Major Questions for Development of Immunotherapies Requiring Animal Models

Workshop on Challenges, Insights, and Future Directions for Mouse and Humanized Models in Cancer Immunology and Immunotherapy

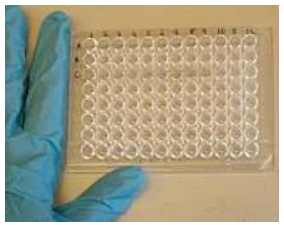
SITC

Washington, DC

November 10, 2016

Biological molecule

Role in Immunity



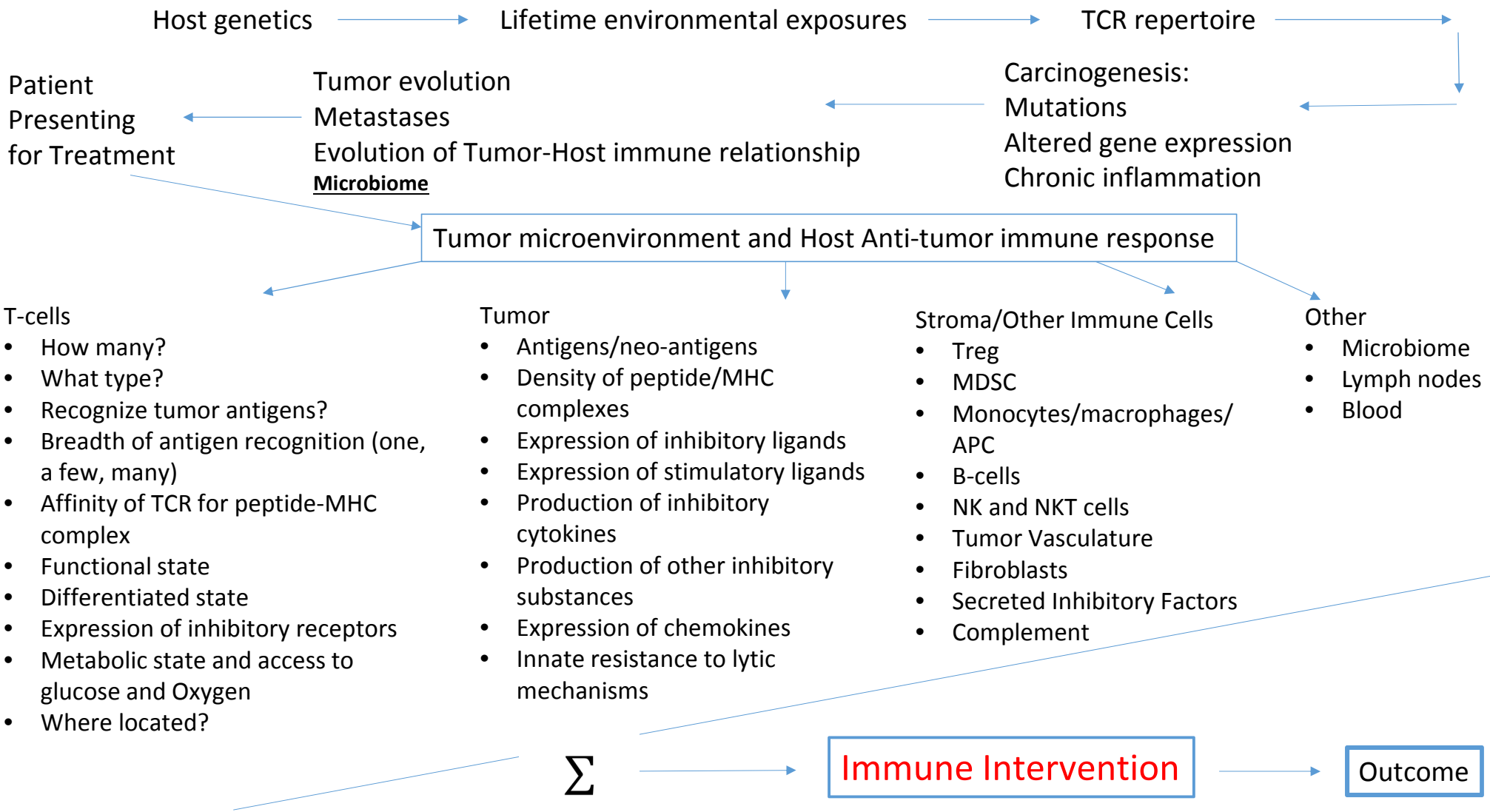
Relevance to Human Immunity

Role in Anti-Tumor Immunity
Pharmacology

Toxicity

Human Trial:

- Toxicity
- Dose/Schedule
- Immunologic effects
- Effect on immune Tumor Microenvironment
- Anti-Tumor effect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TCR repertoire
Carcinogenesis:
Mutations
Altered gene expression
Chronic inflammation
Tumor evolution
Metastases
Evolution of Tumor-Host immune relationship
Microbiome
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Tumor microenvironment and Host Anti-tumor immune response
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What type?
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Breadth of antigen recognition (one, a few, many)
Affinity of TCR for peptide-MHC complex
Functional state
Differentiated state
Expression of inhibitory receptors
Metabolic state and access to glucose and Oxygen
Where located?
Tumor
Antigens/neo-antigens
Density of peptide/MHC complexes
Expression of inhibitory ligands
Expression of stimulatory ligands
Production of inhibitory cytokines
Production of other inhibitory substances
Expression of chemokines
Innate resistance to lytic mechanisms
Stroma/Other Immune Cells
Treg
MDSC
Monocytes/macrophages/ APC
B-cells
NK and NKT cells
Tumor Vasculature
Fibroblasts
Secreted Inhibitory Factors
Complement
Other
Microbiome
Lymph nodes
Blood
Σ
Immune Intervention
Outcome

Tumor-specific T cells are contained in the PD-1+ TIL population and are functional after in vitro culture

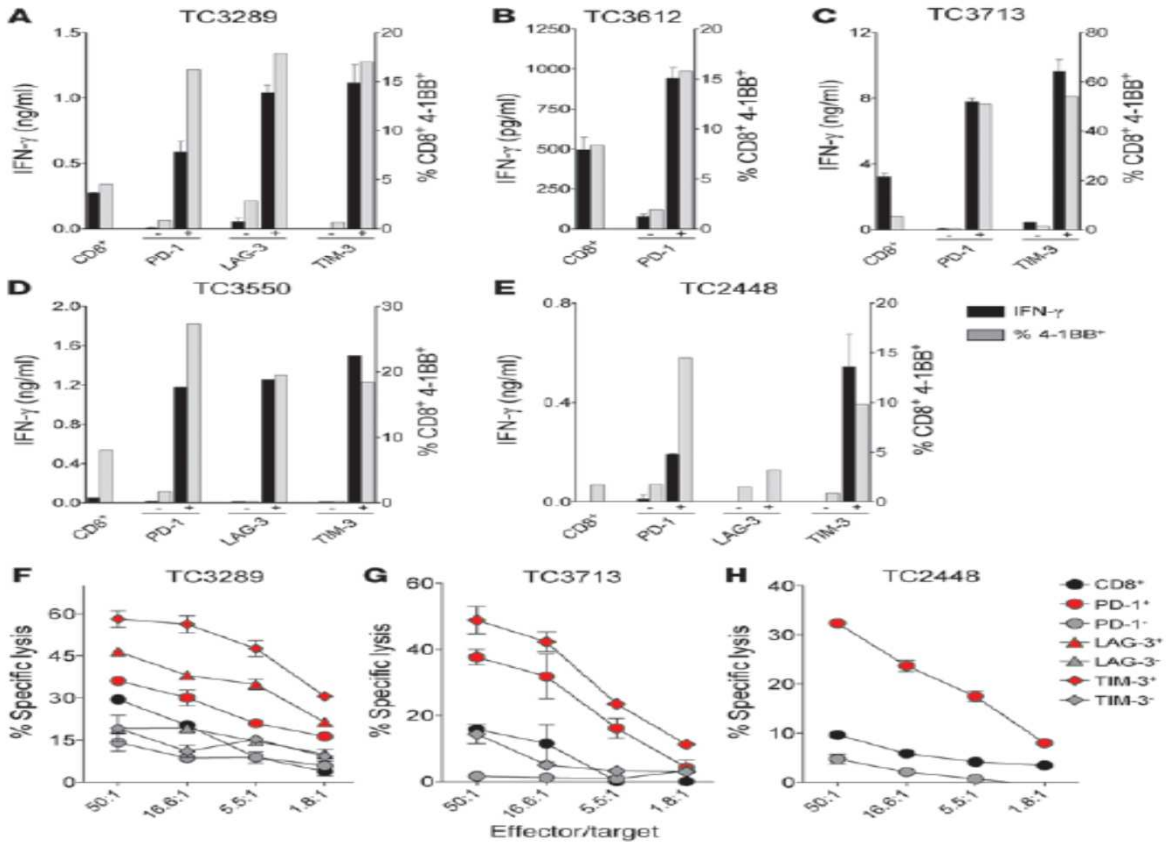
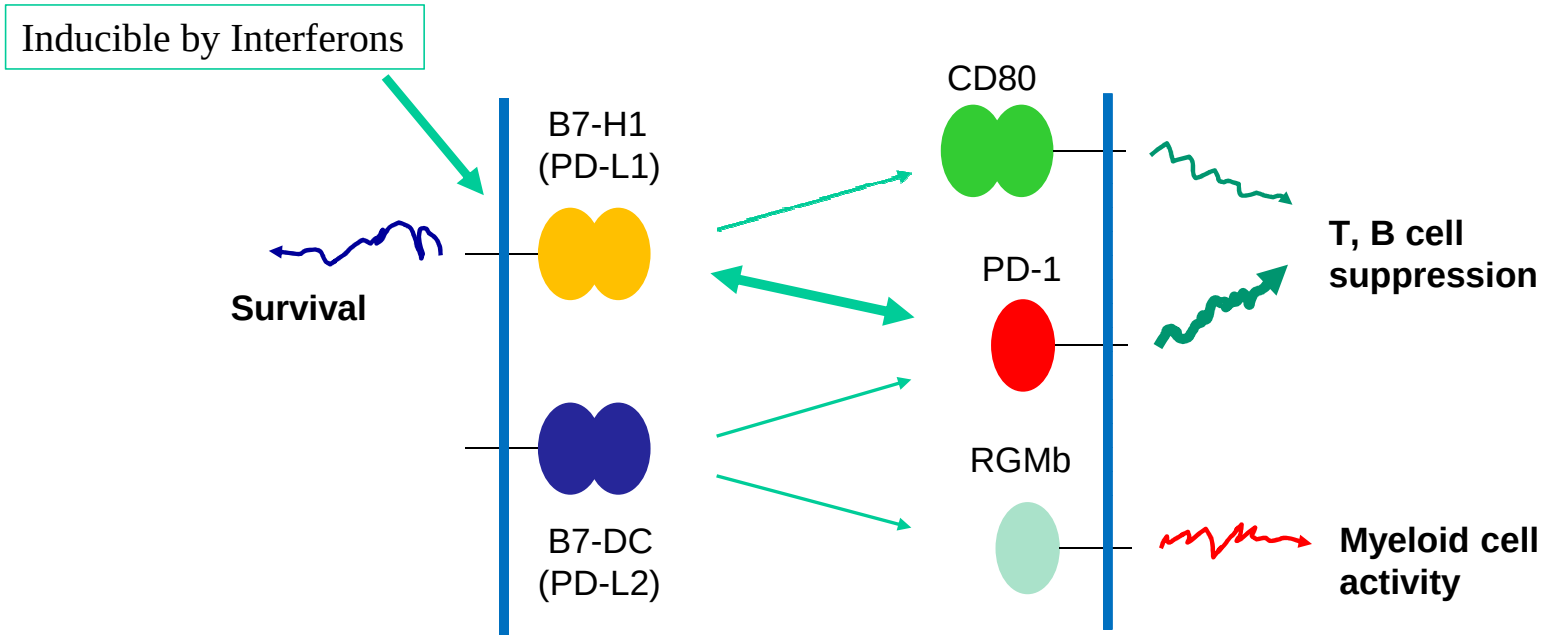


Figure 3
Recognition and lysis of autologous tumor by CD8+ TILs sorted based on PD-1, LAG-3, and TIM-3 expression. Bulk CD3+CD8+ TILs were sorted to high purity from FrTu3289, FrTu3612, FrTu3713, FrTu3550, and FrTu2448 based on positive or negative expression of PD-1, LAG-3 and/or TIM-3, and expanded in vitro for 15 days. (**A**–**E**) Response of fresh tumor–derived TILs to their respective autologous tumor cell lines, TC3289 (**A**), TC3612 (**B**), TC3713 (**C**), TC3550 (**D**) and TC2448 (**E**). Reactivity was assessed by measuring IFN-γ release (duplicates, mean ± SD) and frequency of 4-1BB upregulation. (**F**–**H**) Cytolytic activity of fresh tumor–derived TILs in response to their respective autologous tumor cell lines, TC3289 (**F**), TC3713 (**G**), and TC2448 (**H**). Percentage of specific lysis at different effector/target ratios is shown as mean ± SD.

The PD-L1/PD-1 Pathway



Slide courtesy of Lieping Chen

Spectrum of PD-1/PD-L1 Antagonist Activity

Active

- **Melanoma**
- **Renal cancer (clear cell and non-clear cell)**
- **NSCLC – adenocarcinoma and squamous cell**
- Small cell lung cancer
- **Head and neck cancer**
- Gastric and gastroesophageal junction
- **MMR-repair deficient tumors (colon, cholangiocarcinoma)**
- Bladder
- Triple negative breast cancer
- Ovarian
- Hepatocellular carcinoma
- Thymoma
- Mesothelioma
- Cervical
- **Hodgkin lymphoma**
- Diffuse large cell lymphoma
- Follicular lymphoma
- T-cell lymphoma (cutaneous T-cell lymphomas, peripheral T-cell lymphoma)
- **Merkel cell**

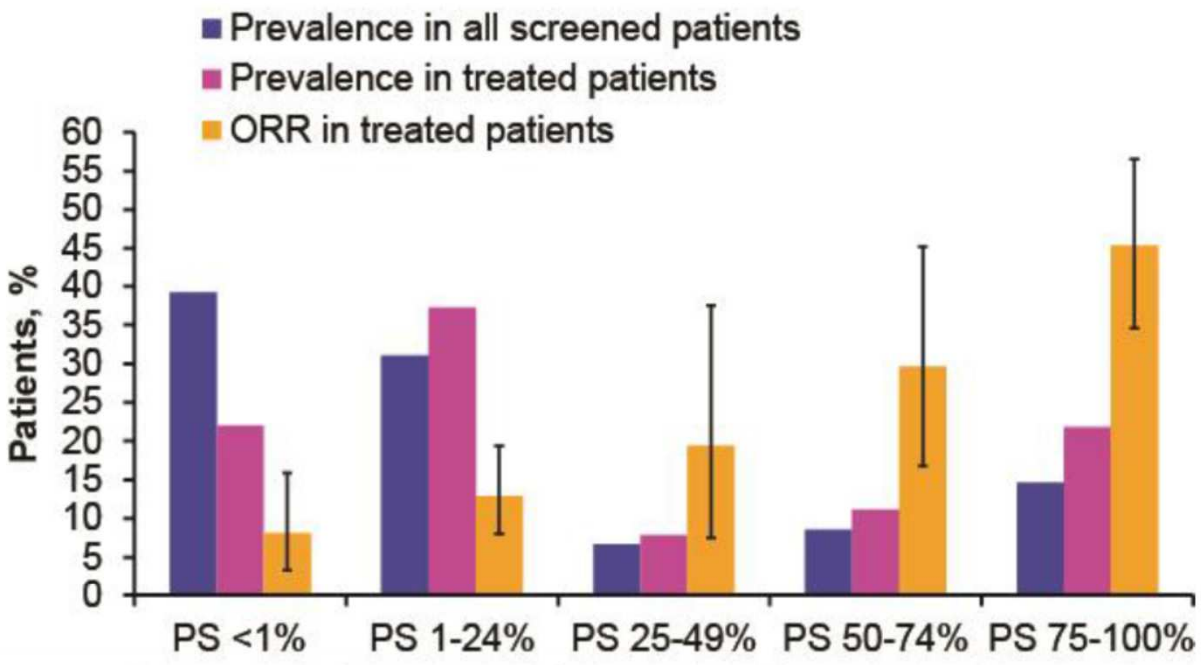
Minimal to no activity

- Prostate cancer
- MMR+ (MSS) colon cancer
- Myeloma
- Pancreatic cancer

Major PD-1/PD-L1 antagonists

- Nivolumab (anti-PD-1)
- Pembrolizumab (anti-PD-1)
- Atezolizumab (MPDL3280, anti-PD-L1)
- Durvalumab (anti-PD-L1)
- Avelumab (anti-PD-L1)

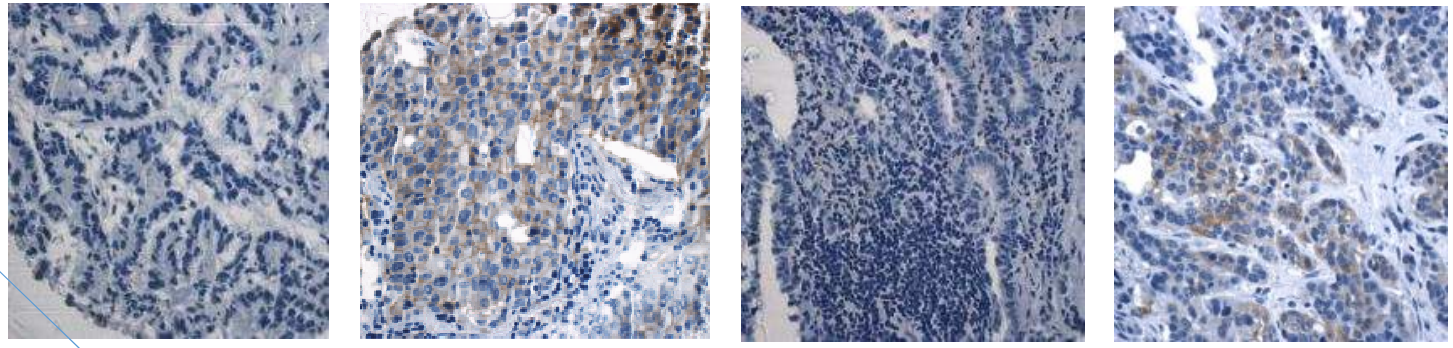
Pembrolizumab ORR in NSCLC by PD-L1 Tumor Expression



All screened patients, n (%)	323 (39.2)	255 (31.0)	55 (6.7)	71 (8.6)	120 (14.6)
All treated patients, n (%)	87 (22.0)	147 (37.2)	27 (6.8)	39 (9.9)	72 (18.2)
ORR in treated patients, n (%) [95% CI]	7 (8.1) [3.3-15.9]	19 (12.9) [8.0-19.4]	6 (19.4) [7.5-37.5]	13 (29.6) [16.8-45.2]	39 (45.4) [34.6-56.5]

Presence of PD-L1 or TILs[1]

PD-L1⁻/TIL⁻	PD-L1⁺/TIL⁺	PD-L1⁻/TIL⁺	PD-L1⁺/TIL⁻



45%	17%	26%	12%
Type 1	**Type 2**	**Type 3**	**Type 4**
45%	41%	13%	1%

NSCLC

Melanoma

Schalper and Rimm, Yale University

Taube et al

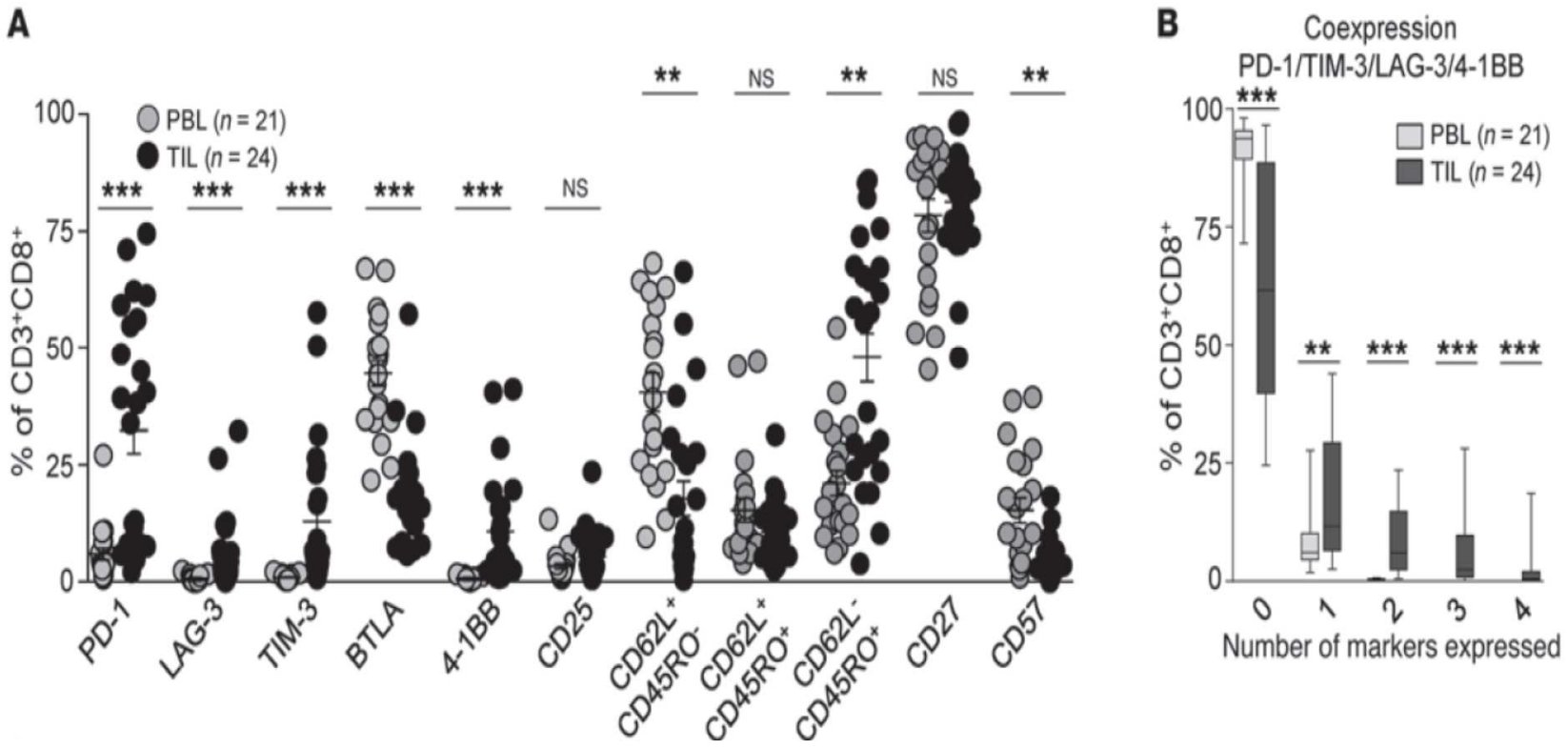
Table 2. Correlation of B7-H1 expression by melanocytes with the presence of immune cell infiltration.

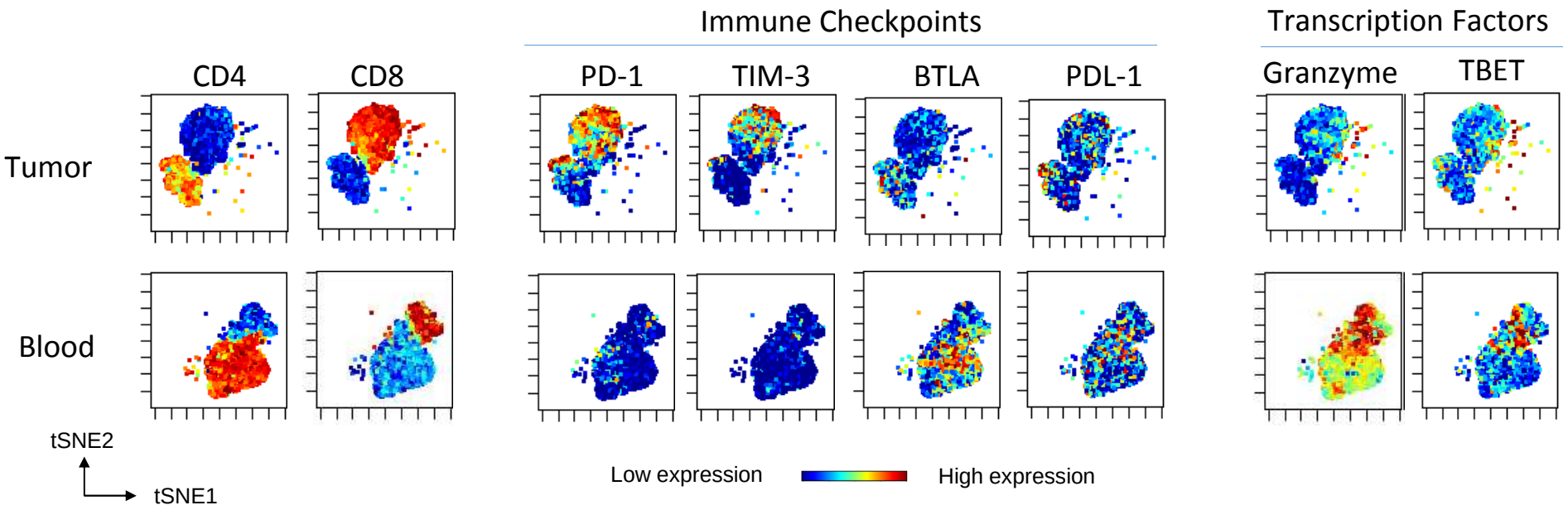
Histology	Total	Number of cases/total cases (%)				*P**
		B7-H1⁺†		B7-H1⁻		
		TIL⁺‡	TIL⁻	TIL⁺	TIL⁻	
Benign nevi	40	14/14 (100)	0/14 (0)	4/26 (15)	22/26 (85)	<0.0001
Primary melanomas (in situ or invasive)	54	19/19 (100)	0/19 (0)	15/35 (43)	20/35 (57)	<0.0001
Metastases	56	23/24 (96)	1/24 (4)	7/32 (22)	25/32 (78)	<0.0001
All	150	56/57 (98)	1/57 (2)	26/93 (28)	67/93 (72)	<0.0001

*Fisher's exact test, two-sided, was conducted on the 2 × 2 matrix defined by B7-H1 (±) expression and TIL (±) for each lesion type. †More than 5% melanocytes with membranous expression on IHC. ‡Including mild, moderate, and severe lymphocyte infiltrates and their associated histiocytes/macrophages.

Melanoma TIL – Expression of Co-inhibitory and Co-stimulatory Receptors (Gros et al)

The Journal of Clinical Investigation http://www.jci.org Volume 124 Number 5 May 2014



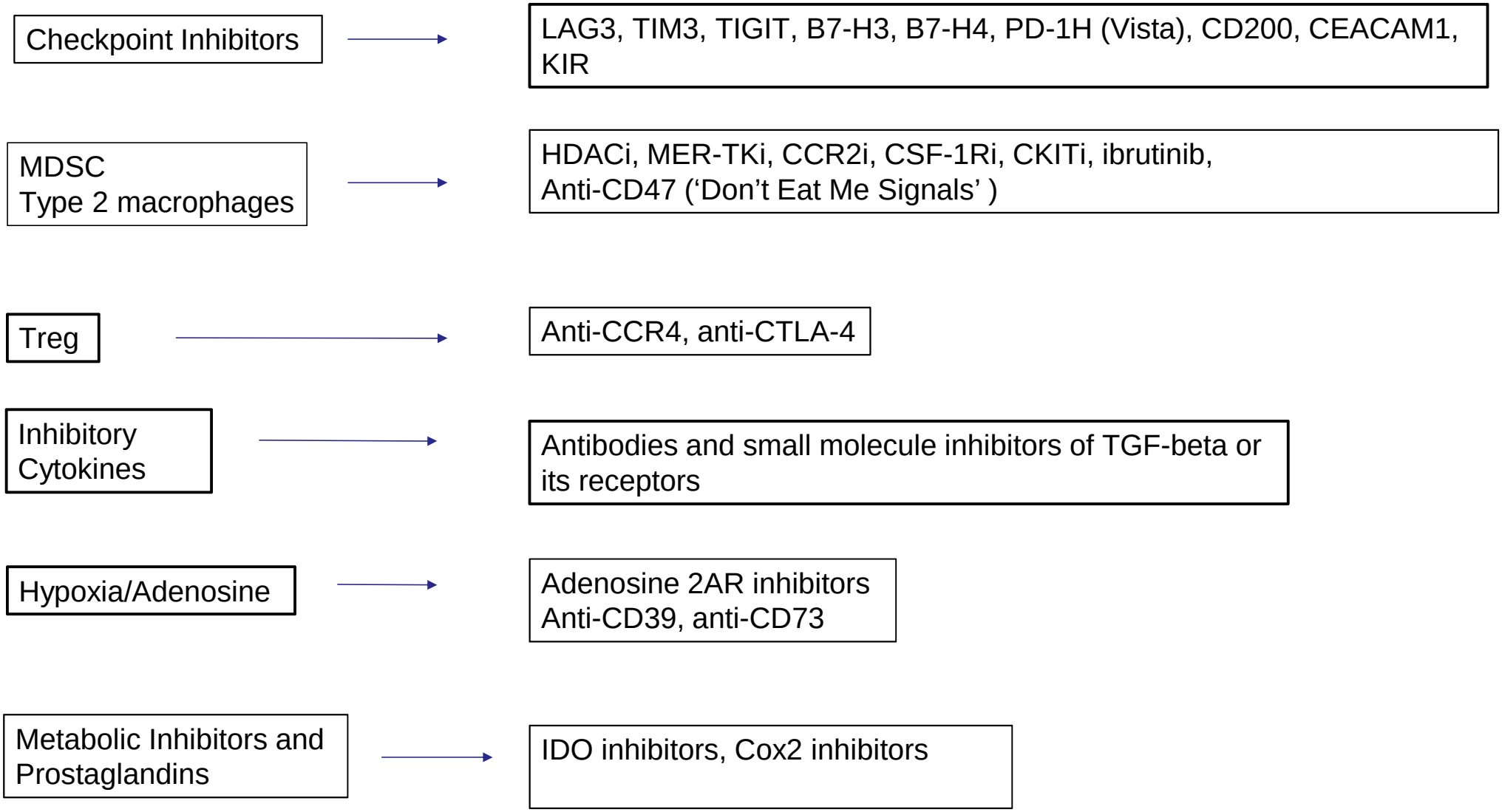


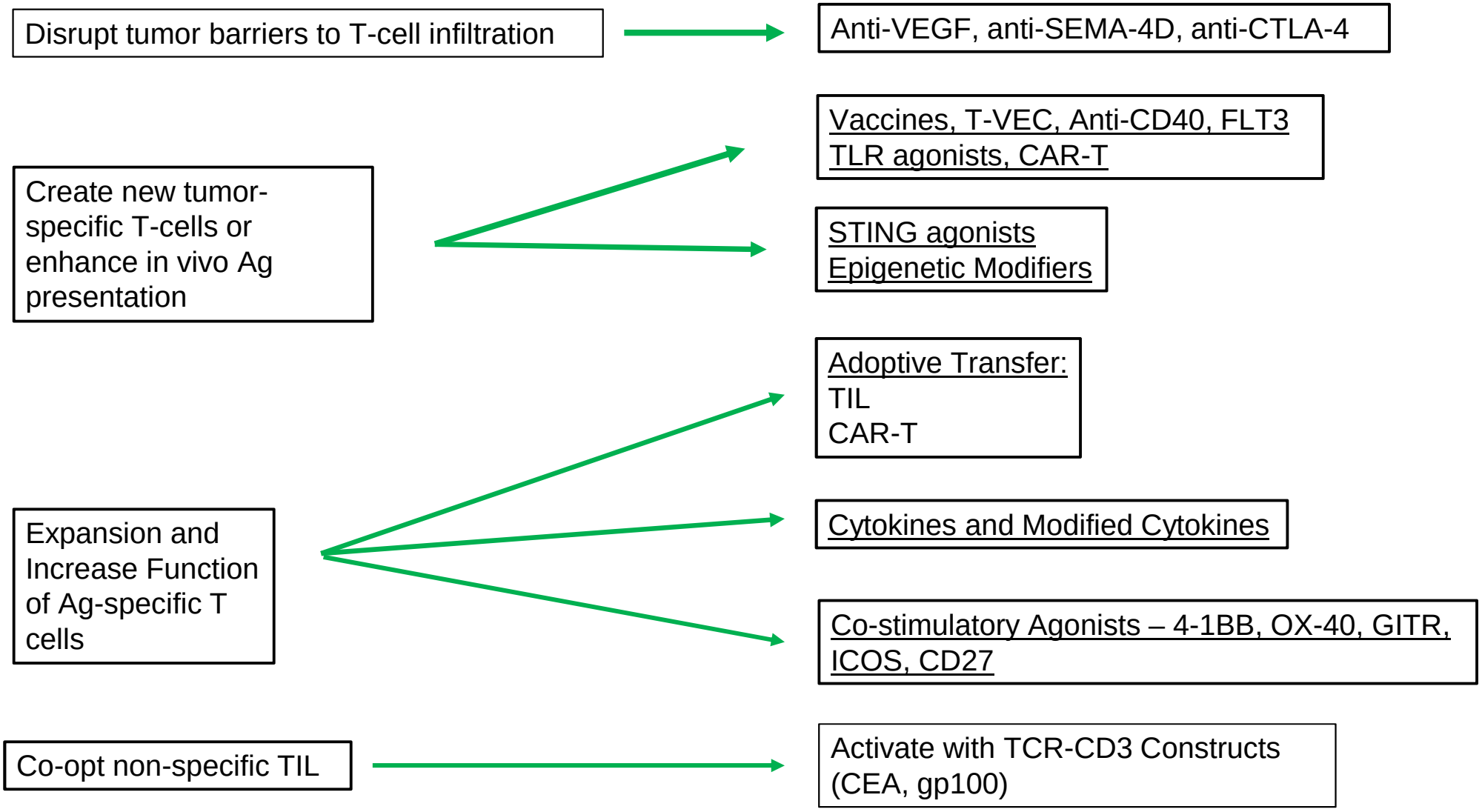
CD3 ViSNE

Provided by Kavita Dhodapkar, Yale University

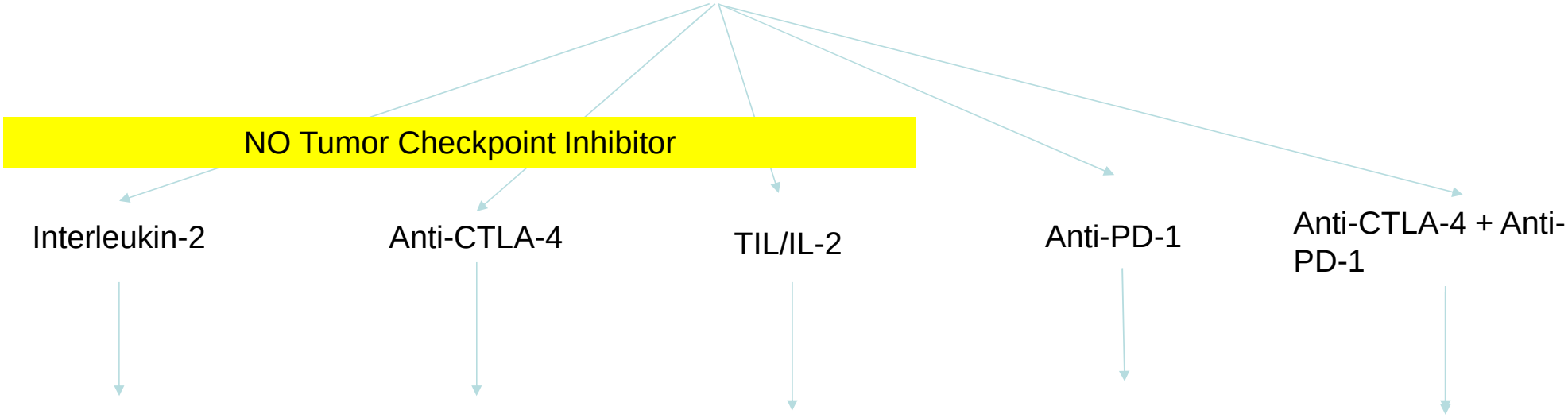
Antigen Presenting Cell or Tumor	T-lymphocyte	Function (excluding Treg)
Peptide-MHC	T cell receptor	Signal 1
CD80/CD86 (B7.1, B7.2)	CD28/CTLA-4	Stimulatory/inhibitory
CEACAM-1	CEACAM-1	inhibitory
CD70	CD27	stimulatory
LIGHT	HVEM	stimulatory
HVEM	BTLA, CD160	inhibitory
PD-L1 (B7-H1)	**PD-1** and CD80	Inhibitory (Th1)
PD-L2 (B7-DC)	PD1 and ?	Inhibitory (Th2) or stimulatory
OX40L	OX40	stimulatory
4-1BBL	CD137	stimulatory
CD40	CD40L	Stimulatory to DC/APC
B7-H3	?	Inhibitory or stimulatory
B7-H4	?	inhibitory
PD-1H (Vista)	?	inhibitory
GAL9	TIM-3	inhibitory
MHC class II	LAG-3	inhibitory
B7RP1	ICOS	stimulatory
MHC class I	KIR	Inhibitory or stimulatory
GITRL	GITR	stimulatory
CD48	2B4 (CD244)	inhibitory
HLA-G, HLA-E	ILT2, ILT4; NKG2a	inhibitory
MICA/B, ULBP-1, -2, -3, and -4+-	NKG2D	Inhibitory or stimulatory
CD200	CD200R	inhibitory
CD155	TIGIT/CD226	Inhibitory/stimulatory

Other Inhibitory Factors
IDO
Treg
MDSC
Macrophages
TGF-beta
IL-10?
VEGF


Checkpoint Inhibitors
LAG3, TIM3, TIGIT, B7-H3, B7-H4, PD-1H (Vista), CD200, CEACAM1, KIR
MDSC
Type 2 macrophages
HDACi, MER-TKi, CCR2i, CSF-1Ri, CKITi, ibrutinib,
Anti-CD47 (‘Don’t Eat Me Signals’)
Treg
Anti-CCR4, anti-CTLA-4
Inhibitory Cytokines
Antibodies and small molecule inhibitors of TGF-beta or its receptors
Hypoxia/Adenosine
Adenosine 2AR inhibitors
Anti-CD39, anti-CD73
Metabolic Inhibitors and Prostaglandins
IDO inhibitors, Cox2 inhibitors


Disrupt tumor barriers to T-cell infiltration
Anti-VEGF, anti-SEMA-4D, anti-CTLA-4
Create new tumor-specific T-cells or enhance in vivo Ag presentation
Vaccines, T-VEC, Anti-CD40, FLT3 TLR agonists, CAR-T
STING agonists
Epigenetic Modifiers
Expansion and Increase Function of Ag-specific T cells
Adoptive Transfer:
TIL
CAR-T
Cytokines and Modified Cytokines
Co-stimulatory Agonists – 4-1BB, OX-40, GITR, ICOS, CD27
Co-opt non-specific TIL
Activate with TCR-CD3 Constructs (CEA, gp100)

Metastatic Melanoma



- ORR –15%
- CR – 5%
- 2-yr survival =20-25%

- ORR ~15%
- Median survival 10-11 months
- 2-year survival 30-35%

- ORR ~50%
- Durable CR= 20%

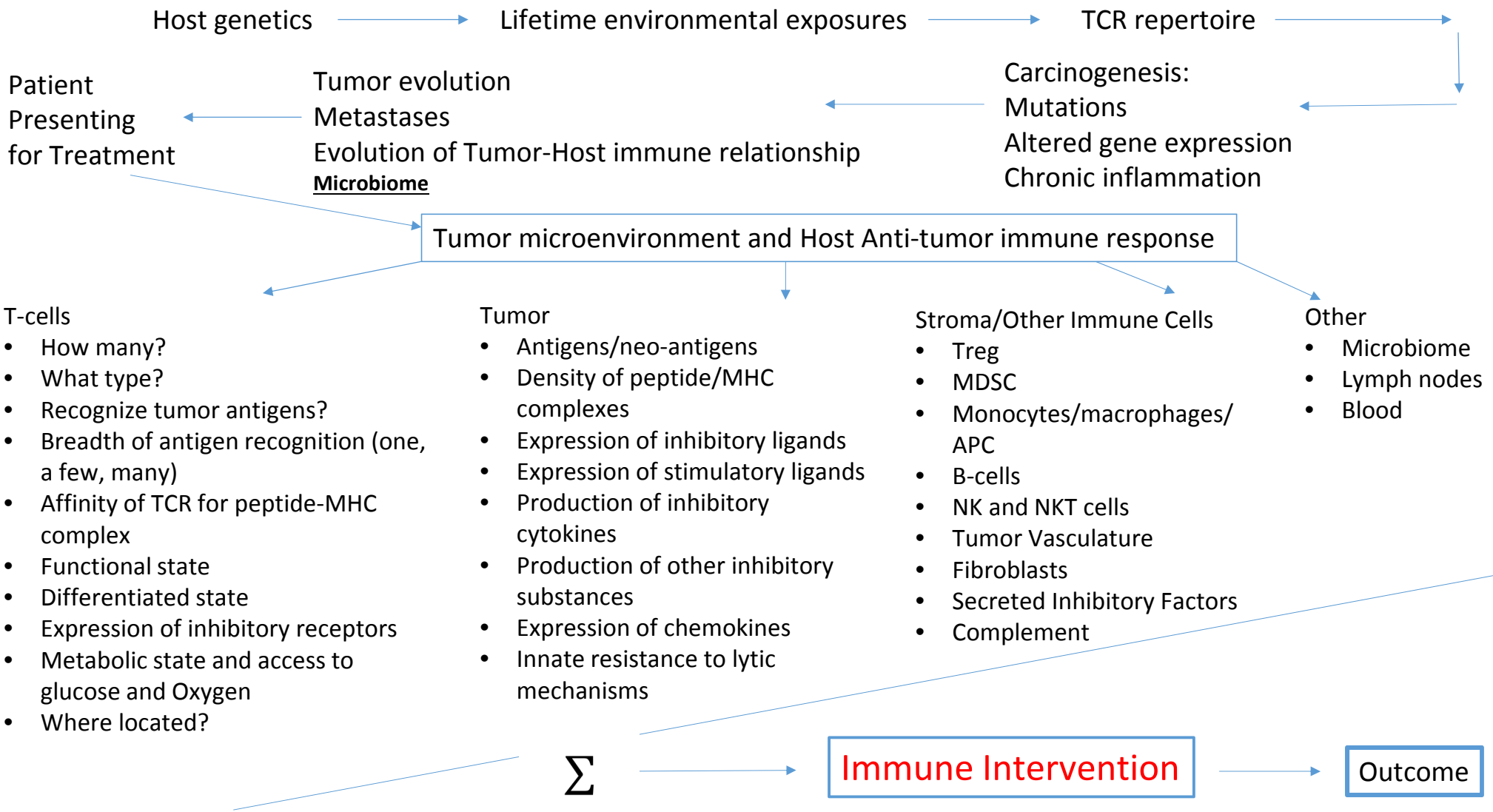
- ORR~40%
- 2-yr-survival = 55%

- ORR ~55%
- 2-yr survival ~ 70%

A few critical questions for the future

- What are the relevant anti-tumor T-cells recognizing?
  - mutations, stem cell Ag, developmental Ag, tissue specific Ag
  - Are certain relevant tumor antigens ignored during tumor development?
- If no or minimal T cells in tumor, why?
  - not present in body, or can't get in?
- How does tumor biology control/determine anti-tumor immune response?
  - Type and number of mutations, signaling pathways (Sting, WNT/beta catenin, RTK, PI3K/AKT, RAS-RAF, IFN-g, epithelial to mesenchymal transition), vascularity and production of angiogenic cytokines, effects on stromal cells, antigen processing and presentation, resistance to lysis, pattern of organ metastases
- How do host factors influence developing an active anti-tumor immune response
  - Genetic polymorphisms, microbiome

- What are the critical T-cell features to mediate anti-tumor effect and what controls them? What is missing?
  - Proliferation and regenerative capacity, cytotoxicity, cytokine production, chemokine production, vascular effects, migration, ability to kill serially, formation of long-term memory (which subsets are most effective)
  - Magnitude of T-cell response?
  - T-cell receptor affinity for target peptide/MHC complex
  - Breadth of response (multiple antigens)?
- What are the most critical mechanisms for inhibiting T-cell activity within the tumor?
  - Checkpoints, hypoxia, glucose metabolism, essential amino acids, Treg, MDSC/type 2 macrophages, cytokines
- What other signals do T-cells need to function optimally and how best to deliver (even after checkpoint blockade)?
  - Cytokines, co-stimulatory ligands, functional APC
  - Multi-valent, requirement for Fc, cis versus trans, duration
- Are other types of cells (NK, NKT, monocytes, eos, granulocytes) or antibodies important?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Immune Intervention
Outcome

Human Tumor Immunone Project

Baseline

Perturbed states

Tumor Type A

Tumor Type B

Tumor Type C

Tumor Type D

Tumor Type E

Tumor Type F

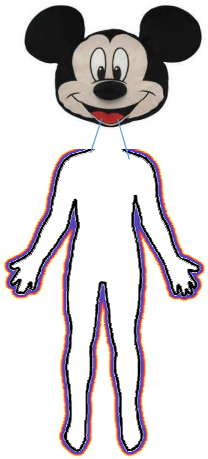
Tumor Type G

Tumor Type H

Tumor Type I

Mouse Tumor Immunone Project

Match to Mouse Model



Therapeutic Intervention

Mechanisms of Toxicity for Immune Checkpoint Blockade or Co-Stimulatory Agents

- Mostly unknown
- May be epitope dependent (4-1BB)
- Cross-reactivity of Ab with normal tissue
- Activation of prior subclinical auto-immunity (recognition of self-Ag)
  - Prior genetic predisposition
  - Epitope spread
  - Cross-reactivity of tumor and normal tissue Ag
  - Increased effector cell function (Th1, Th2, Th17, other)
  - Reduced Treg function
- Cytokines may play role in pathology
- Role of antibody-dependent toxicity (serologic responses)
- Role of microbiome