Novel biomarkers: pitfalls, limitations, emerging options.

Bernard A. Fox
Harder Family Endowed Chair for Cancer Research
Laboratory of Molecular and Tumor Immunology
Robert W. Franz Cancer Research Center
Earle A. Chiles Research Institute
Providence Portland Medical Center

UbiVac

Department of Molecular Microbiology and Immunology; and
Knight Cancer Institute, OHSU, Portland, Oregon , USA

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Bernard A. Fox, PhD

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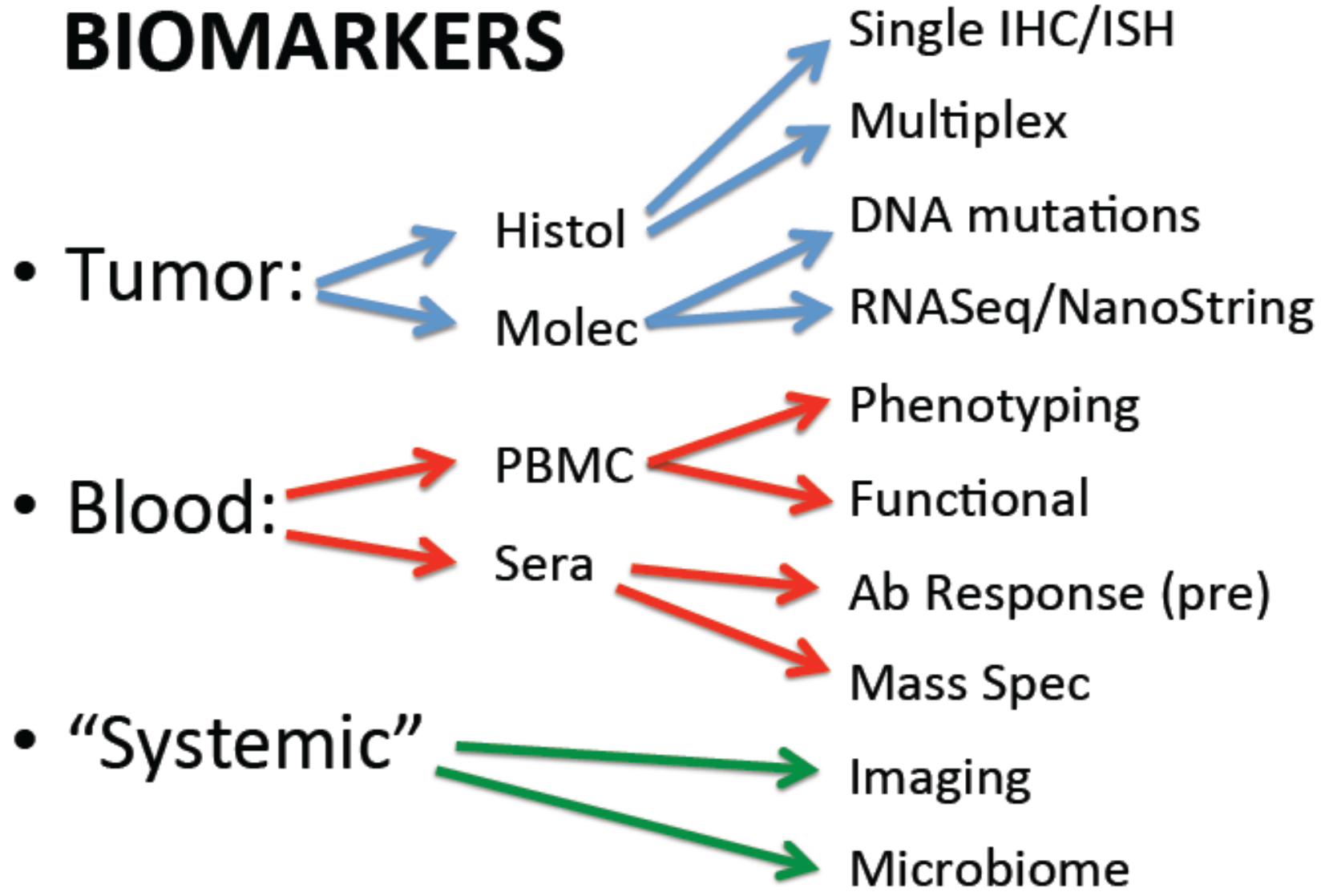

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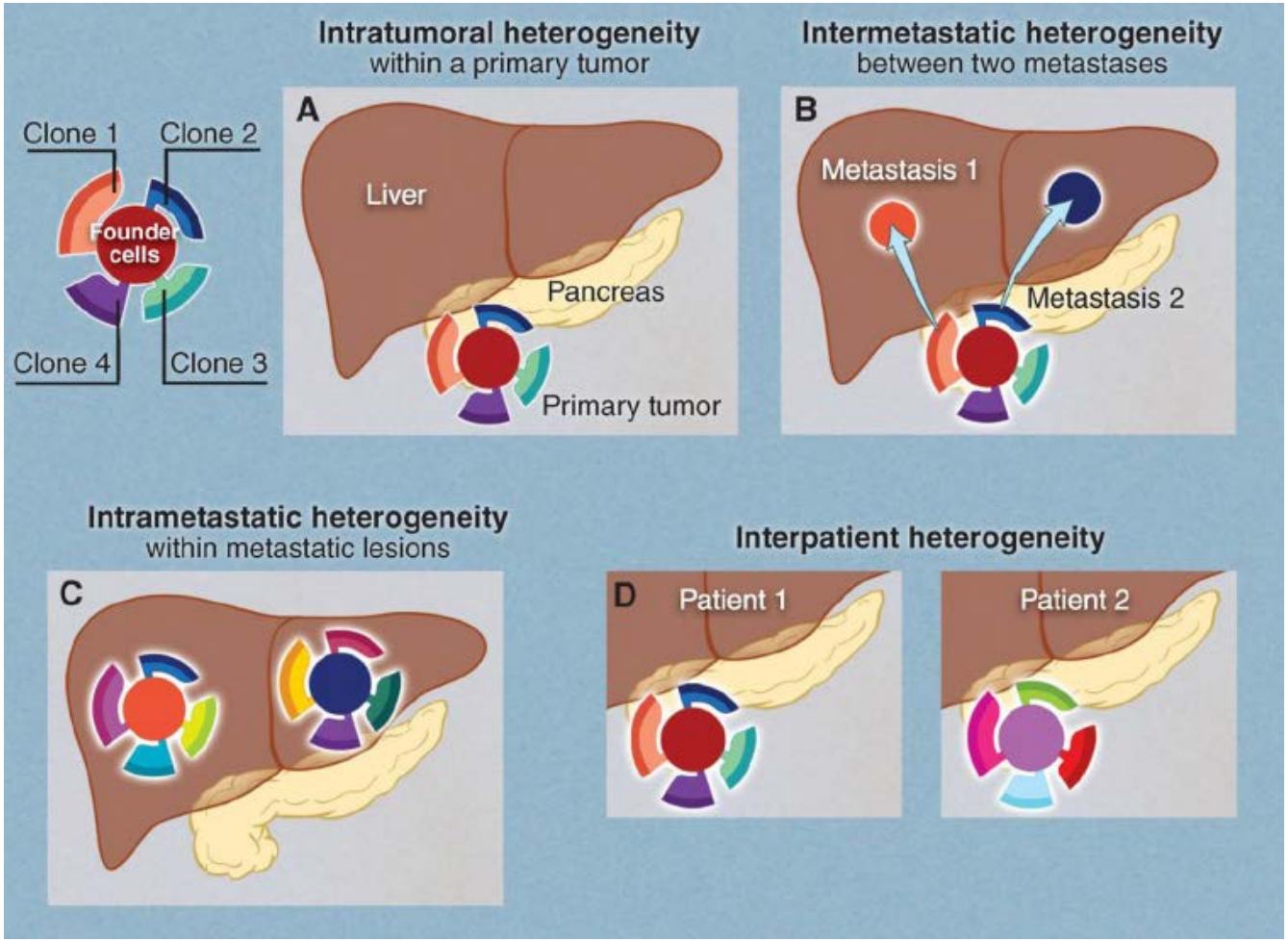
“Hurdles”

- Tumor Heterogeneity (antigenic)

- Checkpoint blockade and Costimulatory Abs only effective against immunogenic tumor (preclinical)

Cancer Heterogeneity Mandates Broad Immunity



Hypothesis:

Effective treatment of metastatic cancer will require an immune response to many antigens

Vogelstein B, Science 339:1546, 2013

de Bruin, EC, Science 346, 251, 2014 (NSCLC)

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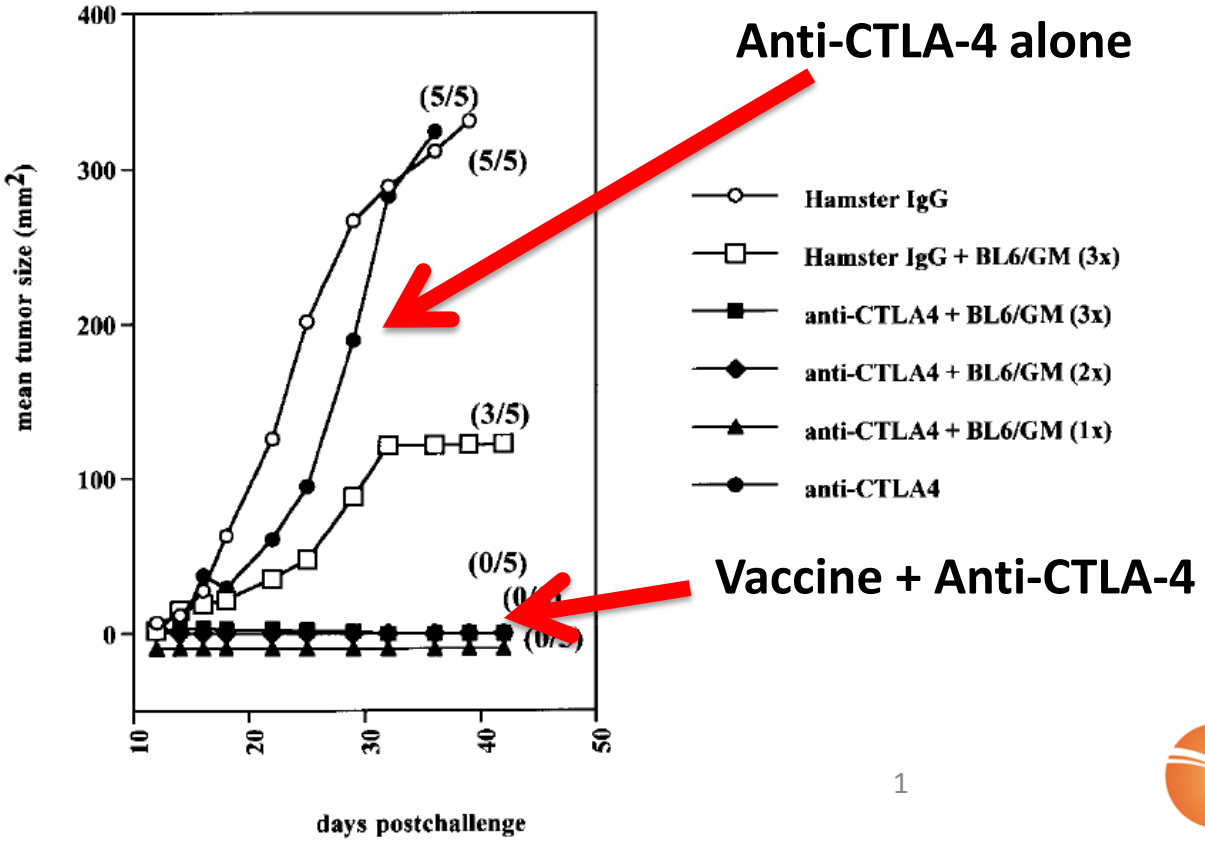
Many Cancers not seen by immune system

Combination Immunotherapy of B16 Melanoma Using Anti–Cytotoxic T Lymphocyte–associated Antigen 4 (CTLA-4) and Granulocyte/Macrophage Colony-Stimulating Factor (GM-CSF)-producing Vaccines Induces Rejection of Subcutaneous and Metastatic Tumors Accompanied by Autoimmune Depigmentation

By Andrea van Elsas, Arthur A. Hurwitz, and James P. Allison

J. Exp. Med 190 (3) : 355-366, 1999



Hypothesis:

Tumors that are less immunogenic need something to prime anti-cancer immunity.

- **Vaccines**
- Chemo/Rad
- Antibodies
- BiTEs / DARTs
- Oncolytic viruses

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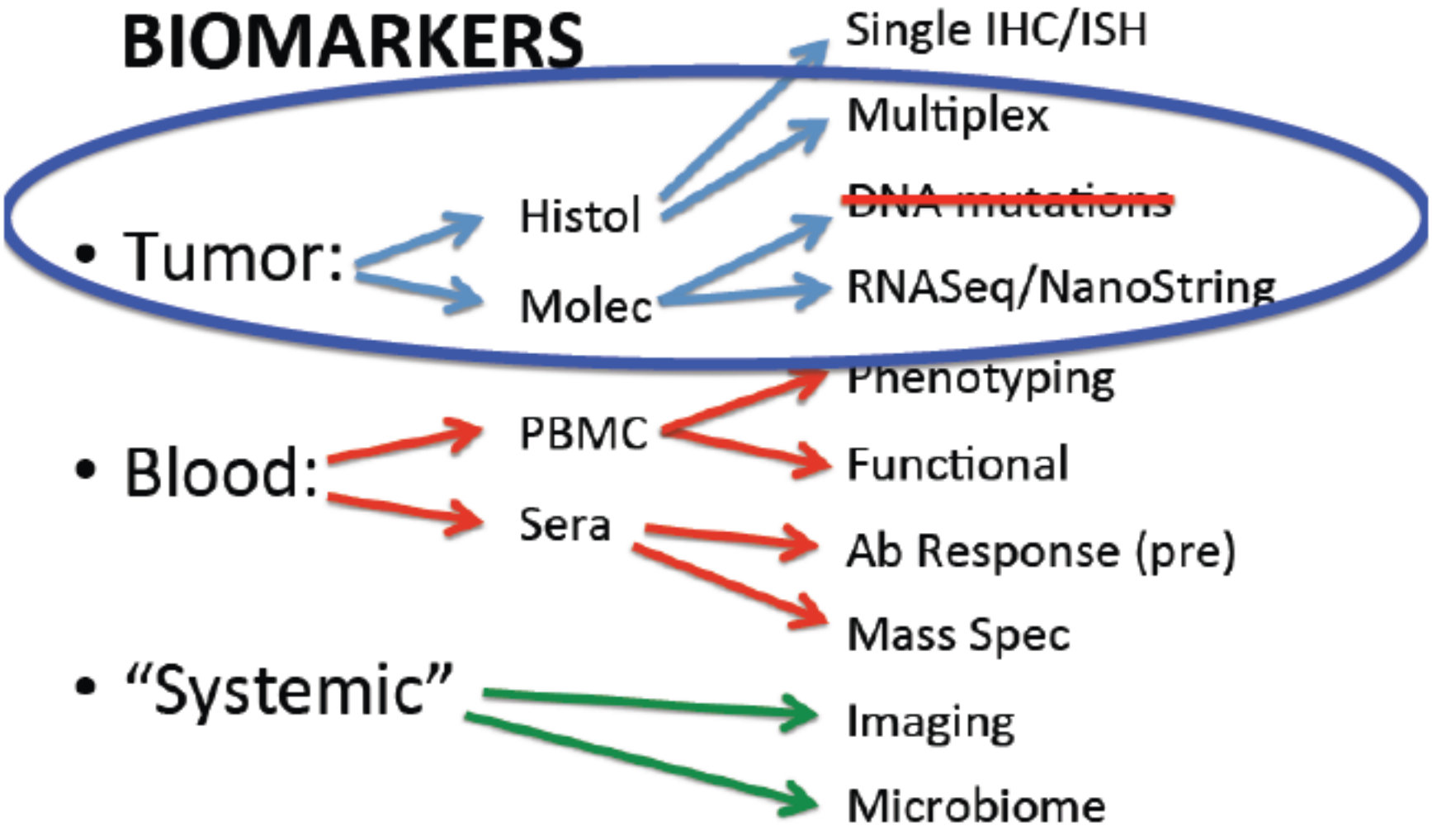
Cancer Immunogenicity and heterogeneity important

- Underscores limitations of sampling

- Gives direction for “Future” – More effective treatments with potential to CURE.



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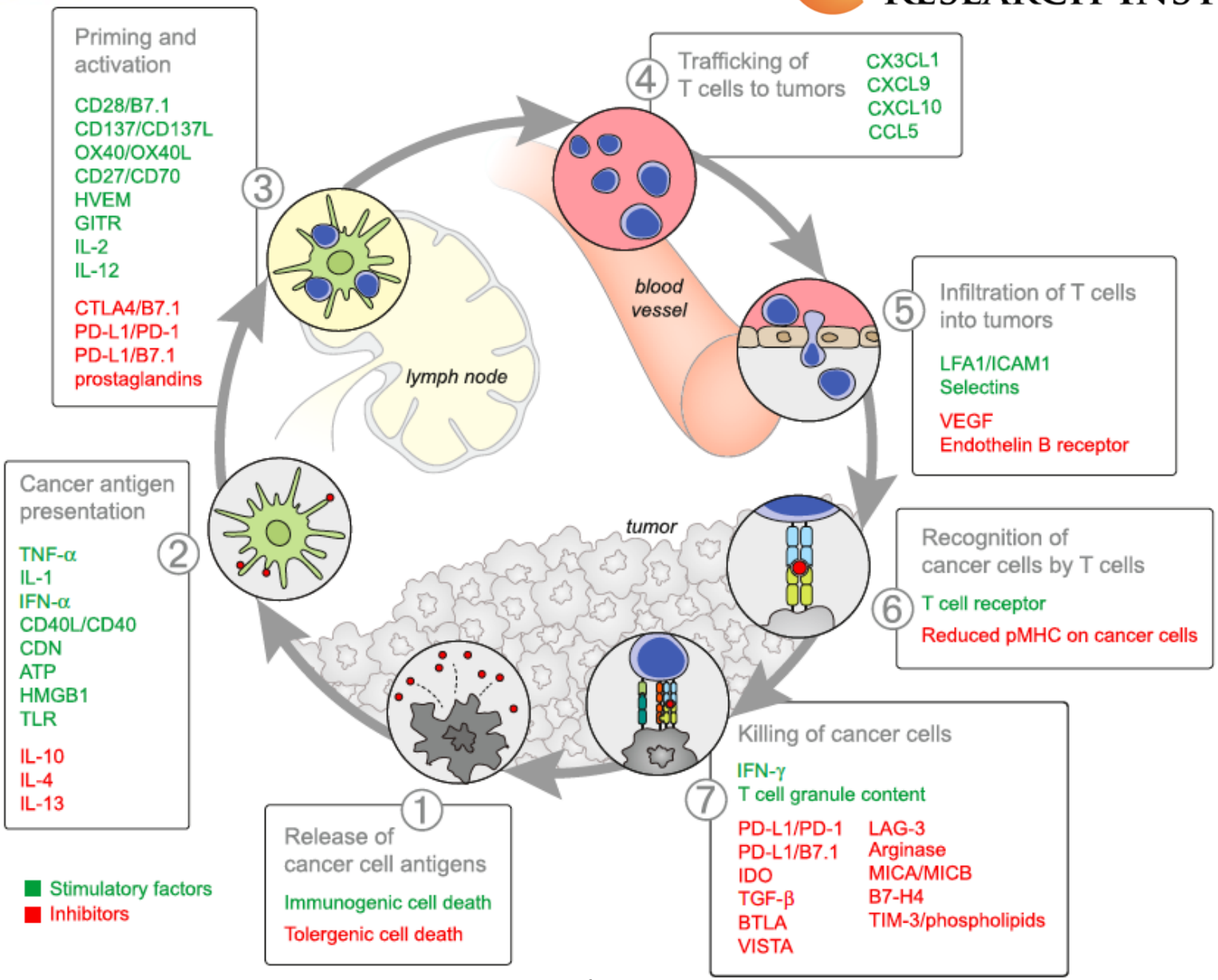
Immunity
Review



Chen and Mellman Immunity 39, July 25, 2013

Zipei Feng

red = PD-L1
yellow = CD8
green = Foxp3
purple = CD20
aqua = melanoma cocktail
pink = CD163
blue = DAPI

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Biomarkers **Not Predictive** of tumor-specific TIL cells in the tumor

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NOT PREDICTIVE

Number of CD3

Number of CD8

Number of FoxP3

Feng *et al. Journal for ImmunoTherapy of Cancer* (2015) 3:4
DOI 10.1186/s40425-015-0091-z

Journal for ImmunoTherapy of Cancer

The CD8:FoxP3 ratio is predictive

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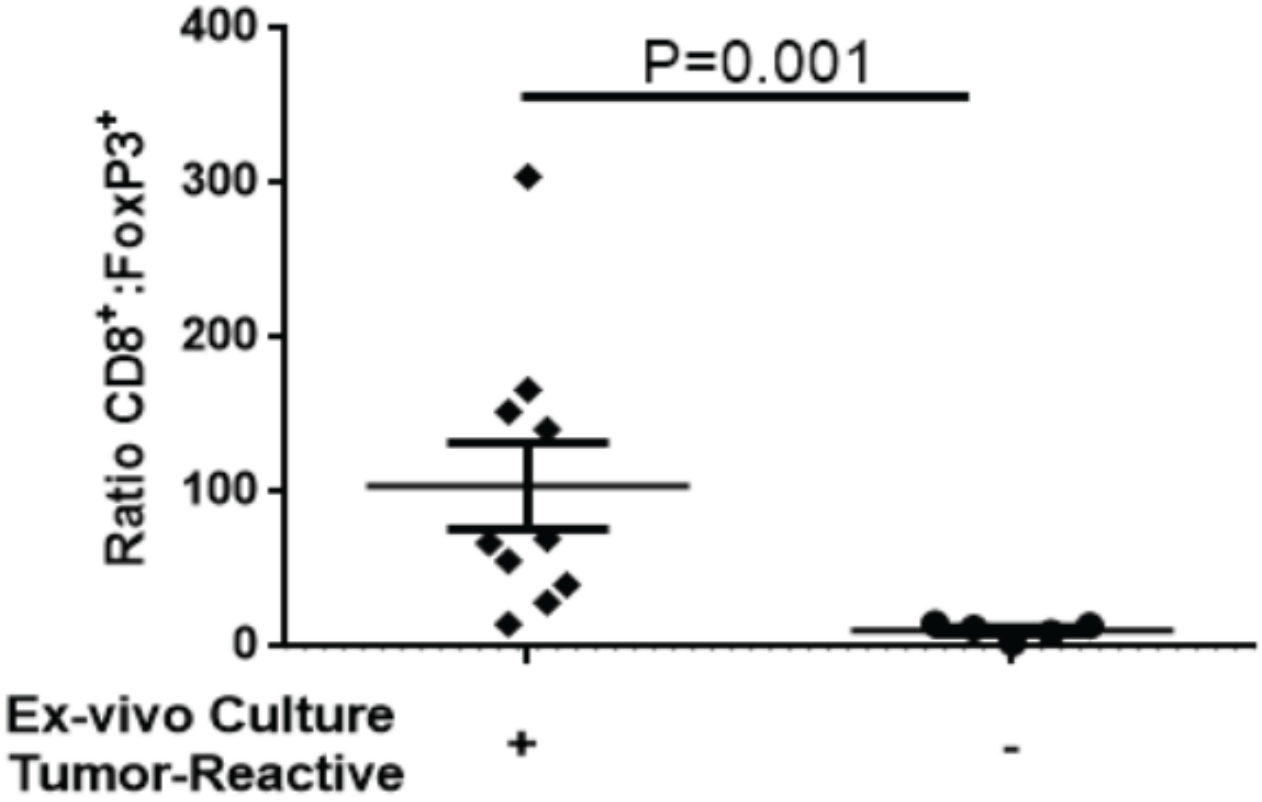

Feng *et al. Journal for ImmunoTherapy of Cancer* (2015) 3:4
DOI 10.1186/s40425-015-0091-z

Journal for ImmunoTherapy of Cancer

Combining CD8:FoxP3 Increases Power to Predict TIL

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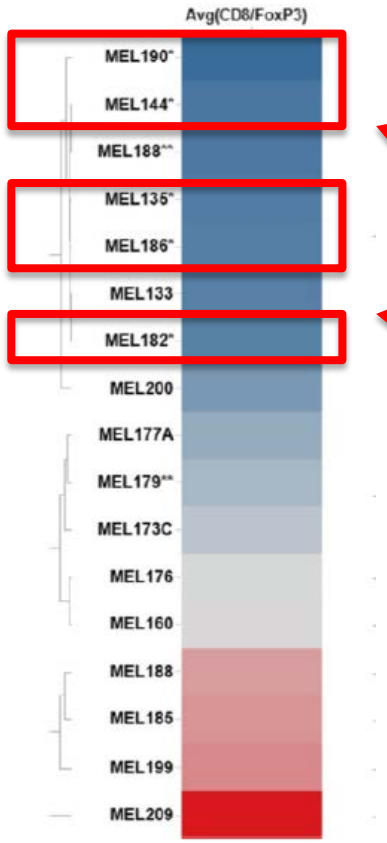

Red box Identifies tumors that fail to grow TIL

Feng *et al. Journal for ImmunoTherapy of Cancer* (2015) 3:4
DOI 10.1186/s40425-015-0091-z

Journal for ImmunoTherapy of Cancer

Combining Both CD8:FoxP3 and CD8:PD-L1 Increases Predictive Power - Melanoma

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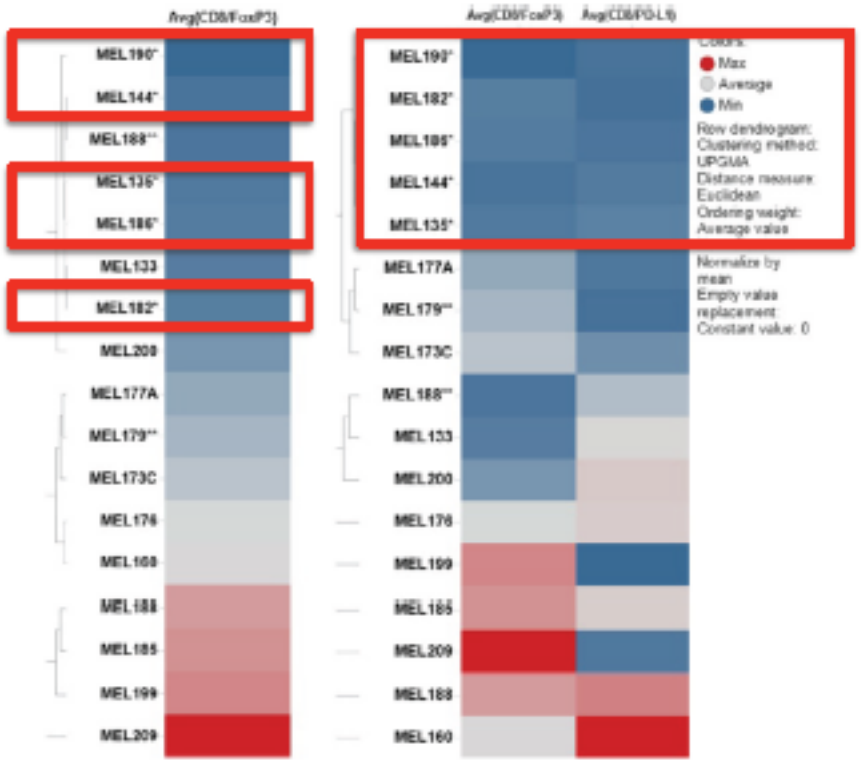

Red box Identifies tumors that fail to grow TIL

**Collaboration with Cliff Hoyt (PE)*

**Applying to NSCLC and HNSCC*

Feng *et al. Journal for ImmunoTherapy of Cancer* (2015) 3:4
DOI 10.1186/s40425-015-0091-z

Journal for ImmunoTherapy of Cancer

How to think about relationships?

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How to think about relationships?

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Effectors

Suppressors

Relationships are complex

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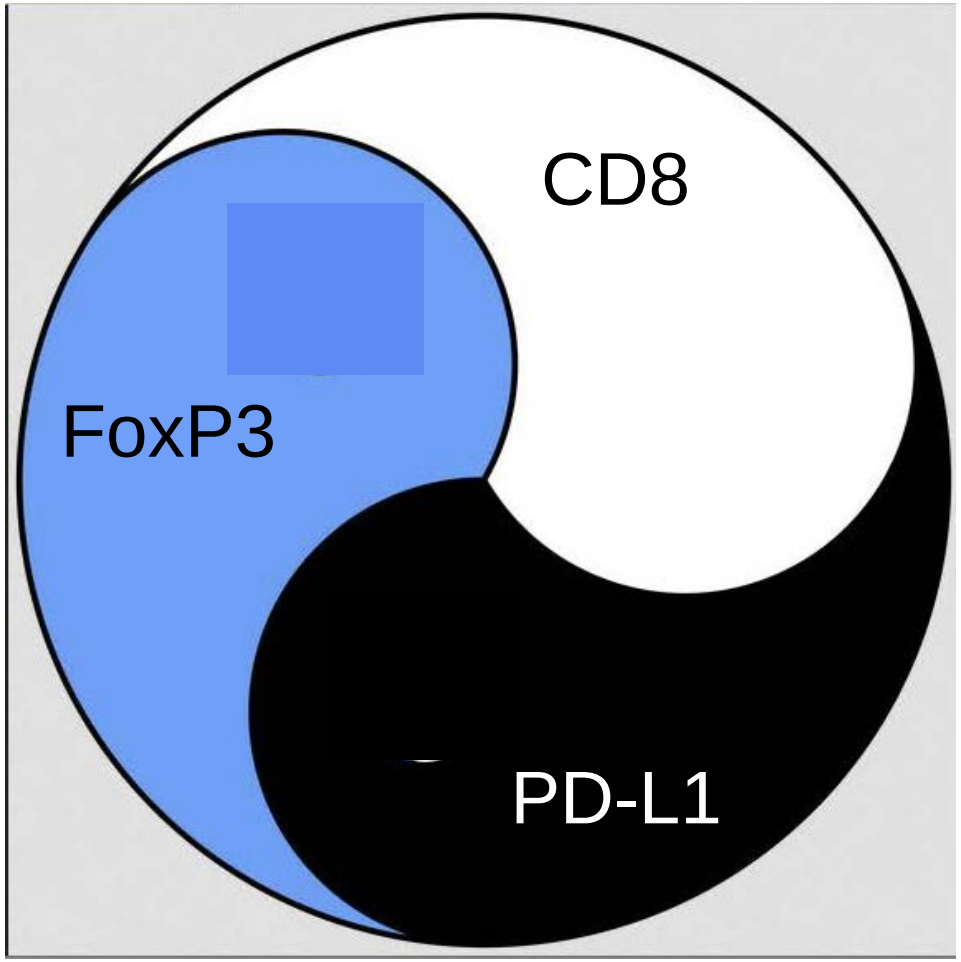

Relationships are complex

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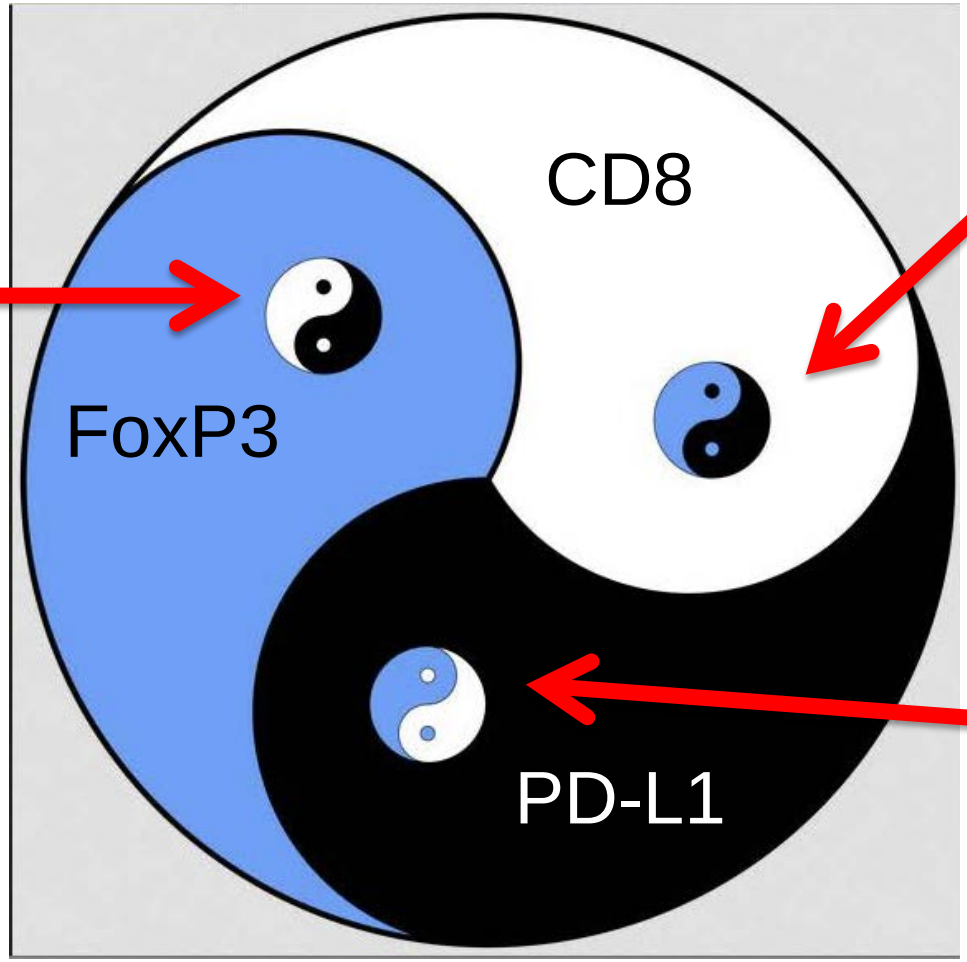
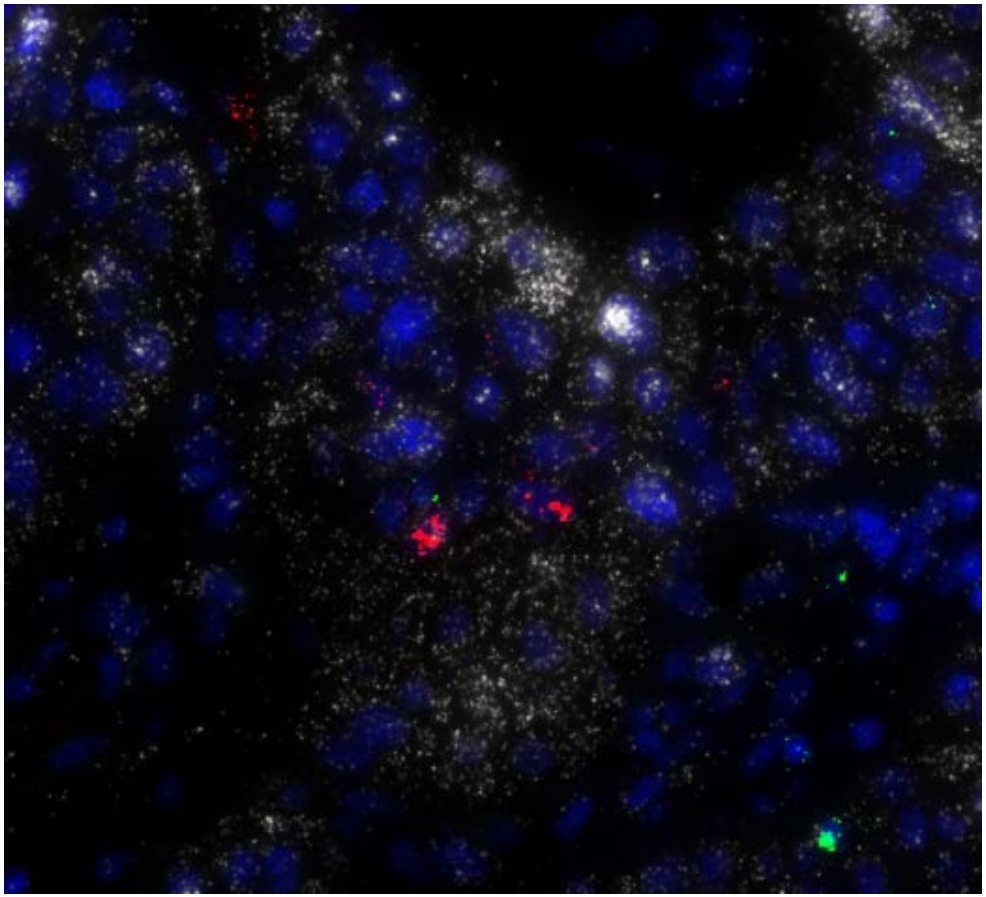
Ratio to CD4s / Tu – induced vs Natural
Where are they located? Perimeter / Center



TCR Specificity / Maturation status
CM, EM, Eff
IFN, TNF, …

Where expressed?
What other inhibitors?

Use ISH to Identify Functional Properties – Collaboration with ACD Bio



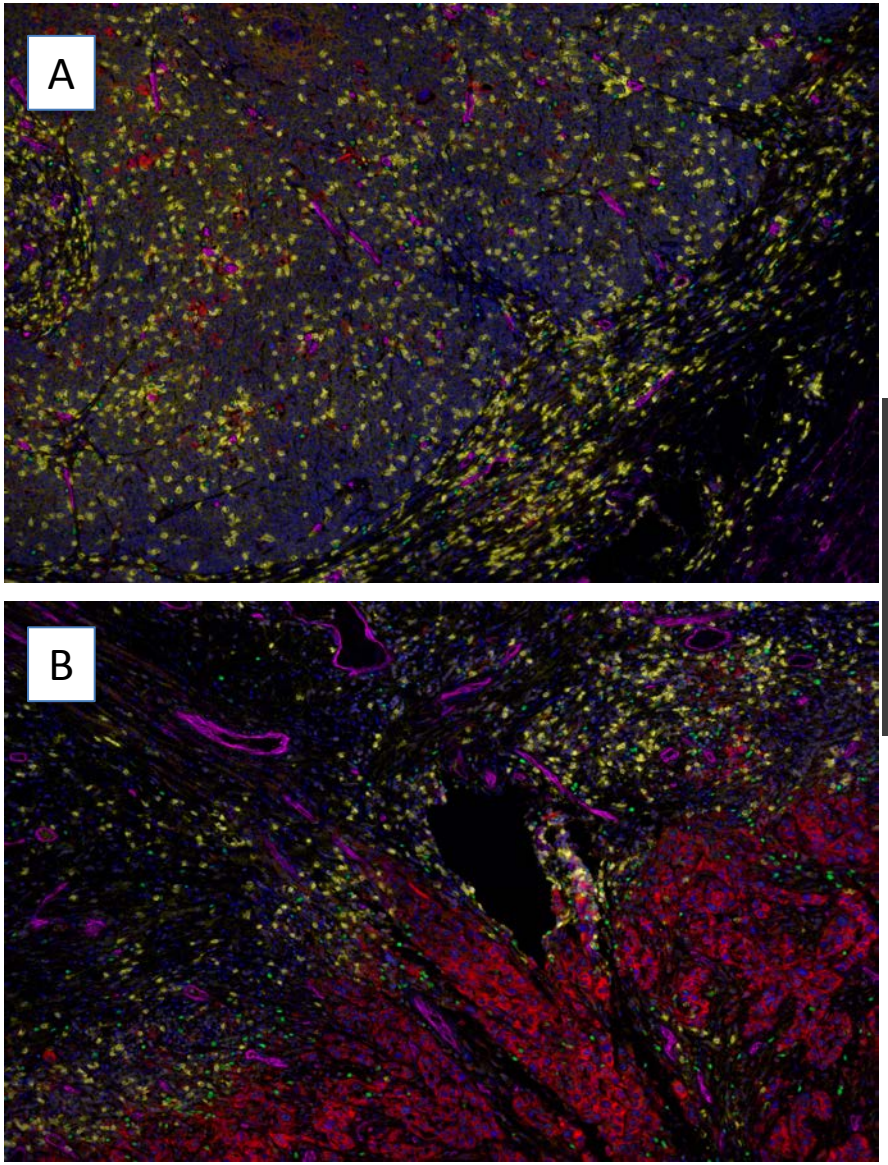
White – TGFβ

Red – TNFα

Green – IFNγ

**Collaboration with Emily Park & Xiao-Jun Ma (ACD Bio)*

NanoString Gene Expression Immune Profiling Analysis Performed on Two OHNSCC



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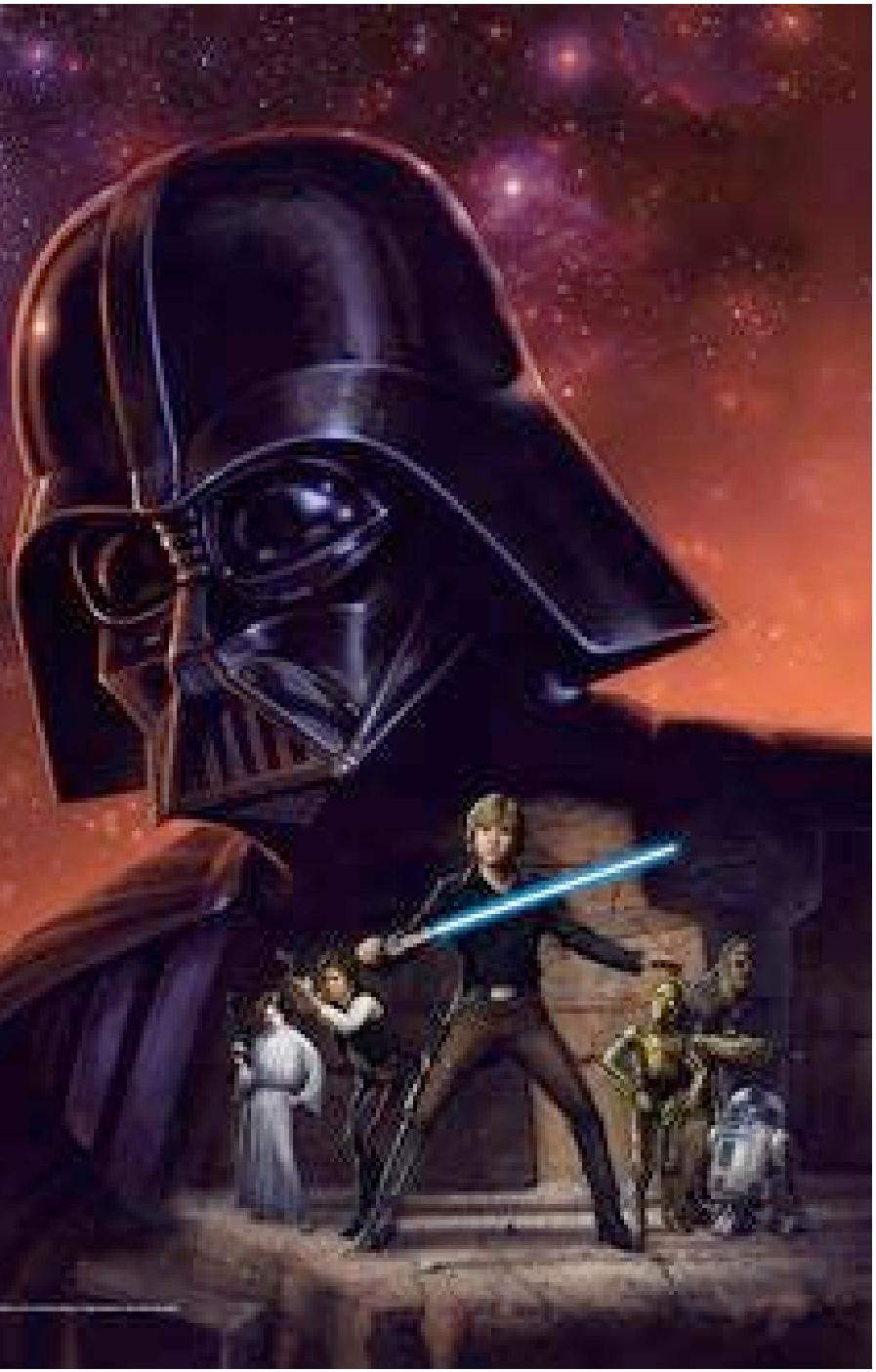

Possible Today: Use Multispectral

- *Assess tumor biopsies*
  - *T cell infiltrates*

Could Stratify patients

Currently some CLIA platforms – LDT (PE)

Not to distant FUTURE

- *10-25? markers (PD-L1, TIM3, VISTA, other)*

Tailor Therapy

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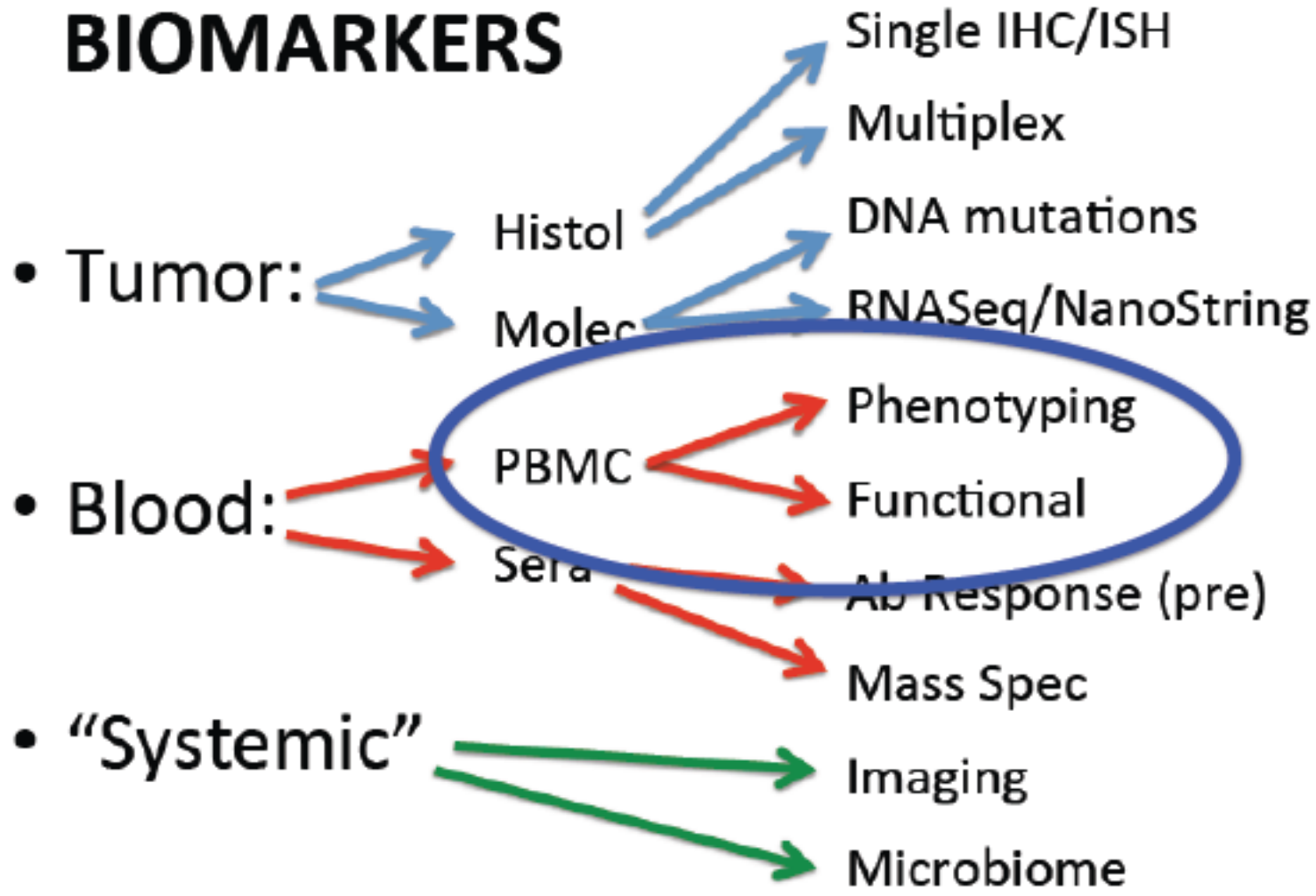

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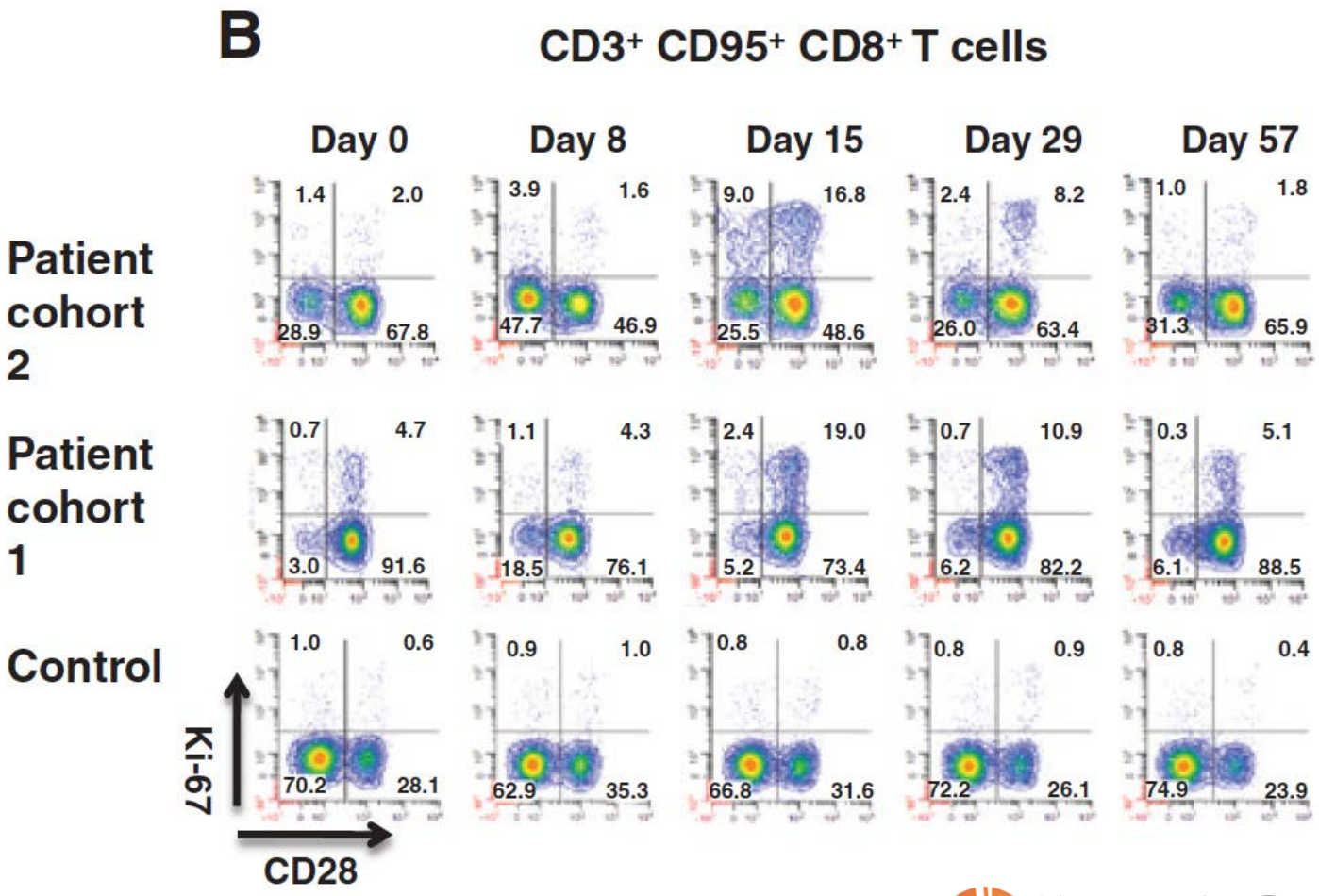
Blood – Phenotypical changes to treatment: Anti-OX40 administration induces qualitative changes in cycling CD8 T cells



Curti B.D., Can Res 73: 7189 2013

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Blood – Functional : Pre-existing or Treatment induced Immunity

Idea: Immunoscore in the "Blood"

Method: Use DC-Targeted microvesicles containing viral ag or >100 over-expressed cancer proteins / NCI-Prioritized Ags / CRA

- **Demonstrated to viral antigen (CMV)**
  Ye, W. J. Transl. Med 12:100, 2014

- **Evidence also in Prostate Cancer**
  van de Ven, R., Manuscript in preparation

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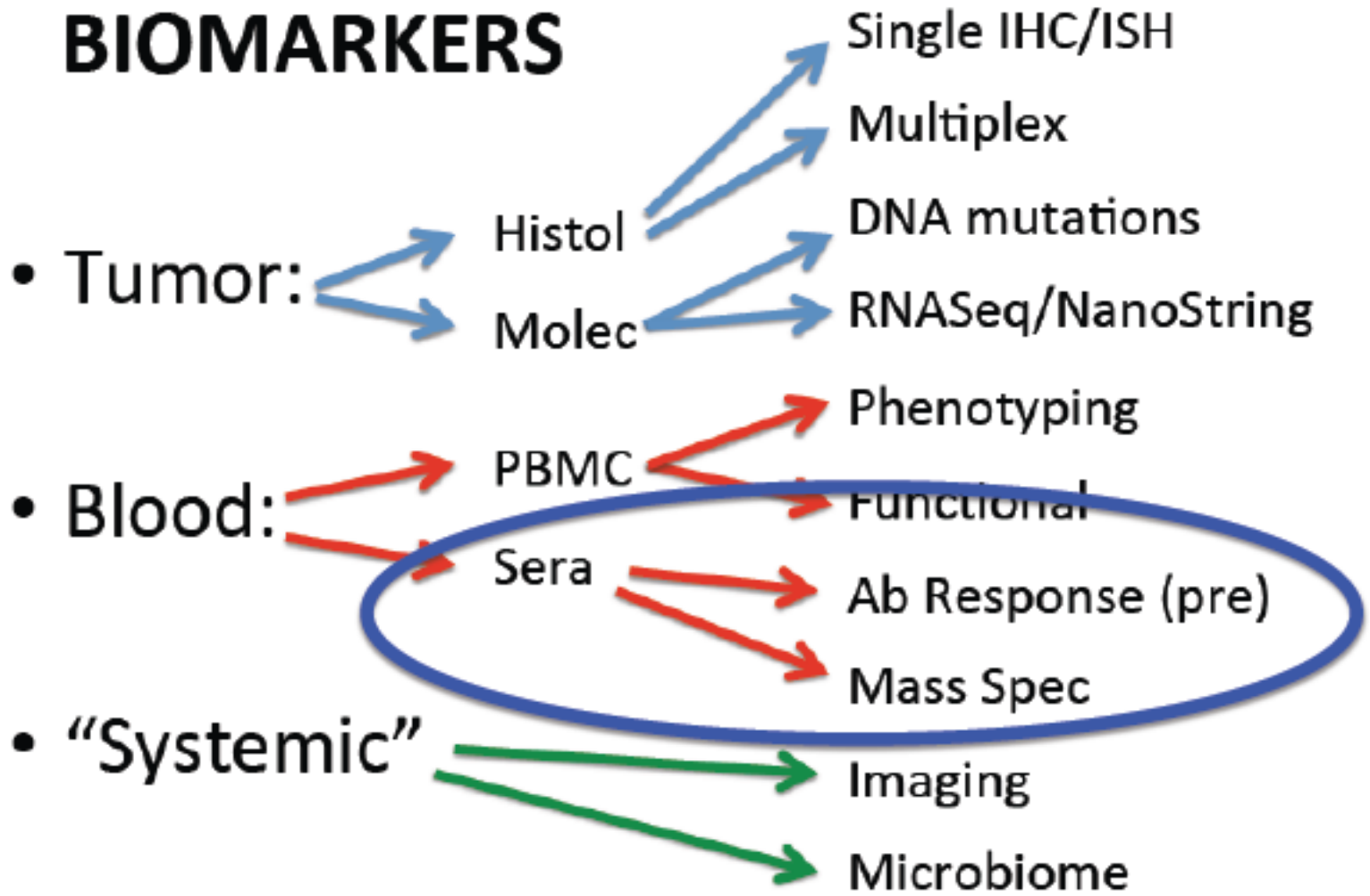

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Sera: Abs or Other Proteins

Hypotheses:

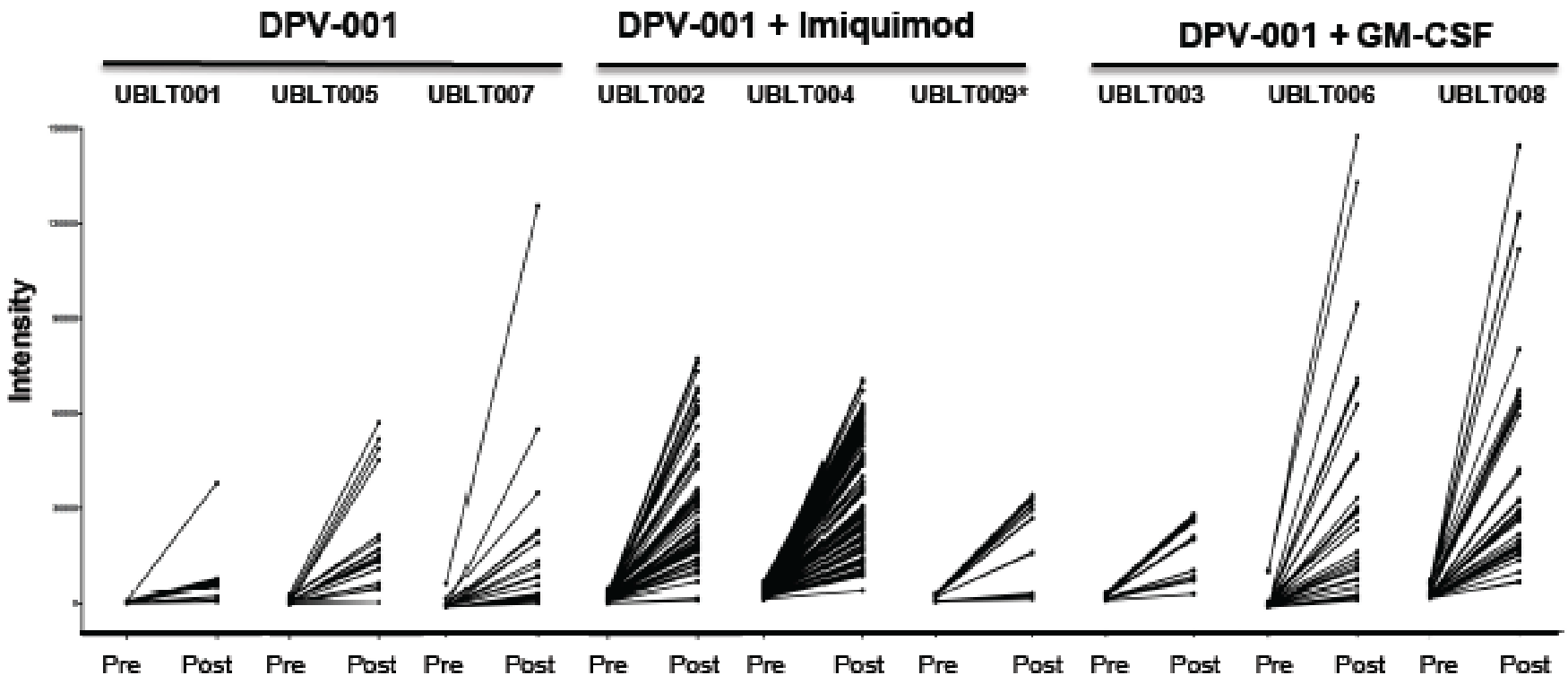
- Abs to “certain” targets ID patients with “therapeutic” immunity
- Inflamatory/other proteins in sera ID patients with ongoing anticancer immunity

Method:

- Protein Arrays
- Mass Spec / Deep Maldi Approach

Vaccinated patients make strong immune response (10 fold) to cancer antigens.

- Majority against non-mutated epitopes



Sanborn, R., Journal for ImmunoTherapy of Cancer 2015, 3(Suppl 2):P435

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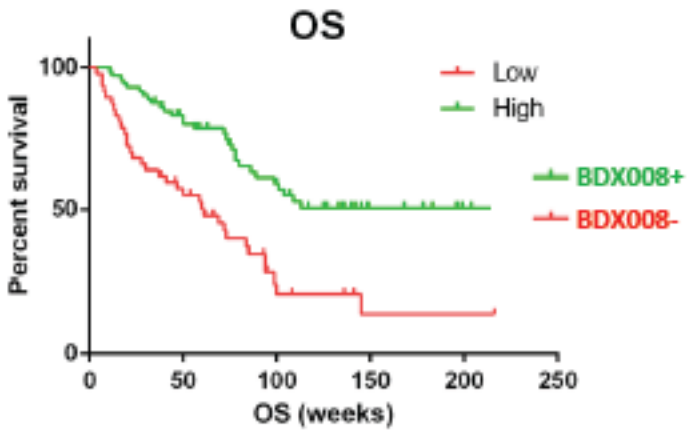
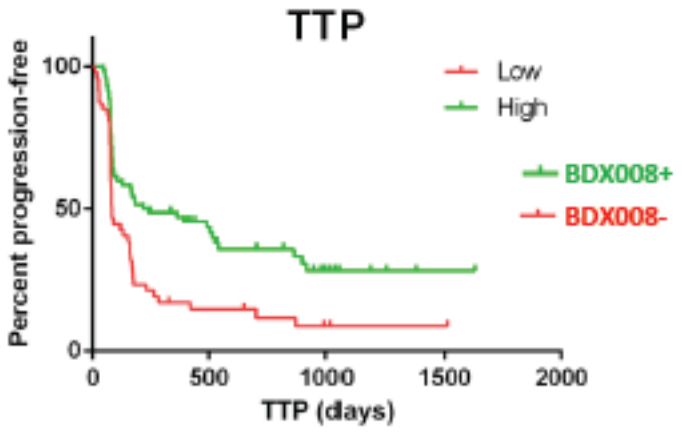
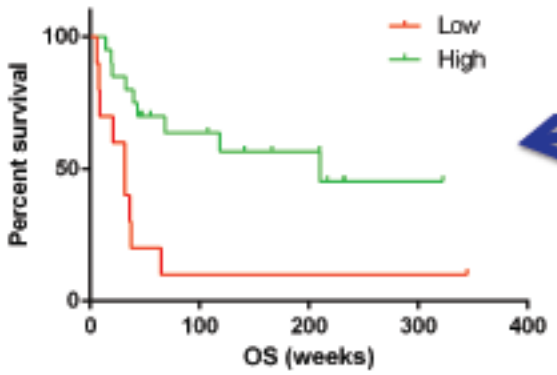
Sera: Mass Spec / Deep Maldi Approach
Melanoma – Anti-PD-1 Cohort







- Predictive biomarker
  - Significant correlation in validation set
- Pattern of Inflammatory proteins (?)

Weber, J.S. Journal for ImmunoTherapy of Cancer 2015, 3(Suppl 2):P103 (4 November 2015)

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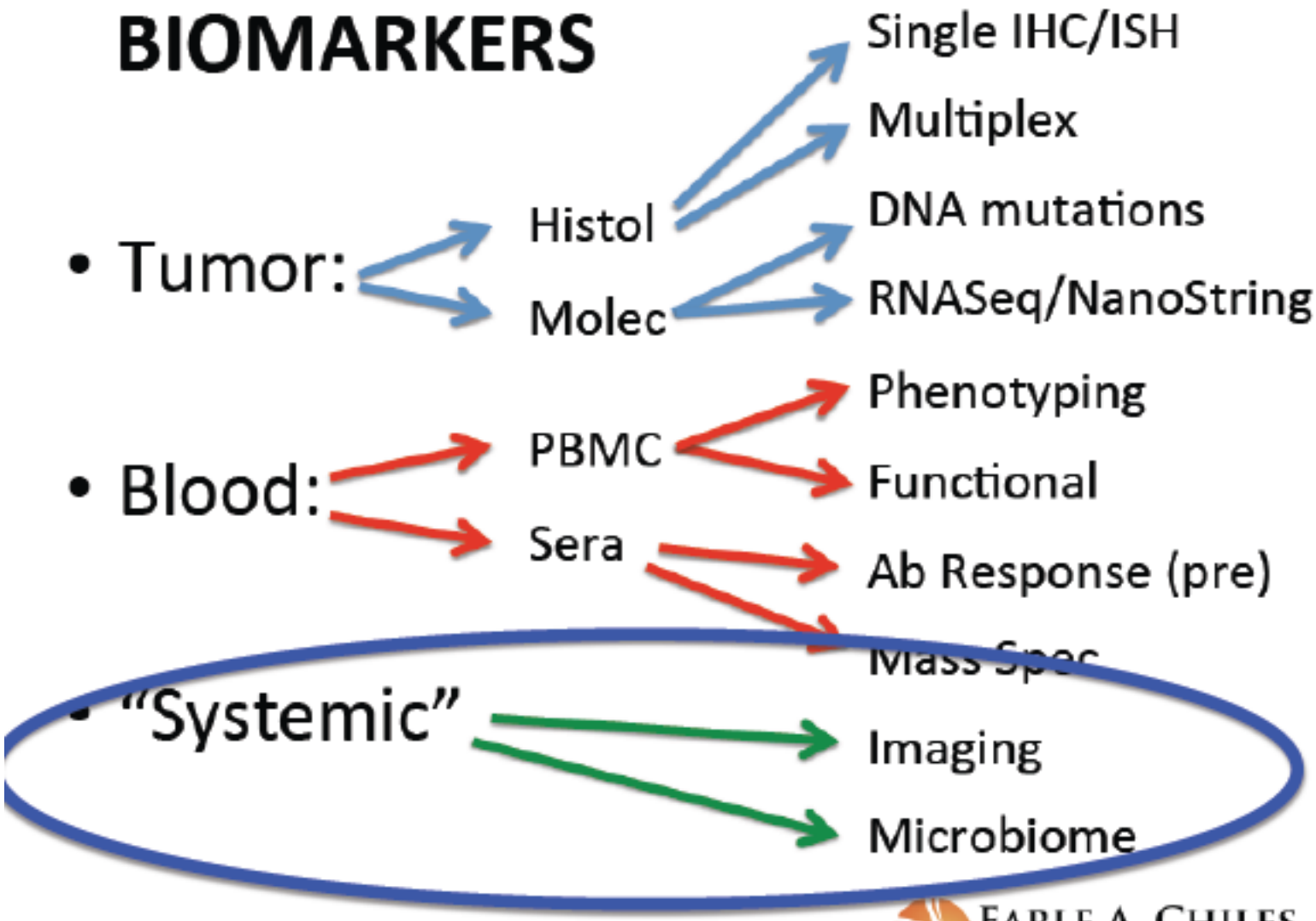

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1

Future:

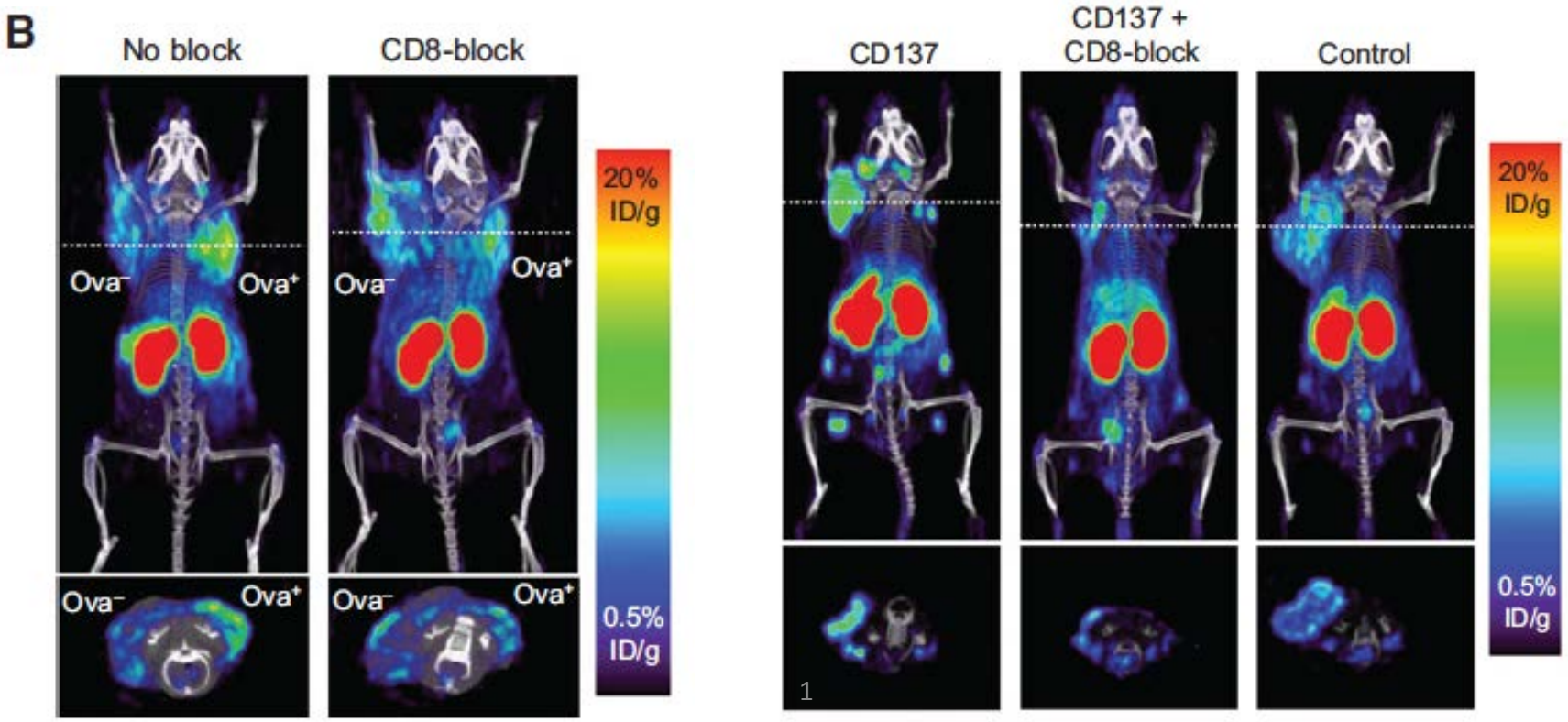
Microenvironment and Immunology

Cancer Research

An Effective Immuno-PET Imaging Method to Monitor CD8-Dependent Responses to Immunotherapy

OnlineFirst November 16, 2015;

Richard Tavaré[1,2], Helena Escuin-Ordinas[3], Stephen Mok[2], Melissa N. McCracken[2], Kirstin A. Zettlitz[1,2], Felix B. Salazar[1,2], Owen N. Witte[2,4,5,6], Antoni Ribas[2,3,7,8,9], and Anna M. Wu[1,2,7]



"Never, ever, think outside the box."

THE NEW YORKER

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Must Evaluate the MICROBIOME!!

- Call to archive “relevant” microbiome of all patients enrolled on clinical trials
- Need to develop a TCGA for Microbiome of patients on “Immunotherapy” trials

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Fox Lab:
Shawn Jensen
Sachin Puri
Tarsem Moudgil
Chris Twitty
Sarah Church
Michael LaCelle
Christopher Paustian
Michael Afentoulis
Rieneke van den Ven
Christopher Dubay
David Messenheimer
Tyler Hulett
Zip Feng

Hong-Ming Hu
Keith Bahjat
Andy Weinberg
Michael Gough
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MLU-Halle
Barbara Seliger
Daniel Bethmann
Alexander Eckert
Matthias Kappler
Claudia Wickenhauser

CBER, FDA
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Guys Hospital
Edward Odell
Selvam Thavaraj

PerkinElmer
Cliff Hoyt
Kent Johnson
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Paolo Ascierto

Definiens

ACD Bio
Emily Park
Xiaojun Ma
Lichong Wang

Patients and their families

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