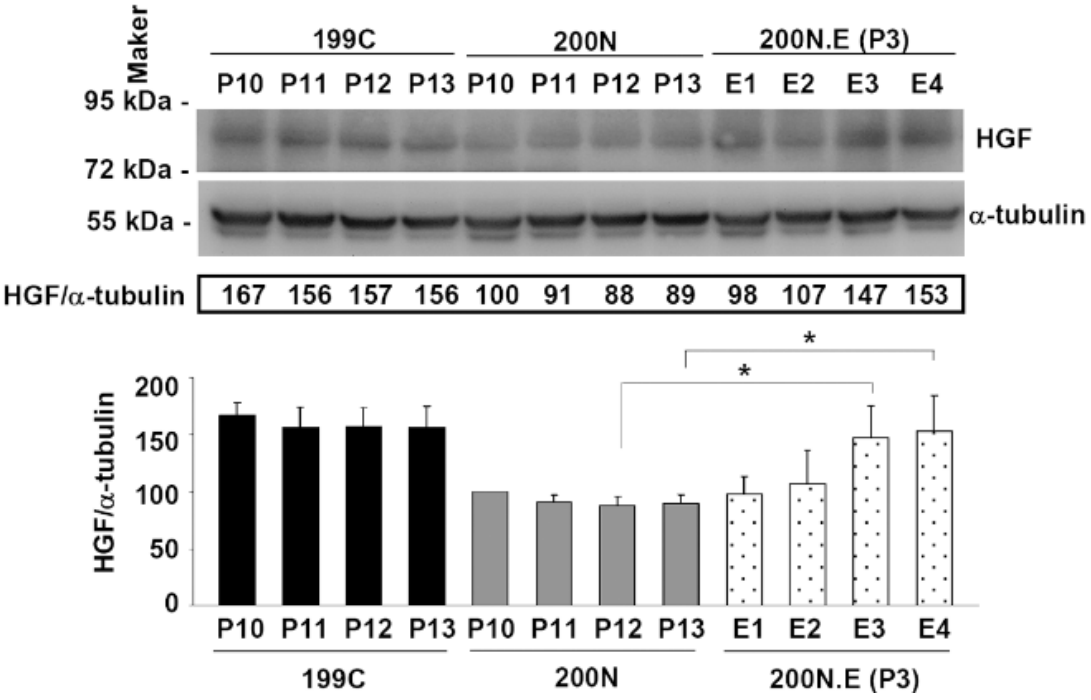
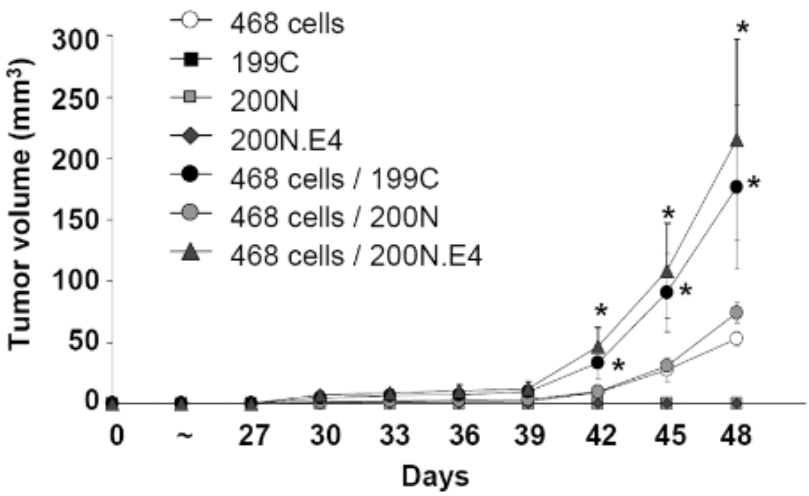
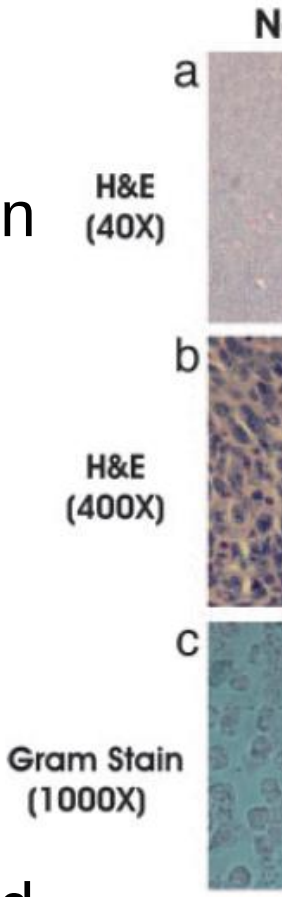
- MDA-MB-468 tumors cells secrete factors which facilitate NAF conversion to a tumor supporting cell type after 4 passages
- This conversion was associated with increased HGF expression, and significant increases in tumor colony formation.

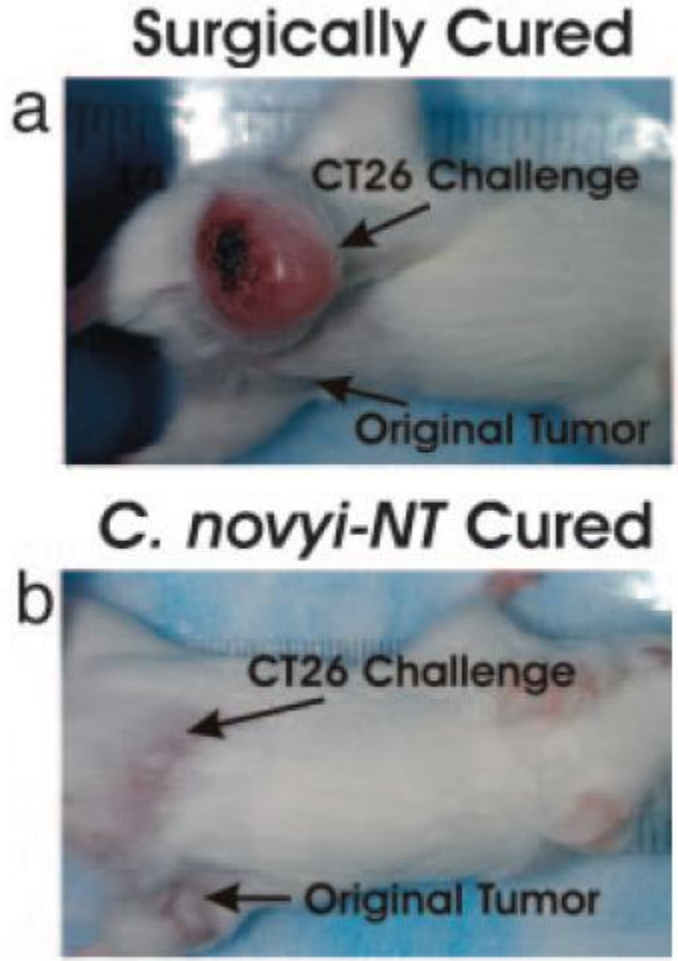




Failure to Clear, or Failure to Simulate?

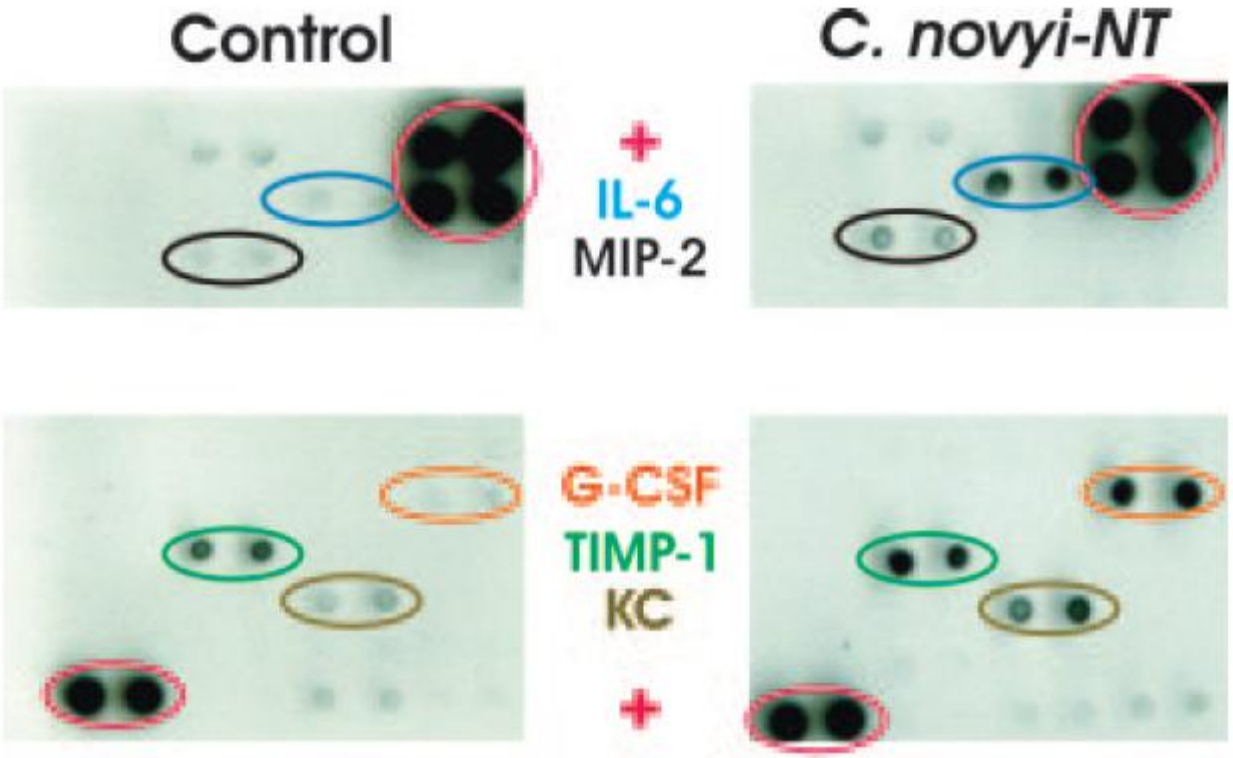
- Colorectal tumors create a hypoxic tumor center, which bacterial spores can germinate in
- Injection of spores results in hemorrhagic necrosis of tumor
- 1/3 of mice full clear the tumor with no recurrence
- Does clostridium spores kill enough tumors for the immune system to clean up the rest, or rather stimulate the immune system to full clear the tumor and develop memory?





Immunological Conversion

- Surgical excision of tumor was insufficient to promote tumor clearance, suggesting spores were stimulatory in nature.
- Suggests an immunological memory response.
- So, what signals were involved in tumor clearance?



Antitumor Immunity Development

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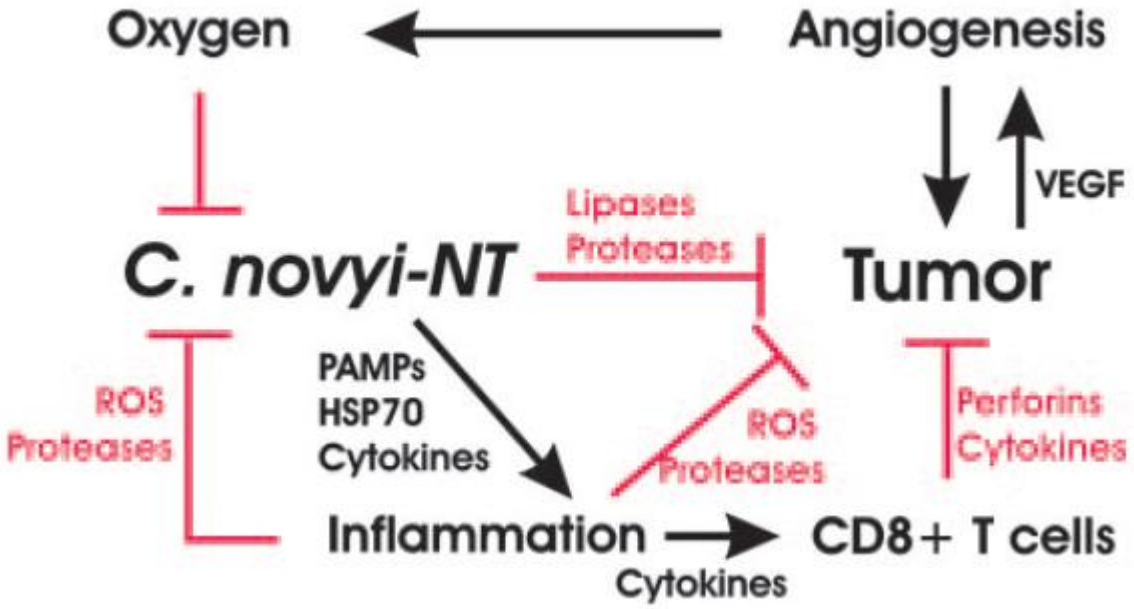
- Increases in neutrophil chemotatic cytokines, coupled with the tumor destruction from the bacterial spores, facilitates tumor clearance
- Clearance occurs alongside development of an anti-tumor cellular response



Immunologic Cancer Progression

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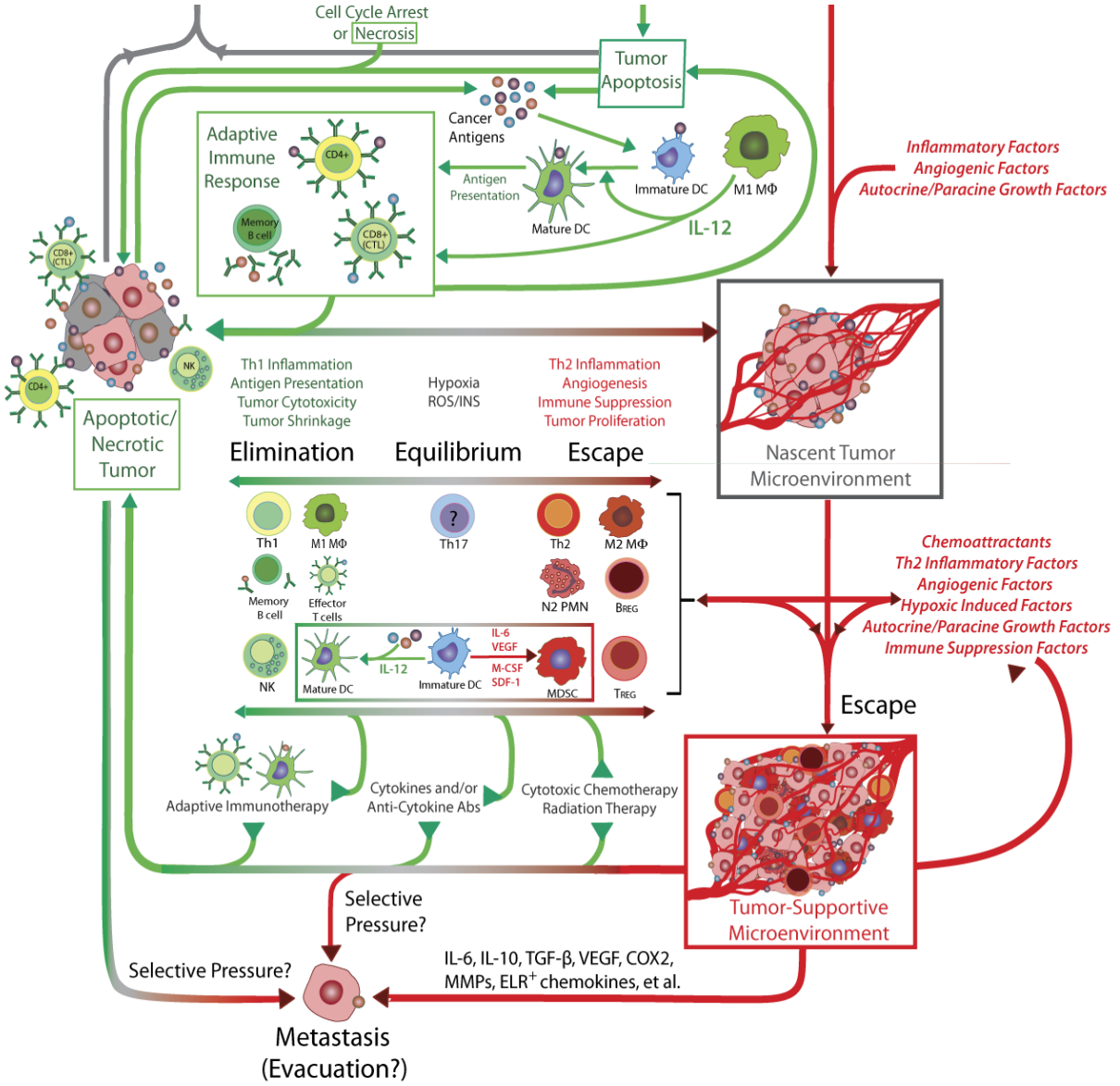
- Tumor escape involves the eventual subversion of immunological recognition and/or response
- Tumor escape related to many factors, but largely associated with T_H2 cytokine profile and Type 2 Mφ and Nφ
- VEGF, IL-6, SDF-1, and other factors are critical in inhibiting the anti-tumor T_H1 and CTL immune response



Model of Tumor-Induced Immunosuppression

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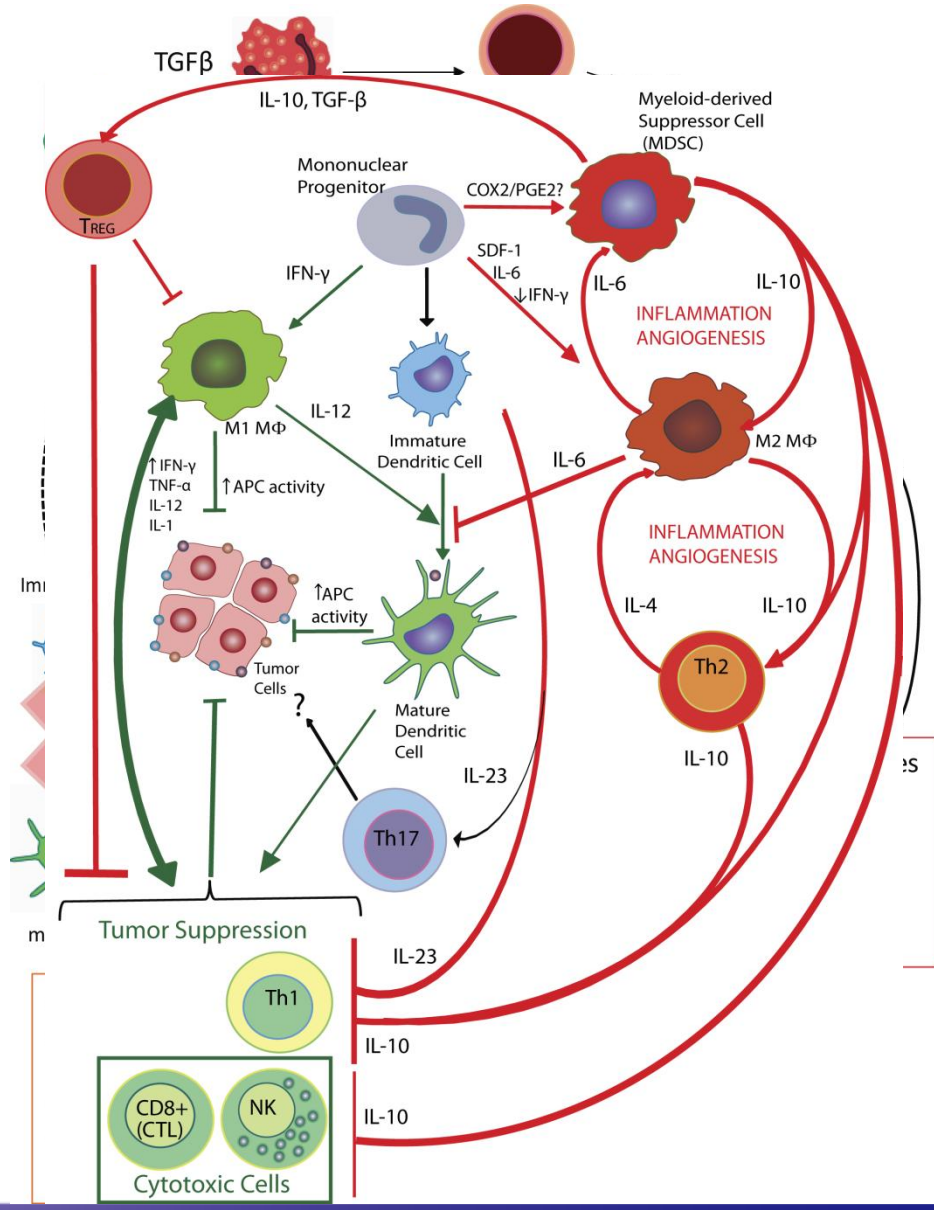
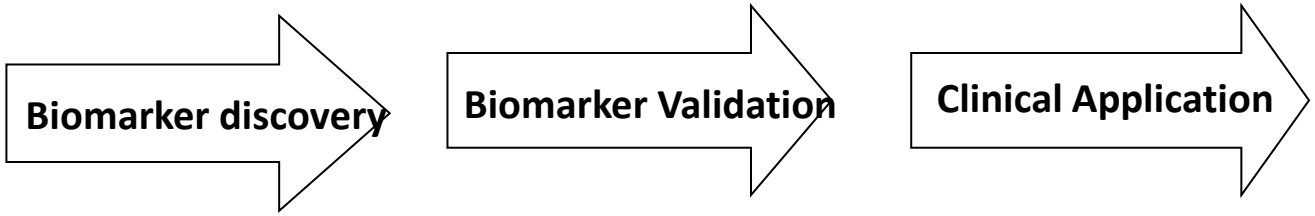
- Inhibition of inflammatory cell and T cell derived IFNγ and IL-12 limit anti-tumor immune response
- Lack of IL-12 and IFNγ facilitate drive to TH2 profile of IL-4, IL-10, TGFβ, and promote VEGF and MMP production in the TME
- This promotes pro-umor inflammation, angiogenesis, and metastasis.
- Also, the T_H2 environment reduces NK and CTL mediated killing, decreased cytolytic capacity of NKs and Mφs, and decreases tumor antigenicity



A Multidimensional Approach for Biomarker Discovery

Antibody arrays
Protein arrays

- Protein expression profile
- Glycosylation
- Phosphorylation
- Auto-antibody
- Protein-protein interaction
- Kinase activity
- Phosphatase activity
- Enzyme activity



Biomarker discovery	Biomarker Validation	Clinical Application
Secretome ab arrays	ELISA	ELISA
High density sandwich ab arrays	Quantitative arrays	Quantitative arrays
Glycoprotein arrays		
Protein arrays		
Phosphorylation ab arrays		

Thanks to the RayBiotech Family

Questions?