Immunophenotyping to Differentiate Responder and Non-responder Patients in Cancer Immunotherapy

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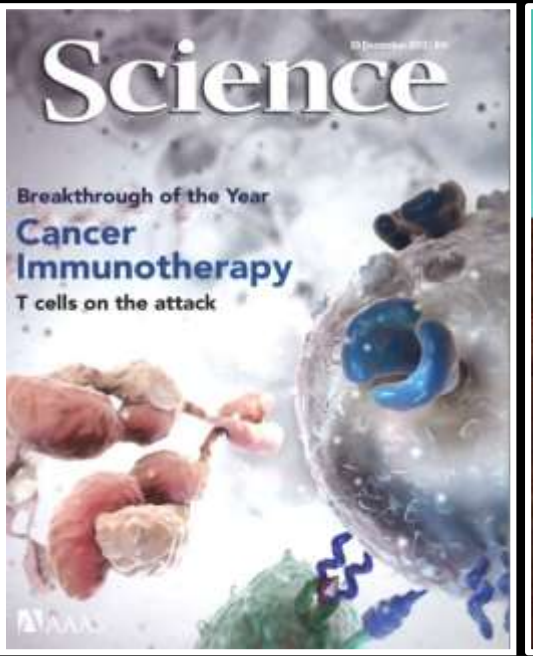
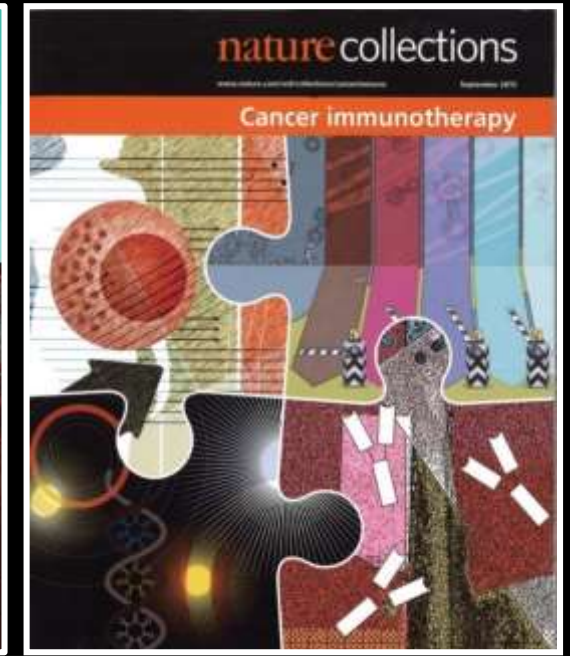
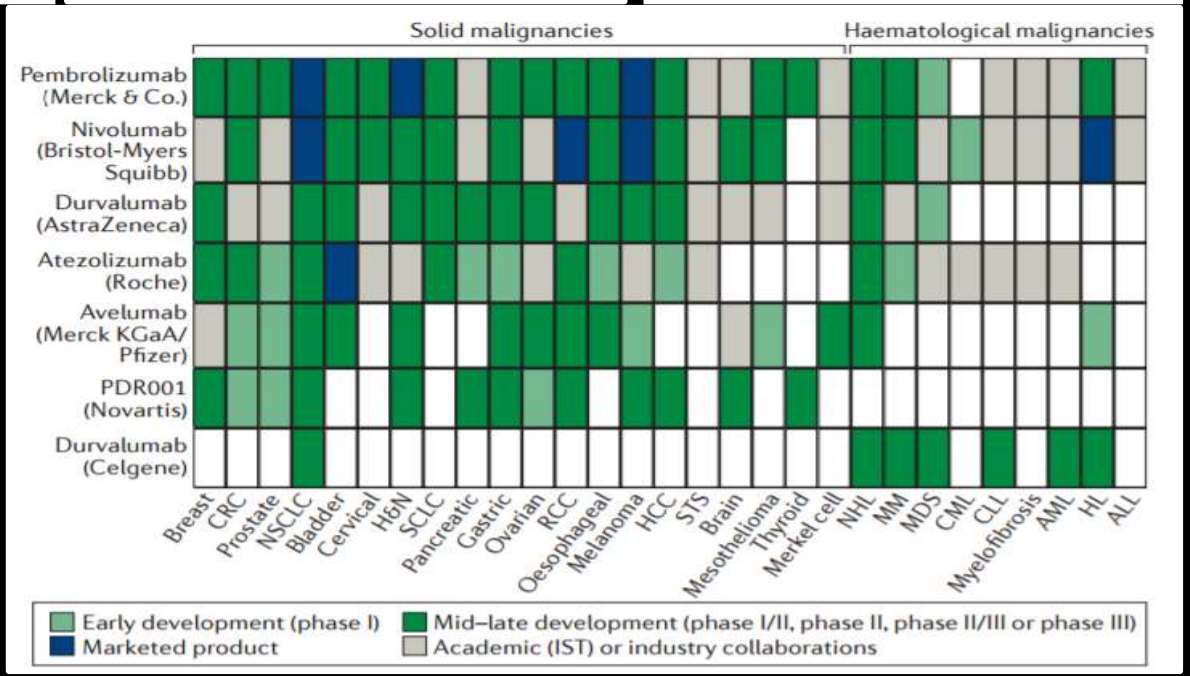




The Promise of Immuno-therapy

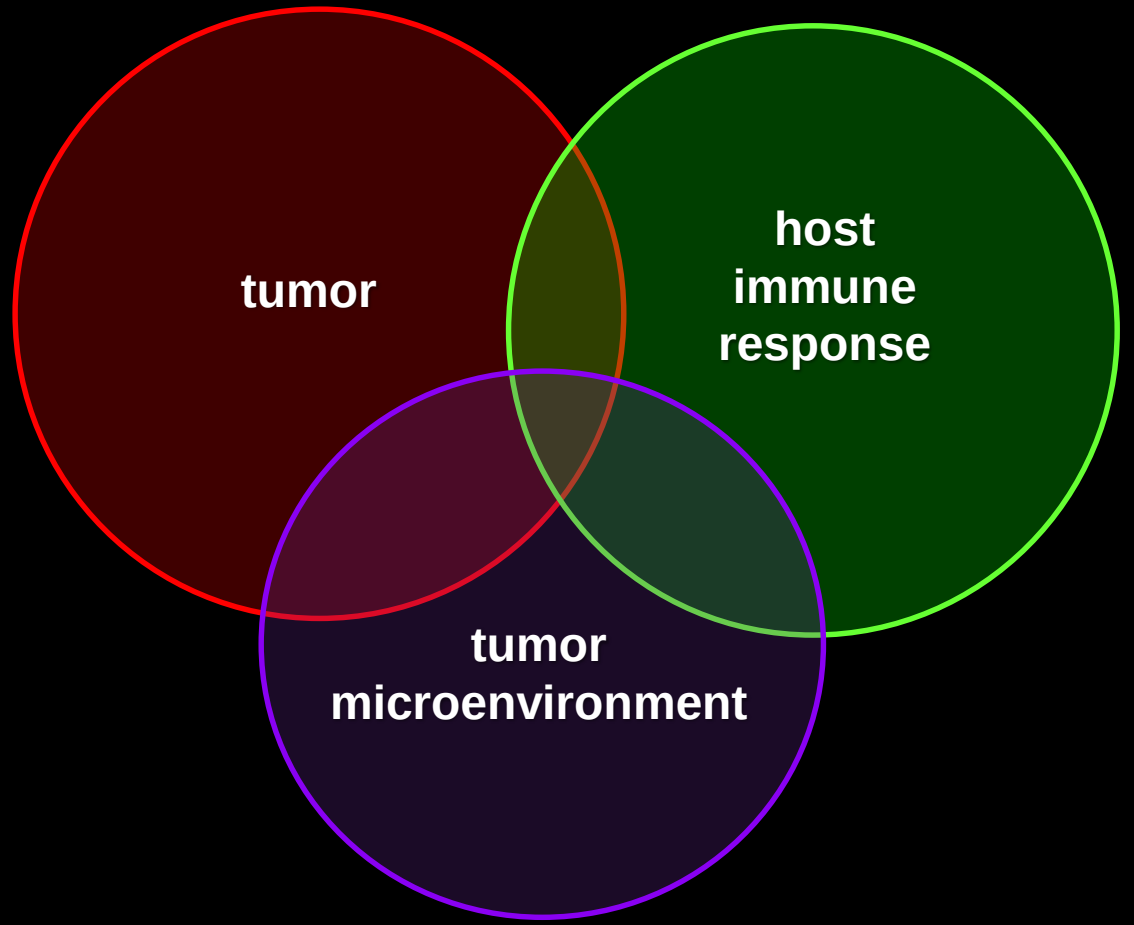


Cancer Immunotherapy

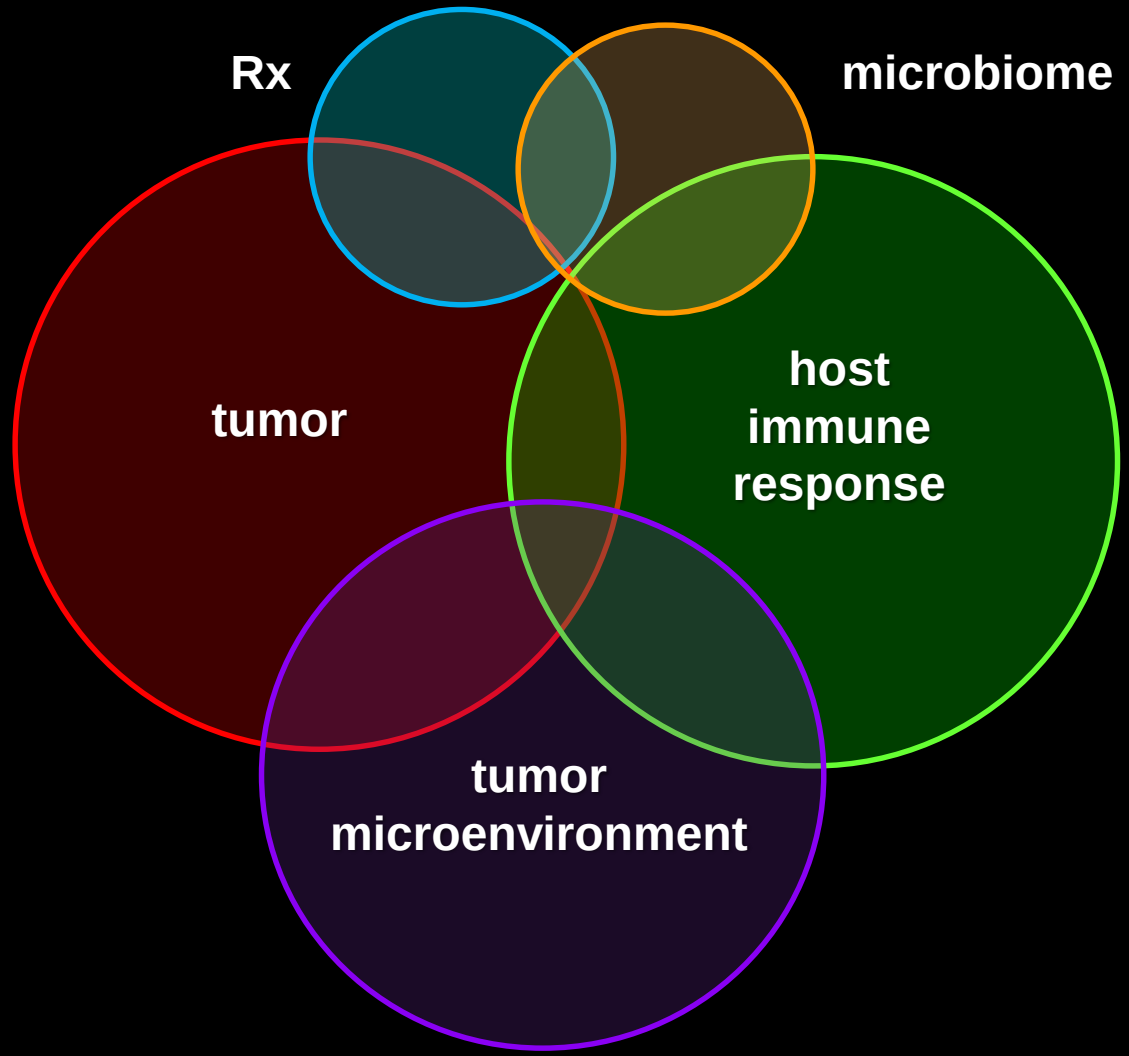
- significant variation in response rates between and within tumor types
- need for more sophisticated immunophenotypic methods for predictive and prognostic profiling of responder (R) and non-responder (NR) patients
- high cost of futile therapy in NR cohort plus

  indirect care costs for management of toxicity /AEs
- need for more informed rationale for combination regimens
  - doses, sequence
  - Rx classes

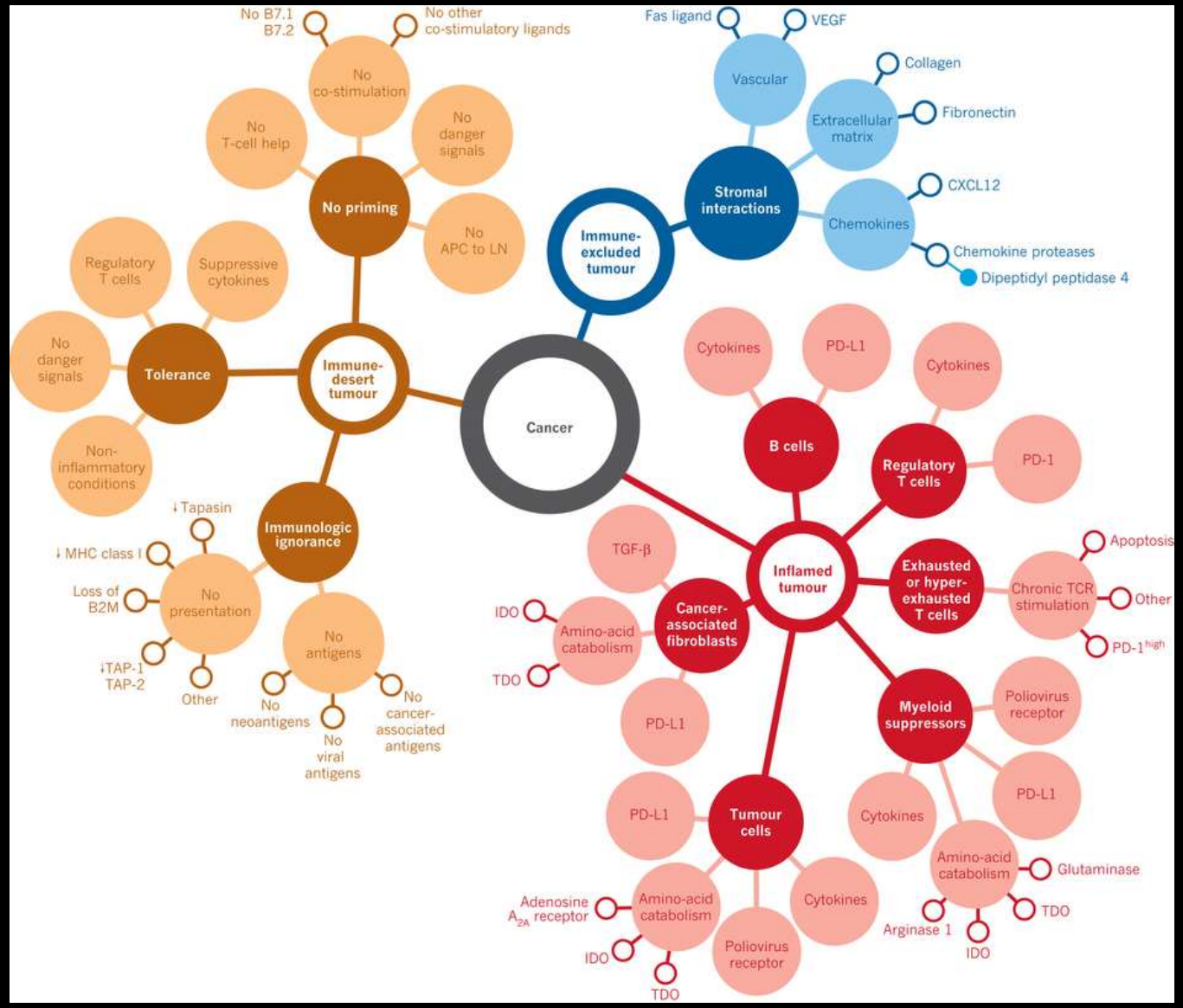
Deconvolution of the Complex, Multi-Dimensional Matrix of Immuno-Oncology Therapeutics



Deconvolution of the Complex, Multi-Dimensional Matrix of Immuno-Oncology Therapeutics

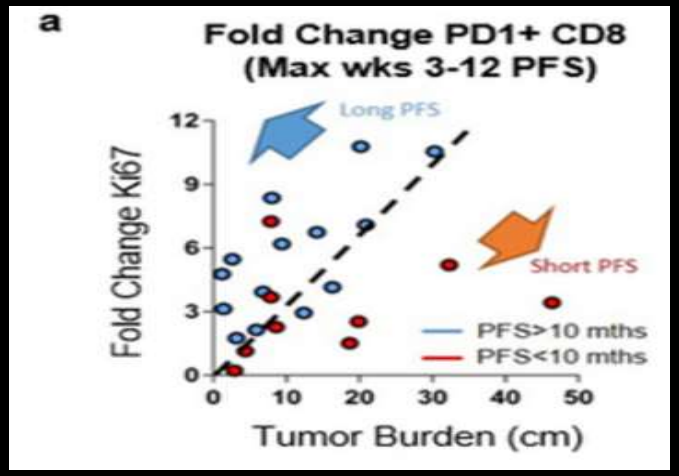


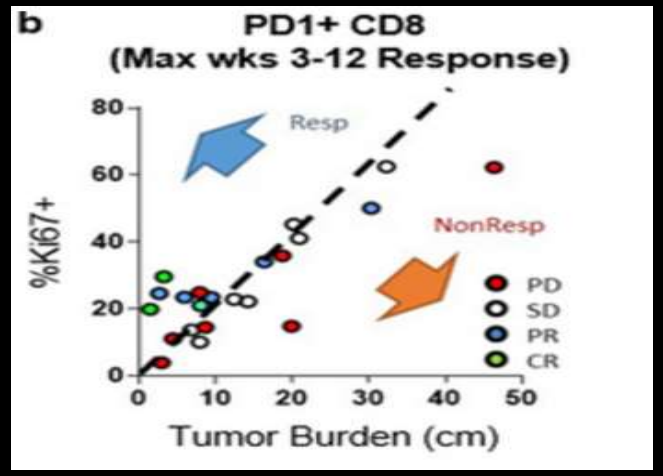
The Complexity of Cancer-Immune Phenotypes

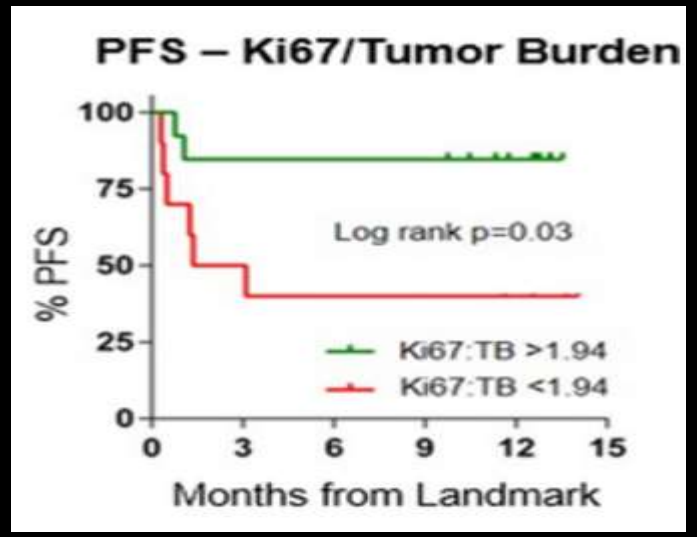


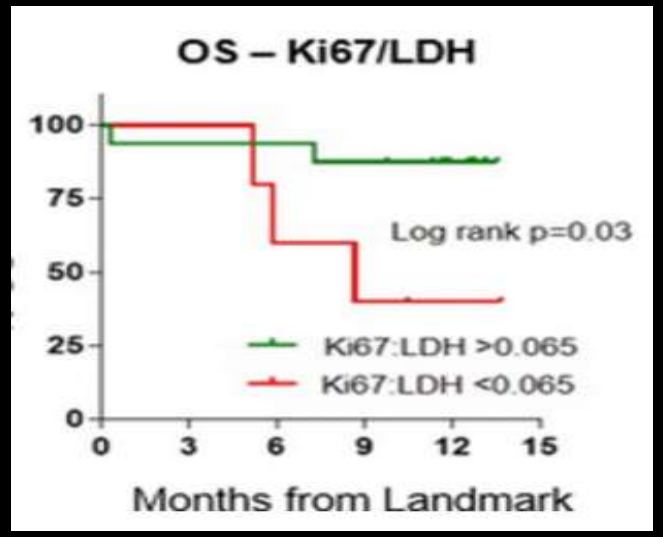
From: D. S. Chen and I. Mellman (2017) Nature 541, 321

Relationship of Tumor Burden, PFS and OS to Enhanced Expression of Ki67 as $CD8^{+}$ Proliferation Marker in Pembrolizumab Anti-PD-1 Therapy of Stage IV Melanoma (n=23)



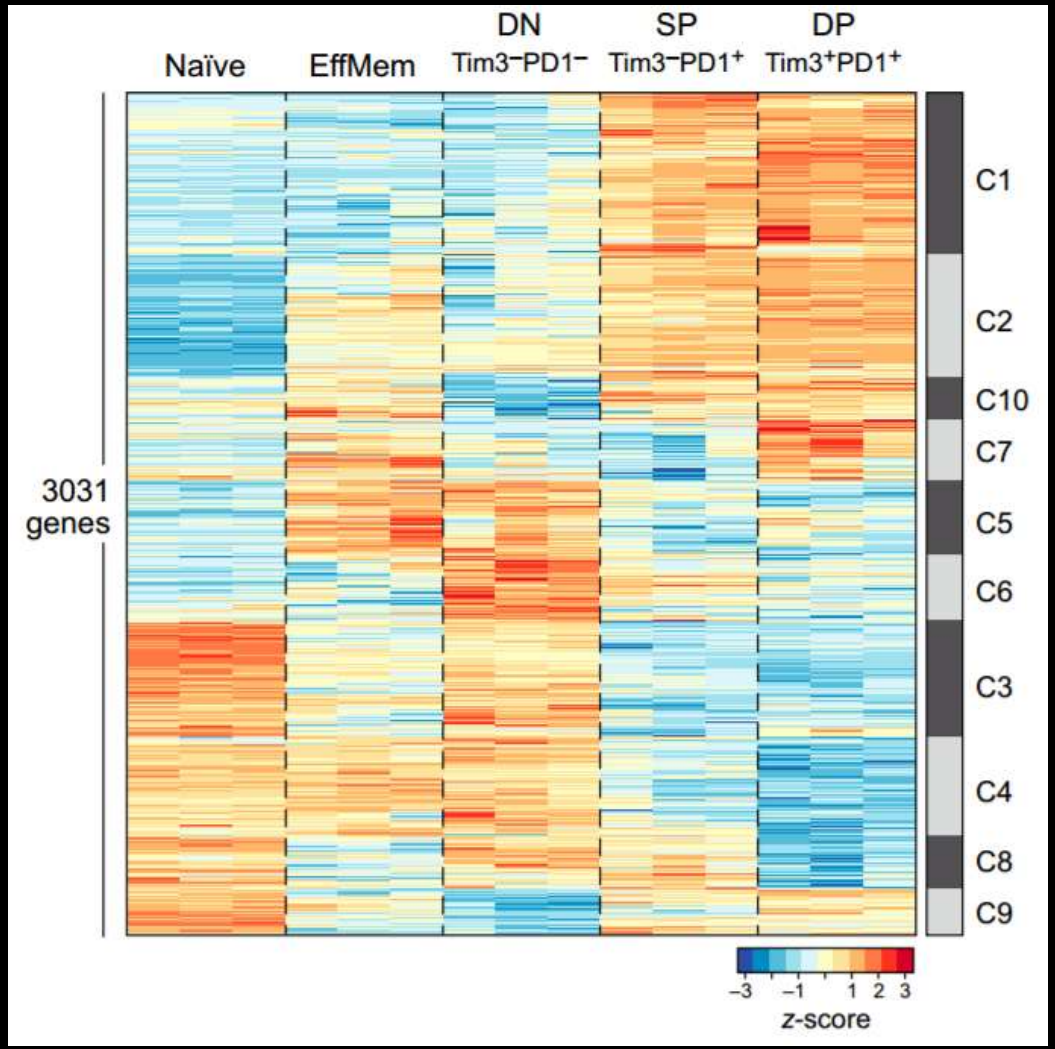






From: A. C. Huang et al. Nature (2017) doi:10.1038/nature22079 Supplemental Data Fig. 9

Heatmap of Differentially Expressed Genes in Tumor Infiltrating Lymphocytes and Identification of Activation and Dysfunction (Exhaustion) Modules

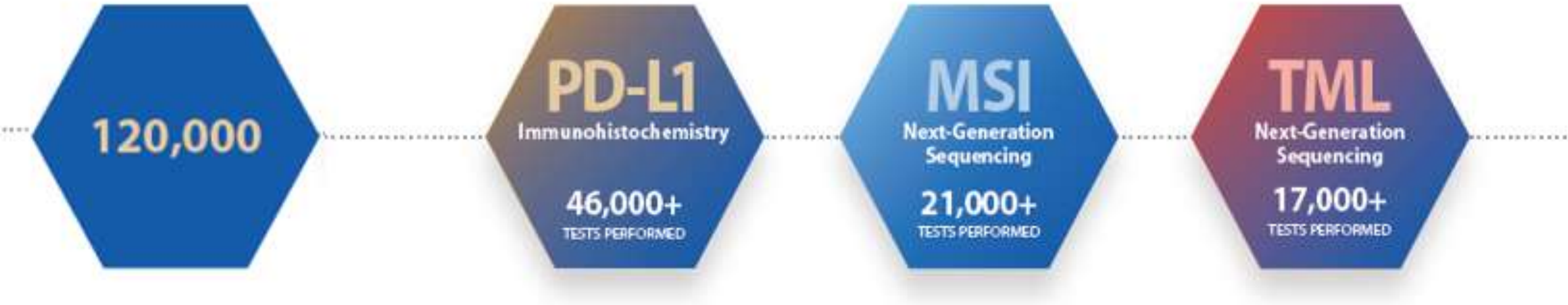


M. Singer et al.(2016) Cell 166, 1500

The Complex Metabolic Landscape of the TME and Potential Effects on Anti-Tumor Immune Functions

- competition of tumor cells and activated immune cells for tryptophan, arginine and glutamine
- over expression of indoleamine 2, 3-dioxygenase (IDO) by tumor cells
  - tryptophan metabolites (e.g. kynurenines) down regulate TCR and induce $FOXP3^{+}$ Tregs
- arginine depletion
  - impaired T cell function, induce MDSC generation
- glutamine depletion
  - promotes generation of Tregs
- CD73 expression on tumor cells and adenosine accumulation
  - impaired T and NK cell activity, recruitment of MDSC

Large Scale Profiling of Markers Relevant To Immune Checkpoint Blockade Therapy



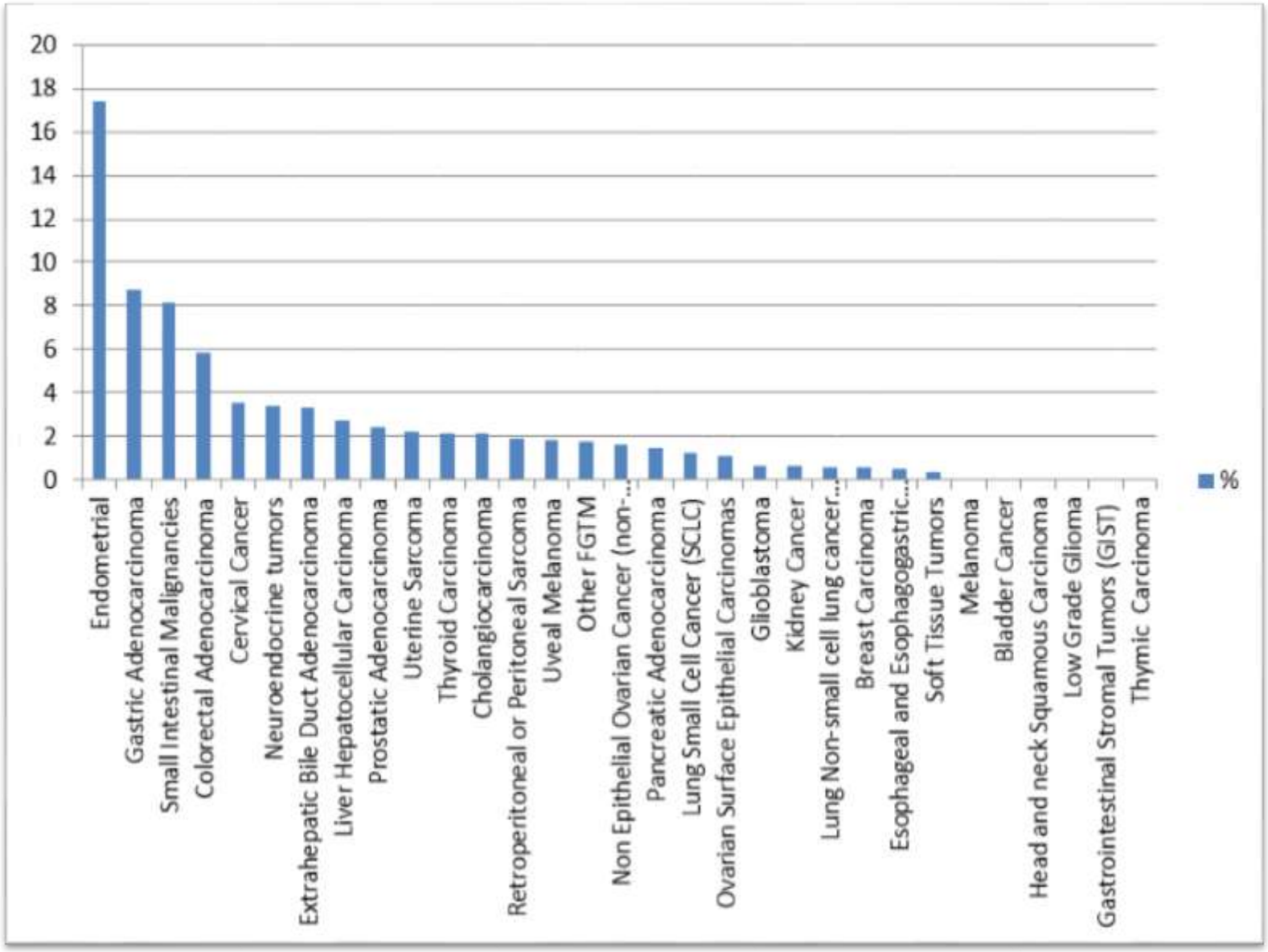
Cancer Patients Profiled

Programmed Cell Death-Ligand 1

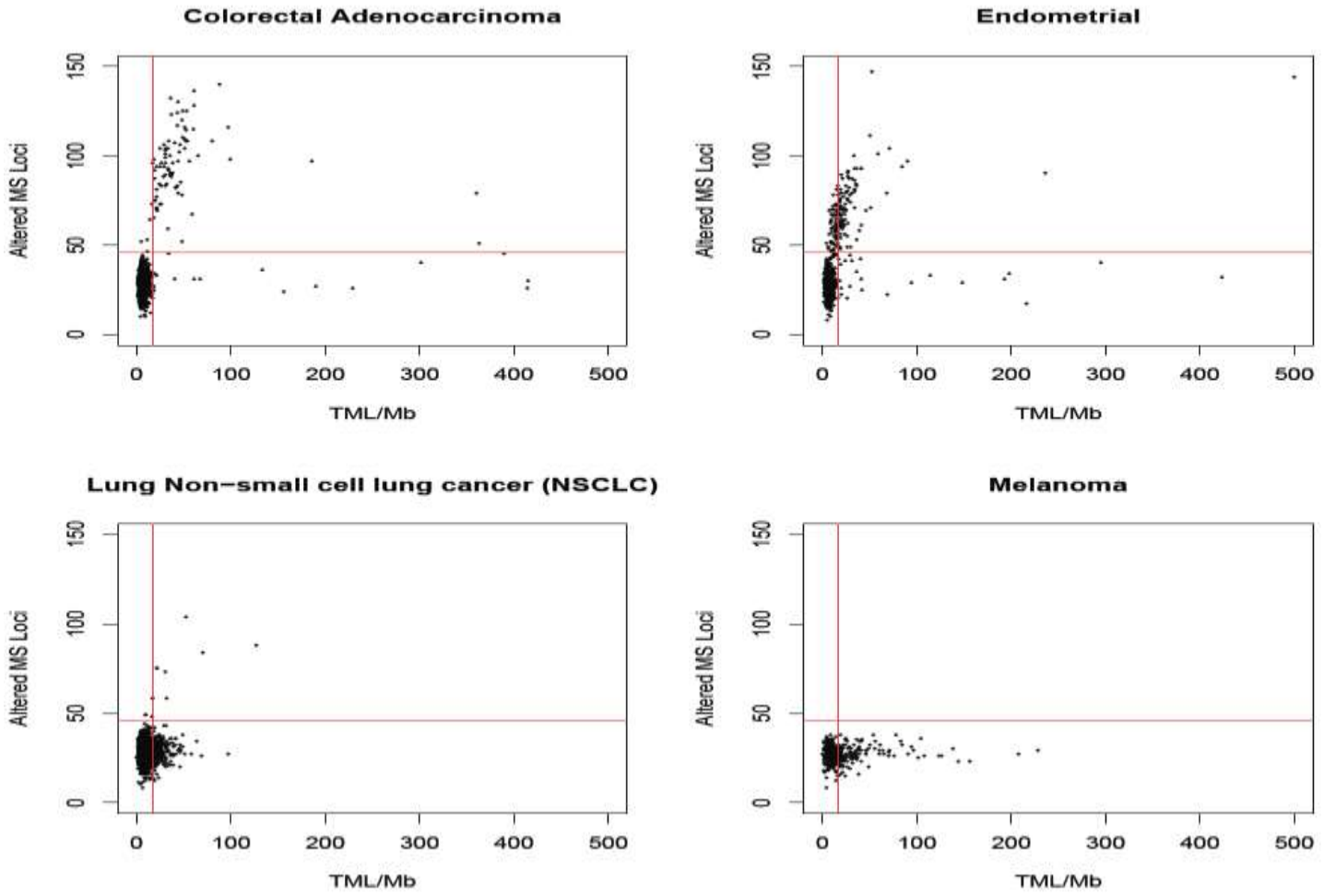
Microsatellite Instability

Total Mutational Load

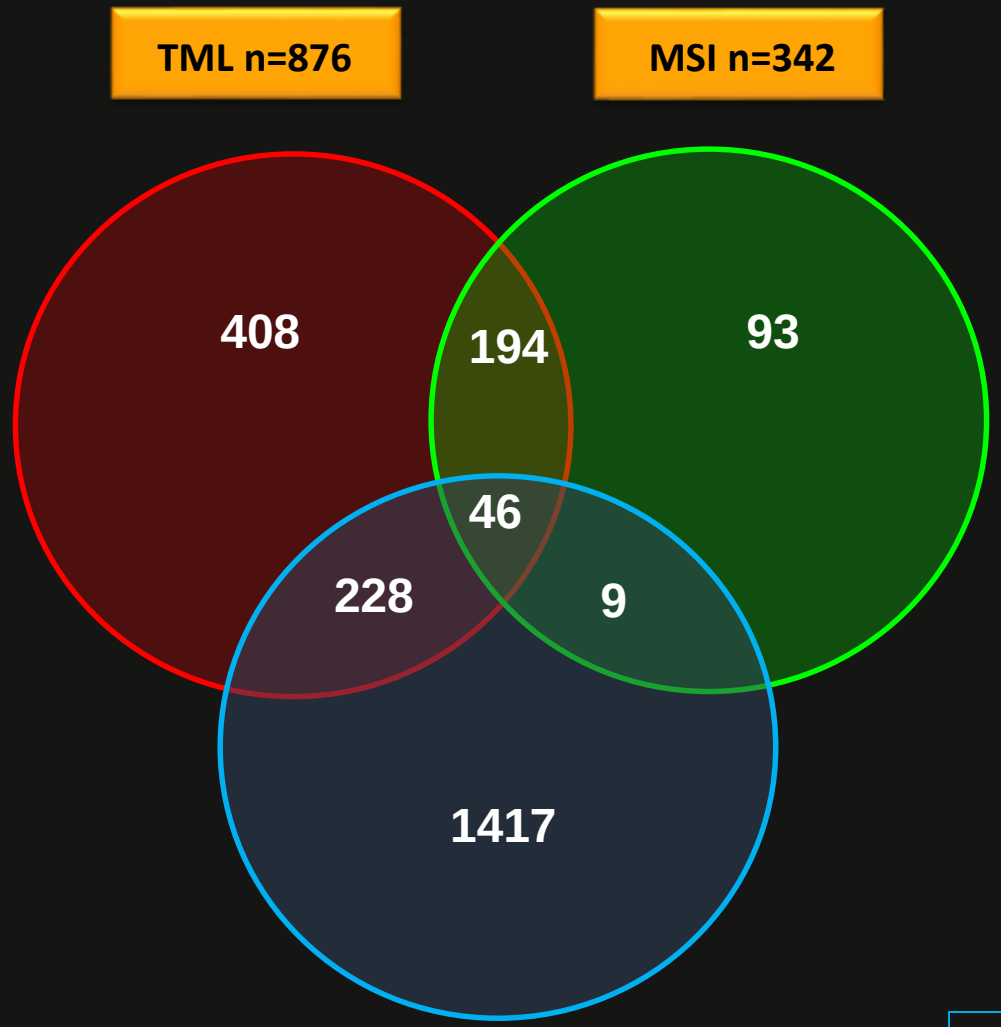
Frequency of MSI-High Profiled by NGS in 31 Cancers (n =11, 251)



Scatter plots comparing MSI determined by NGS and TML per megabase for colorectal adenocarcinoma (n = 1267), endometrial cancer (n = 667), NSCLC (n = 964), and melanoma (n = 175)



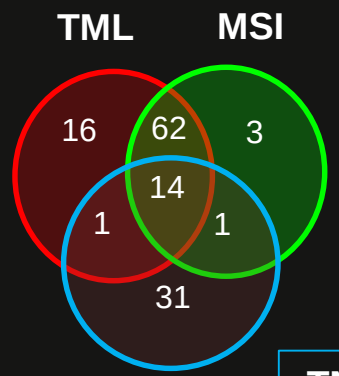
Overlap of TML, MSI and PD-L1 Markers in 2,918 Patients In Cohort of Total 11,780 Patients



Triple negative
n=8952

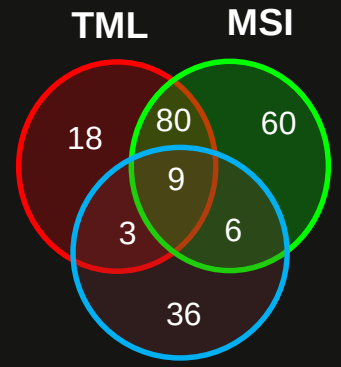
Overlap of TML, MSI and PD-L1 Markers in Four Major Cancers

Colorectal Adenocarcinoma



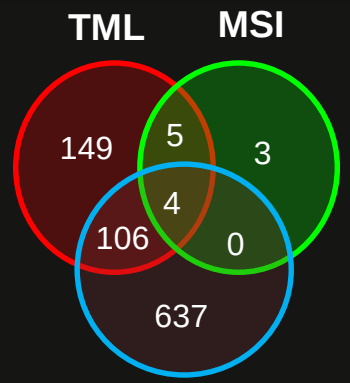
TNeg 1267

Endometrial



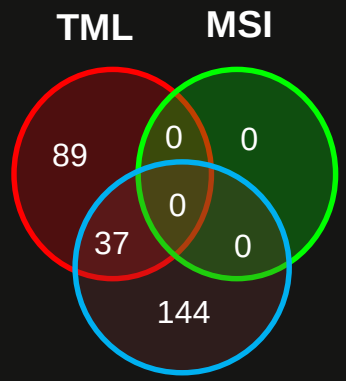
TNeg 667

Non-small cell lung cancer



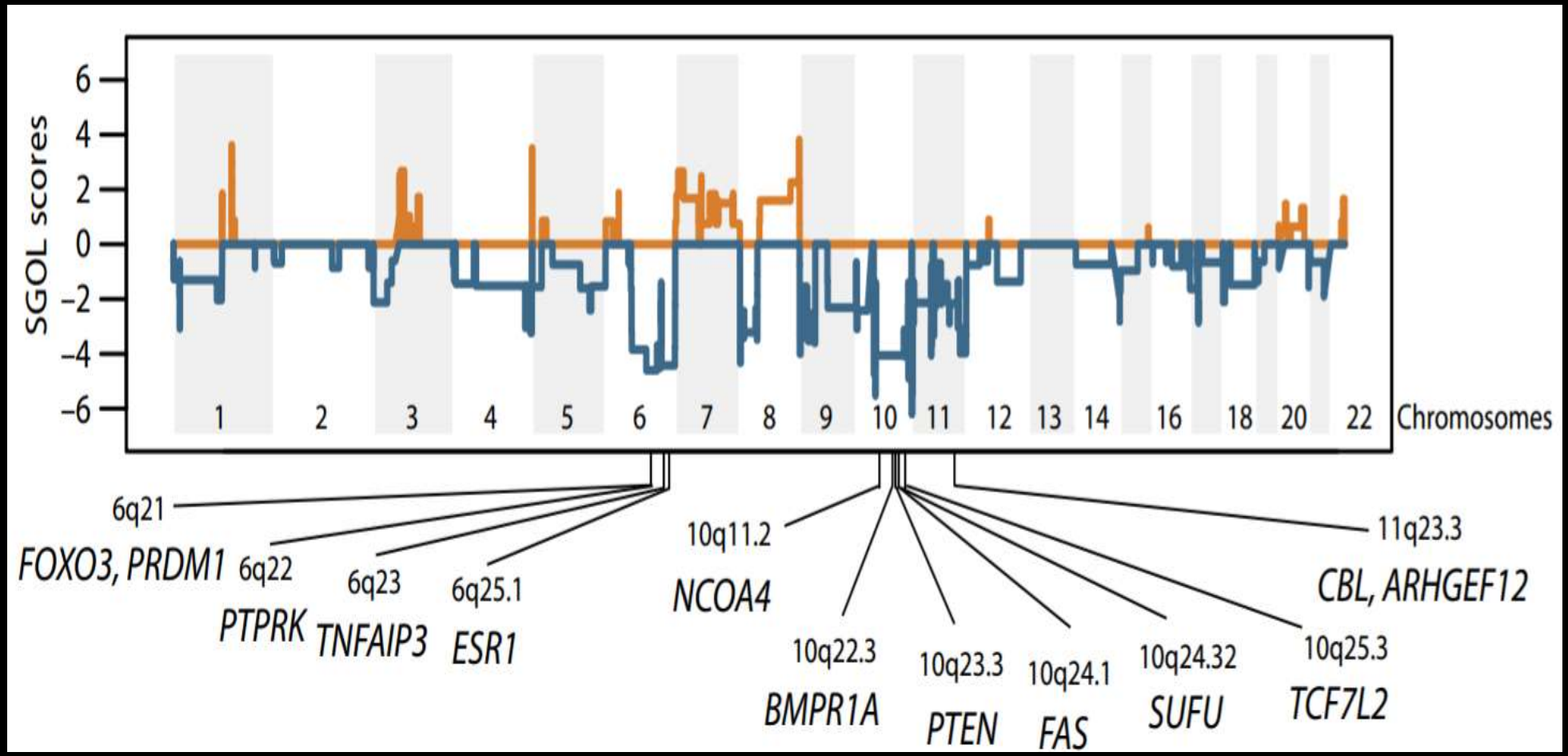
TNeg 964

Melanoma



TNeg 175

Copy Number Loss (>2000) as a Potential Resistance Phenotype for Double Non-Responders to Sequential CTLA-4 and PD-L1 Blockade and Loss of Tumor Suppressor Genes on Chromosomes 6q, 10q, and 11q23.3



From: W. Roh et al. (2017) Sci. Trans. Med. 9, eaah3560

Nature (2017) 544, 3

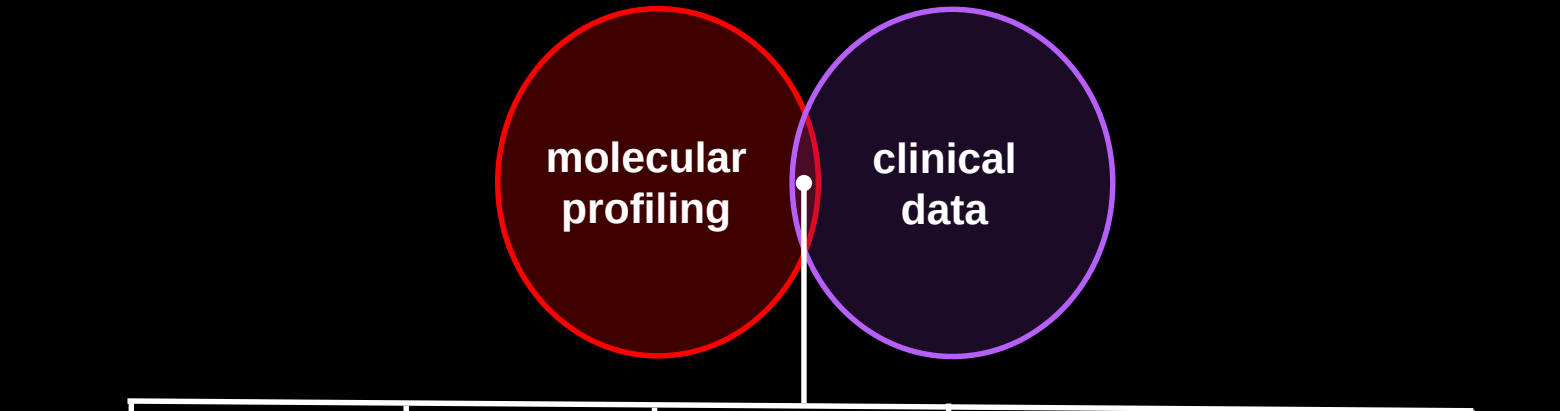
KENNETH K. LAW/BALTIMORE SUN/GETTY

Immunotherapy offers hope to some people with hard-to-treat cancers — but it can backfire.

IMMUNOTHERAPY

Cancer drugs may speed tumours in some people

The Quest for Precision Immuno-oncology (I/O) Therapy



WGS/WES Ig/TCR sequencing

SNP assays

RNA Seq

Rx Regimen(s)

adverse events

PFS and OS

tumor profiling

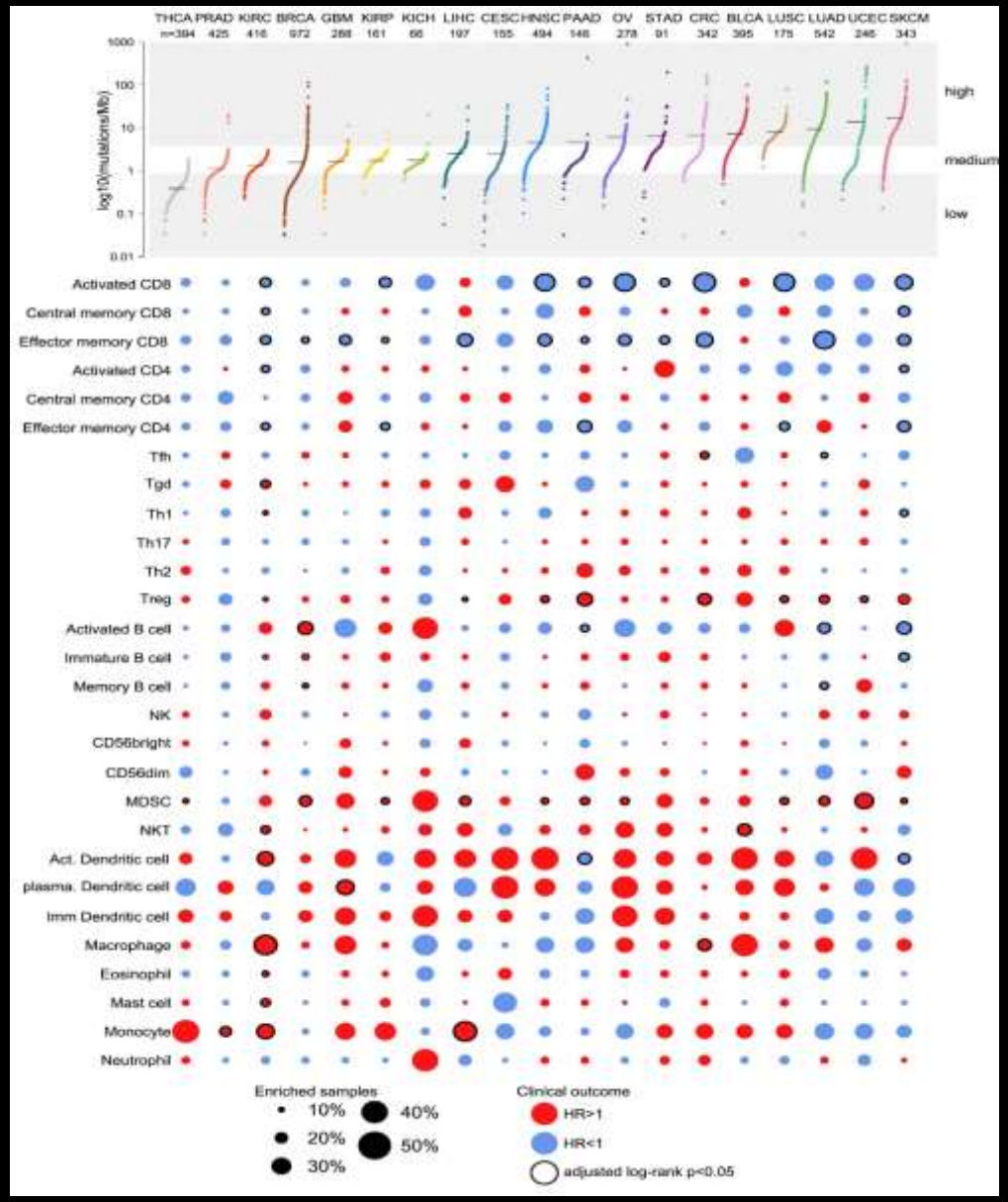
- mutanome
- neoantigens
- cancer germ-line antigens
- HLA alleles
- clonal/subclonal mutations

immuno-phenotypes

- local (infiltration)
- systemic
- Rx response
- Rx resistance

- single I/O
  - dose
- combination
  - dose
  - sequence
- prior Rx

Characteristics of Immune Cell Infiltrates in 19 Solid Tumor Types



P. Charoentong at el. (2017) Cell Reports 18, 248.

Predictive Biomarkers for Response to Immune Checkpoint Blockade and Other Immunostimulatory Anti-Tumor Therapies

- high negative predictive value
  - reliable identifier of NR cohort
- pre-treatment marker desirable but improved response prediction early in therapy would still be valuable
  - efficacy (R), safety and avoid cost of futile therapy (NR)
- minimally invasive to enable dynamic monitoring
- utility in different cancers
- availability/regulatory approval for all markets in which Rx is sold

Biomarkers for Immunophenotyping

- multiplex panels
- “biomarker positivity”: present, absent or graduated?
- how will reagents, assays and cut-off thresholds be standardized and validated?
- if pre-I/O-Rx (baseline) tissue biopsy is not a robust prediction for R or NR status when and how should post-I/O-Rx profiling be done?
- can immunophenotyping in blood accurately mirror intratumoral events in disseminated metastases with heterogeneous TMEs?
- is there an ‘over-arching’ immunophenotype characteristic for ‘R’ patients that will be valid for diverse I/O-Rx classes with different MOAs?

Need for New Minimally-Invasive Assays for Monitoring Patient Responses to Immunotherapy

- ‘static’ snapshot of immune profile in resected lesions/biopsies versus longitudinal monitoring of dynamic changes with tumor progression /Rx responses
- how far does the immune profile assayed in blood (liquid biopsy) mirror Intratumoral events in anatomically dispersed metastases?
  - immune cell subsets?
  - cytokines?
  - ctDNA?
  - exosomes?

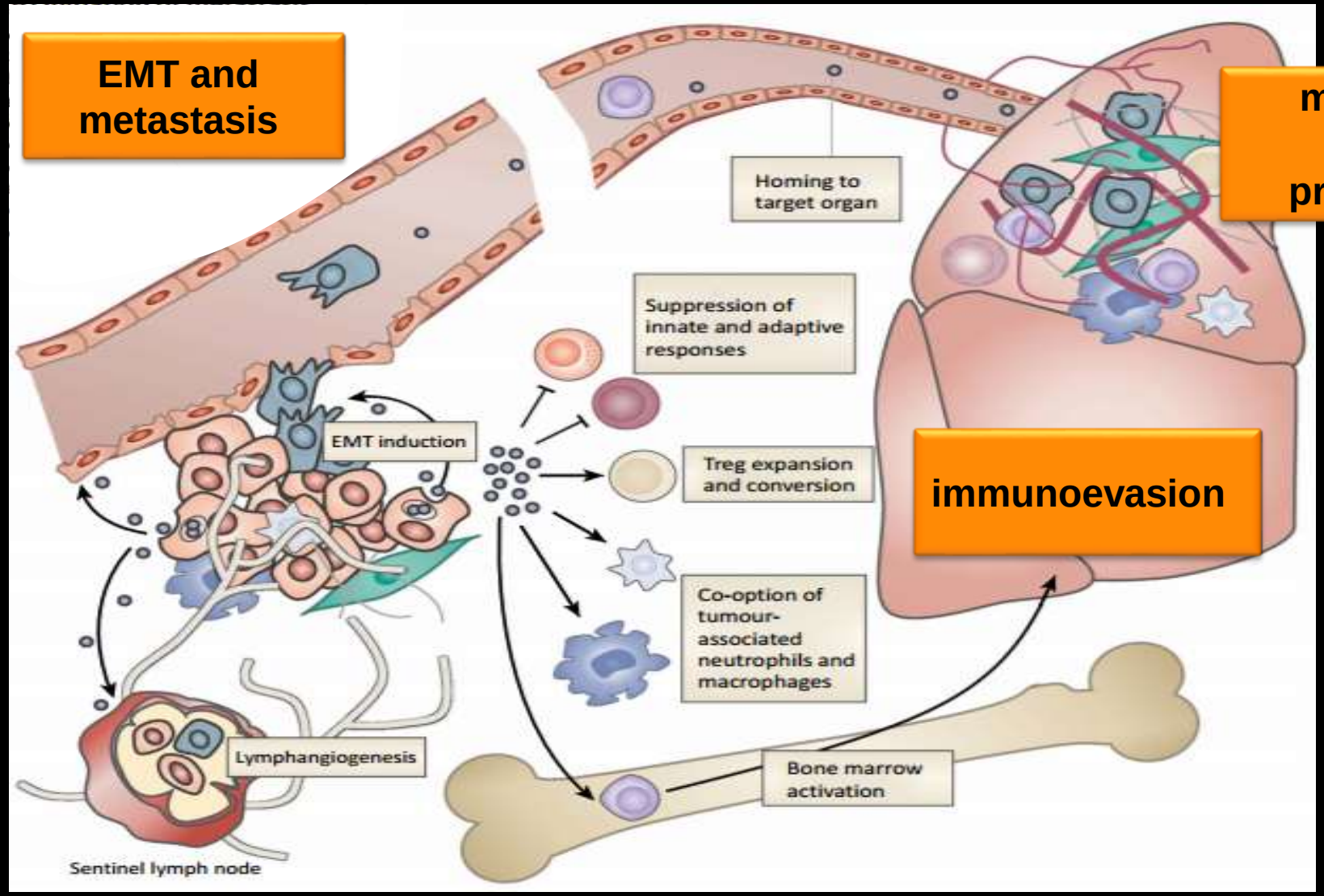
Liquid Biopsy: CTCs and/or ctDNA/RNA

Tumor-Derived Exosomes as a Potential Molecular Profiling Platform to Assess Variation in Immunotherapy Efficacy

Exosomes and Modulation of Immune Functions

An Emerging Component in the Complex Balance Between Immune Activation and Suppression in Tumor-Host Interactions

Putative Roles of Tumor-Derived Exosomes in Cancer Metastasis and Immuno-evasion



Adapted from N. Syn et al. (2016) Trends in Pharmacological Sciences 37, 606

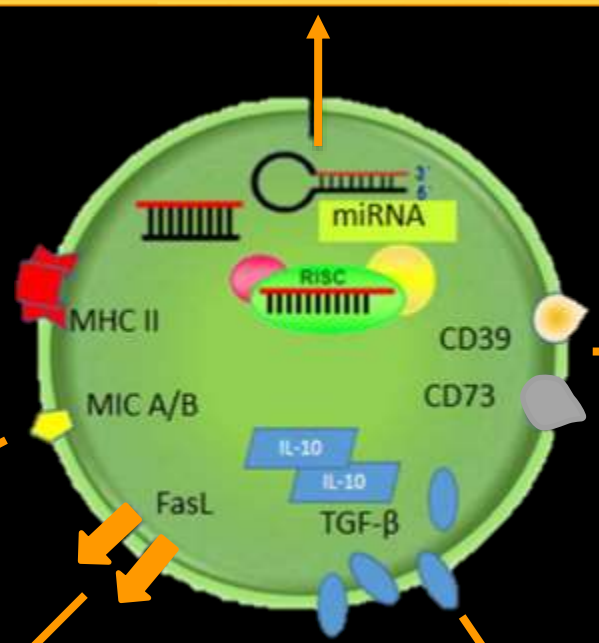
Immune Stimulation by Cancer-Derived Exosomes

- direct activation of effector T cells by MHC class I and II complexes on vesicle membrane
  - T cell priming required
- transfer of tumor neoantigens to dendritic cells (DCs) and other antigen-presenting cells (APCs)
- exosomes stimulation of naïve $CD4^+$ T cells by DC –derived exosomes
- stimulation of pro-inflammatory macrophage M1 phenotype

Cancer–Derived Exosomes and Suppression of Anti-Tumor Immune Functions

Changes in gene expression;
Inhibition of NK cell function;
DC and T cell dysfunction;
M1→M2 conversion of macrophages

Induction of antigen-specific tolerance



Generation of extracellular adenosine

Decoy NKG2D ligands

Apoptosis induction through death receptors

Cytokine-mediated immunosuppression: induction of Treg cells; promotion of MDSC differentiation

Adapted from L. Czernek and M. Düchler(2017) Arch. Immunol. Ther. Exp. S00005-016-0453-3.

Profiling of Host Immune Cell-Derived and Tumor-Derived Exosomes as Potential Prognostic/Predictive Markers in Immunotherapy

Plasma Tumor-Derived Exosomes

- exosome fraction elevated in cancer patients
- literature reports of up to 150µg protein/ml plasma versus <15µg/ml for normal controls
- preclinical and clinical literature indicating correlation of exosome levels with tumor stage and grade
- emerging evidence in evaluation of response to therapy
  - exosome reduction in long term AML remission plus shift in molecular profile to resemble normal controls
  - reduction in immunosuppressive markers

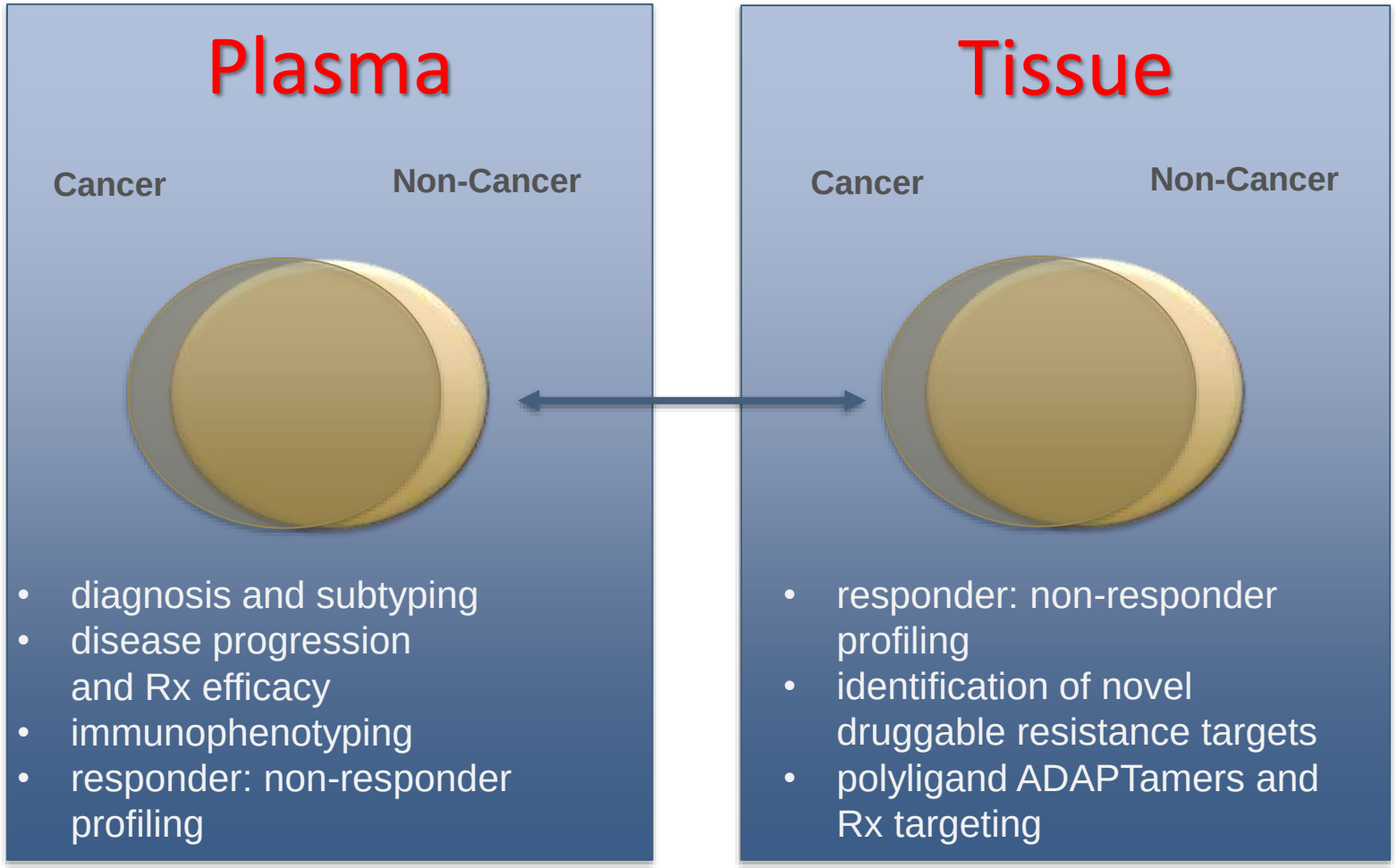
Blood-based Exosome Profiling

Profiling Biological Systems In Their Natural State(s)

Unbiased Target Identification

- **10^{12} exosomes/ml**
- **proprietary aptamer oligonucleotide multiplex assay**
  - **NGS readout of selective binding**
- **identify cell-of-origin**
- **quantitative and qualitative disease-associated changes**
  - **identified by ADAPTamer libraries**
  - **MDx and Rx target identification**

ADAPTamer Library Enrichment Schemes



V. Domewnyuk et al(2017) Nature Sci. Reports 42741

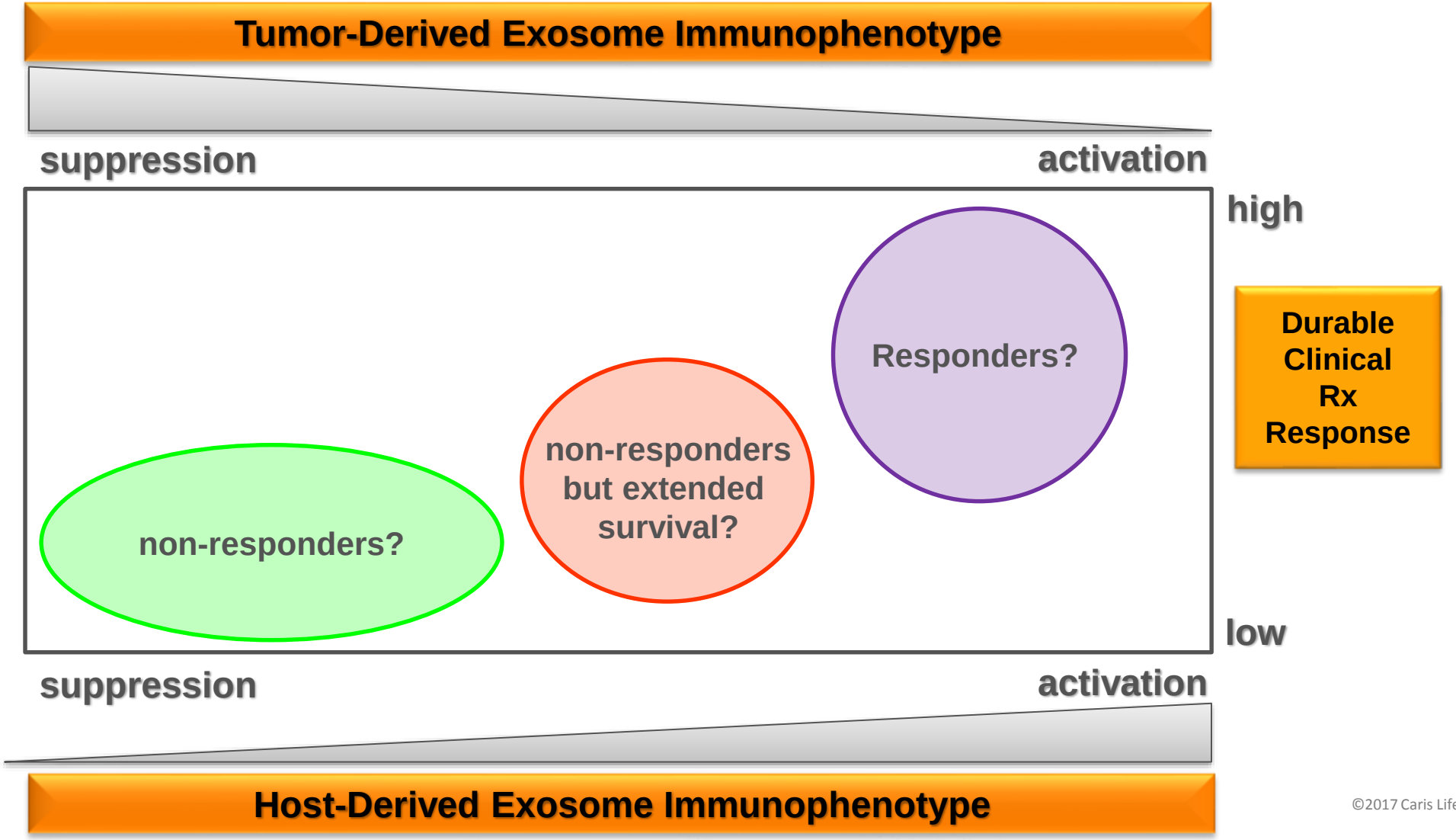
Tumor-Derived Exosomes as a Potential Molecular Profiling Platform to Assess Variation in Immunotherapy Efficacy

- assessment of Exo^{stim} and Exo^{supp} ratio in tumor progression and therapeutic efficacy
  - baseline before immunotherapy
  - effect of prior Rx(1L, 2L) on baseline
  - measure dynamic changes in ratio during immunotherapy
  - patterns and ratios in R and NR cohorts and/or adverse events

Validation of Potential Prognostic-Predictive Utility of Exosome Immune Suppression-Activation Ratio Using Banked Samples from Completed Clinical Trials

- **plasma**
  - **high stability due to small radius of curvature**
  - **5 years at -80°C**

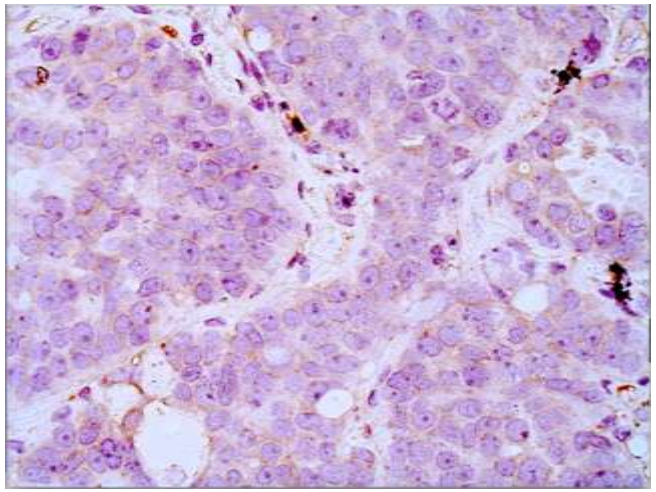
Retrospective Validation of Prognostic-Predictive Utility of Exosome Immune Suppression-Activation Ratio Using Banked Samples from Completed Trials

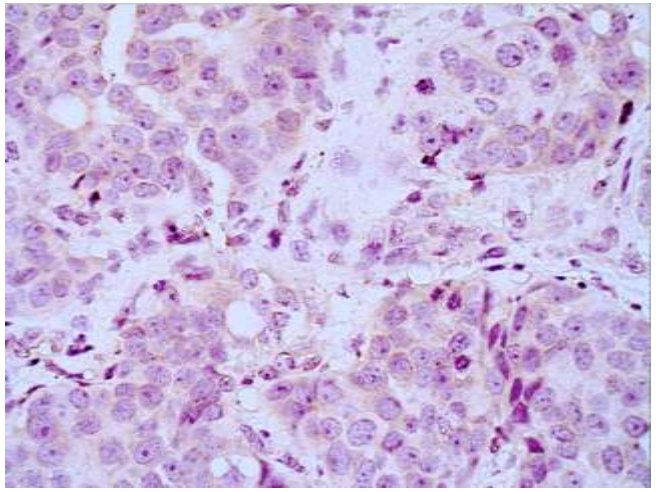


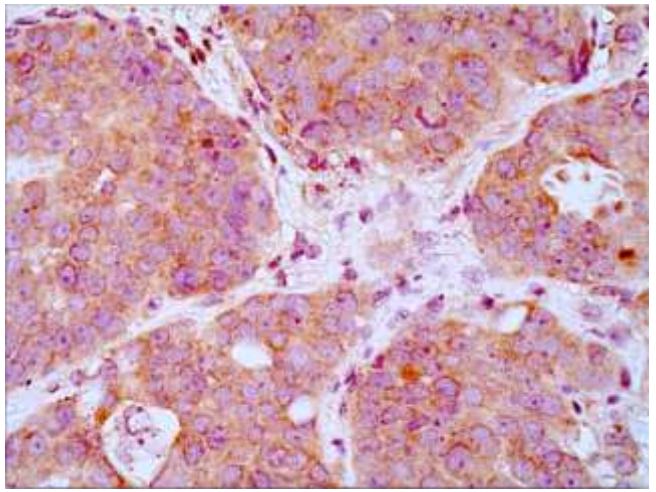
A Major Repository of Consented Tissue Sections From Clinically Annotated Patients

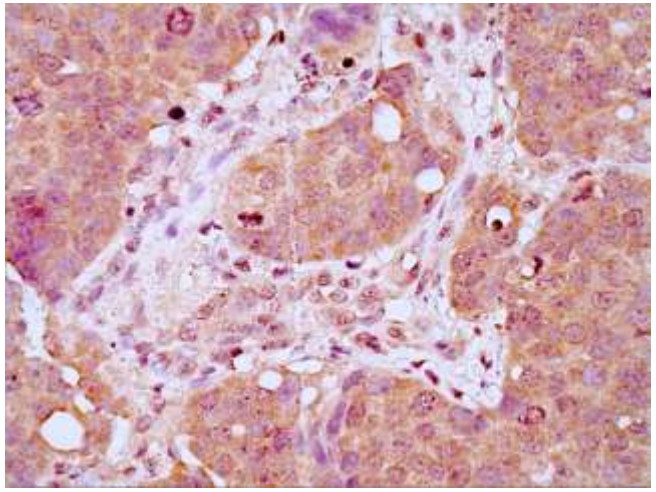
Selection of Breast Cancer-Specific ADAPTamer Libraries

Baseline





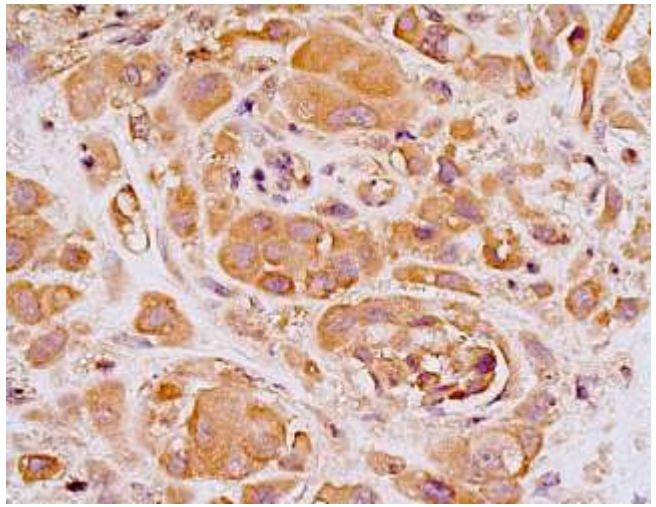


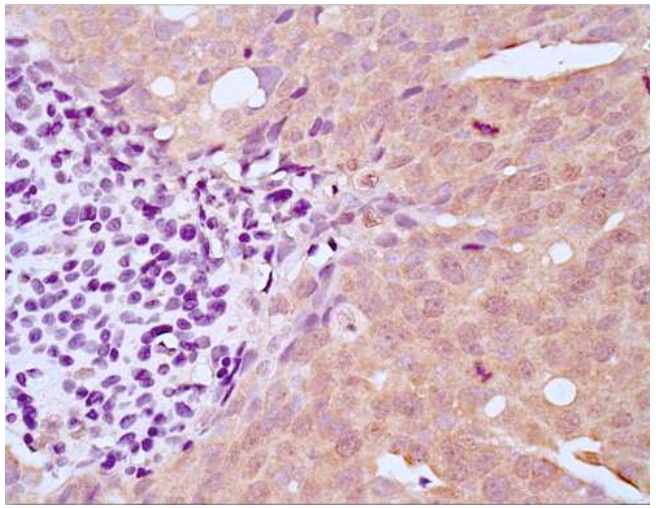


Enriched Libraries

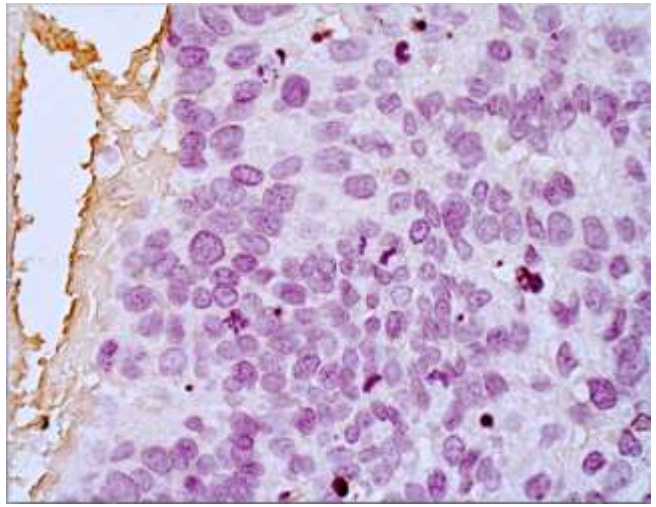
Differential Binding of ADAPTamer Library Discriminates Trastuzumab Responders from Non-responders

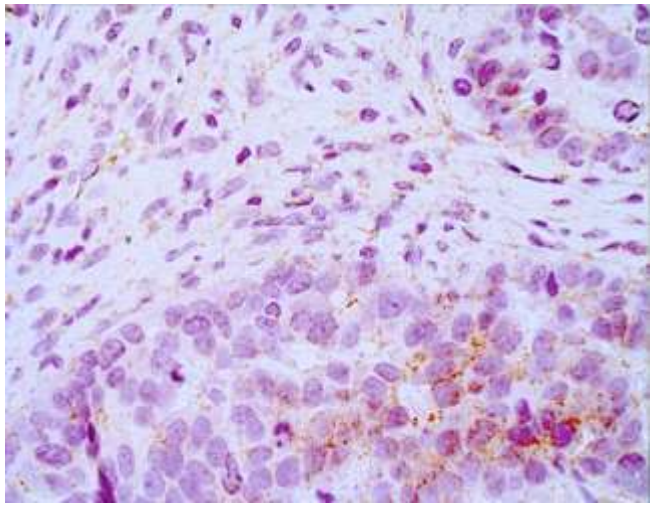
Responder





Non-responder





Trastuzumab Non-Responders Exhibit Strong Nuclear Staining for ADAPTamer Library

Non-responders

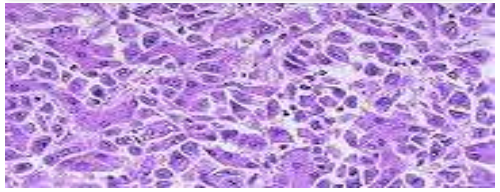
Responders

Identification of Cancer Neoantigens

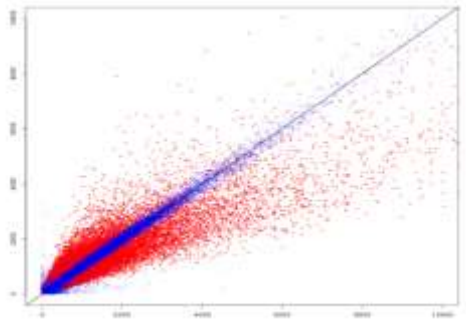
tissue

blood

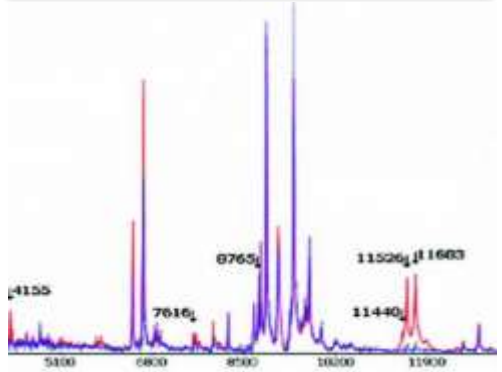
Tumor Samples



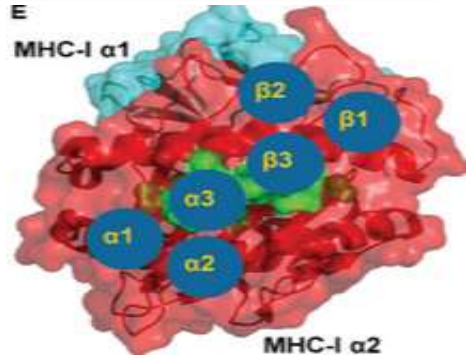
ADAPT Exosome Profiling



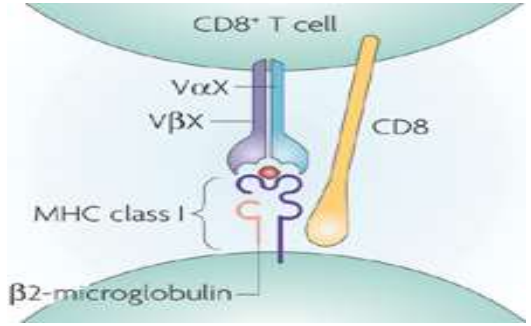
Mass Spectroscopy MHC Epitope Recognition Filter



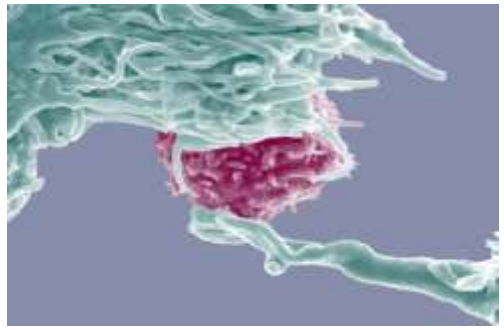
In Silico MHC Epitope Recognition Filter



T Cell Recognition-Activation Functional Assays



Activation/Suppression of Dendritic Cells



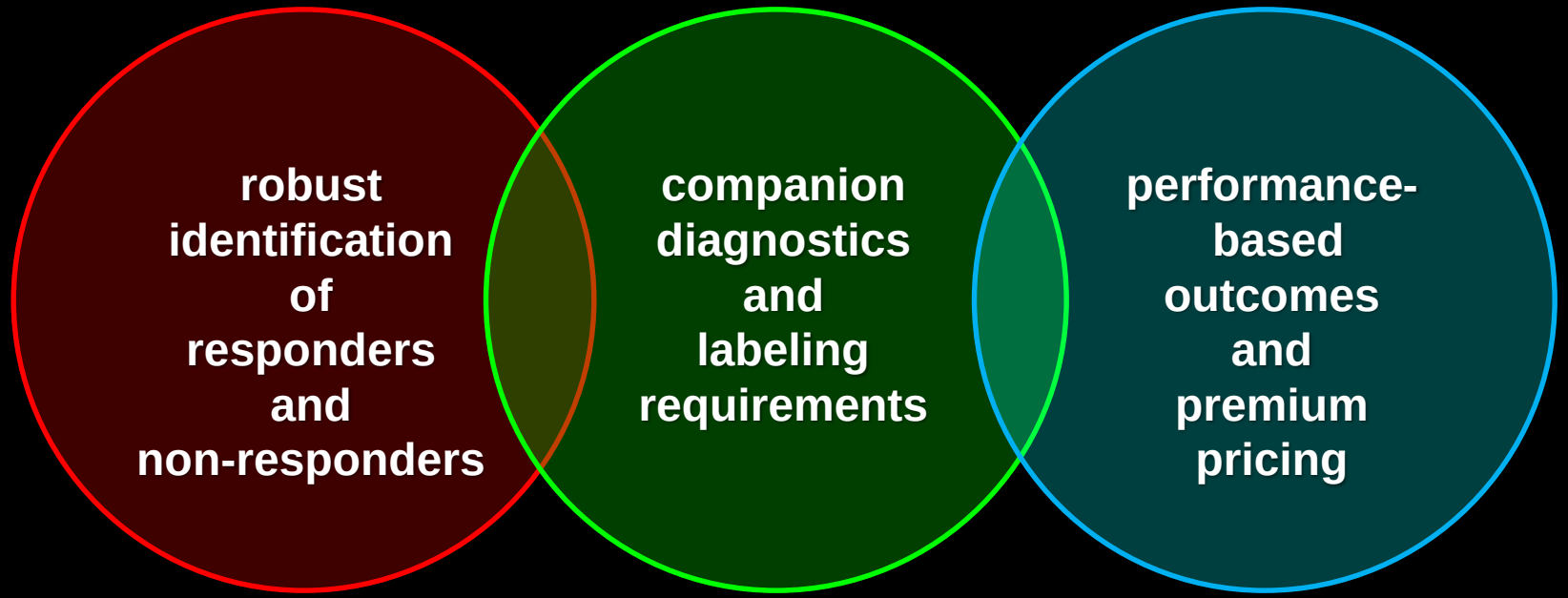
Is Widespread Adoption of Immunotherapy Economically Feasible?

- direct Rx cost and futile Rx cost
- indirect care cost of clinical care
- escalating cost of combination regimens (> $200K)
- extravagant cost of cell-based therapies ($500K - $1.5 million)
- high non-responder fraction complicates reaching QALY threshold of $150K in responders

The Evolving Trajectory for Payer Policy for Cancer Therapeutics

- performance – based pricing
- indication – based pricing
- reference – based pricing

Performance-Based Contracts and Pricing: The Inevitable Future Landscape for Immunotherapy?



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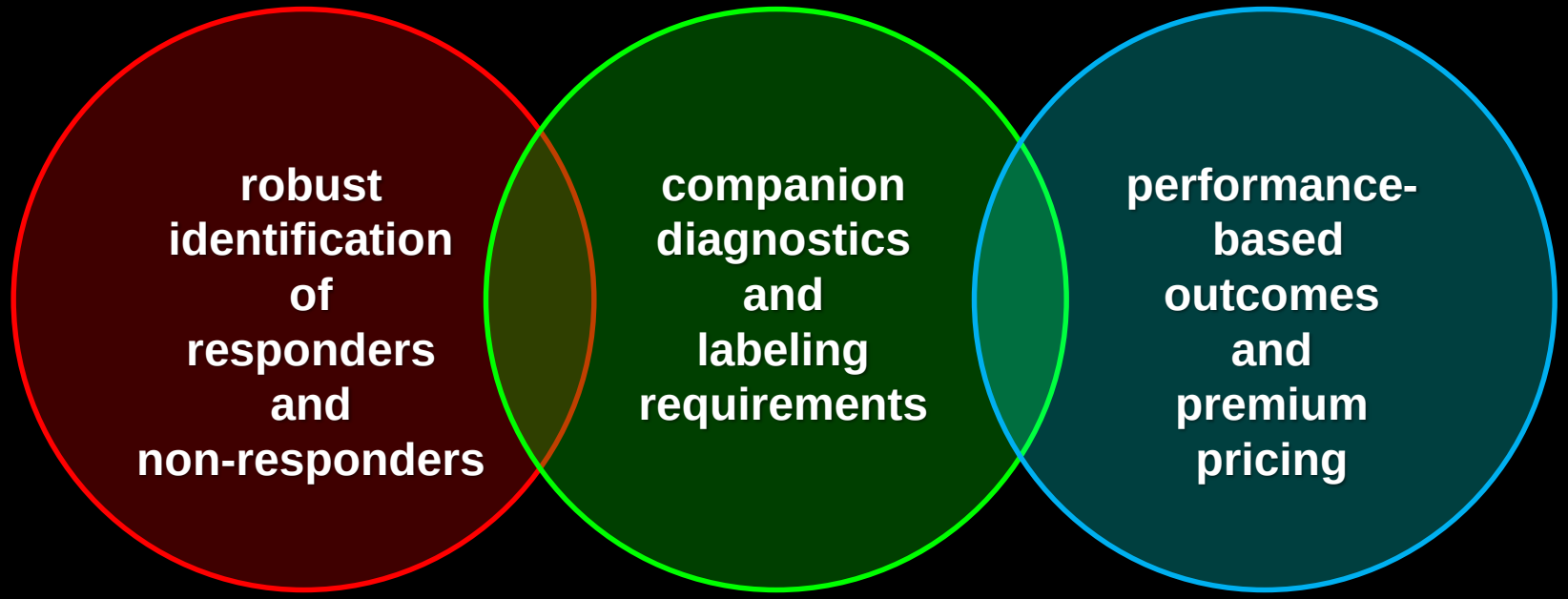

integration of R:NR immunophenotypes into clinical trials and registration dossier

risk sharing