Faculty

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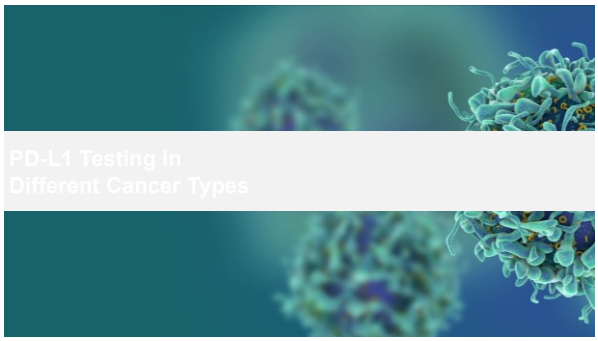
Outline

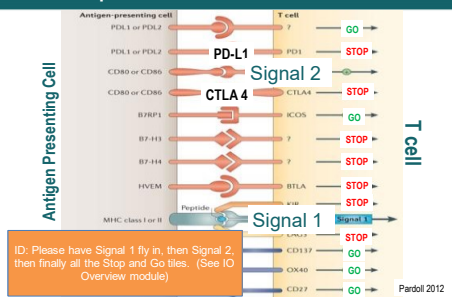
- PD-L1 testing in different cancer types
- Expanded role of MSI/MMR status in multiple cancers
- Tumor mutational burden (TMB) as an evolving IO biomarker
- Relationship between TMB and PD-L1
- Expanding use of checkpoint inhibitors to treat different cancer types
- Biomarker landscape



PD-L1 Basics

- Programmed death ligand 1 (PD-L1) is a normal physiologic immune response inhibitor
- Dampens the proliferation and function of T cells
- Functions at the immune synapse
- Cancers use PD-L1 to inhibit the endogenous anti-tumor immune response
- PD-L1 is targeted by infusing antibodies that block PD-L1 function on tumor cells
- Antibodies that block PD-L1 "inhibit the inhibitor," ultimately leading to immune stimulation and tumor clearance
- Many other immune inhibitor molecules are being targeted but none have shown the efficacy of anti-PD-L1 therapy yet

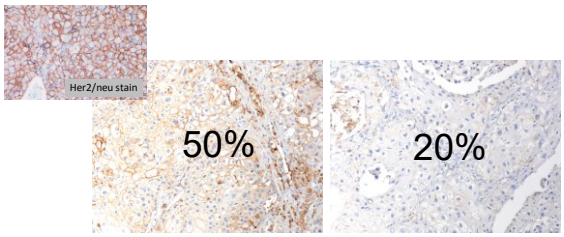
Immune Checkpoints



ID: Please have Signal 1 fly in, then Signal 2, then finally all the Stop and Go tiles. (See IO Overview module)

Pardoll 2012

Expression of Targeted Proteins as a Predictive Biomarker



Companion vs. Complementary PD-L1 Testing

Companion diagnostic test

- Used in conjunction with a therapeutic drug to determine its applicability to a specific person
- Test result ***must be positive*** in order for the patient to receive the drug

anti-PD1/L1 therapy — GO — + Test Result — − — STOP — anti-PD1/L1 therapy

Complementary diagnostic test

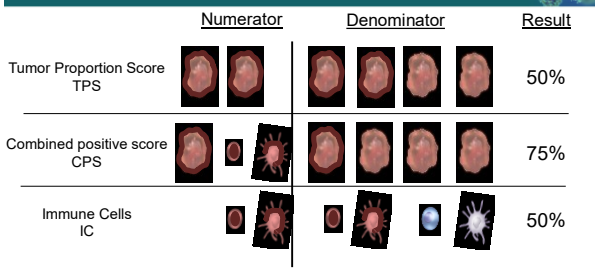
- Identifies patients who respond particularly well to a drug
- Is ***not a pre-requisite*** for receiving the drug

anti-PD1/L1 therapy — GO — + Test Result — − — GO? — anti-PD1/L1 therapy

Evaluation of PD-L1 Expression

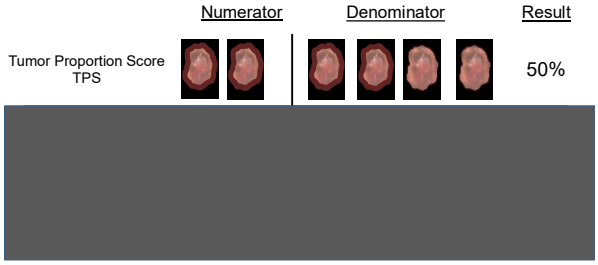
Cell Type	Cellular Location	Percentage
Histiocytes, Lymphocytes, tumor cell	Cell Membrane, Cell Cytoplasm	25%

Evaluation of PD-L1 Expression



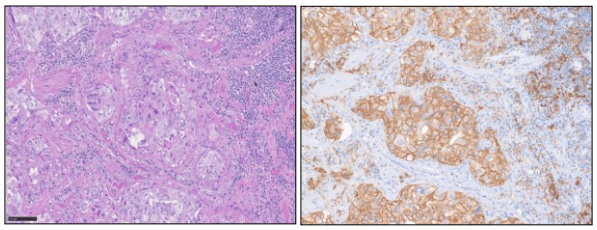
http://captodayonline.com/scoring-gastric-gej-cancers-pd-l1-expression/

Evaluation of PD-L1 Expression



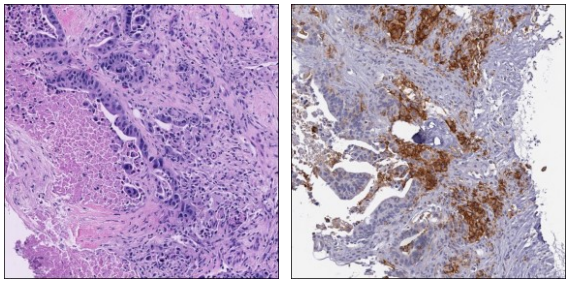
http://captodayonline.com/scoring-gastric-gej-cancers-pd-l1-expression/

Example: Squamous Lung Cancer

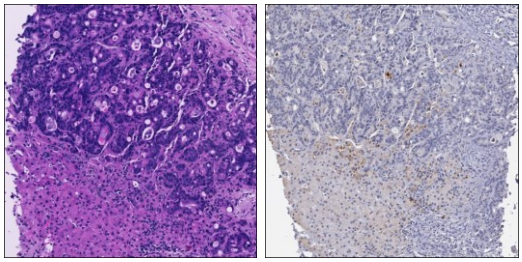


Images courtesy of Daphne Wang, Johns Hopkins

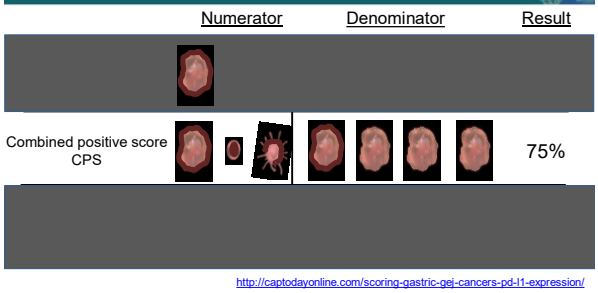
Example 1: Metastatic Lung Adenocarcinoma



Example 2: Metastatic Lung Adenocarcinoma

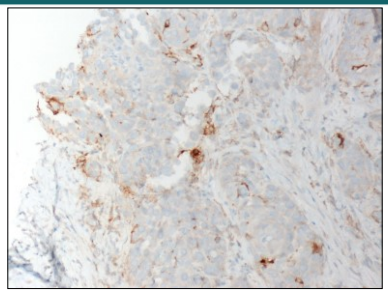


Evaluation of PD-L1 Expression



http://captodayonline.com/scoring-gastric-gej-cancers-pd-l1-expression/

Example: Gastric Carcinoma



Evaluation of PD-L1 Expression

Numerator	Denominator	Result
Immune Cells IC		50%

http://captodayonline.com/scoring-gastric-gej-cancers-pd-l1-expression/

Example: Urothelial Carcinoma

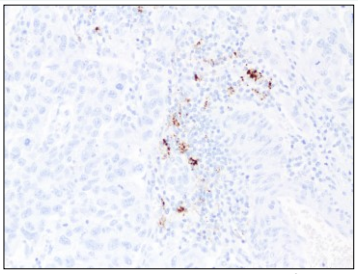


Image courtesy of Dr. Andres Matoso, Johns Hopkins

Pathologist Performance

JAMA Oncology | Original Investigation

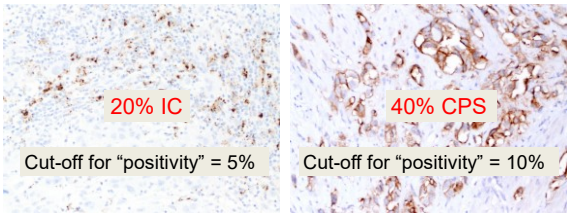
A Prospective, Multi-institutional, Pathologist-Based Assessment of 4 Immunohistochemistry Assays for PD-L1 Expression in Non-Small Cell Lung Cancer

David L. Rimm, MD, PhD; Gang Han, PhD; Jamie M. Taube, MD; Eunhee S. Yi, MD; Julia A. Bridge, MD; Douglas B. Flieder, MD; Robert Homer, MD, PhD; William W. West, MD; Hong Wu, MD; Anja C. Roden, MD; Junya Fujimoto, MD; Hui Yu, MD; Robert Anders, MD; Ashley Kowalewski, MS; Christopher Rivard, PhD; Jamaal Rehman, MD; Cory Batenchuk, PhD; Virginia Burns, PhD; Fred R. Hirsch, MD, PhD; Ignacio I. Wistuba, MD, PhD

ICC for pathologists by each antibody in Tumor Cells						
	22c3	28-8	SP142	E1L3N	Summary	
All, N=90	0.882	0.832	0.869	0.859	0.86(0.02)	← TPS
ICC for pathologists by each antibody in Immune Cells						
	22c3	28-8	SP142	E1L3N	Summary	
All, N=90	0.207	0.172	0.185	0.229	0.19(0.03)	← IC

Same Cancer, Different PD-L1 Antibodies, Different Cut-Offs for "Positivity"

FDA-approved companion PD-L1 immunohistochemistry



Images courtesy of Dr. Andres Matoso Johns Hopkins

PD-L1 Immunohistochemistry: A New Challenge for Pathologists

Test variables

- Which PD-L1 antibody?
- Which staining pattern (TPS, CPS, IC)?
- Which cutoff value?
- Which of the patient's tumor samples (primary biopsy, post-treatment, metastasis)?
- Which pathologist?

Most challenging IHC interpretation ever!

Case Presentation #1

- 48 year old patient with metastatic gastric cancer
- Oncologist requests microsatellite instability (MSI) status and PD-L1 testing

Statistics: Gastric Cancer

JAMA Oncology | Original Investigation

Safety and Efficacy of Pembrolizumab Monotherapy in Patients With Previously Treated Advanced Gastric and Gastroesophageal Junction Cancer
Phase 2 Clinical KEYNOTE-059 Trial

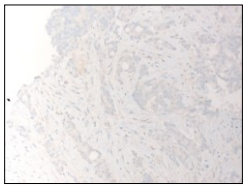
Charles S. Fuchs, MD, MPH; Toshihiko Doi, MD, PhD; Raymond W. Jang, MD, MSc, FRCPC; Ilai Muro, MD; Tarek Safesh, MD; Weiling Sun, MD; Shadia I. Jalal, MD; Manish A. Shah, MD; Jean-Philippe Metges, MD; Marcelo Garrido, MD; Talia Golan, MD; Zev A. Wainberg, MD; Daniel V. Catenacci, MD; Atsushi Ohtsu, MD; Kohei Shitara, MD; Basil Geva, MD; Jonathan Bleeker; Geoffrey Ku, MD; Philip Philip, MD, PhD, FRCP; Peter C. Enzinger, MD; Yung-Jue Bang, MD, PhD; Diane Levitan, PhD; Jiang Wenxi Rosales, MD, PhD; Rita P. Dalal, MBBS, MPH; Harry H. Yoon, MD

Table 1. Objective Tumor Response

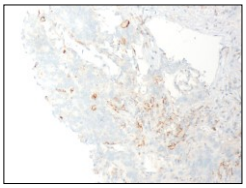
Best Overall Response[a]	Participants (n = 259) No.	% (95% CI)
Objective response (CR+PR)	30	11.6 (8.0-16.1)
Disease control (CR+PR+SD ≥2 mo)	70	27.0 (21.7-32.9)
CR	6	2.3 (0.9-5.0)
PR	24	9.3 (6.0-13.5)
SD	42	16.2 (11.9-21.3)
Progressive disease	145	56.0 (49.7-62.1)
Nonevaluable	7	2.7 (1.1-5.5)
No assessment[b]	35	13.5 (9.6-18.3)
Duration of response, median (range), mo		8.4 (1.6+ to 17.3+)[c]

PD-L1 Expression in Gastric Cancer

42% PD-L1 negative
6.4% overall response rate

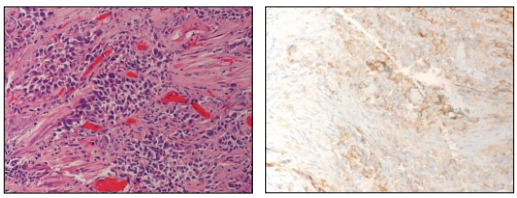


58% PD-L1 positive
15.5% overall response rate



MSI/PD-L1 Testing of the Biopsy

- PCR testing: All 5 microsatellites intact
- FDA-approved *companion* PD-L1 immunohistochemistry



PD-L1: What We Know Right Now

PD-L1 is an targetable immune inhibitor
- Offers improved response rates in patients with aggressive cancers

It is an imperfect but useful predictive biomarker

IHC is used in both companion and complementary tests

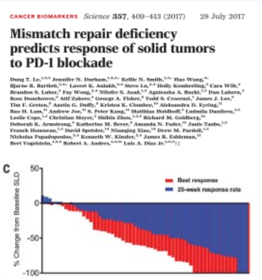
IHC interpretation fraught with variables

Expanded Role of MSI/MMR Status in Multiple Cancers

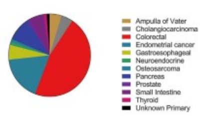
Tests for MSI and MMR

- Microsatellite testing uses DNA and is performed by PCR
- Mismatch repair proteins are detected by immunohistochemical stains
- Next generation sequencing is an emerging DNA-based test

Landmark Study Results



86 patients
9/2013 to 9/2016
12 tumor types

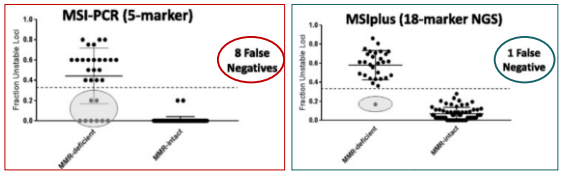


ORR 53%
CRR 21%

Case Presentation #2

- 70-year-old patient with metastatic prostate cancer
  - High-grade, Gleason pattern 5+4
  - Prominent tumor-infiltrating lymphocytes
- Oncologist requests MSI testing

Prostate Cancer and MSI Testing by PCR



J Immunother Cancer. 2018;6:29.

MSI/MMR Testing: What We Know Right Now

Oncologists are now requesting this for *many* different tumors

- Not just colorectal and endometrial cancer

Neither PCR nor NGS is FDA-approved

NGS not widely available

Tumor Mutational Burden (TMB): An Evolving Immuno-Oncology Biomarker

Tumor Mutational Burden (TMB)

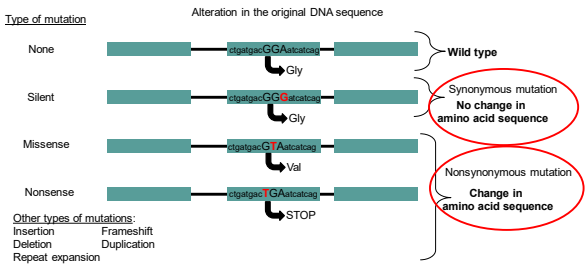
The number of DNA mutations in a cancer cell

DNA sequencing of all coding region (exons) of the entire genome

Calculated value reported as number of mutations per megabase

- >10 mutations/Mb considered "high TMB"

What is a DNA mutation?

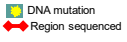


DNA Sequencing Approaches

~180,000 exons in the human genome
30 million bases

- **Whole exome sequencing**: Evaluates all bases of all coding regions (100% of exons)

- **Gene panel**: Evaluates only portions of the exome (50% of exons)



Tumor Mutational Burden (TMB) Testing

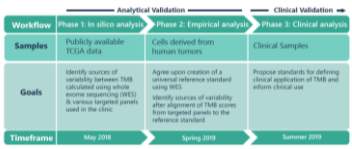
Whole Exome Sequencing	Target Gene Panels
• Gold standard • Expensive • Generates a large amount of data • Requires more DNA	• Uncertainty about minimum necessary coverage • Reduced cost • Generates less data • More easily-integrated into hospital labs • Requires less DNA

Tumor Mutational Burden

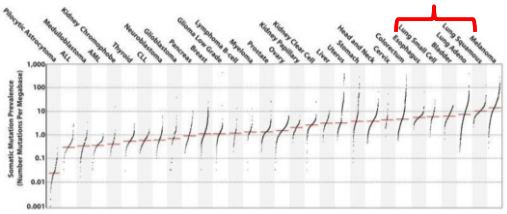
- **No single definition of which types of mutations to include**
- **No recommendation on the percentage of exons to sequence**
- Multi-stakeholder harmonization effort
  - Government, Academia, Diagnostics, Industry, Operational
- Publish universal best practices defining TMB
- Analytic validation
- Reference standards

FRIENDS of CANCER RESEARCH

http://www.focr.org/tmb

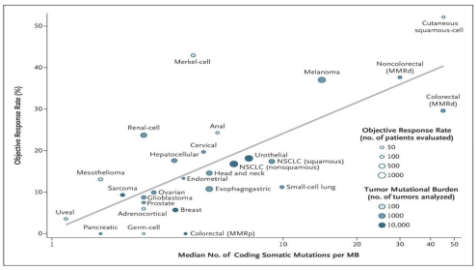


TMB and Response to Immune-Based Therapy



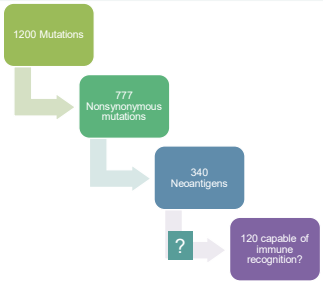
Branca MA et al. *Nat Biotechnol.* 2016;34:1019-1024.

Correlation Between TMB and Overall Response Rate in 27 Tumor Types

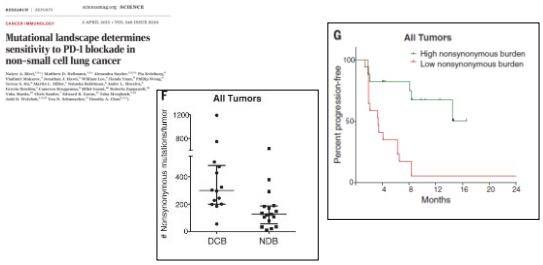


Yarchoan M et al. *N Engl J Med*. 2017;377:2500-2501.

TMB and Immune Response: A Possible Mechanism

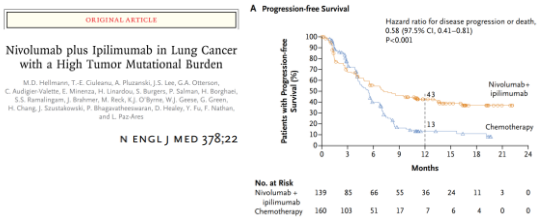


Early Evidence for TMB and Clinical Benefit



Rizvi NA et al. *Science*. 2015;348:124-128.

More Evidence of TMB Correlating with Clinical Benefit


ORIGINAL ARTICLE

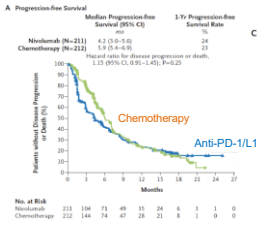
Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden

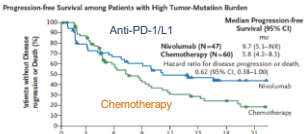
M.D. Hellmann, T.-E. Ciuleanu, A. Pluzanski, J.S. Lee, G.A. Otterson, C. Audigier-Valette, E. Minenza, H. Linardou, S. Burgers, P. Salman, H. Borghaei, S.S. Ramalingam, J. Brahmer, M. Reck, K.J. O'Byrne, W.J. Geese, G. Green, H. Chang, J. Szustakowski, P. Bhagavatheeswaran, D. Healey, Y. Fu, F. Nathan, and L. Paz-Ares

N ENGL J MED 378;22

Patients with high TMB defined as 10 mutations/Mb.
PFS declined when patients with low TMB were included in analysis.

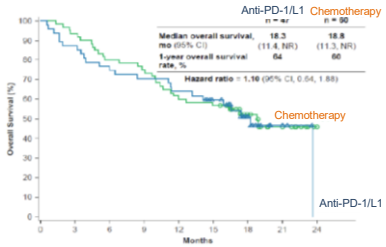
More Evidence that TMB Might be Useful, continued





Carbone. *N Engl J Med*. 2017;376:2415-26.

But No Difference in Overall Survival



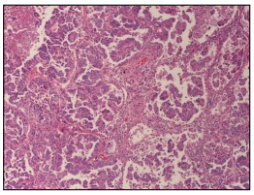
Carbone. *N Engl J Med*. 2017;376:2415-26.

TMB: What We Know Right Now

- A mechanism driving anti-cancer immune elimination
- Testing needs standardization
- Predicts progression-free survival
- May not predict overall survival
- Further studies required to define clinical benefit

Case Presentation #3

- 57-year-old patient, former smoker, 4.5 cm lung mass on CT scan
- Pathology
  - Adenocarcinoma with pleural involvement
  - Lymph node positive for metastatic disease
  - Stage IV (T2bN1M1a)
- Oncology referral



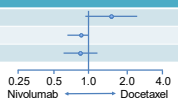
Anticipated Testing

- Mutational analysis
  - *EGFR*
  - *ALK*
  - *ROS1*
  - *BRAF*
- PD-L1 immunohistochemistry
- Tumor mutational burden?

Lack of Benefit of PD-1 Blockade vs. Docetaxel in *EGFR*-Mutant NSCLC (PFS)

EGFR Mutation Status	n	Unstratified hazard ratio*
Positive	82	1.46 (0.90-2.37)
Not detected	340	0.83 (0.65-1.06)
Not reported	160	0.83

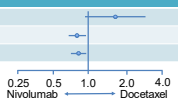
Progression free survival, CheckMate -057



Subgroup Analysis of PFS

EGFR Mutation Status	n	Unstratified hazard ratio*
Positive	70/86	1.79 (0.94-3.42)
Not detected	660/875	0.83 (0.71-0.98)
Not reported	778/1033	0.85 (0.73-0.98)

Progression Free Survival, Keynote-010



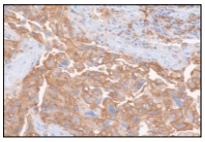
* 95% confidence interval

Borghaei H. *N Engl J Med* 2015;373:1627-1639. Herbst RS, et al. *Lancet* 2016;387:1540-1550.

Test Results

- Mutation analysis
  - *BRAF*
  - *EGFR*
  - *ALK*
  - *ROS1*

  No mutations detected
- PD-L1 immunohistochemistry 90% Tumor Proportional Score
- Tumor mutational burden 18 mutations/Mb

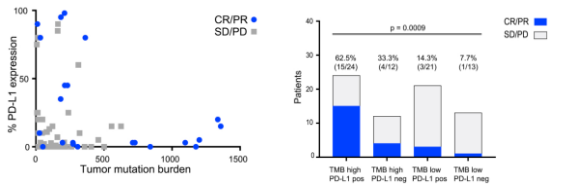


Is this patient a candidate for anti-PD-1/L1 blockade?

TMB: What We Don't Know Right Now

- Definition of TMB
- How/when do we test?
  - Validation of testing methods
- Role of TMB in the presence of other established biomarkers (eg *EGFR*)
- Are TMB and PD-L1 independent complementary biomarkers?

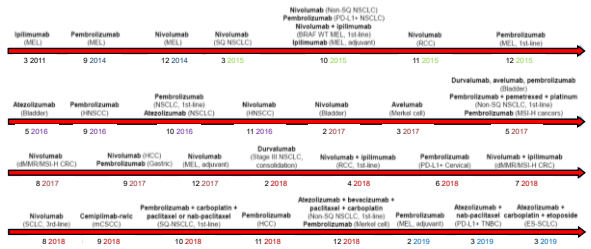
Relationship Between TMB and PD-L1



Hellmann MD, et al. *Cancer Cell*. 2018;33:843-852.

Expanding Use of Checkpoint Inhibitors to Treat Different Cancer Types

FDA-Approved Checkpoint Inhibitors for Solid Tumors

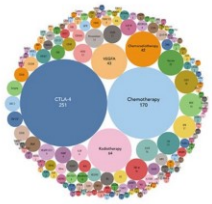


Source: https://www.fda.gov/drugs/informationondrugs/approveddrugs/ucm279174.htm, accessed 3/23/2019.

Number of PD-1/L1 Combo Trials

Number of PD-1/L1 Combo Trials Using Common Strategies:

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



The Anne-Marie Rolan Clinical Accelerator
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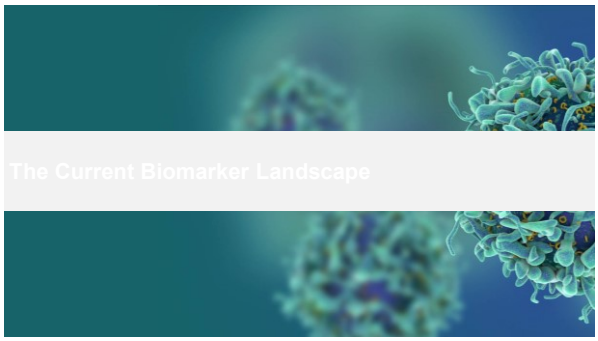
Other Immuno-Oncology Strategies

- Checkpoint inhibitors
- Engineered/ adoptive T-cells
- Immune system modulators
- Cancer vaccines
- Oncolytic virus



Biomarkers in Immuno-Oncology

Current Clinical Practice

- PD-L1 immunohistochemistry
- MSI/MMR deficiency
- Tumor infiltrating lymphocytes in breast cancer

Emerging

- Tumor mutational burden

Developing

- Multiplex immunohistochemistry/immunofluorescence
- Plasma circulating tumor cell/TMB measurements ("Liquid biopsy")
- Multiple gene expression (mRNA) profiles
- Microbiome composition
- Myeloid-derived suppressor cells
- Other immune-modulating molecules (LAG-3, IDO, etc)

Summary

- PD-L1 testing in different cancer types
- Expanded role of MSI/MMR status in multiple cancers
- TMB as an evolving immuno-oncology biomarker
- Relationship between TMB and PD-L1
- Expanding use of checkpoint inhibitors to treat different cancer types
- Biomarker landscape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Update on Immuno-Oncology Biomarkers
Thank You