ASU Complex Adaptive Systems Initiative
Arizona State University

Cambridge HEALTHTECH Institute

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

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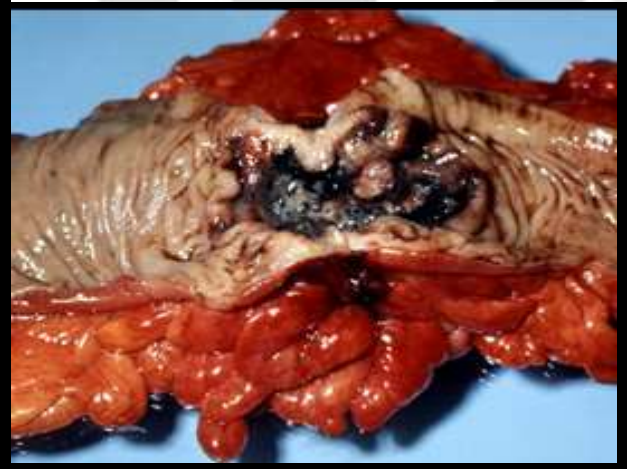
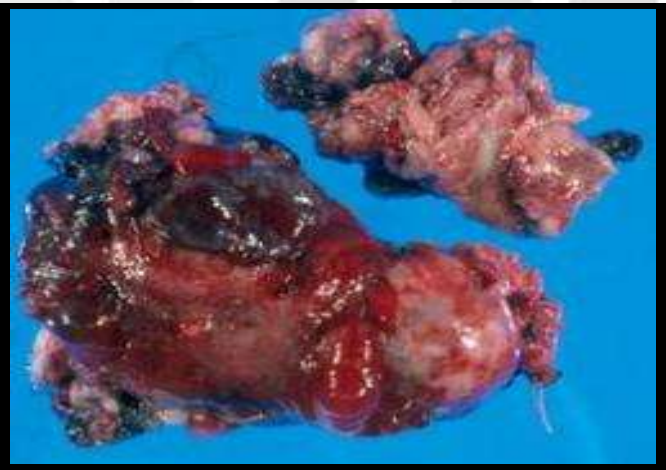
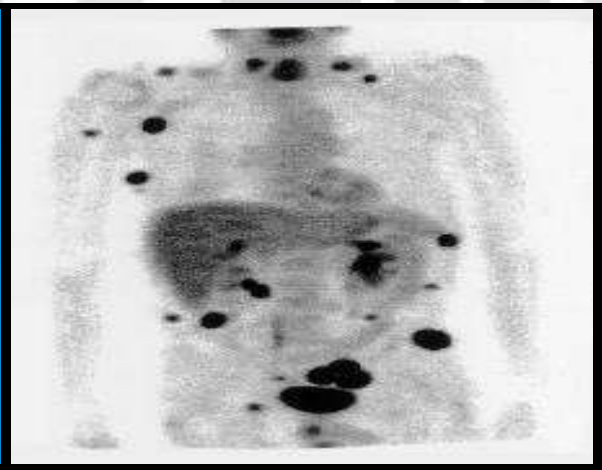
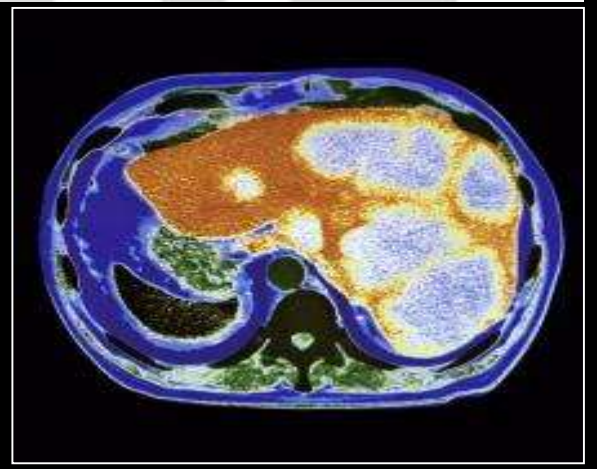
Confronting the Clinical, Economic and Human Toll of Cancer

Cancer (2017): New Diagnoses 1.68 million; Deaths: 600,920

Projected Increase in Incidence of 20% by 2020 and 30% by 2030

The Promise of Cancer Immunotherapy

- immune checkpoint modulation
- adoptive T cell therapy and T cell engineering
- antibodies and antibody drug conjugates
- fractionated radiation
- cancer vaccines
- oncolytic viruses
- combination therapies

Realizing the Promise of Cancer Immunotherapy

- wide variation in Rx response rates
  - only 20-40% positive responses even in most responsive malignancies
  - even lower percentage of clinically durable responses (KM-“long tail”)
- improve response rates across all malignancies
- management of serious AEs (CRS, autoimmune risk)
- will I/O combination regimens increase response rates?
- rationale for selection of combination regimens
- improved preclinical models for new I/O agent discovery and combination testing

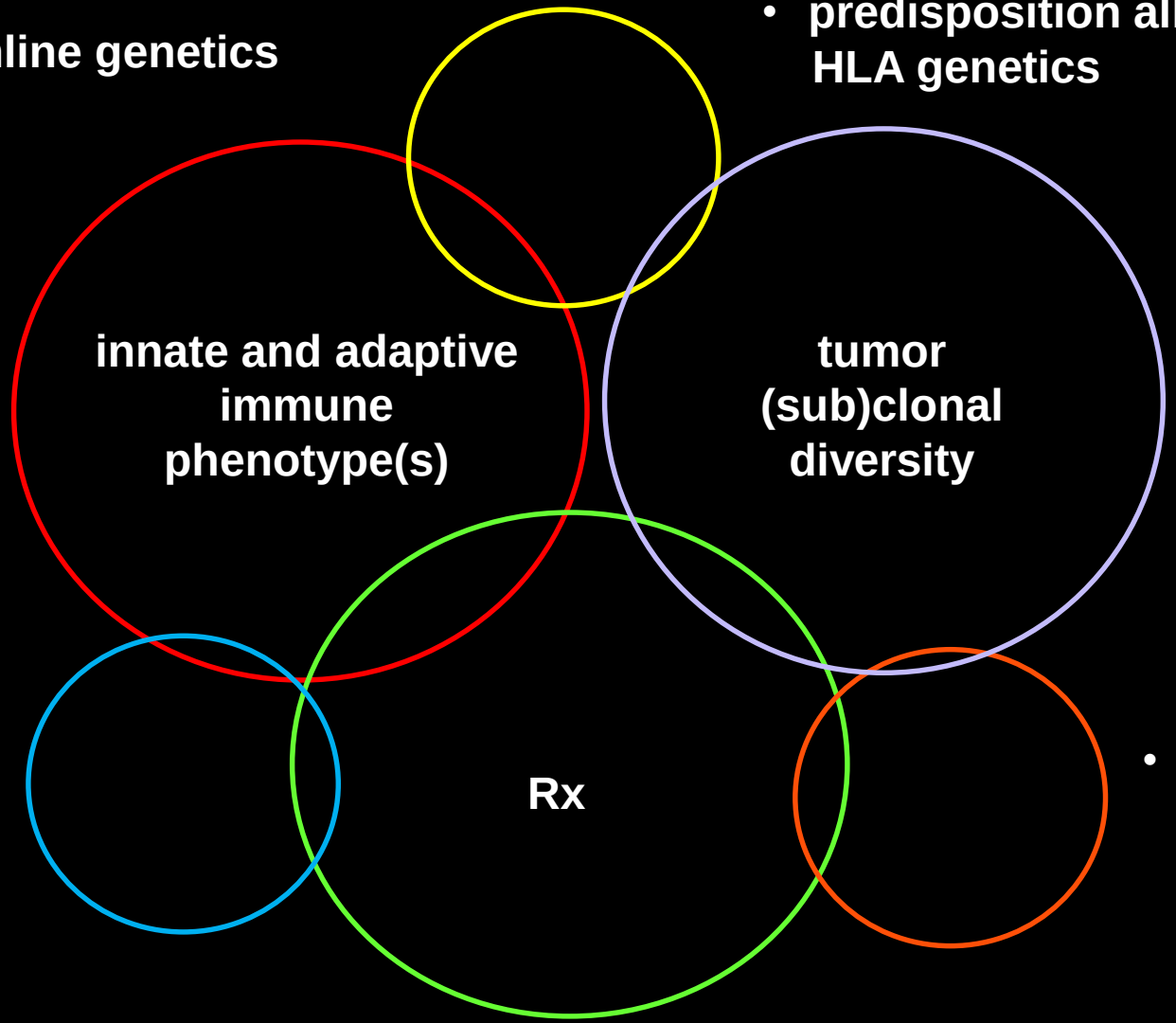
urgent need for multi-parameter immunophenotyping to reliably predict responder vs non-responder patients (immunoscore : immunopredictor : immune response index)

Cancer as a Complex Adaptive System

The Requirement for Holistic, Systems-Based Approaches to Improve Patient Selection for Immunotherapy and Optimize Therapeutic Outcomes and Cost-Effectiveness

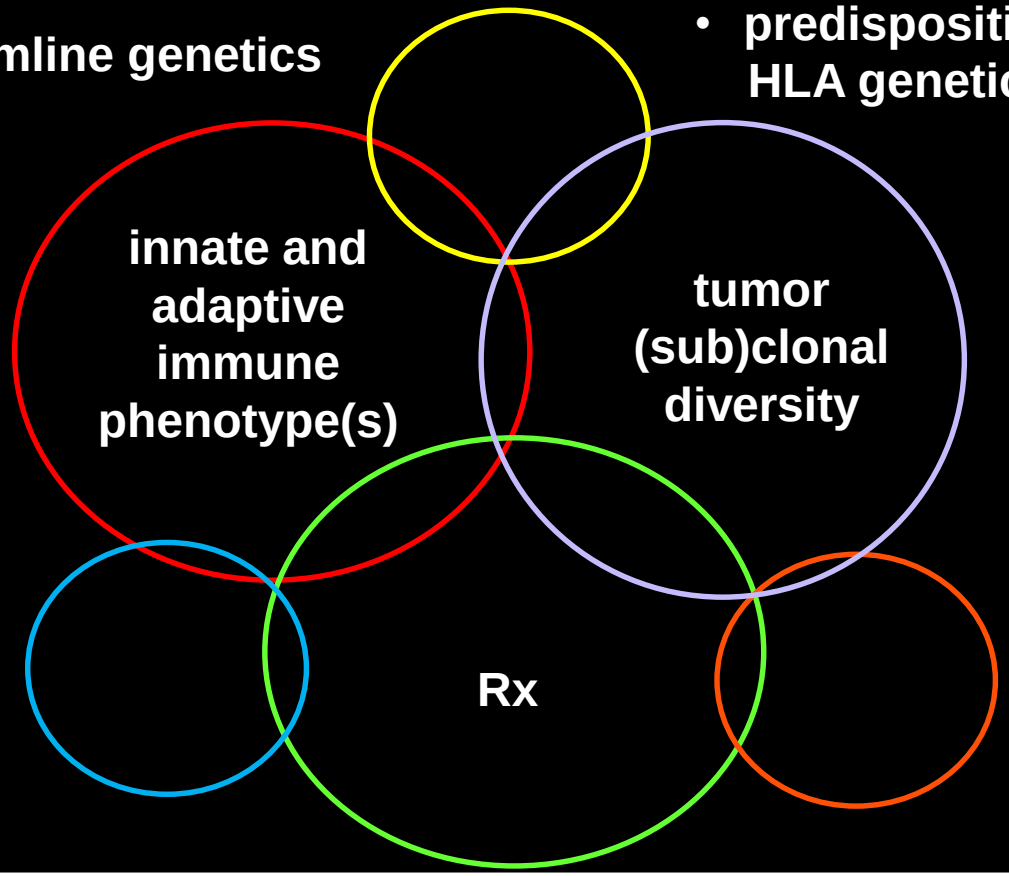
Mapping the Complex Spatio-Temporal Dynamics of Adaptive Co-evolution in the Tumor-Immune Ecosystem

- germline genetics
- predisposition alleles
  HLA genetics



- local and systemic
- inter-patient/ intra-patient
- mutagen burden and neoantigen profile
- immuno-editing
- microbiome
- intrinsic and acquired Rx resistance

Cancer as a Complex Adaptive System



- germline genetics
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  HLA genetics
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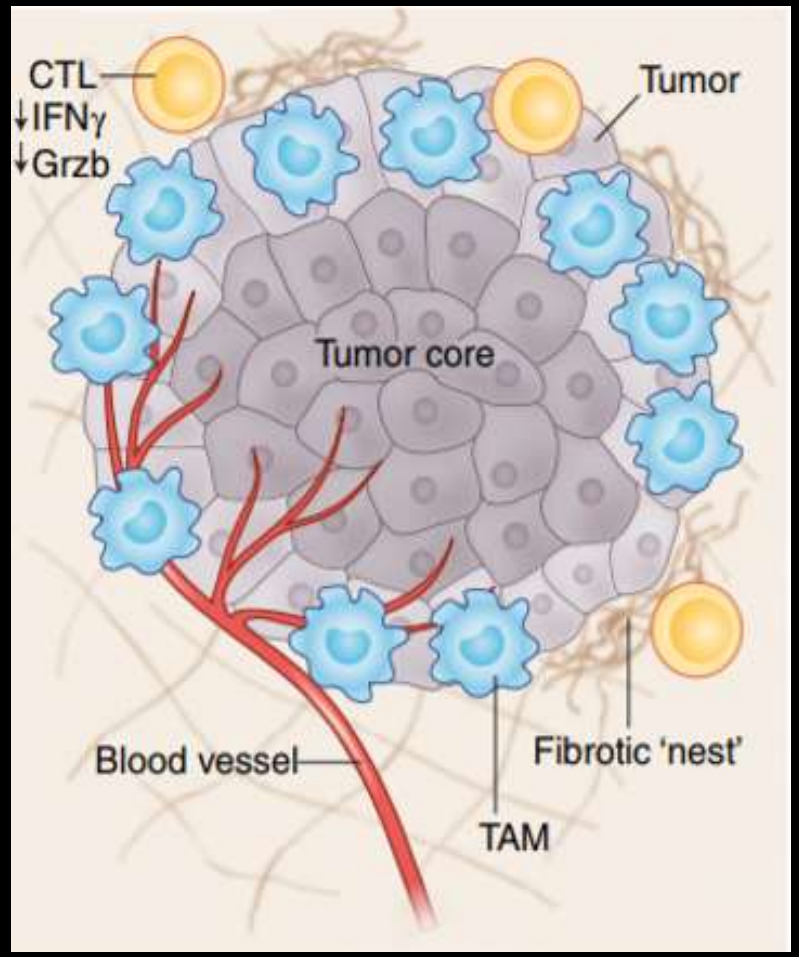
ill-defined systemic factors affecting disease risk, progression and Rx responses

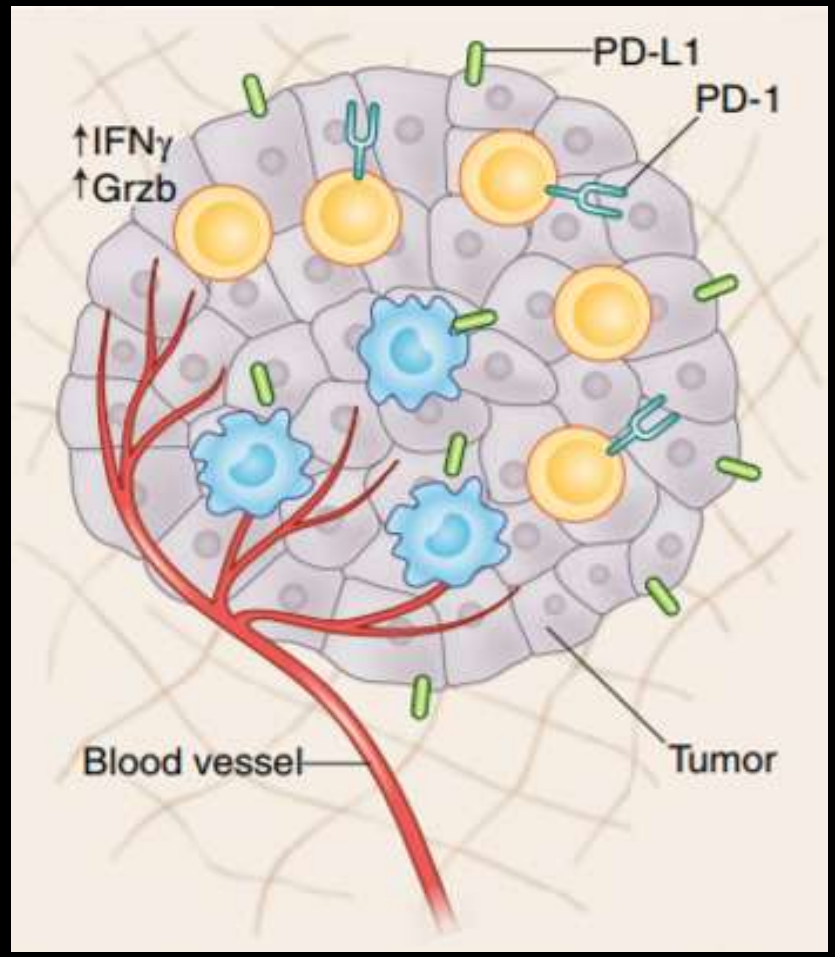
- age
- gender
- ethnicity
- environmental risk factors/lifestyle
- adiposity
- comorbidities
- chronic, subclinical inflammation
- selection pressure of prior Rx regimen(s)

General Classes of Tumor-Immune Microenvironments (TIME)

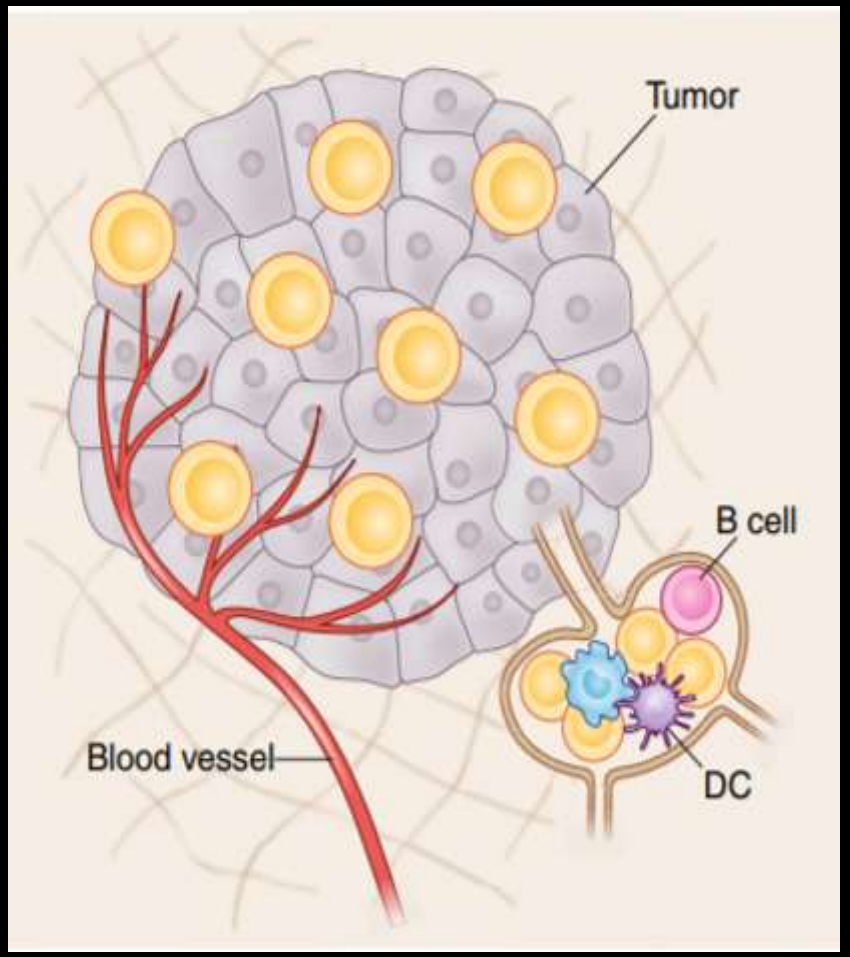
Infiltrated-excluded



Infiltrated-inflamed



Infiltrated-TLS



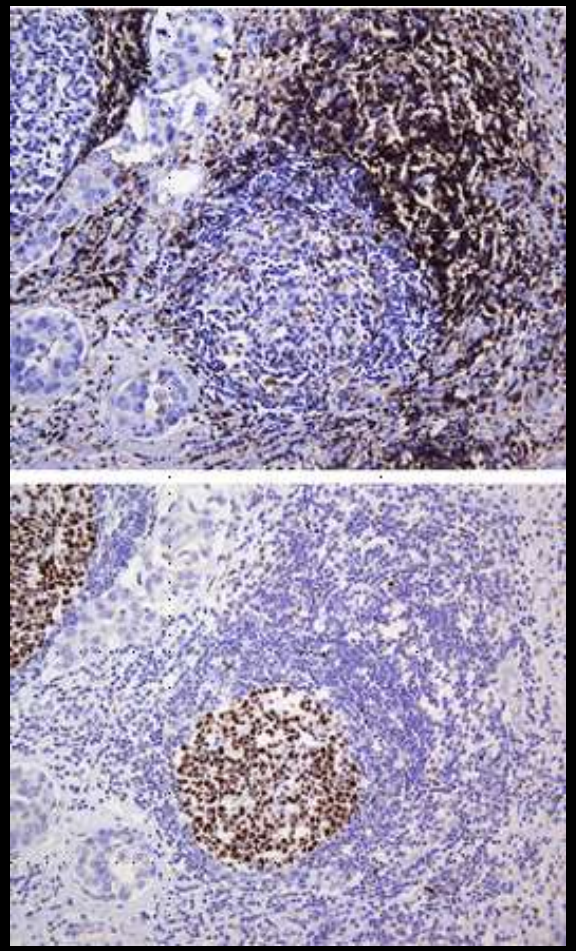
Adapted from: M. Binnewies et al. (2018) Nature Med. 24, 541

Tumor Immunophenotypes Associated With Responsiveness to Immune Checkpoint Blockade

"Hot", "Inflamed", "Immunogenic"

- high tumor mutation burden
- high tumor-infiltrating $CD8^+$ cytotoxic cells
- activation of tissue-resident T cells (Tres)
- long lived memory T cells (Tem)
- high Th1 and T17 helper cells
- CD21+DCs
- high expression of Th1 stimulating cytokines
- higher expression of TIL checkpoint receptors
- M1 phenotype tumor-associated macrophages (TAM)
- low Tregs, MDSCs, low TGF-β

Formation of Ectopic/Tertiary Lymphoid Intra-tumoral and Peri-tumoral Structures(TLS) and Lymphoid Cell Aggregates and Better Patient Survival



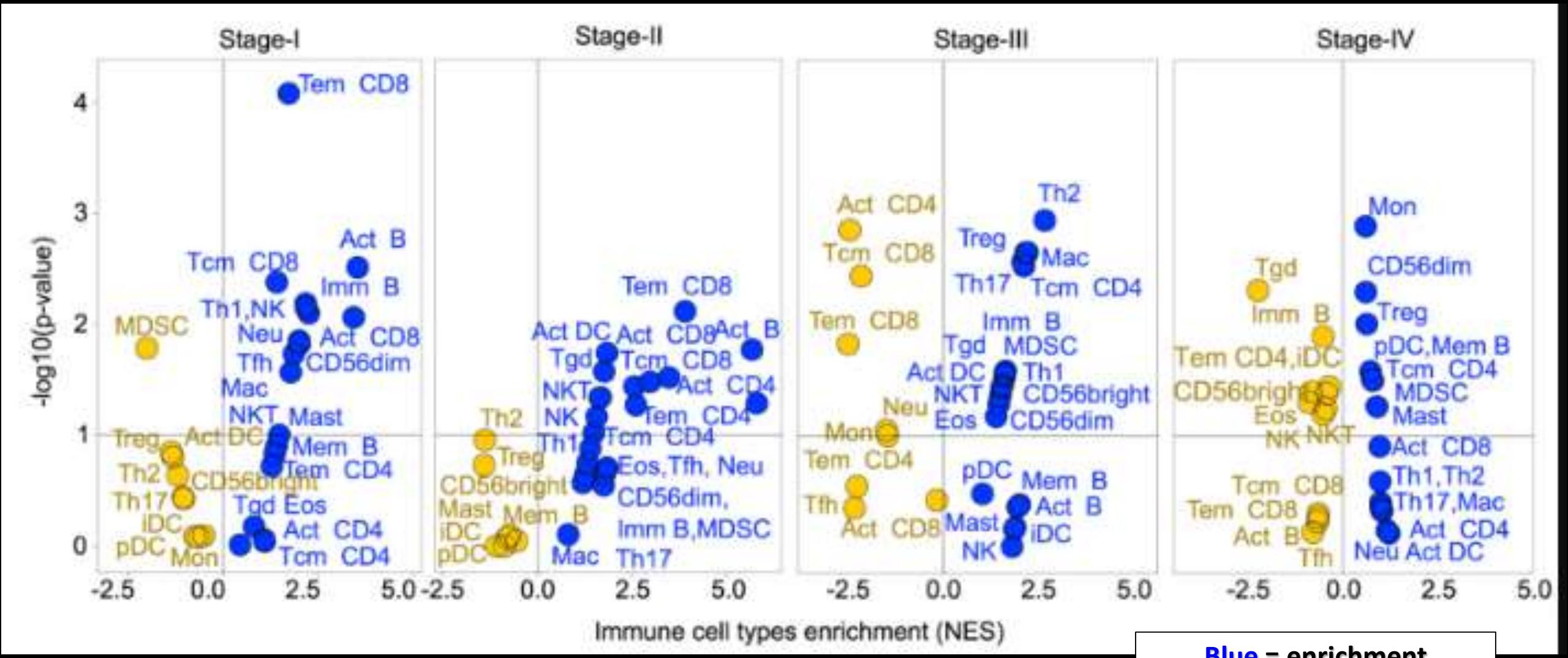
- detected in CRC, ovary, lung tumors (plus autoimmune diseases and chronic inflammation)
- proliferation of B and T cells
- CD21+ dendritic cells in germinal centers
- 12 gene cytokine gene expression involved in T cell homing and TLS development
  - CCL3, 4, 5, 8, 18 and CXCL 9, 10, 11, 13, 19, 21
  - no expression of CCL1, 20 and 22 involved in recruitment of Tregs

Tumor Immunophenotypes Associated With Non-Responsiveness to Immune Checkpoint Blockade

"Cold": "Non-Inflamed", "Non-Immunogenic", "Immune Desert"

- low mutational and neoantigen burdens
- low $CD8^+$ effector cells
- high Tregs and MDSCs, low Th1
- immunoediting and loss/down-regulation of neoantigens
- impaired antigen presentation
  - loss/downregulation of MHC
  - JAK 1/2 and β2 microglobin mutations in MHC1
- increased levels of immunosuppressive cytokines
  - PTEN loss and increased CCL2, VEGF and reduced T cell infiltration
  - β-catenin/Wnt mutations and reduced CCL4 chemokine production and dendritic cell recruitment
- CCR2/CCL2 and M2 phenotype TAMs
- IPRES (innate PD-1 resistance) gene signature
  - immunosuppressive cytokines, EMT-TFs and pro-angiogenic factors

Shifts of Intratumoral Immune Cell Populations to Immunosuppressive Phenotypes with Tumor Progression



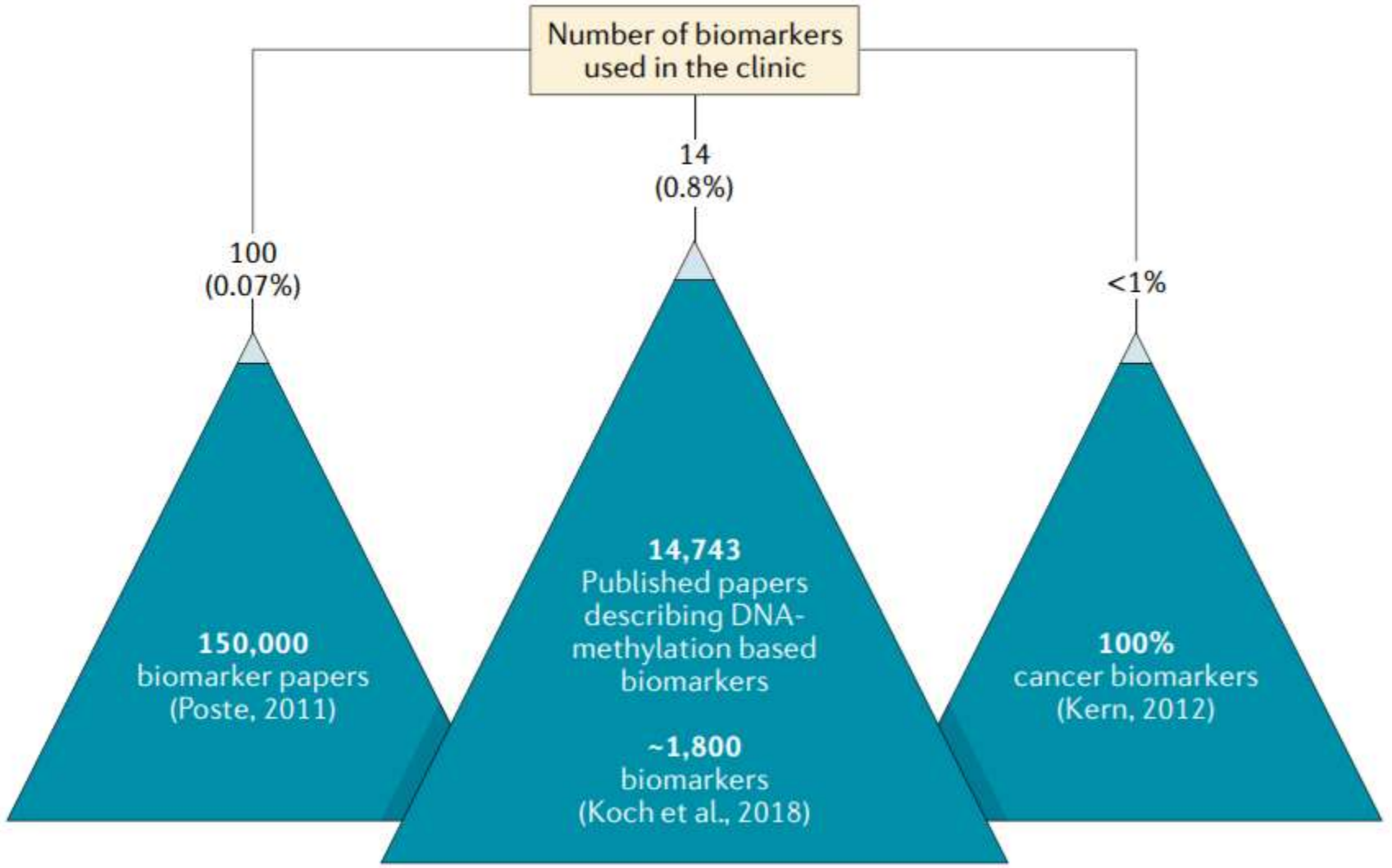
Blue = enrichment
Yellow = depletion

P. Charoentong et al. (2017) Cell Reports 18, 248.

The Cancer Biomarker Challenge

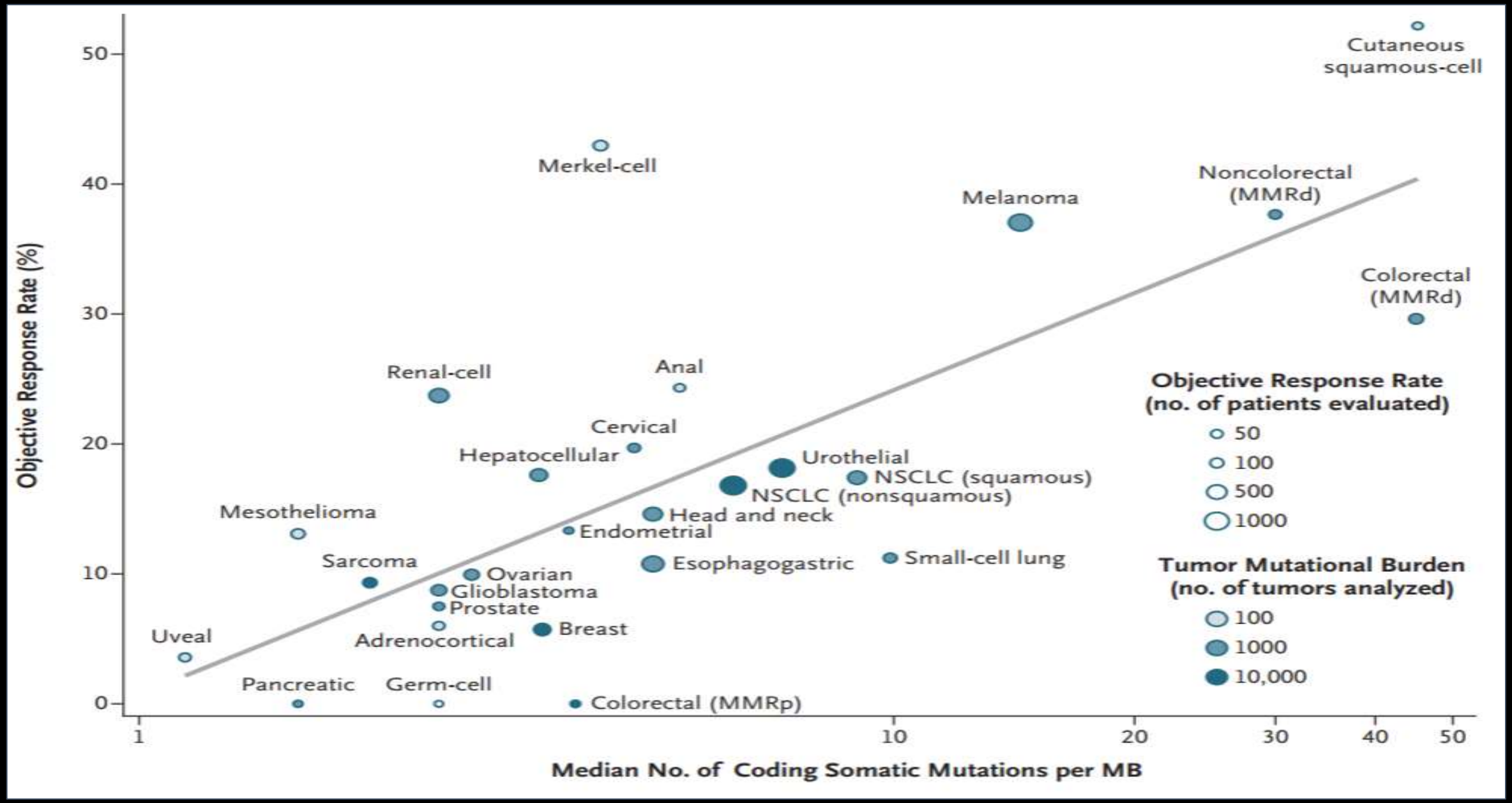
The Publish and Vanish Syndrome

- low success rates in the clinical validation of cancer biomarkers



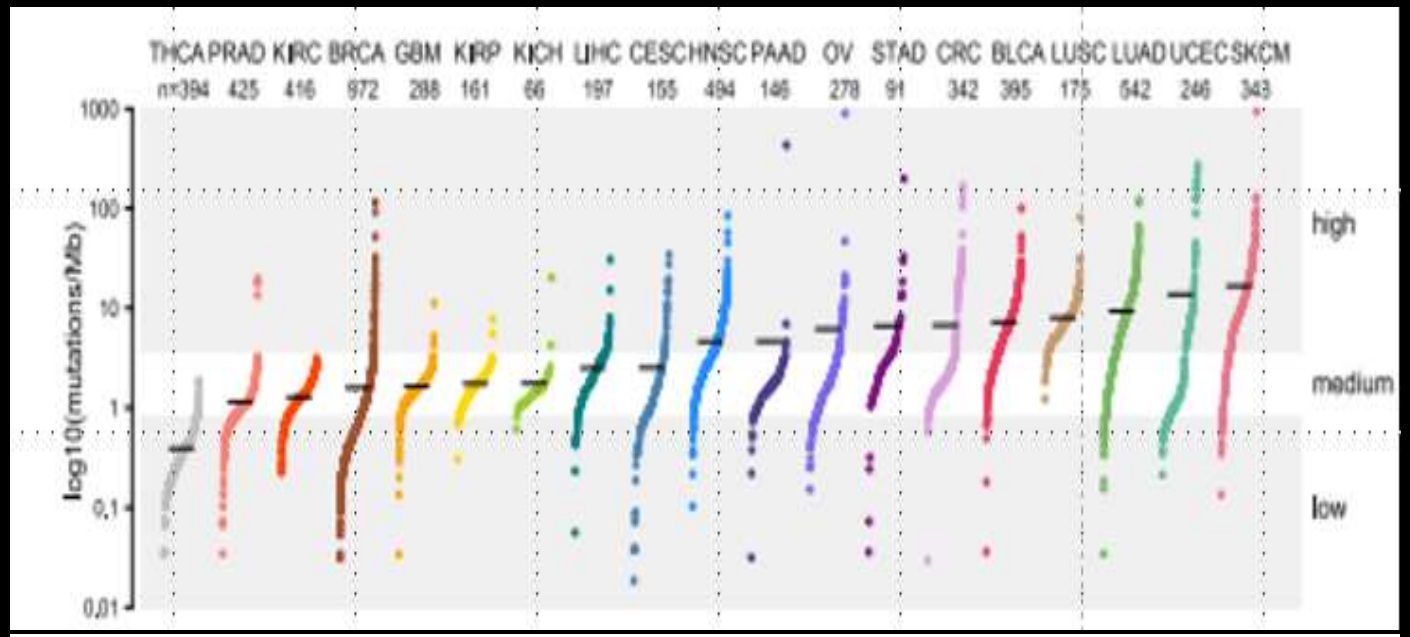
From: A. Koch et al. (2018) Nat. Rev. Clin. Oncol. 15, 459

Tumor Mutational Burden and Response to Immune Checkpoint Blockade

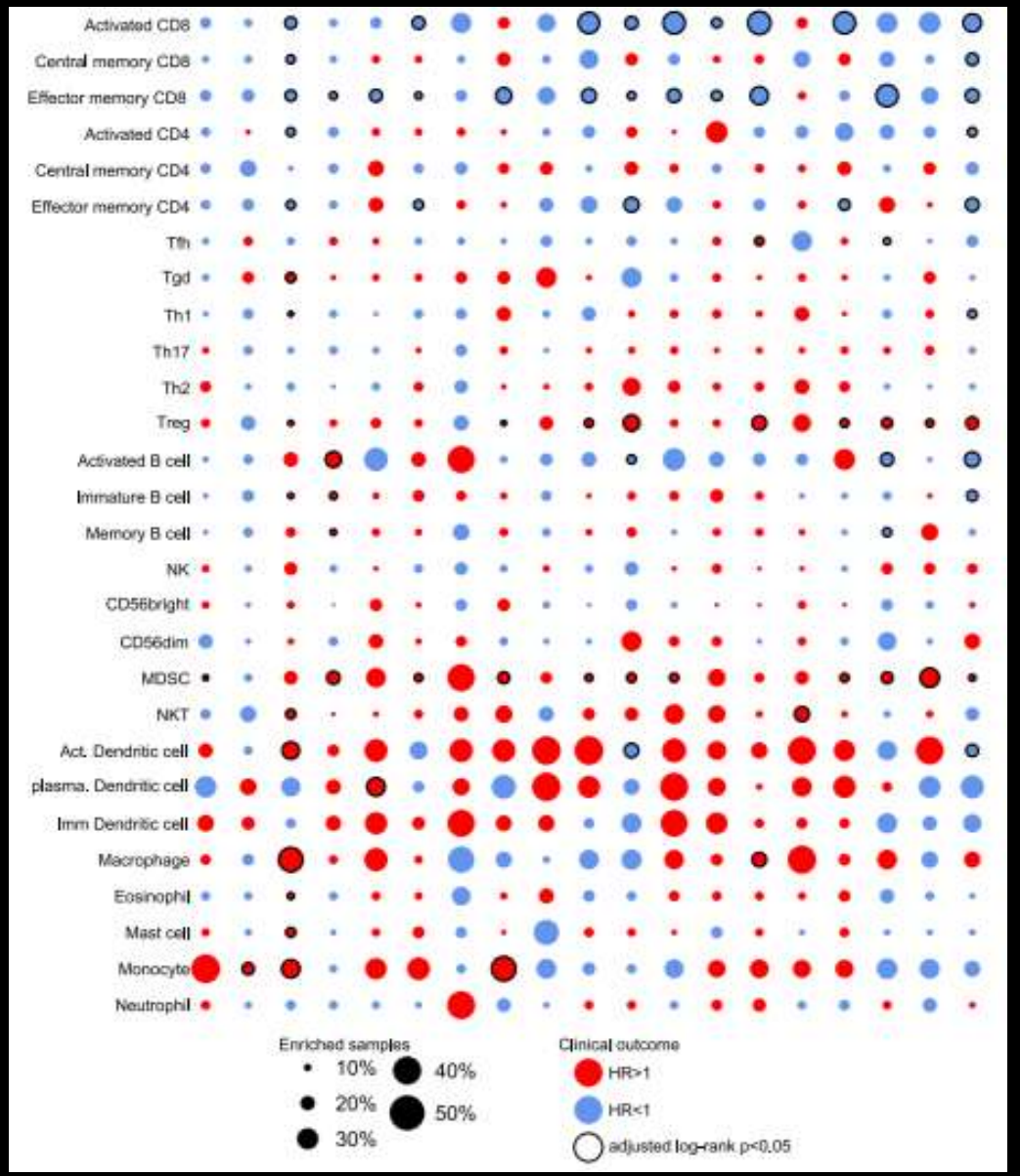


From: M. Yarchoan et al. (2017) NEJM 377, 2501

Immune Profiling Across 19 Solid Cancers Sorted by Mutational Load, Adaptive and Innate Immune Subpopulations and Clinical Outcomes



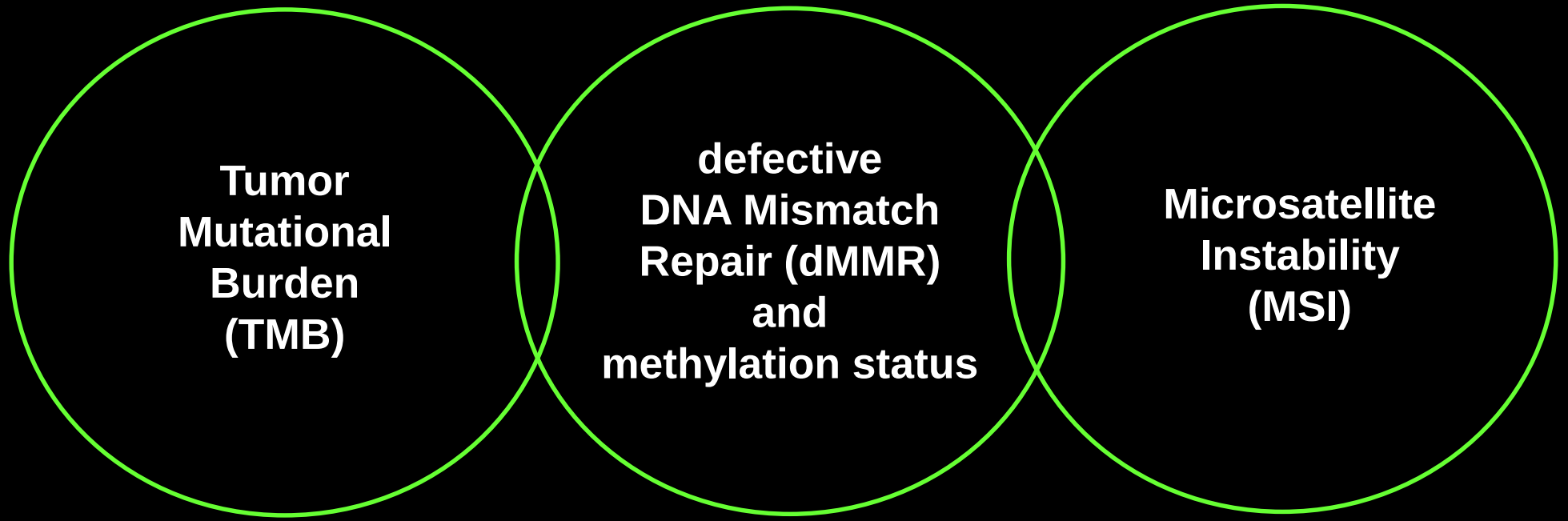
THCA: Thyroid carcinoma
PRAD: Prostate adenocarcinoma
KIRC: Kidney renal clear cell carcinoma
BRCA: Breast invasive carcinoma
GBM: Glioblastoma multiforme
KIRP: Kidney renal papillary cell carcinoma
KICH: Kidney chromophobe
LIHC: Liver hepatocellular carcinoma
CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma
HNSC: Head and neck squamous cell carcinoma
PAAD: Pancreatic adenocarcinoma
OV: Ovarian serous cystadenocarcinoma
STAD: Stomach adenocarcinoma
CRC: Colon-rectum adenocarcinoma
BLCA: Bladder urothelial carcinoma
LUSC: Lung squamous cell carcinoma
LUAD: Lung adenocarcinoma
UCEC: Uterine corpus endometrial carcinoma
SKCM: Skin cutaneous melanoma



Adapted from: Charoentong et al., 2017, Cell Reports 18, 248–262

Neoantigen Burden and Prediction of Responsiveness to Immune Checkpoint Blockade (ICB)

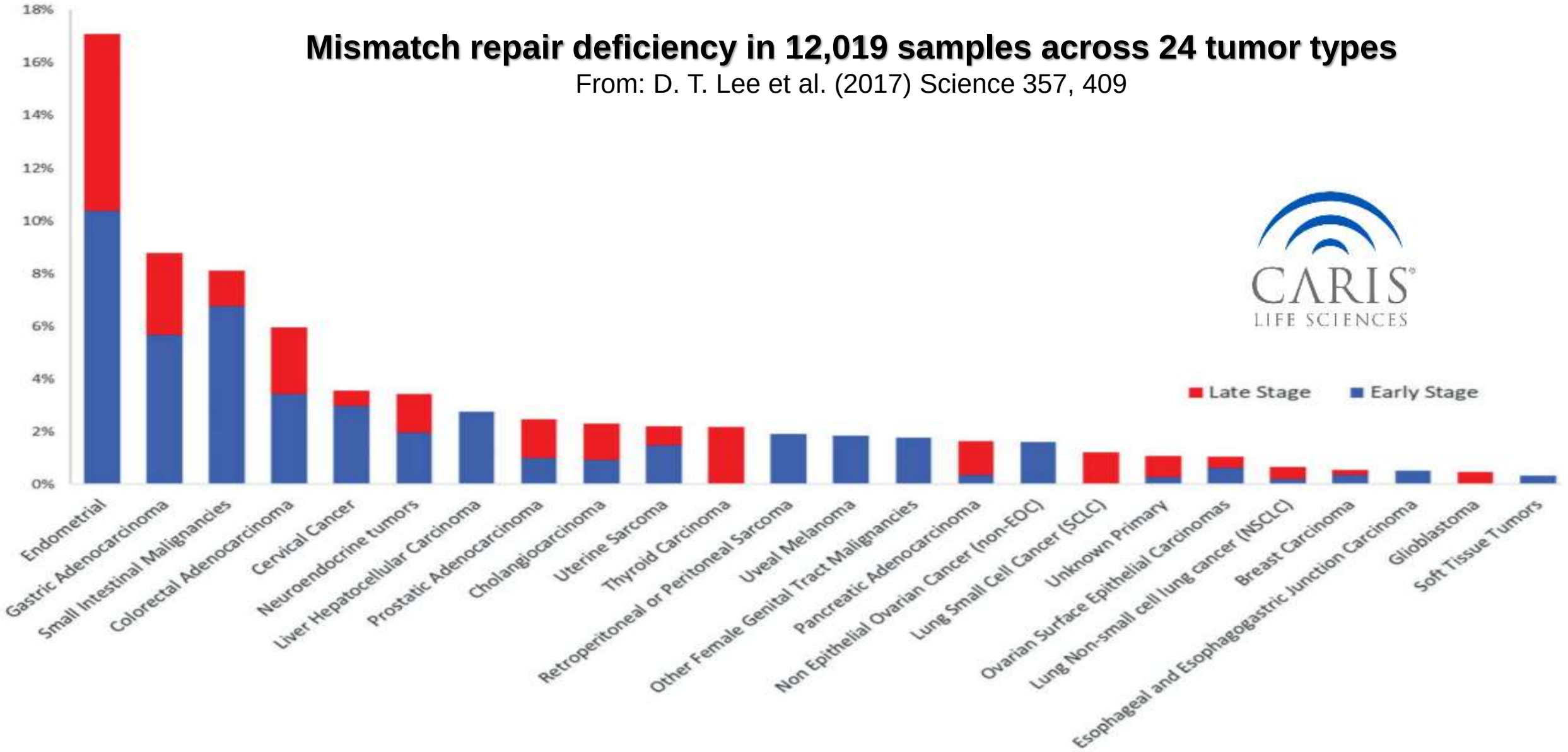
enhanced neoantigen expression



association with responsiveness to ICB

non-synonymous single nucleotide variants versus indel/frameshift mutations

Mismatch Repair Deficiency Predicts Response of Solid Tumors to PD-1 Blockade



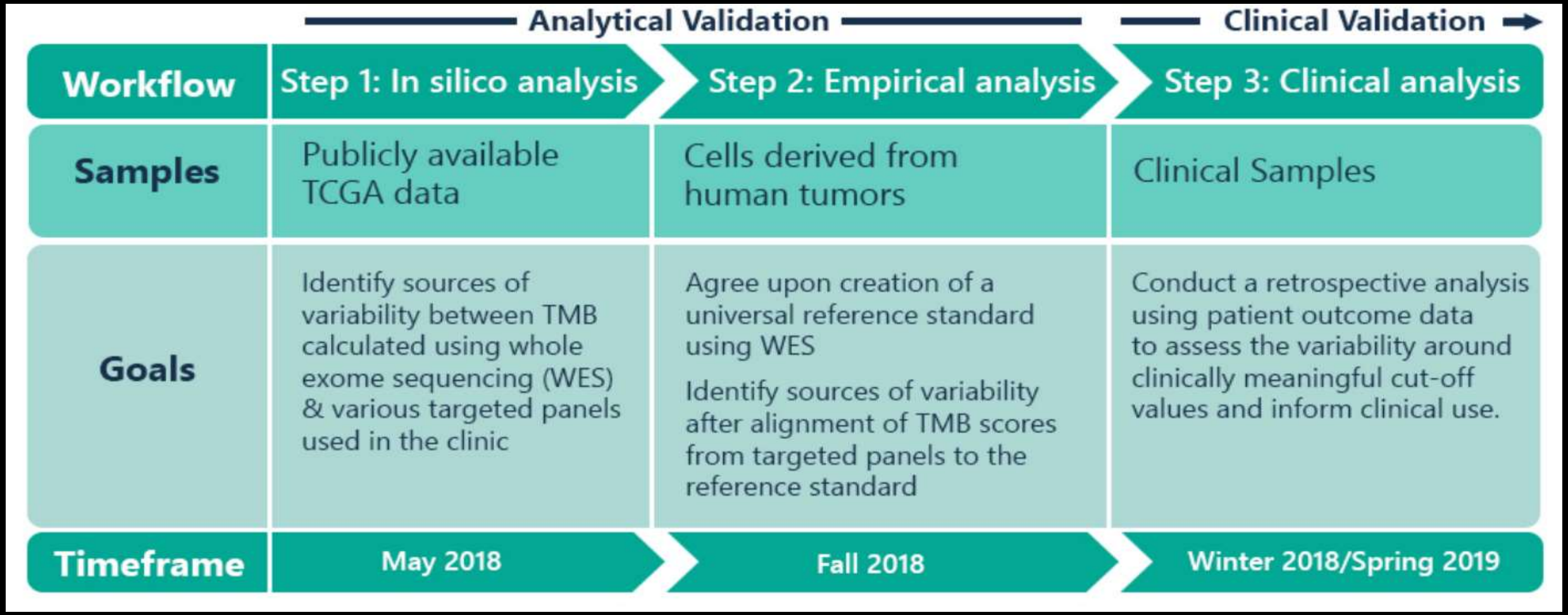
MSI-H/dMMR as ‘Cancer Agnostic’ Marker for Patient Stratification for anti-PD-1 Therapy

TMB Profiling

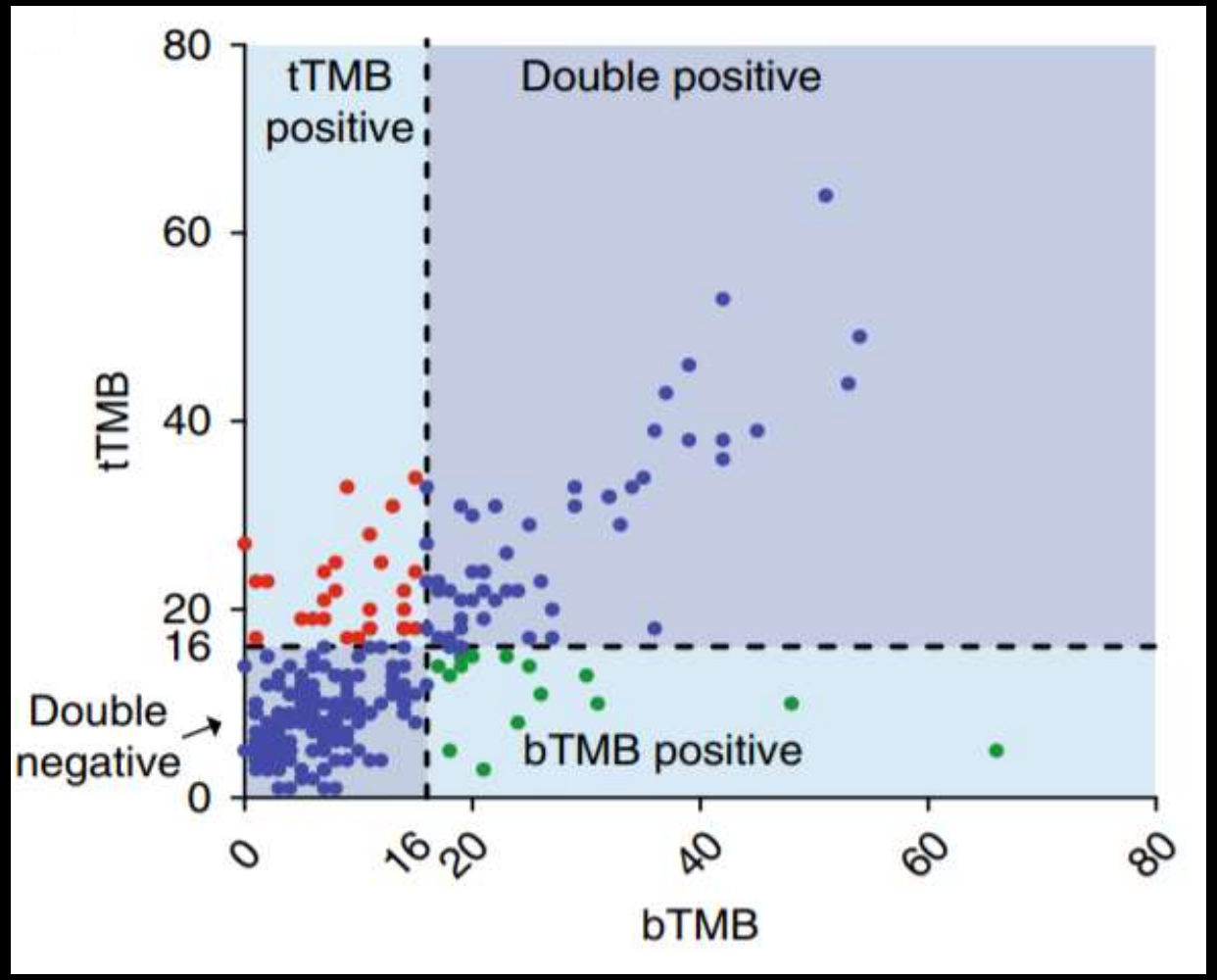
- limitations on tissue and/or poor quality of extracted DNA
  - Hellman et al (2018) NEJM 378, 2093 only 1004/1739 pts suitable
- sample
  - FNA biopsy or resection ?
- multiple TMB test protocols
  - WES or targeted sequencing panels
  - depth of sequencing
  - lack of concordance between NGS panels of different sizes
  - different cut-off points
  - subtraction of clonal hematopoiesis mutations
- TAT and cost

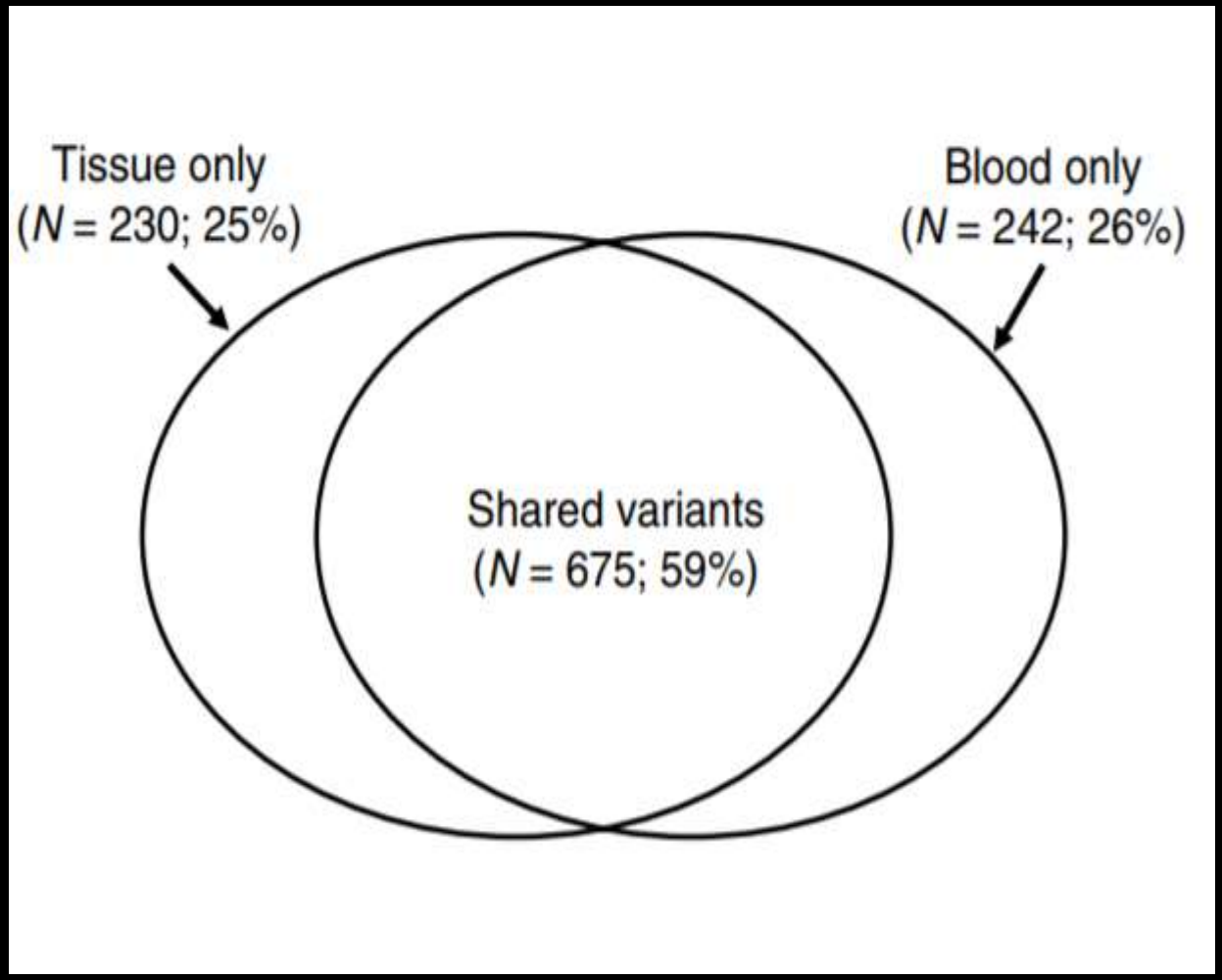
FRIENDS of CANCER RESEARCH

Tumor Mutational Burden (TMB)



Analysis of Tissue and Blood-Based Tumor Mutational Burden in NSCLC

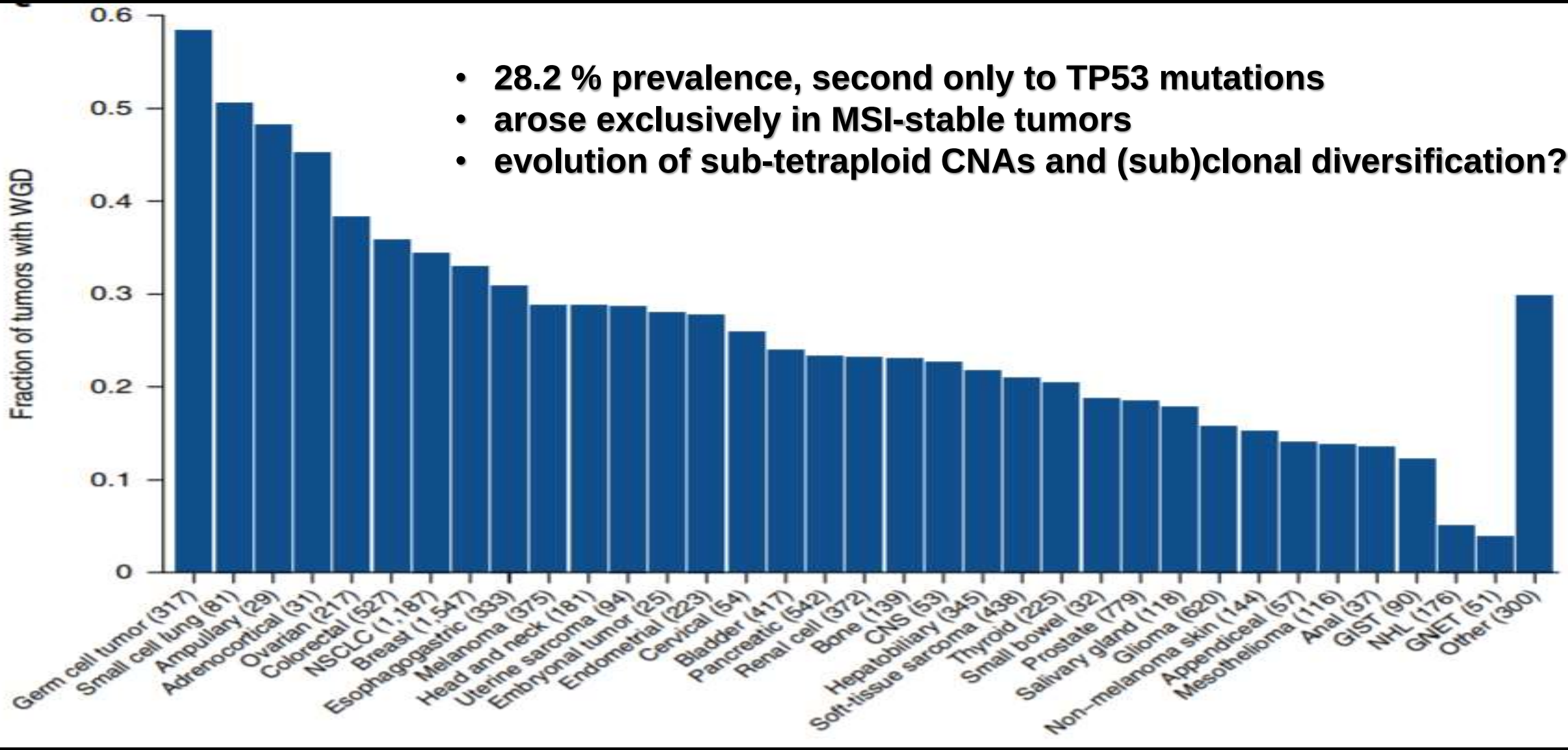




259 patient samples:
OAK and POPLAR trials
Spearman correlation = 0.64

From: D.R. Gandara et al. (2018) Nature Medicine 0134-3

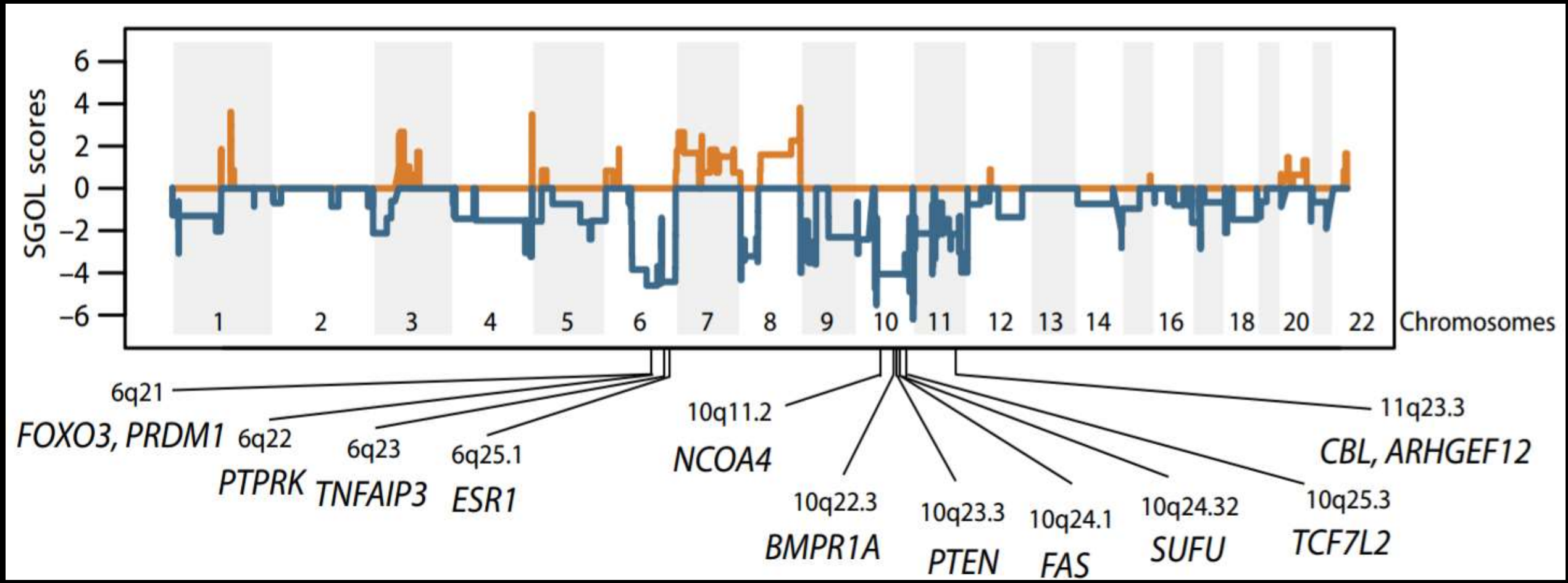
Genome Doubling in 9,692 Cancers and Association with Worse OS Outcomes



- **28.2 % prevalence, second only to TP53 mutations**
- **arose exclusively in MSI-stable tumors**
- **evolution of sub-tetraploid CNAs and (sub)clonal diversification?**

From: C.M. Bielski et al. (2018) Nature Genetics 0165-1

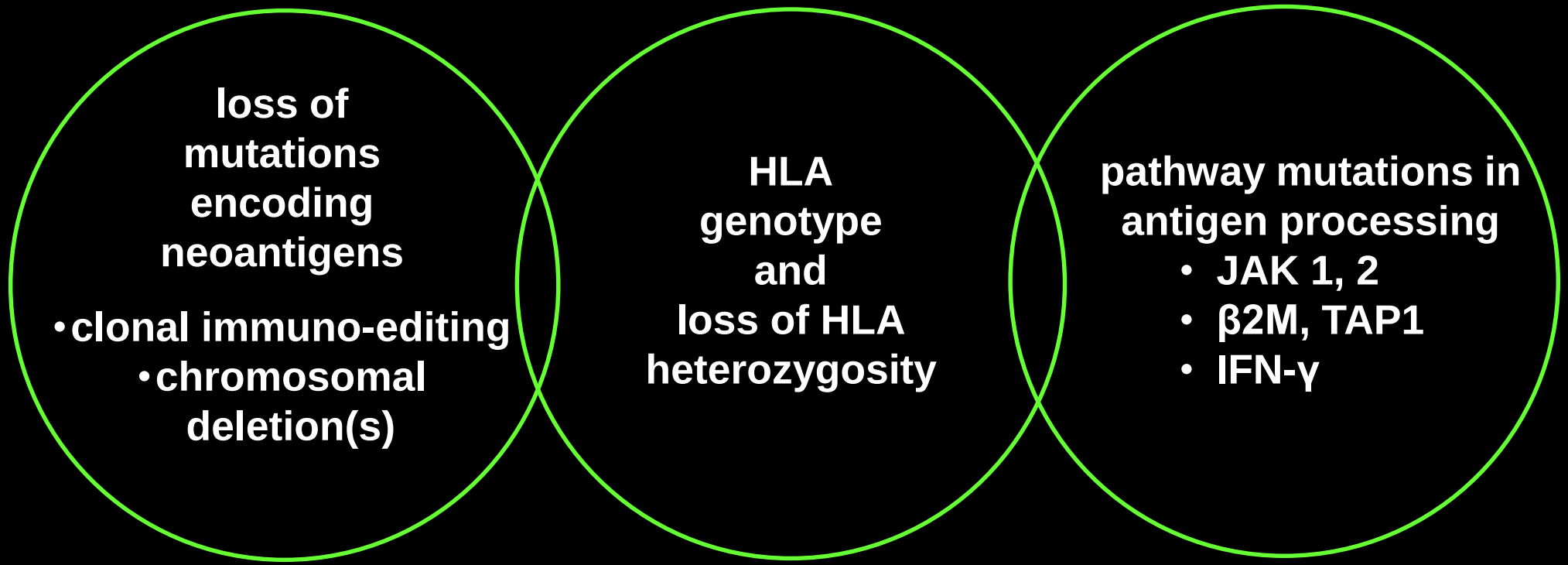
Copy Number Loss (>2000) as a Potential Resistance Phenotype for Double Non-Responders to Sequential CTLA-4 and PD-L1 Blockade and Loss of Tumor Suppressor Genes



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

Neoantigen Expression and Detection in Prediction of Responsiveness to Immune Checkpoint Blockade (ICB)

- antigen loss/reduced expression
- impaired immune recognition and antigen processing



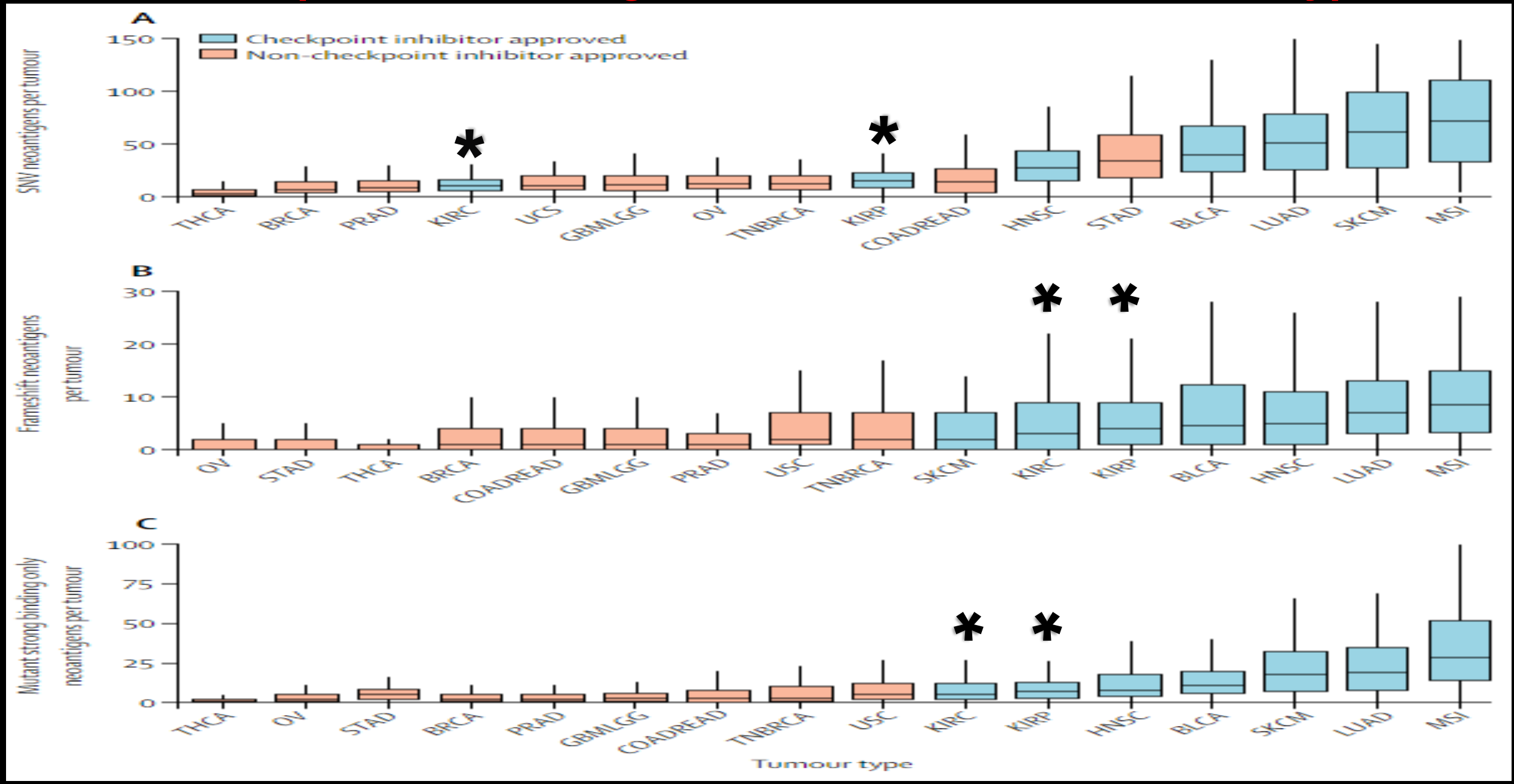
intrinsic and acquired resistance to ICB

Tumor Neoantigen Landscape Is Diverse and Sparse

P. Charoentona et al (2017) Cell Reports 18, 248

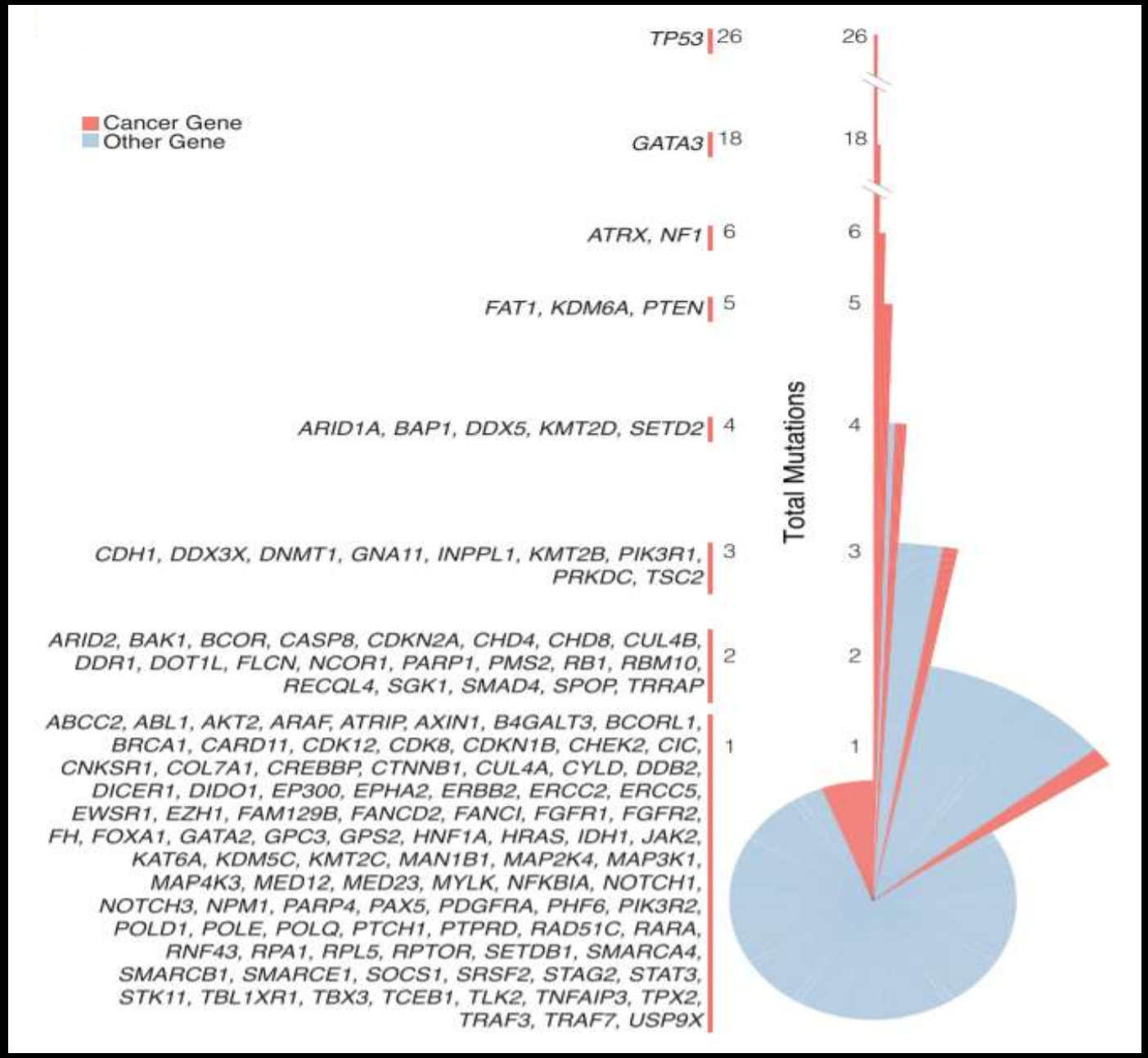
- 8243 samples from 20 solid tumor types
- 1 petabyte of genomic data ⟶ 50 gigabytes of structured immunogenomic data
- number of neoantigens correlated with mutational load
- 933,954 expressed nsSNV neoantigens (911,548 unique) originating from 893,960 somatic point mutations
- < 10% derived from driver genes and bulk from passenger genes
- only 24 of the 911,548 unique antigens were shared in at least 5% patients or more than one cancer type

Tumor-Specific Neoantigen Burden Across 19 Cancer Types



Adapted from: S. Turajlic et al. (2017) Lancet Oncology 18, 1009

Splice-Site Mutations Across Genes and 8656 TCGA Tumor Types

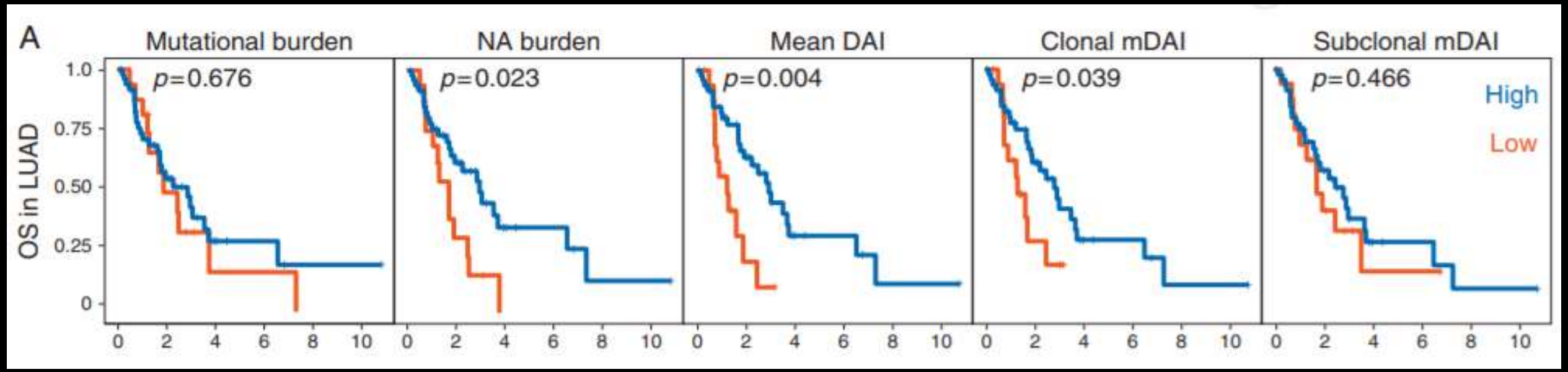


- **predicted higher MHC binding affinities (Net MHC4, NetMHCpan-3.0) for splice variants**
- **higher number of neoantigens (2-3x) in splice variants than nsSNV neoantigens**
- **elevated expression of PD-1, PD-L-1 in tumors with higher splice burden**

Adapted from: R. E. Jayasinghe et al. (2018) Cell Reports 23, 270

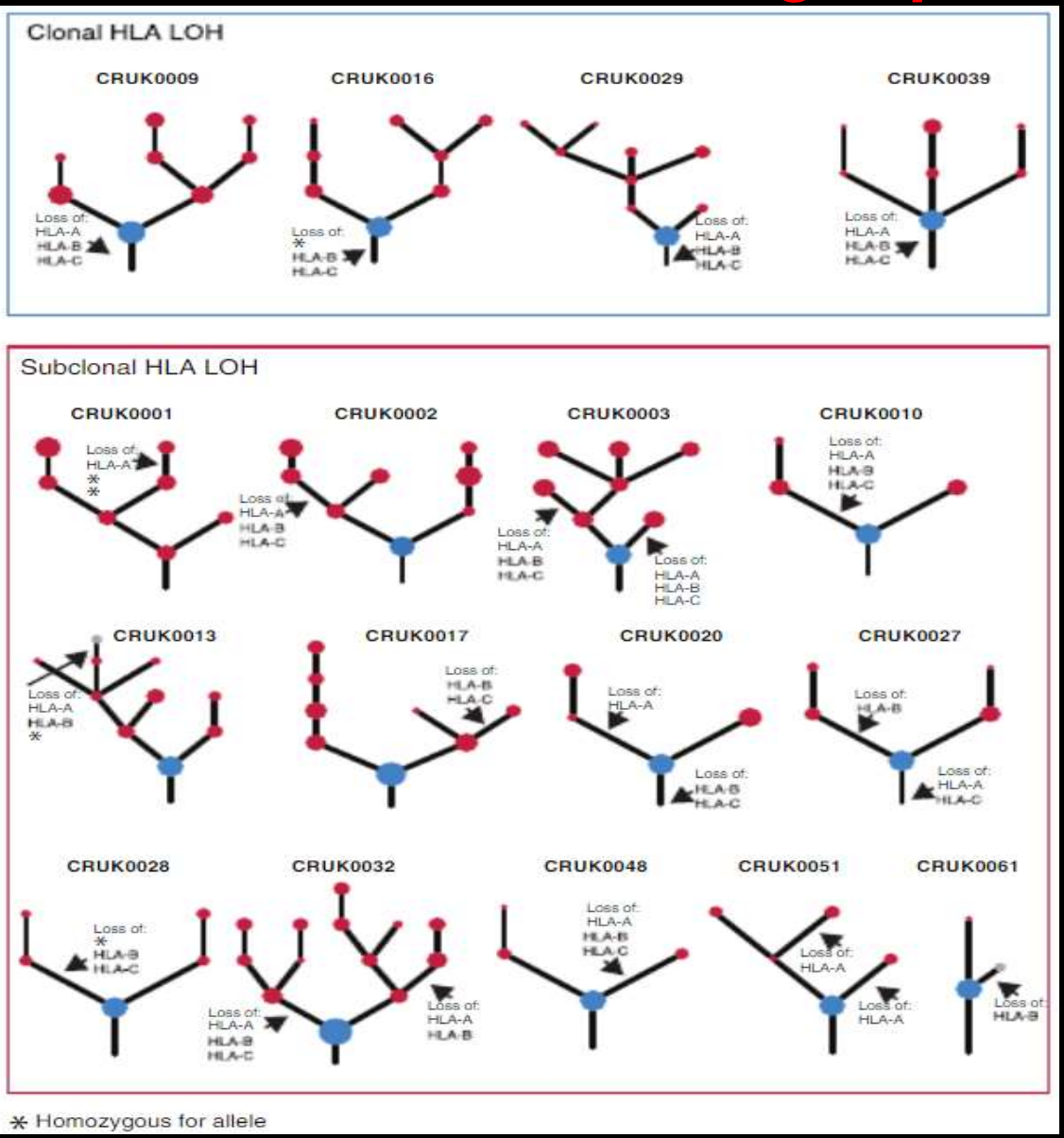
Mapping of Tumor Neoantigen Affinities for MHC Binding

- strong-binding neopeptides with MHC-I affinities <500nM associated with better response rates to ICB in NSCLC and melanoma
- differential agretopicity index (DAI= difference in binding affinities of wt/mutated peptides) as a potentially superior index of peptide immunogenicity than standard single neopeptide-MHC affinity profiling?



From: E. Ghorani et al. (2018) Annals of Oncology 29, 271-279

HLA Loss of Heterozygosity in Lung Adenocarcinoma and Lung Squamous Cell Carcinoma



- HLA loss of heterozygosity and failure to recognize antigenic peptides no longer presented on the lost allele
- enrichment of neoantigens predicted to bind with high affinity to lost vs retained HLA alleles
- higher frequency of LOH in subclones at metastatic sites
  - role of immune microenvironment(s) as a selective force in shaping branched clonal evolution?

From: N. McGranahan et al. (2017) Cell 171, 1259

The Need for Systems-Level Characterization of Immune-Response Networks in Different Cancer Types, Different Metastases and Different I/O Regimens

New Analytical Platforms for Increased Granularity for Classification of Immune Cell Subsets, Tumor (Sub)Clonality and Cellular ‘State Spaces’

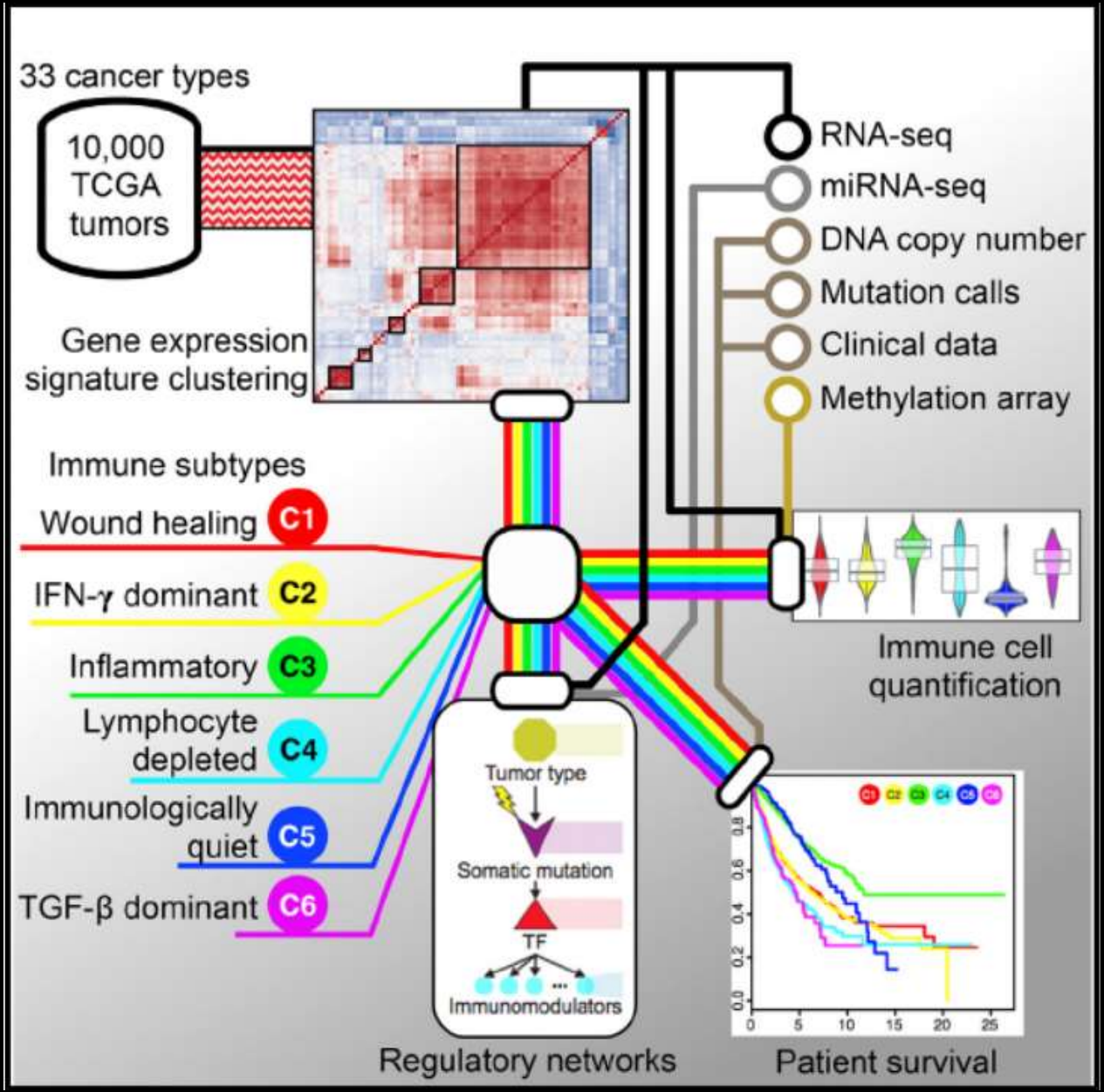
System-Level Characterization of Immune-Centric Networks in Disease

- high dimensional phenotyping on an unprecedented scale
  - from multiOmics profiling to stringent clinical annotation
  - standards: pre-analytical processing, assays, data formats
- daunting level of theoretical ‘large N’ combinatorial interactions
  - tumor (sub)clonal heterogeneity, diversity of immune cell subsets, signaling molecules and intracellular pathways
- linear and non-linear effects in input:output interactions and biological outcomes
- massive data
  - design of experimental protocols to integrate diverse datasets
  - data standards and format for data exchange and meta- analysis

Patient-Paired Sample Congruence (or Lack of!) in Two Commercial Liquid Biopsy Tests for Prostate Cancer

- G. Torga and K.J. Pienta (2018) JAMA Oncol. 4, 868
- blood sample cfDNA profiling by Guardant and Personal Genome Diagnostics Inc. (40 pts)
- concordance achieved in only 35% samples

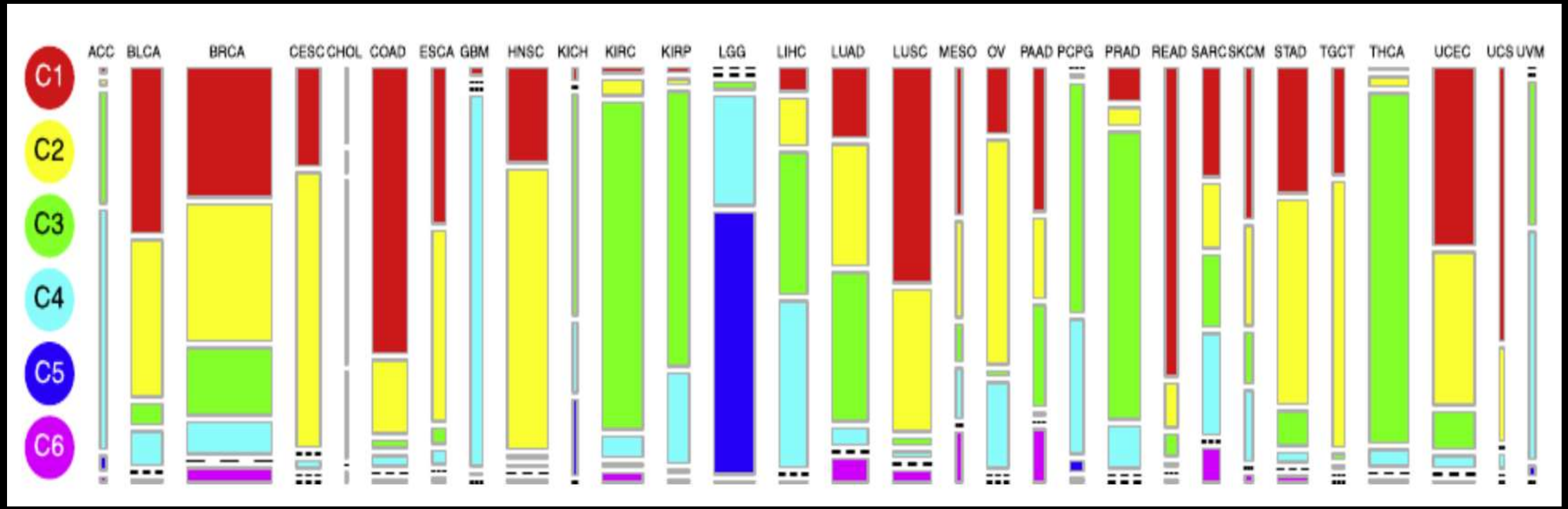
The Immune Landscape of Cancer



- 33 TCGA cancer types (30 solid tumor types)
- 11,180 samples
- patient age range 10-90 years (median 60)
- 22 immune cell subsets
- 166 immune expression signatures
  - 83 cancer context
  - 77 general validity for immune response
- 6 expression cluster groups
  - N = 2416, 2591, 2397, 1157, 385, 180

From: V. Thorsson et al. (2018) Immunity, 48, 812-830

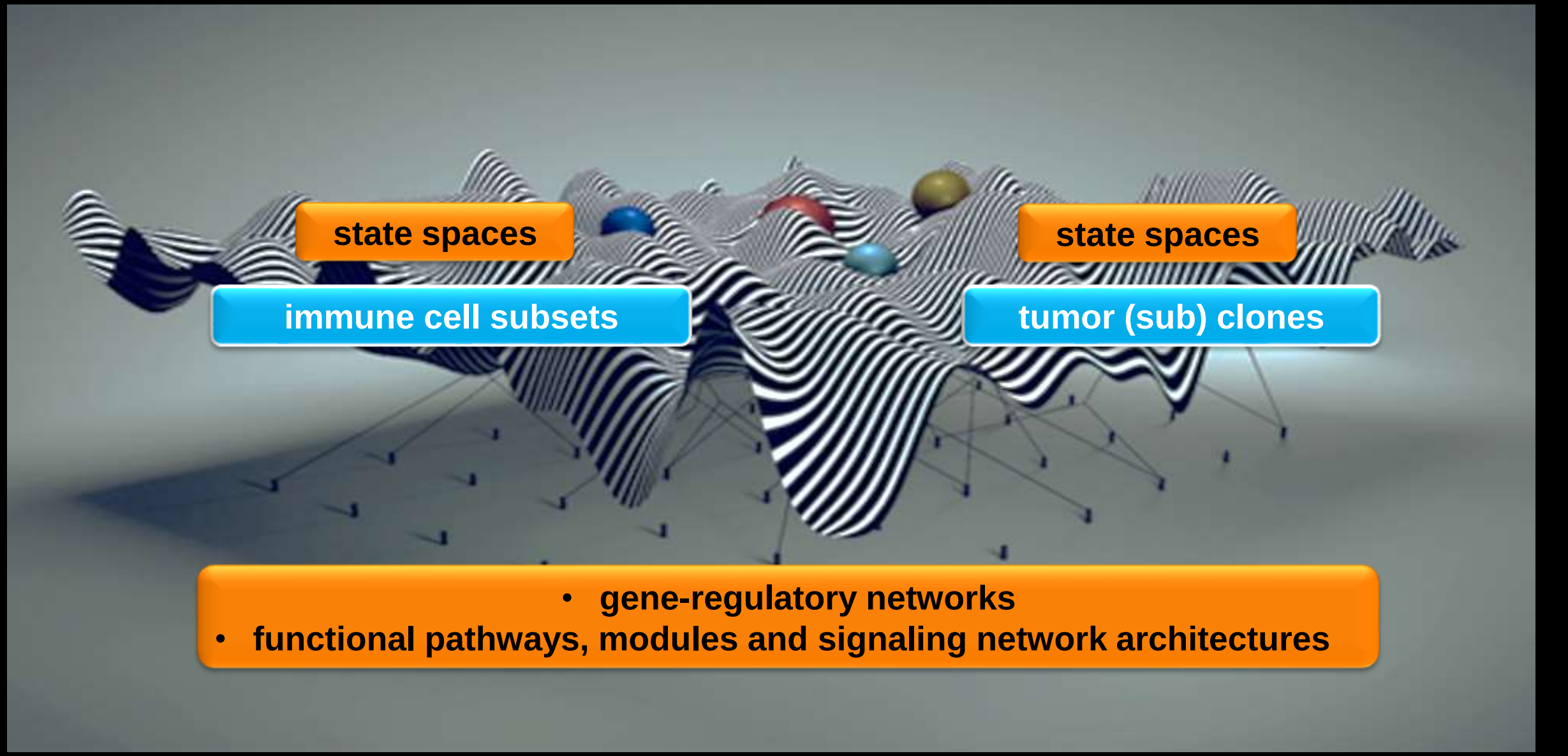
Distribution of Six Co-Clustered Immune Expression Signatures that Span Anatomic Location and Tumor Type in Analysis of 30 TCGA Tumor Subtypes (N=11,180)



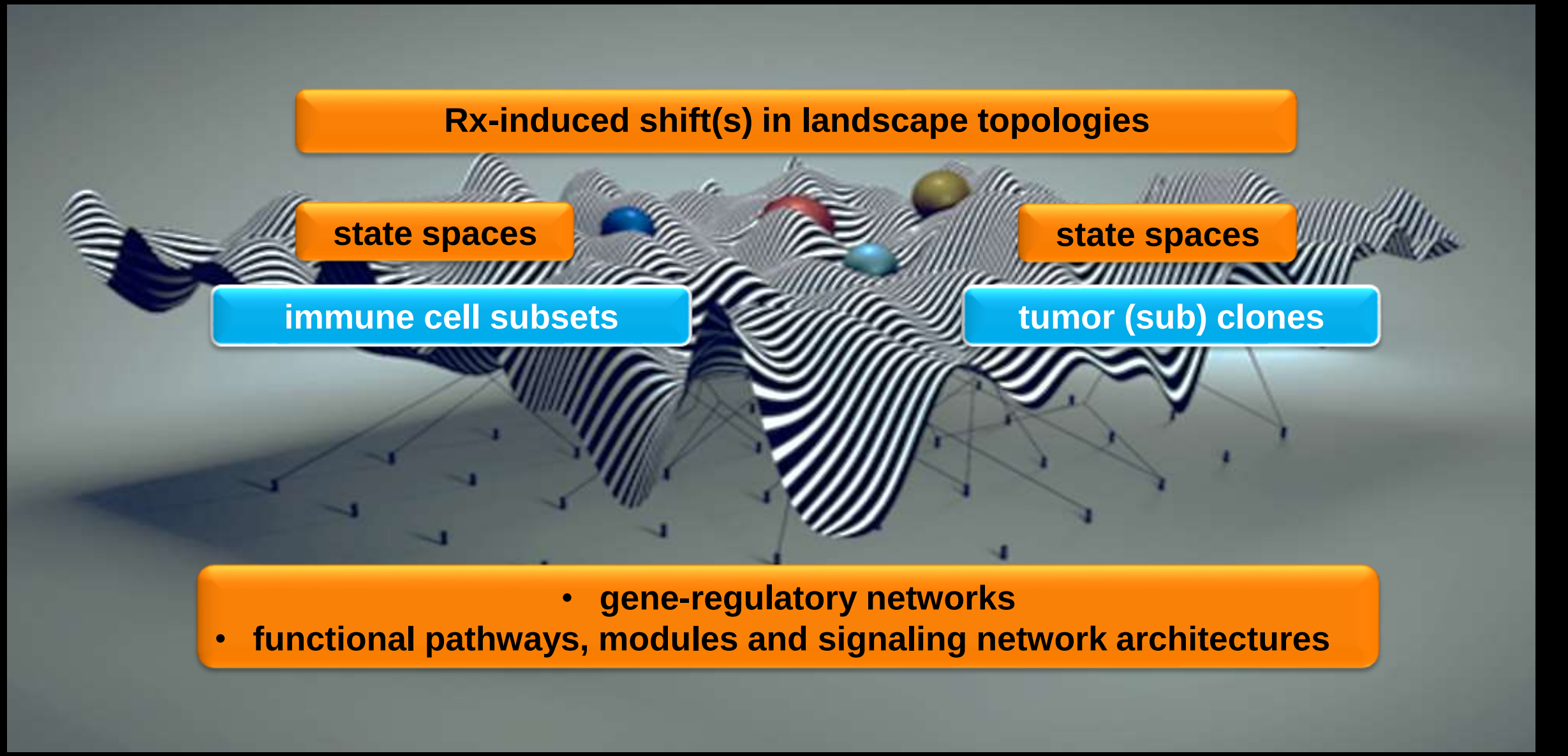
- top third includes cancers most responsive to ICB
  - lung AD, SC; melanoma; kidney (clear and papillary); head-neck; bladder
  - uveal melanoma and adrenocortical carcinoma lowest lymphoid fraction (LF)
  - glioma subtypes greatest LF range (related to microglia content ?)

From: V. Thorsson et al. (2018) Immunity, 48, 812

Multi–Attractor Landscapes, State Space Occupancies amd Co-Evolutionary Pathways in Complex Adaptive Systems



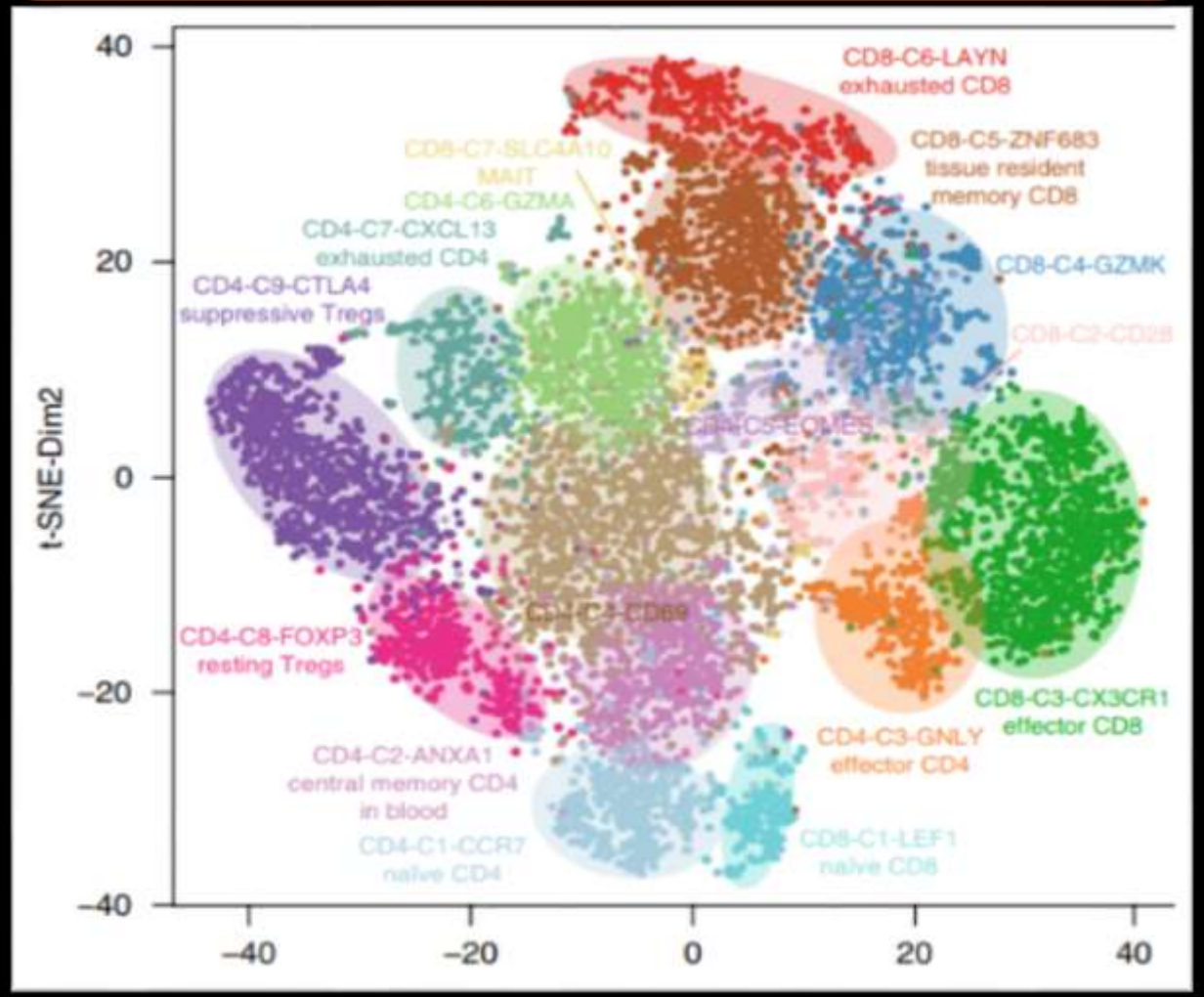
Multi–Attractor Landscapes, State Space Occupancies and Co-Evolutionary Pathways in Complex Adaptive Systems



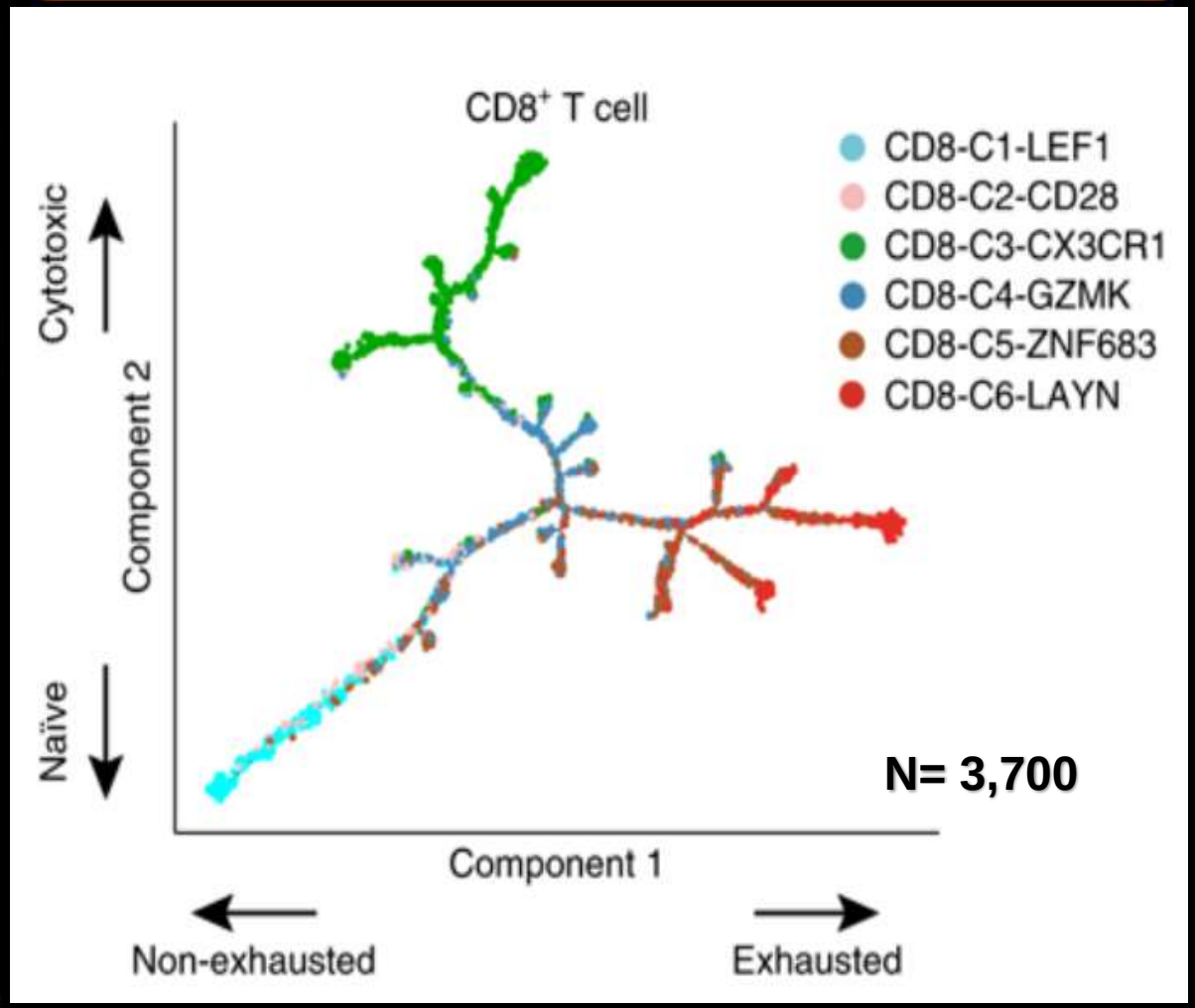
Understanding the (Pre) Exhaustion T Cell Phenotypes Induced by Upregulation of Immune Checkpoint Molecules and Chronic TCR Stimulation

Identification of T Cell ‘State Spaces’ and Responsiveness to Reinvigoration by ICB (and other I/O Rx)

t-SNE Clusters (7) of Single T Cell Subsets in NSCLC

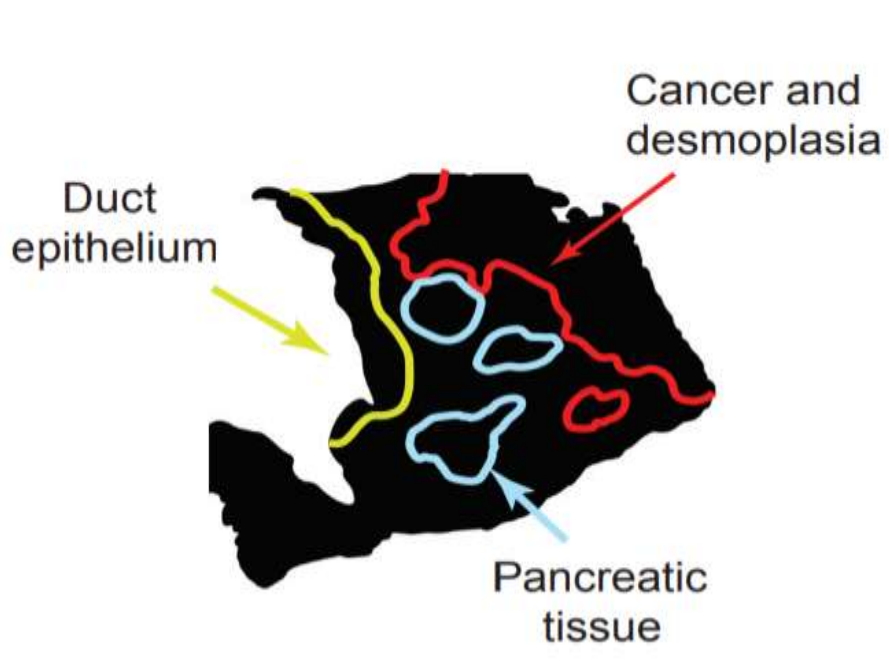


Single Cell Analysis of CD8+ Cell State Transitions in NSCLC

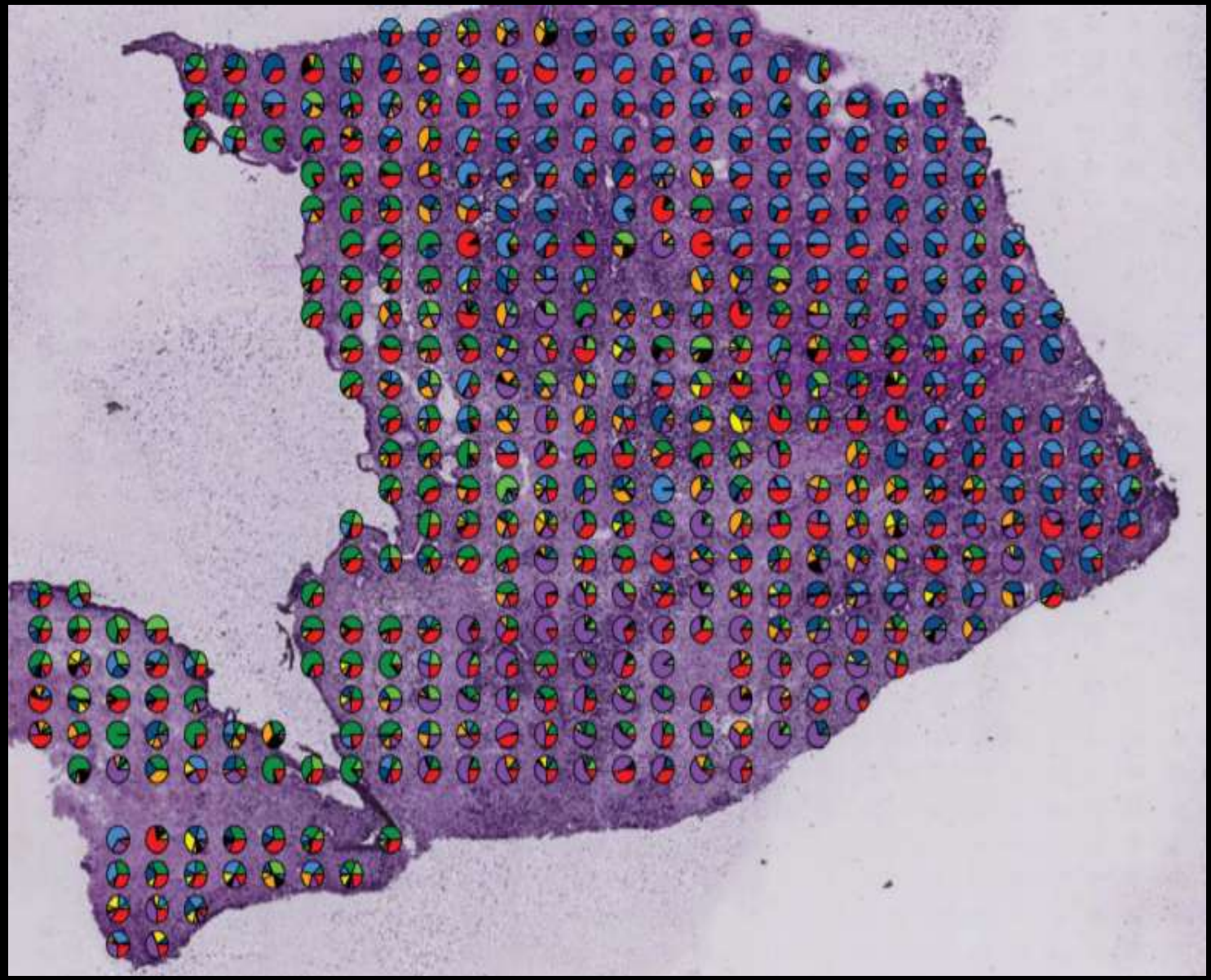


Adapted from X. Guo et al. (2018) Nat. Med. 24, 479

Integration of scRNA Seq and Spatial Transcriptomic in the Analysis of Immune Cell Subsets in Pancreatic Ductal Carcinoma



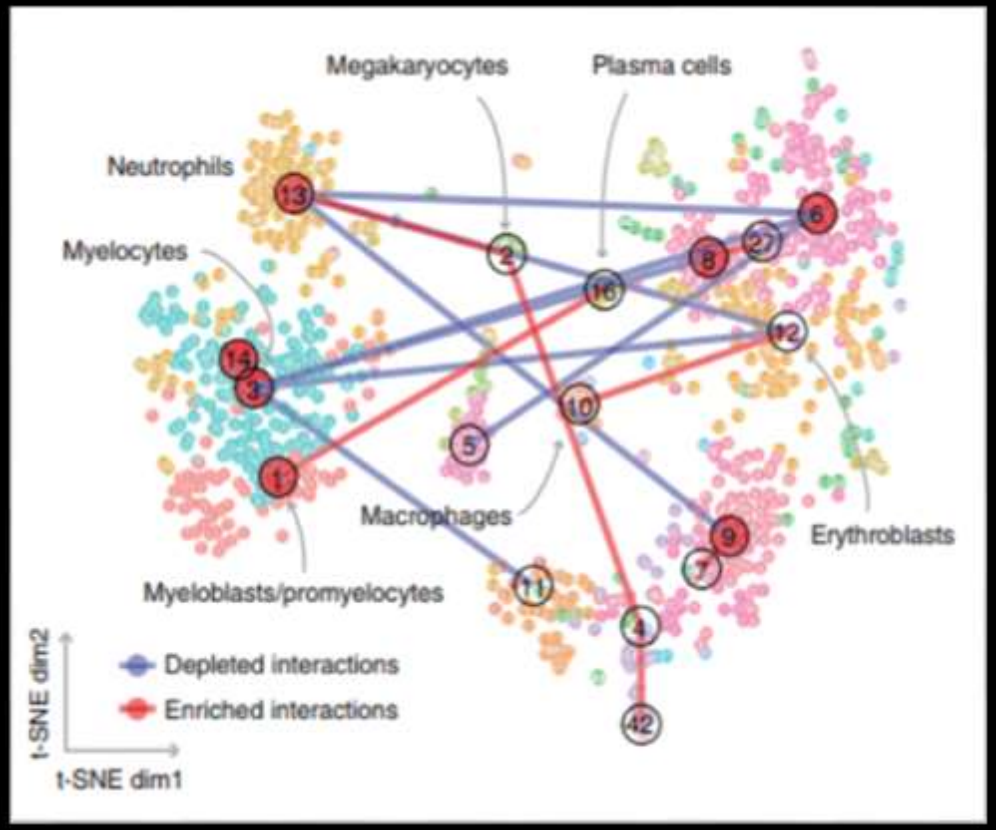
- Cancer cells A
- Cancer cells B
- Cancer cells C
- Fibroblasts
- Macrophages
- Tuft cells
- CD8 T-cells
- Red blood cells
- Acinar cells
- Ductal cells



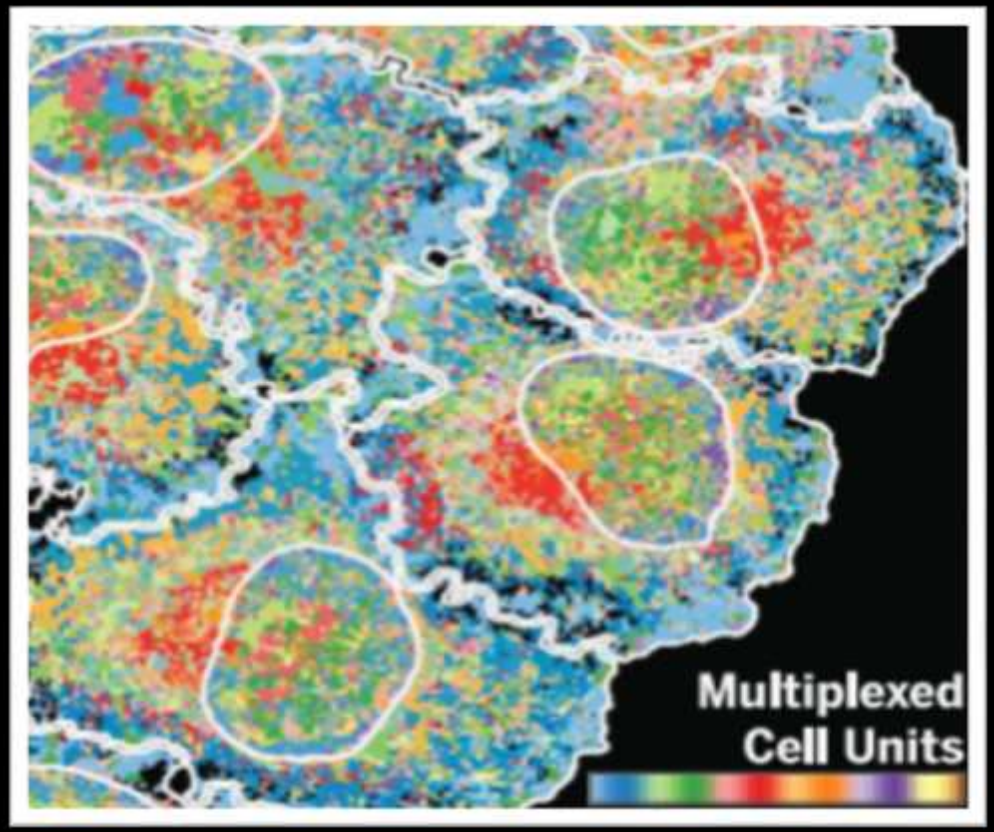
Adapted from: R. Moncada et al. (2018) bioRxiv254375

Spatial Transcriptomics

- microdissection and microfluidics capture of double/triplet cells to define state spaces generated by physical interactions
- profiling from tissue sections
- new automated imaging methods with high (40) multiplex immunofluorescence analysis of frequency of cell type adjacencies and protein expression

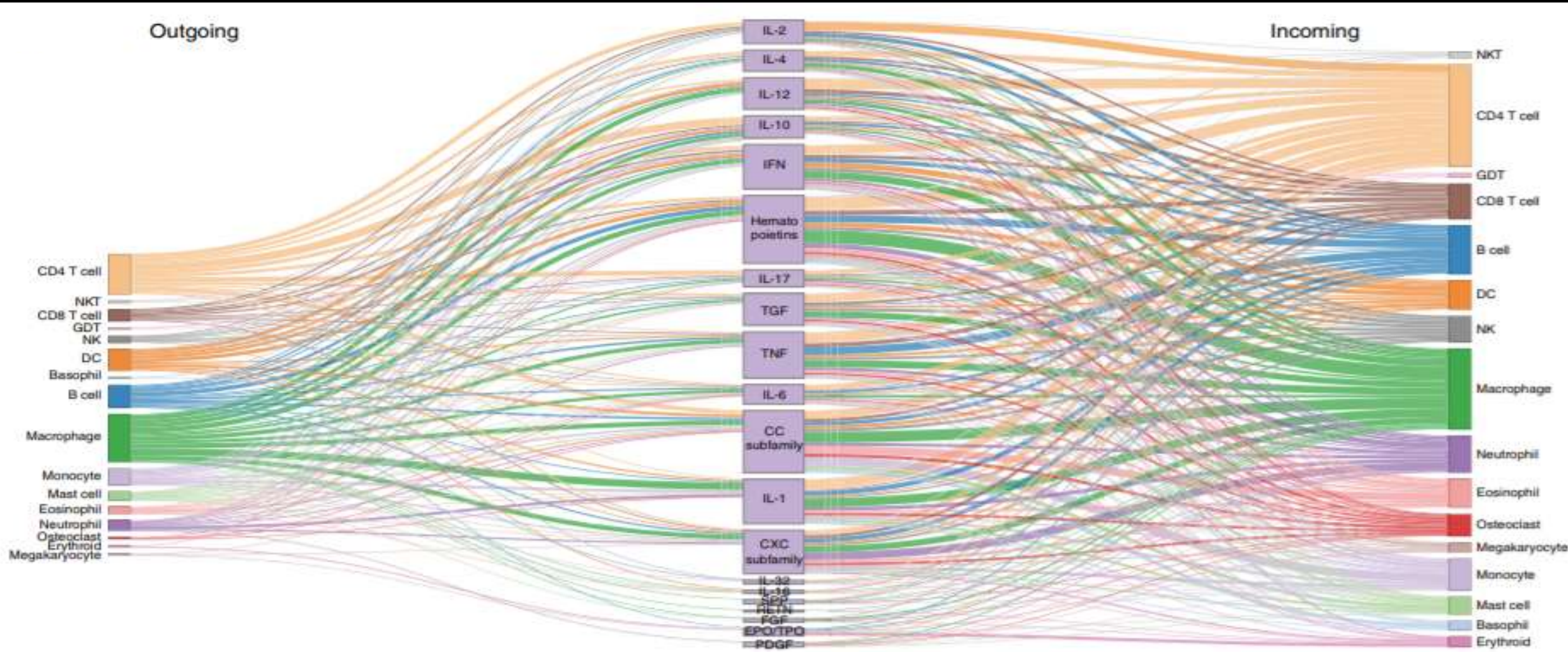


J.C. Boisset et al. (2018) Nat. Methods 15, 547

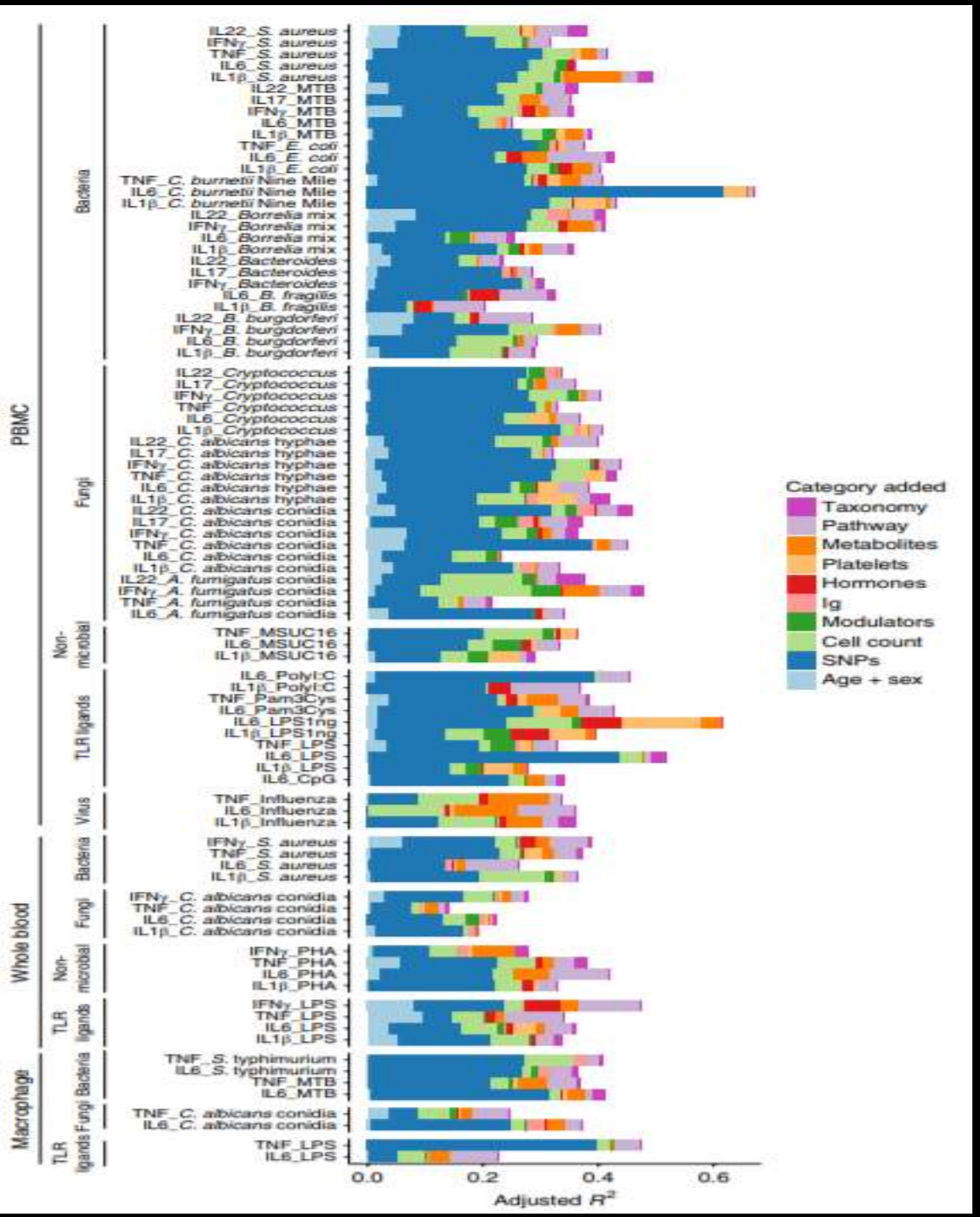


G. Gut et al. (2018) Science 361, 468

System-Level Sankey Plot of Inter-cellular Cytokine-Immune Cell Subset Interactions Derived from Large Scale Literature Analysis (immunoXpresso)



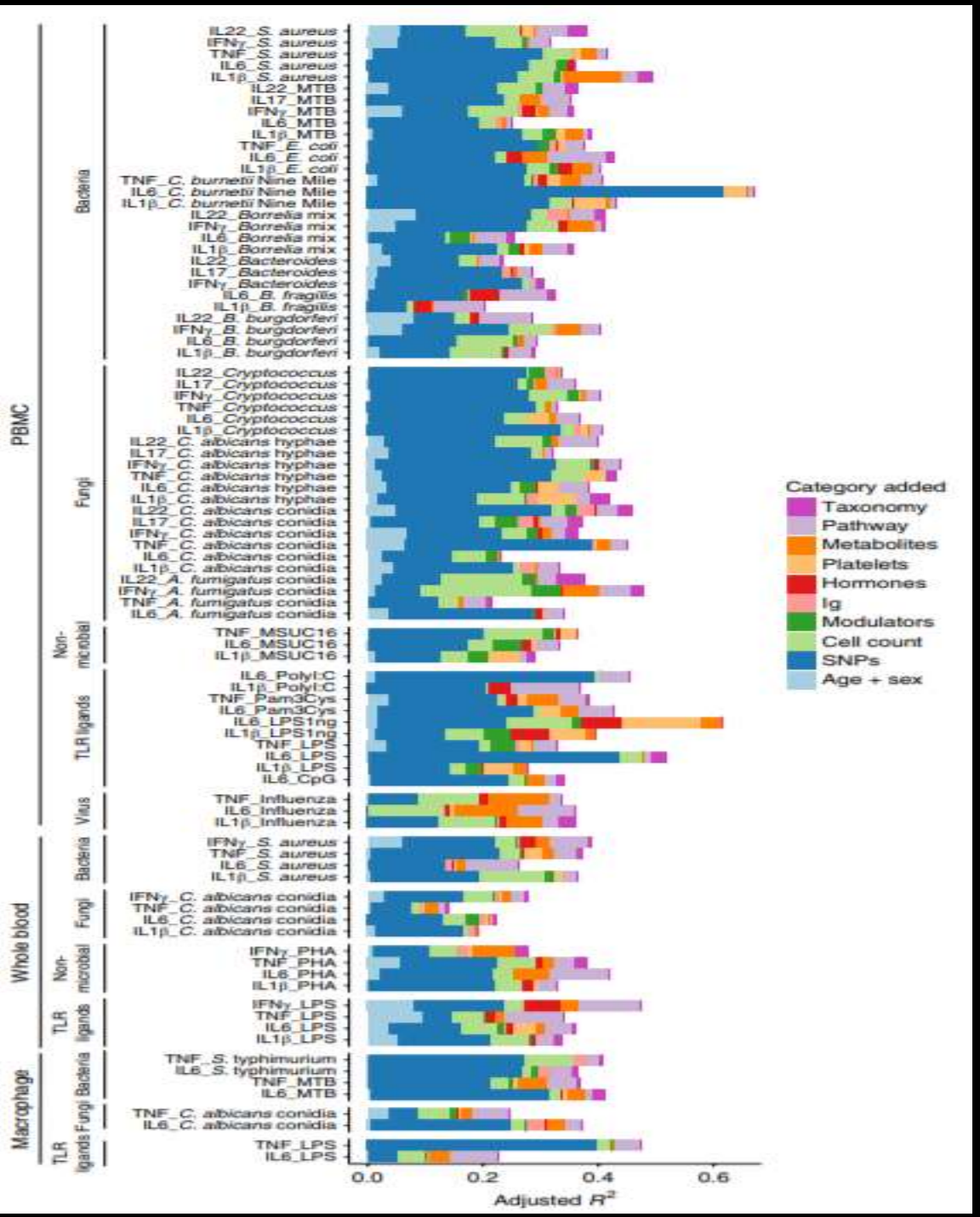
From: K. Kveler at al. (2018) Nature Biotechnol. 36, 651



Deep Phenotyping and ID of Genetic Contributions to Individual Variation in Cytokine Responses

O.B. Bakker et al. (2018) Nature Immunology 19, 776

- 91 cytokine-stimulus pairs in 534 individuals elicited by 20 pathogens
- 70% heritability



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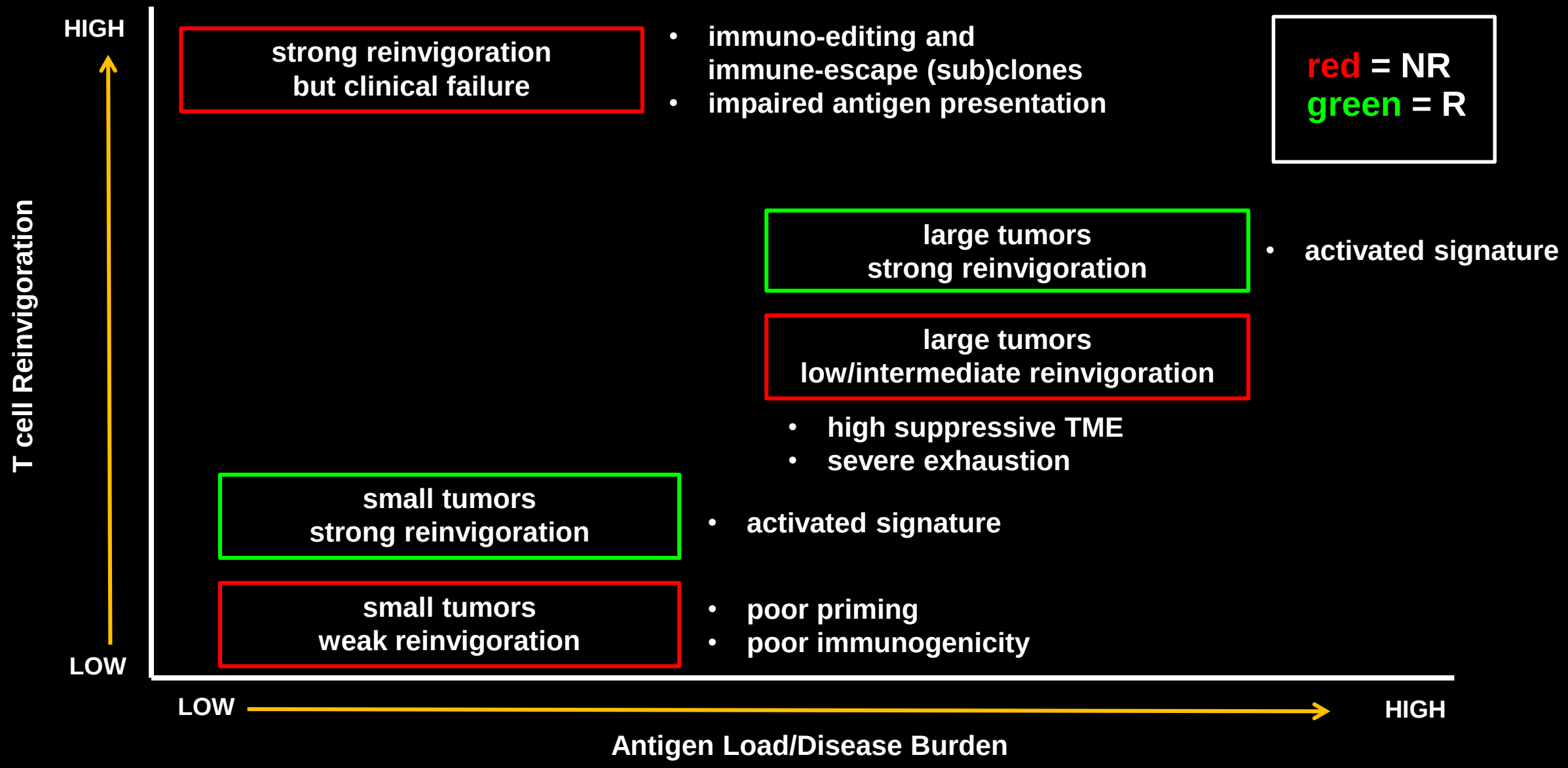
do germ line variants in lymphokine/cytokine affect responsiveness to I/O therapy and AE risk (cytokine release syndrome)?

A Conceptual Framework for Efficacy of ICB Therapy



Adapted from A.C. Huang et al. (20017) Nature 545, 60

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

- handicap of ‘static’ snapshot of immune profile in resected tissue lesions/biopsies versus longitudinal monitoring of dynamic changes in tumor progression/Rx responses
- how far do blood-based (liquid biopsy) assays mirror intra-tumoral events in anatomically dispersed metastases?
  - ctDNA and mutanome profiling?
  - immune cell subsets and trafficking?
  - cytokine signaling networks?
  - exosome species?

Exosomes and Modulation of Immune Functions

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

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
  - B cell-derived exosomes stimulate primed $CD4^+$ T cells but not naïve T cells
- transfer of tumor neoantigens to dendritic cells (DCs) and stimulation of naïve $CD4^+$ T cells
- stimulation of pro-inflammatory M1 phenotype in macrophages

Immune Suppression by Cancer-Derived Exosomes

- exosomal PD-LI suppression of $CD8^+$ T cells
- decreased production of immunostimulatory molecules
  - IL-2, IL-17 and IFN-γ
- increased levels of immune inhibitory proteins
  - TGF-β, IL-10, COX-2
- miR212 induction of reduced MHC II expression
- miR222, miR494 and M1 to M2 shift in tumor associated macrophages
- immunosuppression of B cells by exosome membrane-associated CD39 and CD73 via production of extracellular adenosine
- impaired differentiation of DCs from bone marrow precursors
- enhanced survival of MDSC via activation of STAT3
- inhibition of NK cells by exosome surface MICA/MICB liquids, TGF-LAG and induction of inhibitory TGF-β

Blood-based Exosome Profiling

ADAPT™ BIOTARGETING SYSTEM

V. Domenyuk et al. (2017) Nature Sci. Reports 7, 42741

- 10^{12} exosomes/ml
- proprietary aptamer oligonucleotide library for capture of diverse exosome species based on surface ligands
  - unbiased target identification
  - NGS readout of selective binding
- identify cell-of-origin
- quantitative and qualitative disease-associated changes in membrane proteins and internal cargo

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

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

The Need for Standards in Immuno-Profiling, Data Curation, Annotation and Inter-operable Database Design

- recognition in development of nearly 40 Minimum Information for Biomedical Investigations (MIBBI)
  - https://fairsharing.org/collection/MIBBI
- evolving framework for immune datasets
  - Minimal Information for Adaptive Immune Receptor Repertoire

  F. Rubelt et al. (2017) Nat. Immunol. 18, 1274
- journals have different (or no) policies to standardize nomenclature and access to raw data/analytical computer code(s)

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

Influence of the Gut Microbiome on Immunotherapy Responses

- possible role of dysbiosis in creating chronic inflammation and immunosuppressive phenotype
- limited insight into the roles of diet and microbial metabolites on cancer risk, progression and I/O Rx response
- effect of oral antibiotics on I/O efficacy

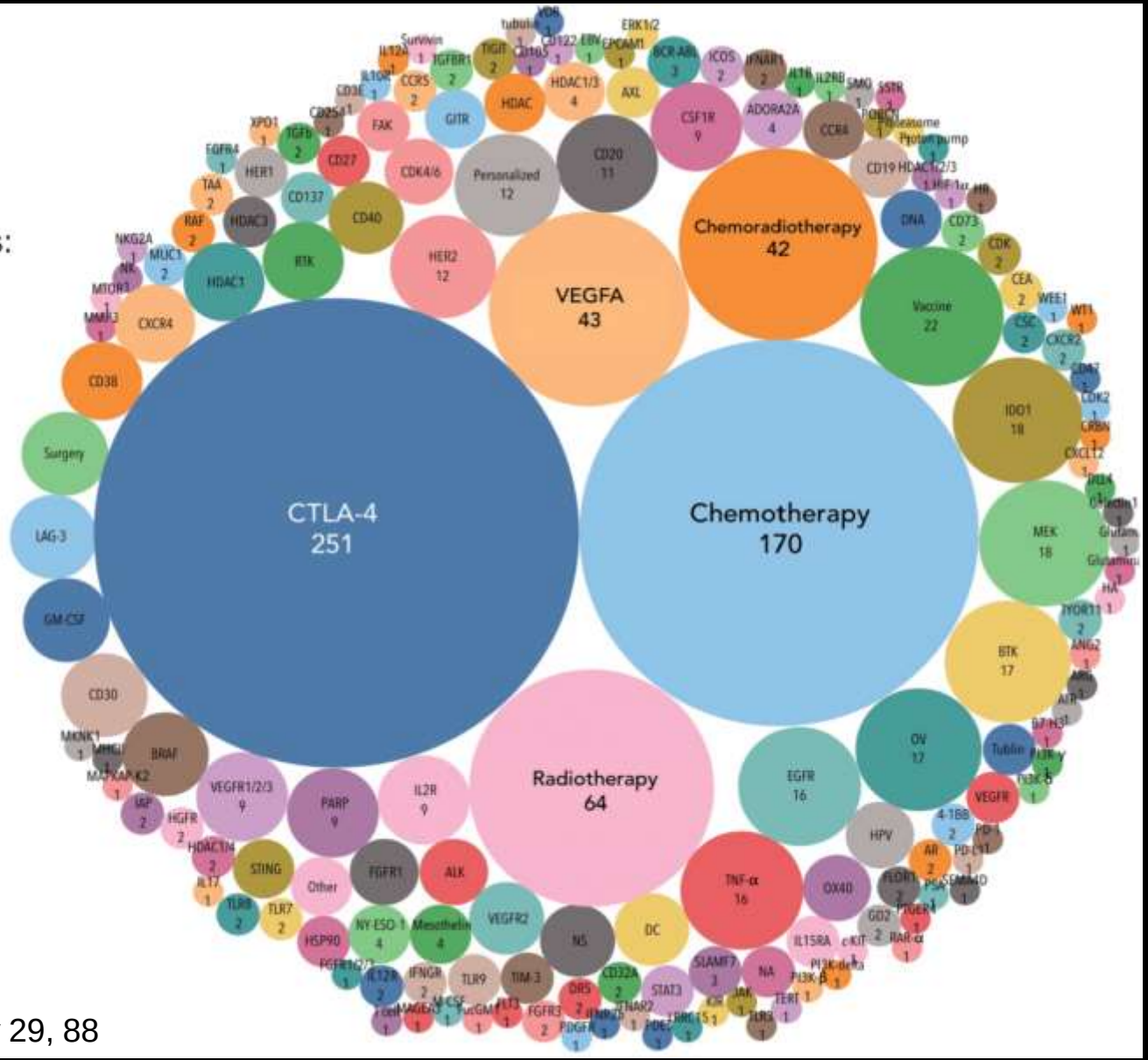
Will Combination Regimens Increase Response Rates in Immuno-Oncology?

- within and across cancer types
- dramatic expansion in combination trials
  - biological rationale for MOAs of the selected agents
  - dosing and sequence, duration, toxicity profile
- limitations of preclinical models for new I/O agent discovery and evaluation of combination regimens
  - organoids, orthotopic human xenografts

Will Combination I/O Regimens Increase Pan-Cancer Response Rates
Targets of Anti-PD-1/PD-L1 Combination Clinical Trials

Numbers of trials using common combo strategies:

1. Anti-CTLA-4 agents: 251
2. Chemotherapies: 170
3. Radiotherapies: 64
4. Anti-VEGFA agents: 43
5. Chemoradiotherapy combos: 42



From: J. Tang et al. (2018) Annals of Oncology 29, 88

Now Comes the Really Hard Part!

meeting public (and political) expectations

- balancing hype versus hope
- realism of likely time frame (and cost) of major gains in next-generation I/O therapies
  - efficacy within and across malignancies
  - combination Rx (current agents)
  - 2/3rd generation I/O agents

ethics

- societal (cost) and individual (futile Rx) implications of unrestricted use of first-generation I/O absent predictive markers to stratify responder and non-responder cohorts

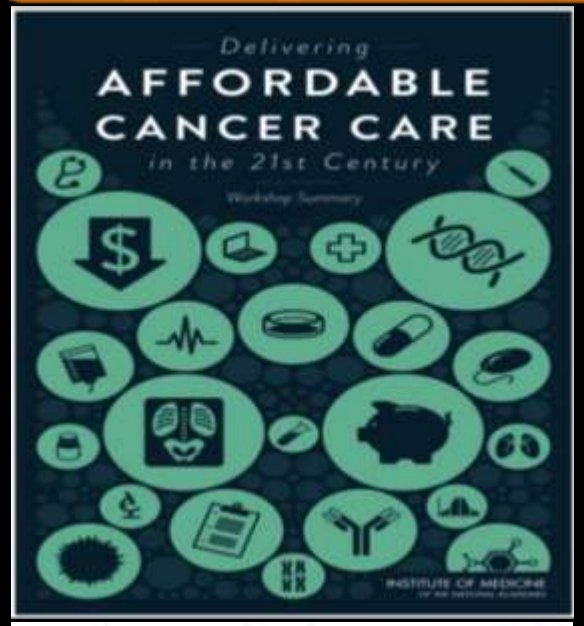
DTC Saturation Advertising Campaigns

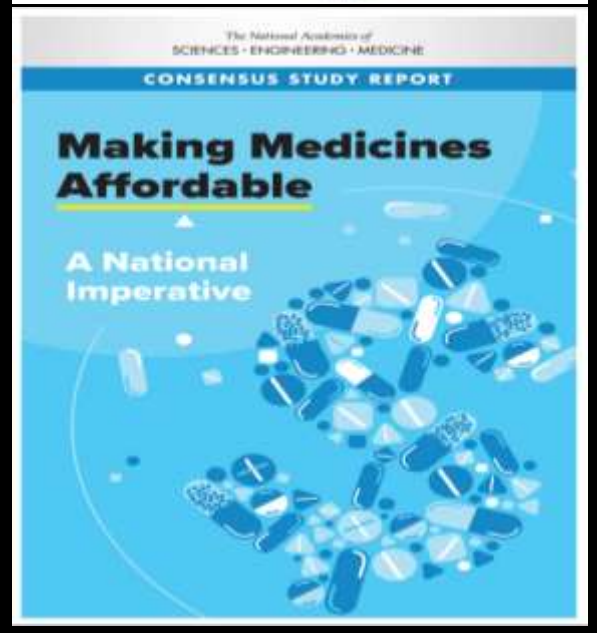
A Delicate Ethical Balance: Come and Be Cured by Us: (Go Elsewhere at Your Peril)!

Immunotherapy: Hype and Hope

- deserved recognition of ICB as major therapeutic advance
- media hype, scale of corporate DTC and AMC advertising campaigns emphasizing responders
  - distorts public awareness that NR>R
  - unrealistic patient expectations of successful outcome
  - potential for serious toxicities
  - cost of futile therapy in NR patients/toxicity risk
- patient demands for immunotherapy despite no evidence of efficacy in their specific malignancy

Cancer Treatment Cost





Value Frameworks for Oncology

ASCO®

ICER
INSTITUTE FOR CLINICAL AND ECONOMIC REVIEW

Drug Pricing Lab
Memorial Sloan Kettering

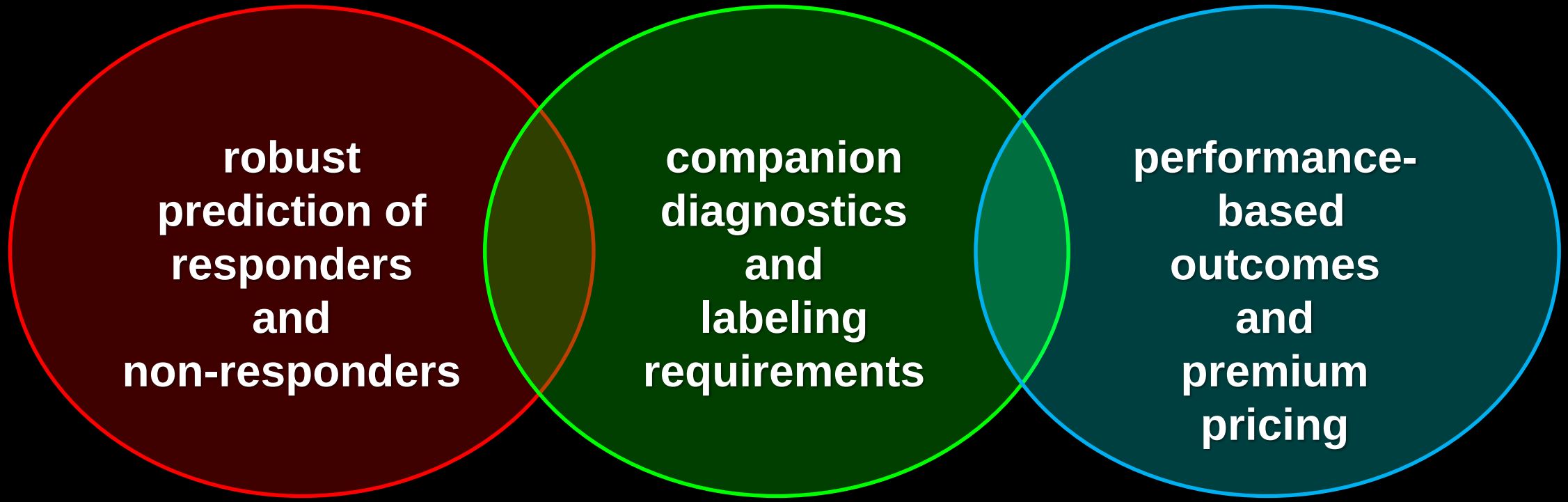
Is the Bar in the ASCO Value Frameworks Too High in Assessing Long-Term Benefits in I/O Therapy?

- JAMA Oncology (2018) 4,326

  - analysis of approved I/O agents (2011 to 8/17)

- 23 indications for 6 I/O agents for metastatic solid tumors
- only 3 gained durable survival bonus points under ASCO framework

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



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

risk sharing

The Urgent Clinical and Economic Imperatives for Predictive Markers to Differentiate Responder and Non-Responder Patients in Different I/O Regimens

- single most important opportunity for the (bio)pharmaceutical industry in advancing rational immunotherapy?
- increased payer pressure for performance-based outcomes and premium pricing?
- proactive industry engagement or reactive response to payer imposition?

Slides Available @ http://casi.asu.edu/presentations