Challenges of Biomarker and Selection

Martin Reck

Department of Thoracic Oncology

LungenClinic Grosshansdorf

Germany

SINGAPORE 2015 ESMO ASIA 18-21 DECEMBER SINGAPORE

Disclosure slide

- Member of Advisory Board: Hoffmann-La Roche, Lilly, MSD, BMS, AstraZeneca, Boehringer Ingelheim, Pfizer, Novartis, Celgene
- Honoraria for lectures: Hoffmann-La Roche, Lilly, MSD, BMS, AstraZeneca, Boehringer Ingelheim, Pfizer

The setting

- Treatment options for patients with non oncogenic addicted NSCLC are limited
- There is urgent need for a new general concept in systemic treatment
- Immunotherapy (Checkpoint Inhibition) has shown superior efficacy compared to chemotherapy in pretreated unselected patients with advanced NSCLC
- A biomarker to identify benefitting patients (or to exclude non benefitting patients) would be highly appreciated

The first problem...

- All attempts to define Biomarkers for immunotherapy have been focussed on response as efficacy marker of immunotherapy
- Immunotherapy ≠ Targeted therapy
- Efficacy of immunotherapy defined by long lasting tumor stabilization

The second problem...

What is the purpose of Biomarker development in immunotherapy:

1. A Biomarker to identify patients with high benefit
2. A Biomarker to exclude patients without benefit

This has to be defined

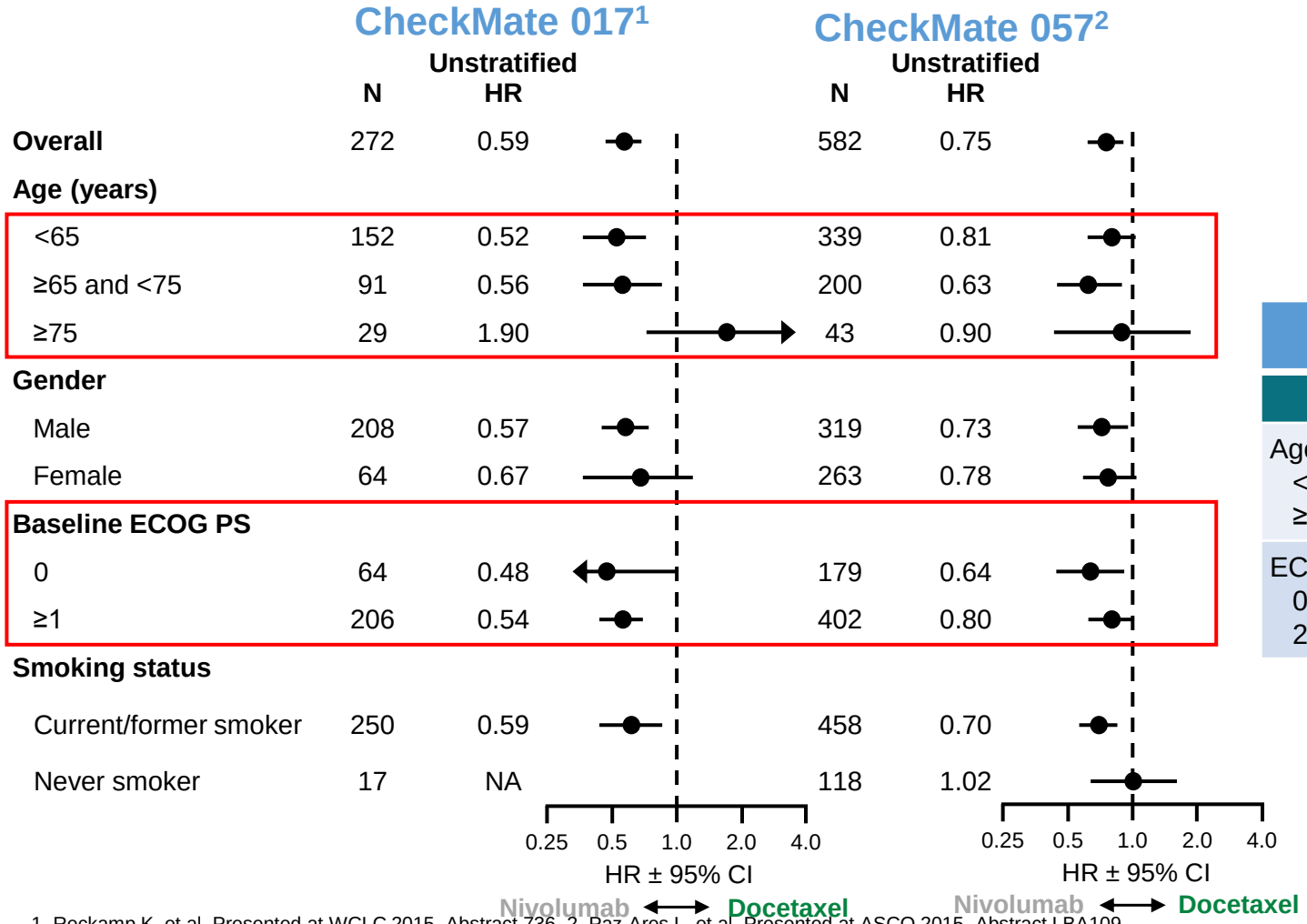
Currently both strategies are under exploration

What data do we have?

- Clinical Factors?

Age and PS as potentials factors?

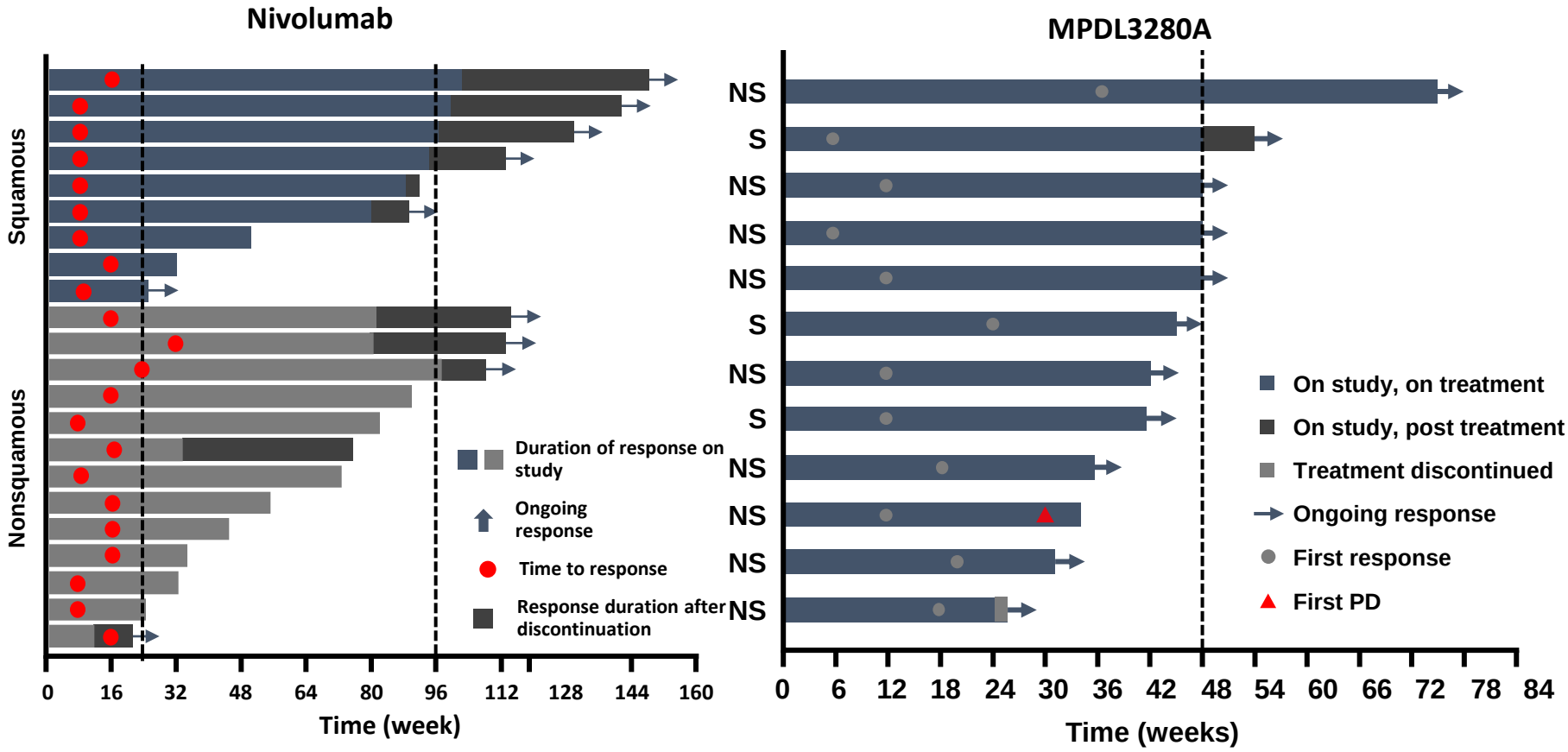
Example Nivolumab



CA209-153[3]	
	ORR, %
Age (years)	
< 70	11
≥ 70	13
ECOG PS	
0–1	11
2	20

1. Reckamp K, et al. Presented at WCLC 2015, Abstract 736. 2. Paz-Ares L, et al. Presented at ASCO 2015, Abstract LBA109.
3. Hussein M, et al. Presented at WCLC 2015, Oral 02.02.

Histology?

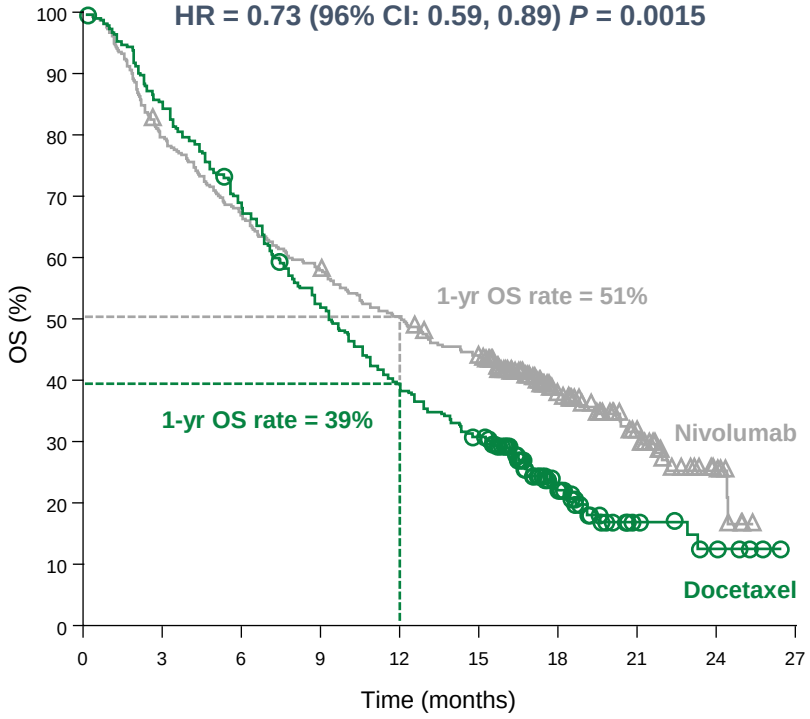


Adapted from Brahmer JR, et al. Mini-Oral presentation at WCLC 2013. *J Thorac Oncol.* 2013;8(Suppl 2):abstract: MO18.03
Horn L, et al. Mini-Oral presentation at WCLC 2013. *J Thorac Oncol.* 2013;8(Suppl 2):abstract: MO18.01.

Histology? – Example Nivolumab

CheckMate 057: nonsquamous[1]

HR = 0.73 (96% CI: 0.59, 0.89) *P* = 0.0015

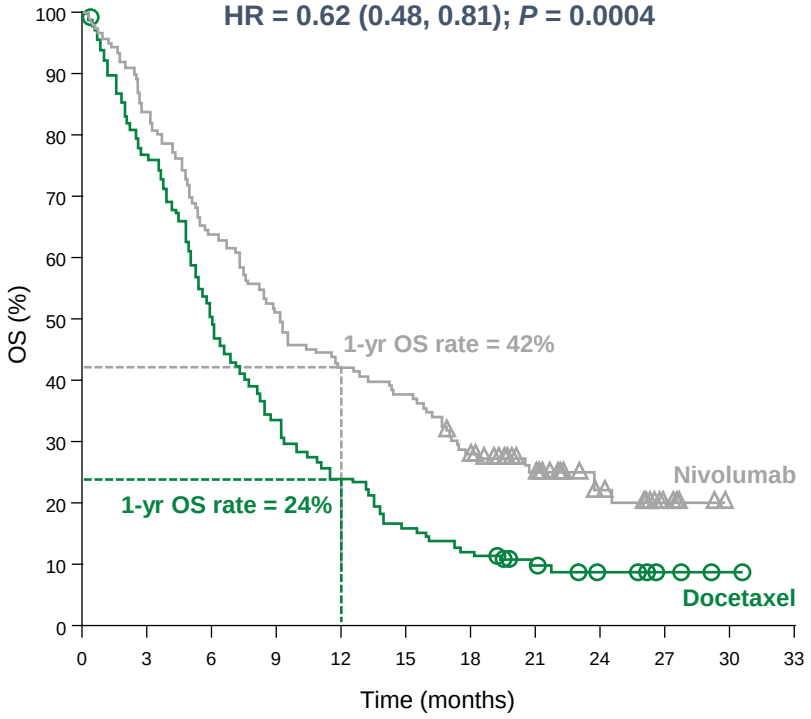


Number of Patients at Risk

Nivolumab	292	232	194	169	146	123	62	32	9	0
Docetaxel	290	244	194	150	111	88	34	10	5	0

CheckMate 017: squamous[2]

HR = 0.62 (0.48, 0.81); *P* = 0.0004



Number of Patients at Risk

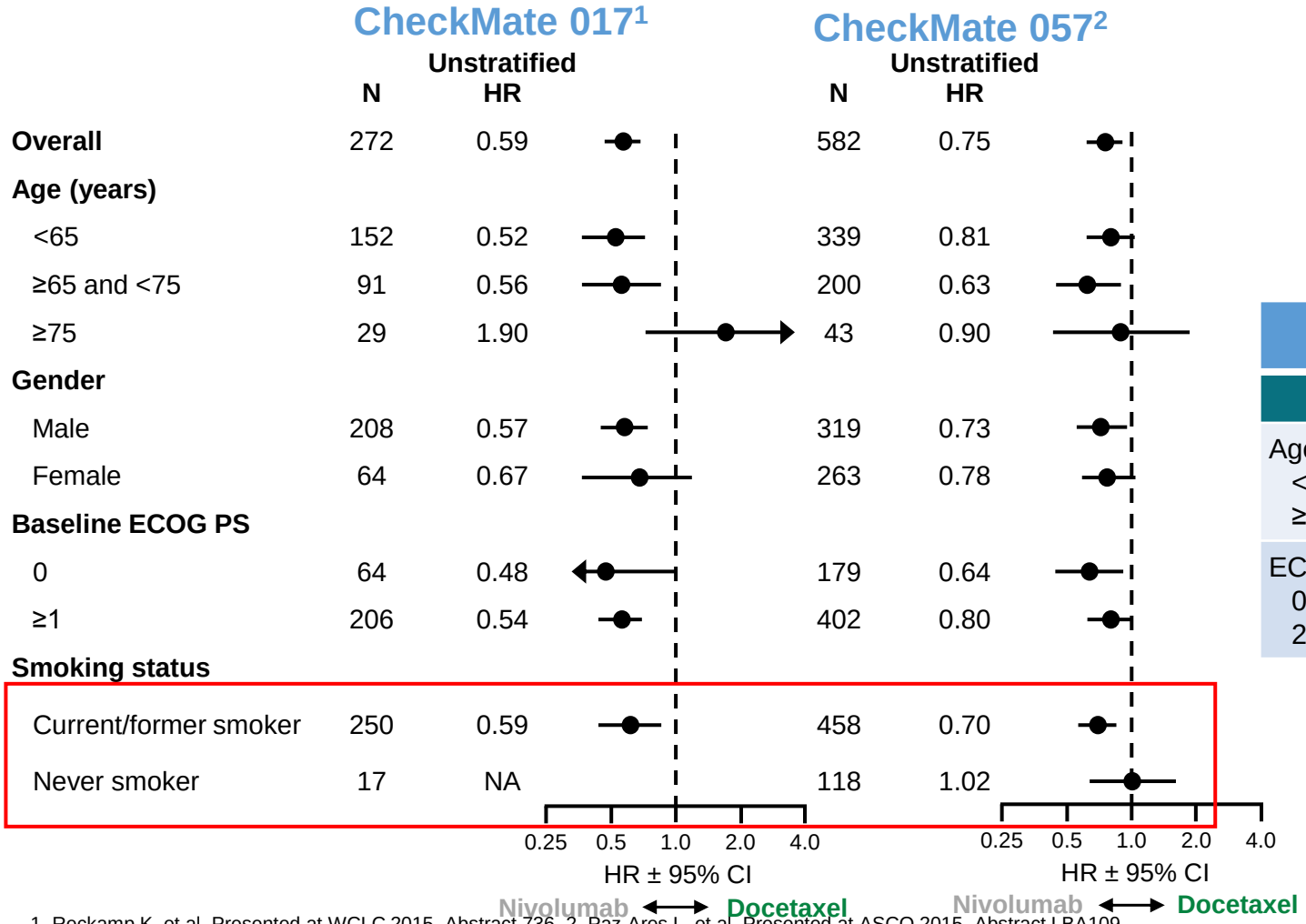
Nivolumab	135	113	86	69	57	51	37	25	14	6	0	0
Docetaxel	137	104	69	46	33	22	17	11	7	3	1	0

Patient characteristics were similar in both studies

1. Paz-Ares L, et al. Presented at ASCO 2015, Abstract LBA109. 2. Reckamp K, et al. Presented at WCLC 2015, Abstract 736.

Smoking?

Example Nivolumab



CA209-153[3]	
	ORR, %
Age (years)	
< 70	11
≥ 70	13
ECOG PS	
0–1	11
2	20

1. Reckamp K, et al. Presented at WCLC 2015, Abstract 736. 2. Paz-Ares L, et al. Presented at ASCO 2015, Abstract LBA109.
3. Hussein M, et al. Presented at WCLC 2015, Oral 02.02.

Smoking?

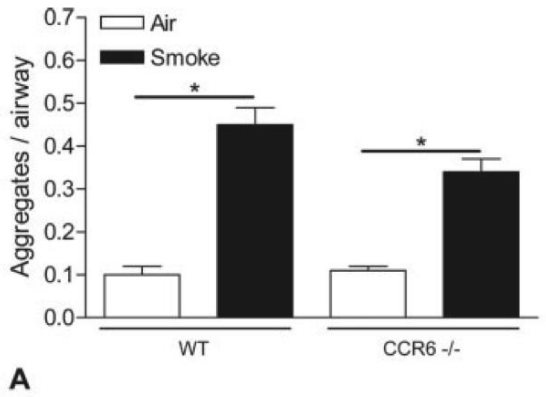
	Response rate (%)		
Sugroup	Nivolumab[1,5] (CA209-003)	Atezolizumab[2,3]	Pembrolizumab[4,6]
Current/former Smoker	27	26	27
Never Smoker	0	10	9

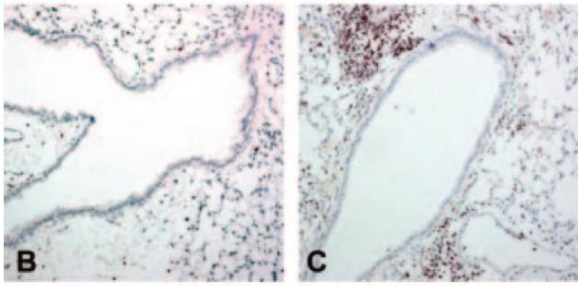
	Pembrolizumab (Keynote 01)[7]	
	Median PFS (mts.)	Med OS (mts)
Current/former Smoker	4.2	14.3
Never Smoker	2.1	8.8

1. Gettinger S, et al. Poster presented at CMSTO 2014. 2. Horn L, et al. Oral presentation at WCLC 2013, Abstract 2347. 3. Soria J, et al. Oral presentation at ECC 2013, Abstract 3408. 4. Hellmann MD, et al. Presentation at WCLC 2015, MINI03.05. 5. Hellmann MD, et al. Poster presented at ESMO 2014, Abstract 6111. 6. Garon E, et al. Oral presentation at ESMO 2014, Abstract LBA43. 7 Hellman MD, WCLC 2015

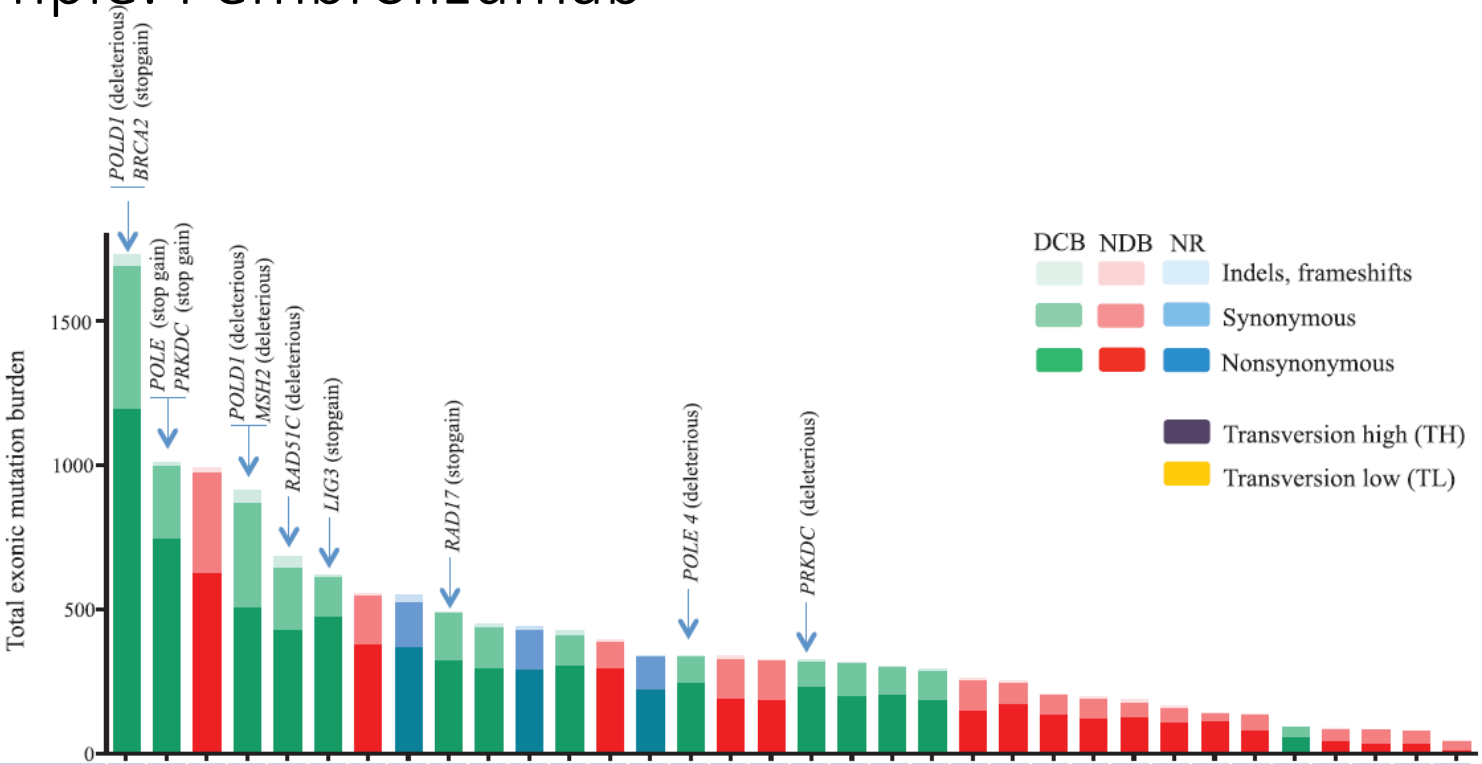
Impact of smoking on immunogenicity

- Induction of pulmonary inflammation and chronic obstructive bronchitis by cigarette smoke (Bracke J, J Clin Immunol 2006)
- Lung cancer of smokers have 10 times as many mutations as those from non-smokers (Vogelstein 2013)
- High mutational load contributes to immunogenicity





Mutational and Smoking status as predictive marker Example: Pembrolizumab



Study ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Cohort	V	D	D	V	V	D	V	V	D	D	V	D	D	V	V	V	D	D	V	V	D	D	D	V	V	V	D	V	V	V	D	V	D	D
Objective Response	PR	PR	PD	SD	PR	PR	PD	SD	PR	PR	PR	SD	PD	PR	SD	PD	PD	PR	PR	PR	SD	SD	PD	PD	PD	PD	PD	SD	SD	PR	SD	PD	PD	SD
PFS (months)	8	14	2	8	14	16	2	4	15	13	4	8	2	4	8	3	3	10	27	10	8	7	2	2	1	2	2	4	6	8	4	2	2	6
Ongoing response	++	++			++	++		++		++	++			++	++			++	++	++														
Smoking signature	TH	TH	TH	TL	TH	TH	TL	TH	TH	TH	TH	TH	TH	TH	TH	TL	TL	TH	TL	TL	TH	TL	TH	TL	TL	TL	TL	TL	TL	TL	TL	TL	TL	TL

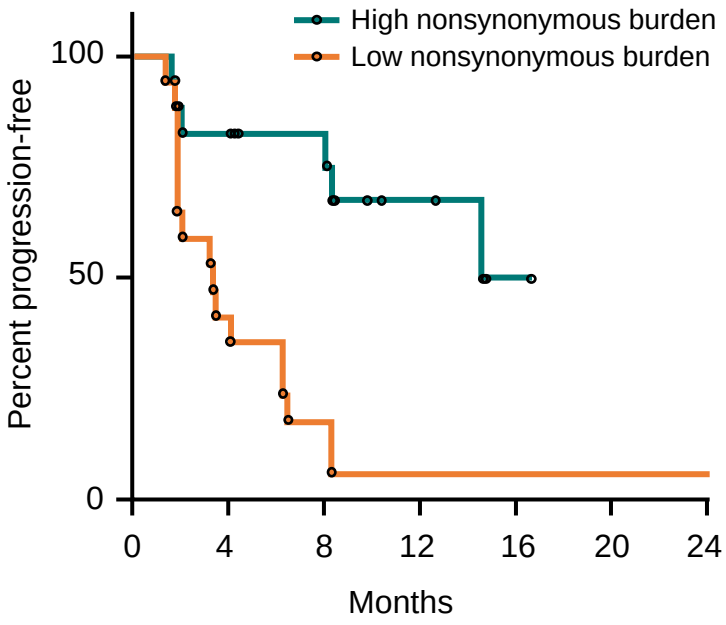
2015 ESMO SINGAPORE

Rizvi Science 2015

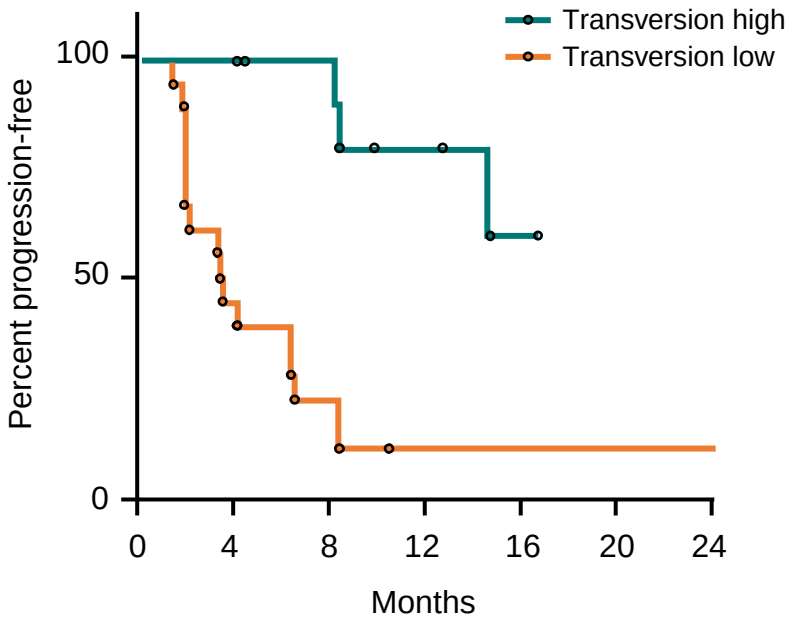
DCB = Durable Clinical Benefit NDB = Nondurable Clinical Benefit NR= Not Reached

Mutational Burden and Sensitivity to IO agents Example: Pembrolizumab

Mutational burden



Molecular smoking signature



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

On the way to an inflammatory signature?

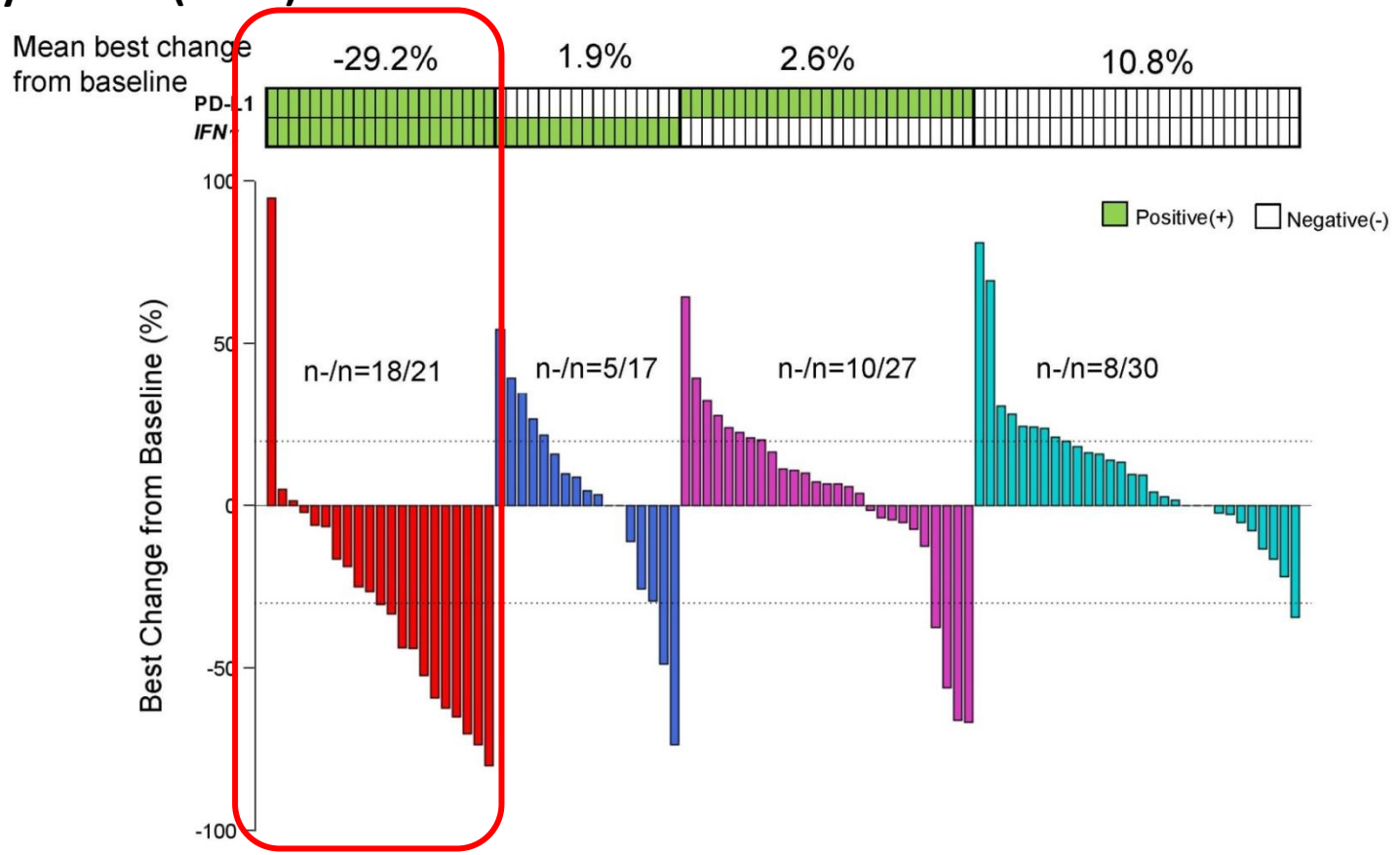
Impact of IFNγ on response Example: Durvalumab

- **Study design**
  - Study 1108 was a non-randomised, open-label, phase 1/2 multicentre study that enrolled patients with stage IIIB/IV squamous and nonsquamous NSCLC
  - A total of 228 NSCLC patients (n=102 squamous; n=126 nonsquamous) were treated with durvalumab 10 mg/kg q2w, most (n=201) were previously treated
    - Of these 200 patients were evaluable for a response
  - Predictive biomarkers were assessed
    - IHC for tumoural PD-L1 was conducted on pre-treatment fresh or archival biopsies (SP263 assay; n=176)
    - Frozen tumour samples with sufficient mRNA quality were profiled (n=122) with 100 pre-selected genes using Fluidigm Biomark™.
    - IFNγ gene expression correlated best with response.
    - Matched mRNA and PD-L1 IHC data were available for 112 patient biopsies

Higgs et al. Ann Oncol 2015; 26 (suppl 6): abstr 15LBA

Impact of IFNγ on response Example: Durvalumab

- **Key results (cont.)**



n=all patients with baseline and ≥1 follow-up scan; n-=number of patients with tumour shrinkage

Higgs et al. Ann Oncol 2015; 26 (suppl 6): abstr 15LBA

Impact of an IFNγ Signature on Efficacy Example Pembrolizumab (Head and Neck C)

Table 3. Association of Immune-Related Gene Expression Signatures and Best Overall Response and PFS in Patients With Head and Neck Cancer[a]

Signature	Nominal 1-Sided *P* Value[b]	
	Best Overall Response N = 40	PFS N = 43
IFN-γ (6 genes)	0.005	<0.001
TCR signaling (13 genes)	0.071	0.002
Expanded immune (18 genes)	0.015	<0.001
De novo (33 genes)	0.018	<0.001

[a]Best overall response and PFS assessed by investigator.
[b]From logistic or Cox regression for overall response and PFS, respectively, using signature scores as a continuous variable.

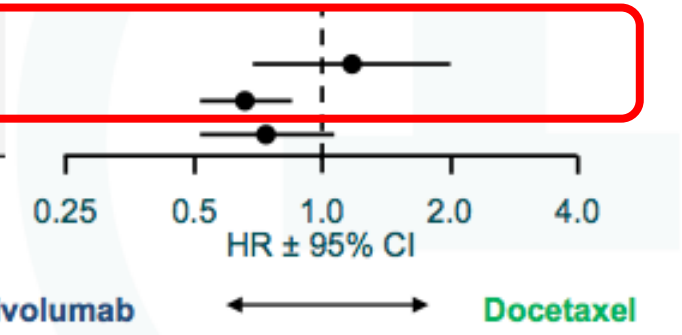
Seiwert TY et al, ASCO 2015

What do we know about tumors with low mutational burden?

- for example tumors with activating EGFR mutations...
- data are really limited and explorative, however...

Efficacy of PD-1/PD-L1 inhibitors in EGFR mutated tumors

***EGFR* mutation status**		
Positive	82	1.18 (0.69, 2.00)
Not detected	340	0.66 (0.51, 0.86)
Not reported	160	0.74 (0.51, 1.06)



	Response rate (%)		
Subgroup	Nivolumab[1,5] (CA209-003)	Atezolizumab[2,3]	Pembrolizumab[4,6a]
EGFR mutated	17%	17%	8%
EGFR wild	20%	23%	22%

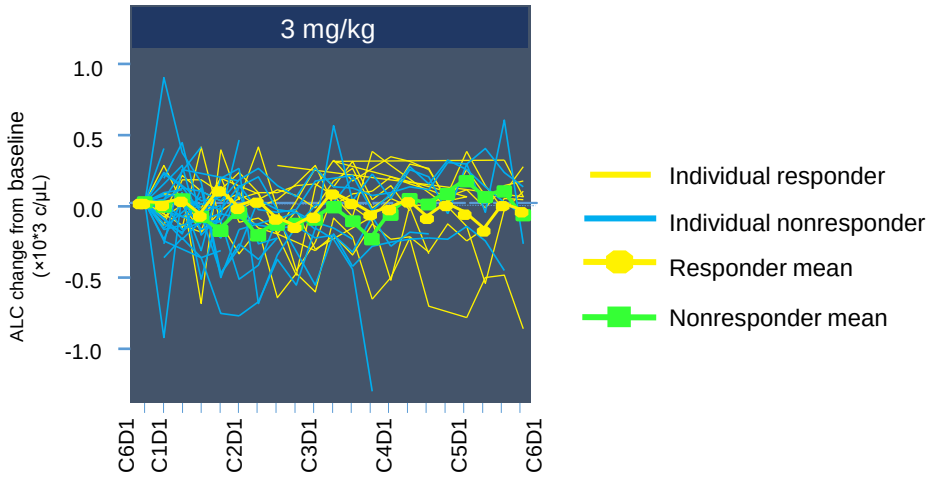
1. Gettinger S, et al. Poster presented at CMSTO 2014. 2. Horn L, et al. Oral presentation at WCLC 2013, Abstract 2347. 3. Soria J, et al. Oral presentation at ECC 2013, Abstract 3408. 4. Hellmann MD, et al. Presentation at WCLC 2015, MINI03.05. 5. Hellmann MD, et al. Poster presented at ESMO 2014, Abstract 6111. 6. Garon E, et al. Oral presentation at ESMO 2014, Abstract LBA43.7. Paz-Ares L, et al. Presented at ASCO 2015, Abstract LBA109.

What data do we have?

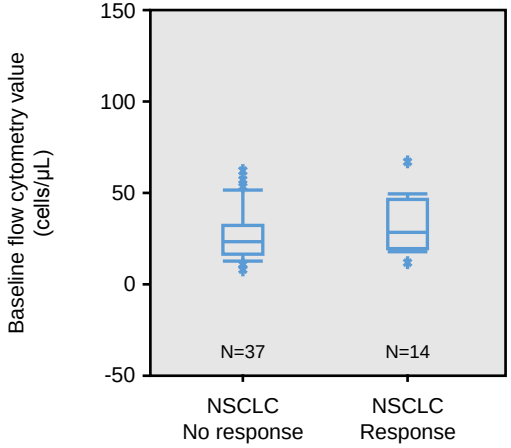
- Clinical Factors?
- Any tissue or blood based factors?

The big disappointment

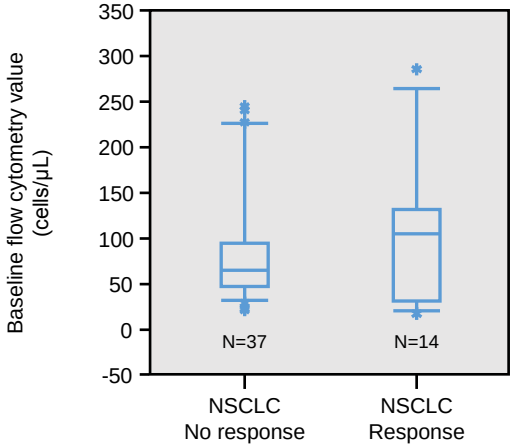
ALC



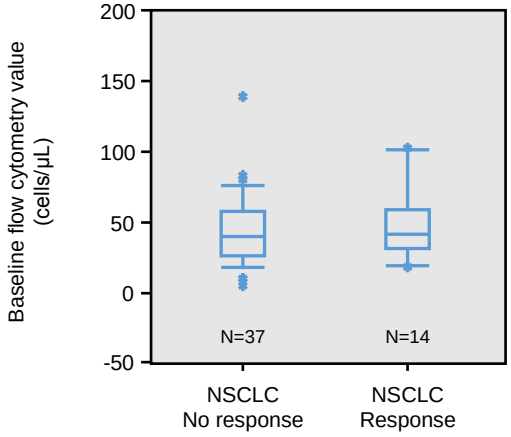
Treg cells



Activated CD8+ T cells



Activated CD4+ T cells



ALC=absolute lymphocyte count

Brahmer J, et al. Poster presented at ELCC 2014, Abstract 96PD.

What data do we have?

- Clinical Factors?
- Any tissue or blood based factors?
- What do we know about PD-L1 expression?
  - Expressed across various tumors on APCs and some non-hematopoetic cells[1]
  - Associated with poor prognosis and decreased T-cell infiltration[1-3]
  - Correlated with response towards PD-1/PD-L1 inhibitors[1,4]
  - Expression independent from molecular marker
  - An easy marker?....

1. McDermott D, Atkins M. *Cancer Med.* 2013;2:662–673. 2. Zou W, Chen L. *Nat Rev Immunol*. 2008;8:467–477. 3. Mu C, et al. *Med Oncol.* 2011;28:682–688. 4 Taube J, et al. *Clin Cancer Res.* 2014;20:5064–5074.

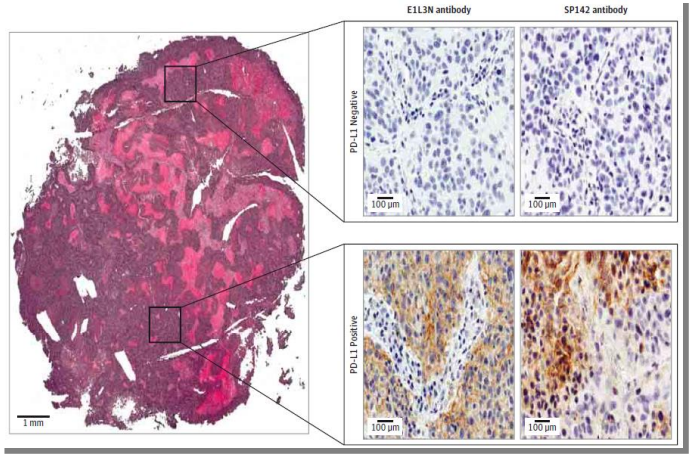
PD-L1 as a Biomarker: A nightmare in complexity

Challenges Surrounding Biomarker

Biology: PD-L1[1-3]

- Inter and intratumor heterogeneity
- Inducible and dynamic (IFN, post-treatment)
- Cell type (immune cell versus tumor versus both)
- Location (membrane versus cytoplasm)

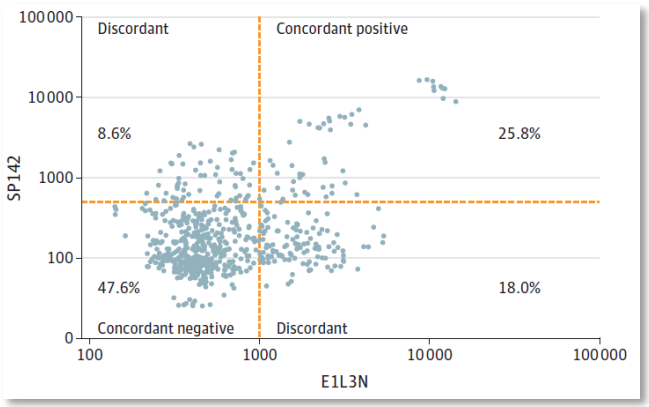
Expression of PD-L1 is heterogeneous[1]



Technical: Assay[1,4,5]

- Epitope stability
- Distribution (patchy versus diffuse)
- Different antibodies and platforms
- Different thresholds for expression
- Interobserver readability

Abs are not identical: >25% discordant[1,6,7]



Logistics: Tissue[1,8,9]

- Interval between tissue and treatment (archived versus fresh)
- Primary versus metastatic disease
- Some circumstances not amenable to obtaining any tissue
- Certain biopsy methods result in poor tissue quality/quantity

IFN = interferon; PD-L1 = programmed death ligand 1.

1. McLaughlin J et al. *JAMA Oncol.* 2015 doi: 10.1001/jamaoncol.2015.3638. [Epub ahead of print]. 2. Heskamp S et al. *Cancer Res.* 2015. [Epub ahead of print]. 3. Pardoll DM. *Nat Rev Cancer*. 2012;12:252-264. 4. Wilson BE et al. *J Immunol Methods*. 1991;139:55-64. 5. Phillips T et al. *Appl Immunohistochem Mol Morphol.* 2015;23(8):541-549. 6. Rimm D et al. *Breast Cancer Res Treat.* 2014;147(2):457-458. 7. Velcheti V et al. *Lab Invest*. 2014;94(1):107-116. 8. Check W. *Cap Today*. 2010. 9. Warth A et al. *Recent Results Cancer Res.* 2015;199:71-84.

PD-L1 Expression Analysis: The issue of method

Agent	Assay	Analysis	Definition of positivity	PD-L1 expression
Nivolumab (anti-PD-1)[1–5]	Dako automated IHC assay (28-8 rabbit antibody) Analytically validated	• Original or new FFPE, tumor cells	• 1% and 5% cutoff among >100 evaluable tumor cells	Pretreated • 56%: 1% cutoff • 49%: 5% cutoff 1st line • 68%: 1% cutoff
Pembrolizumab (anti-PD-1)[6,7]	Dako automated IHC assay (22C3 mouse antibody)	• New tumor biopsy within 60 days before first dose	• Tumor dependent - Melanoma >1% - NSCLC PD-L1+ve: Strong (≥50%) and weak staining (1%–49%) PD-L1-ve: no staining	• ~25%: ≥50% staining • ~45%–70%: ≥1% staining
Atezolizumab (anti-PD-L1)[8–10]	Ventana automated clinical research IHC assay	• Original or new FFPE, immune and tumor cells	• PD-L1+ve IHC 3 (≥10%), IHC 2,3 (≥5%), IHC 1,2,3 (≥1%) • PD-L1-ve IHC 0 (<1%)	• 11%: IHC 3 • 25%: IHC 2 and 3 • 49%: IHC 1,2,3
MEDI-4736 (anti-PD-L1)[11,12]	First-generation or Ventana IHC Automated Assay (in development)	• Original or new FFPE, tumor cells	• Membranous staining in ≥25% of tumor cells at any intensity	• 48%
Avelumab[13] (anti-PD-L1)	NR	• Original or new FFPE	• ≥1% staining at any intensity	• 66%

NR=not reported.

1. Antonia S, et al. Poster presented at WCLC 2013, Abstract P2.11-03. 2. Brahmer J, et al. Poster presented at ASCO 2014, Abstract 8112. 3. Gettinger S, et al. Poster presented at ASCO 2014, Abstract 8024. 4. Topalian S, et al. *N Engl J Med.* 2012;366:2443–2454. 5. 6. Garon E, et al. Poster presented at ASCO 2014, Abstract 8020. 7. Gandhi L, et al. Oral presentation at AACR 2014, Abstract CT105. 8. Soria J, et al. Oral presentation at ECC 2013, Abstract 3408. 9. Rizvi N, et al. Poster presented at ASCO 2014, Abstract TPS 8123. 10. Soria J, et al. Poster presented at ESMO 2014, Abstract 1322P. 11. Brahmer J, et al. Poster presented at ASCO 2014, Abstract 8021. 12. Rizvi NA, et al. Poster presented at ASCO 2015. Abstract 8032. 13. Gulley LJ, et al. Poster presented at ASCO 2015, Abstract 8034.

However...

PD-L1 expression and outcome with PD-1/PD-L1 inhibitors (Pretreated patients)

PD-L1 expression level	Atezolizumab[1] (POPLAR)	Pembrolizumab[2] (2 mg/kg Q3W; KEYNOTE-001)	Avelumab[3]	MEDI4736[4]
	+ve = TC3 or IC3[a] -ve = TC0 and IC0[a]	+ve = ≥50% -ve = <1%	+ve = ≥1% -ve = <1%	+ve = ≥25% -ve = <25%
ORR, %				
Overall	15	15	14	16
PD-L1 positive	38	30	16	27
PD-L1 negative	8	NA	10	5

[a]TC3 or IC3 = TC ≥50% or IC ≥10%; TC0 and IC0 = <1% PD-L1+ respectively.

NR=not reached; IC = immune cells; TC = tumor cells.

1. Spira AI, et al. Presentation at ASCO 2015, Abstract 8010. 2. Flotten O, et al. Presented at WCLC 2015, Abstract MINI03.03.
3. Gulley LJ, et al. Poster presented at ASCO 2015, Abstract 8034. 4. Rizvi NA, et al. Poster presented at ASCO 2015, Abstract 8032.

PD-L1 expression and outcome with PD-1/PD-L1 inhibitors (Pretreated patients)

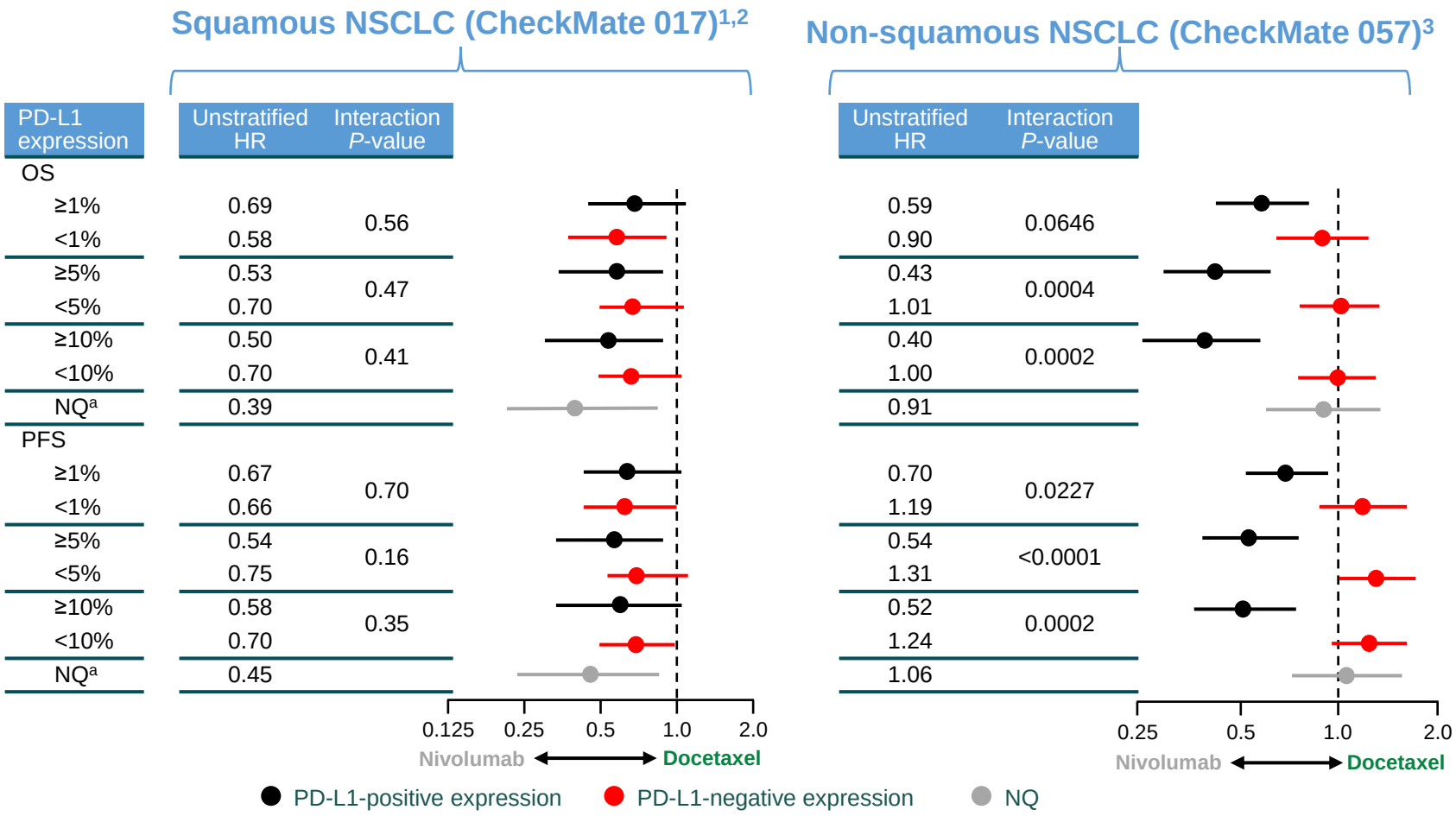
PD-L1 expression level	Atezolizumab[1] (POPLAR)	Pembrolizumab[2] (2 mg/kg Q3W; KEYNOTE-001)	Avelumab[3]	MEDI4736[4]
	+ve = TC3 or IC3[a] -ve = TC0 and IC0[a]	+ve = ≥50% -ve = <1%	+ve = ≥1% -ve = <1%	+ve = ≥25% -ve = <25%
ORR, %				
Overall	15	15	14	16
PD-L1 positive	38	30	16	27
PD-L1 negative	8	NA	10	5
Median PFS				
Overall	2.8 mos	3.3 mos	11.6 wks	NA
PD-L1 positive	7.8 mos	4.2 mos	12.0 wks	NA
PD-L1 negative	1.9 mos	1.5 mos	5.9 wks	NA
Median OS, months				
Overall	11.4	7.6	8.4	NA
PD-L1 positive	NR	NR	8.9	NR
PD-L1 negative	9.7	3.4	4.6	8.9

[a]TC3 or IC3 = TC ≥50% or IC ≥10%; TC0 and IC0 = <1% PD-L1+ respectively.

NR=not reached; IC = immune cells; TC = tumor cells.

1. Spira AI, et al. Presentation at ASCO 2015, Abstract 8010. 2. Flotten O, et al. Presented at WCLC 2015, Abstract MINI03.03.
3. Gulley LJ, et al. Poster presented at ASCO 2015, Abstract 8034. 4. Rizvi NA, et al. Poster presented at ASCO 2015, Abstract 8032.

Different impact of PD-L1 expression related to histology?



[a]at baseline.

1. Brahmer J, et al. *New Engl J Med.* 2015;373:123–135. 2. Spigel DR, et al. Presented at ASCO 2015, Abstract 8009. 3. Paz-Ares L, et al. Presented at ASCO 2015, Abstract LBA109.

PD-L1 expression and outcome in 1st-line NSCLC

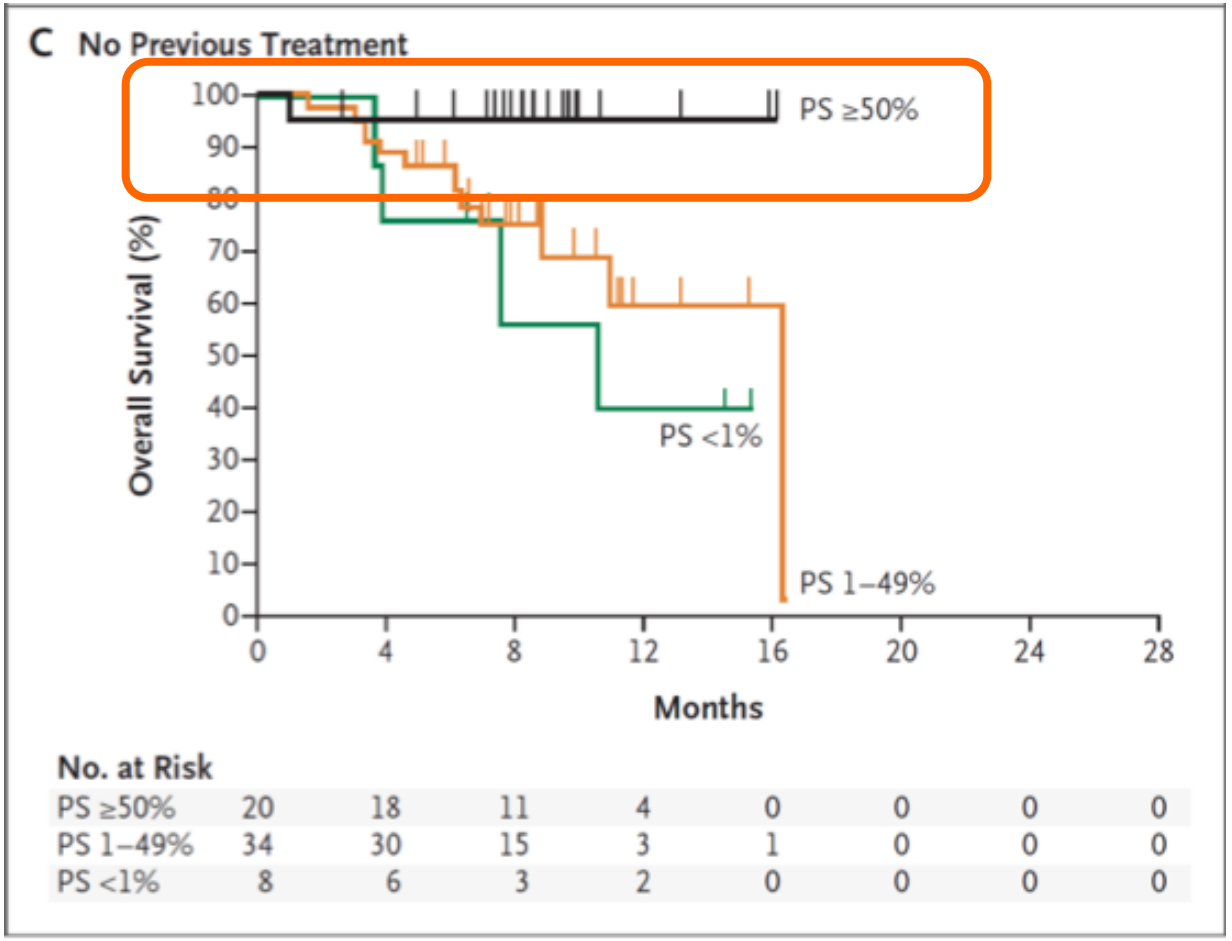
PD-L1 expression level	Nivolumab[1] (CheckMate 012)		Pembrolizumab[2] (KEYNOTE-001)	Atezolizumab[3] (FIR)
	+ve = ≥1% -ve = <1%	+ve = ≥50% -ve = <50%	+ve = ≥50% -ve = <%	+ve = TC3 or IC3[a] -ve = TC0 and IC0[a]
ORR, %				
Overall	23	23	25	29
PD-L1 positive	28	50	50	29
PD-L1 negative	14	15	17	NA
PFS	**Median PFS, weeks**			**24-wk PFS, %**
Overall	15.6	15.6	NA	39
PD-L1 positive	15.1	36.3	NA	43
PD-L1 negative	28.6	10.6	NA	NA
OS	**1-year OS, %**			
Overall	74	74	NA	NA
PD-L1 positive	69	83	NA	NA
PD-L1 negative	86	70	NA	NA

[a]TC3 or IC3 = TC ≥50% or IC ≥10%; TC0 and IC0 = <1% PD-L1+ respectively.

NR=not reached; IC = immune cells; TC = tumor cells.

1. Gettinger S, et al. Poster presented at ASCO 2015, Abstract 8025. 2. Garon EB, et al. *N Engl J Med*. 2015;372:2018–2028. 3. Spigel DR, et al. Presentation at ASCO 2015, Abstract 8028.

Efficacy in untreated patients
Example: Pembrolizumab

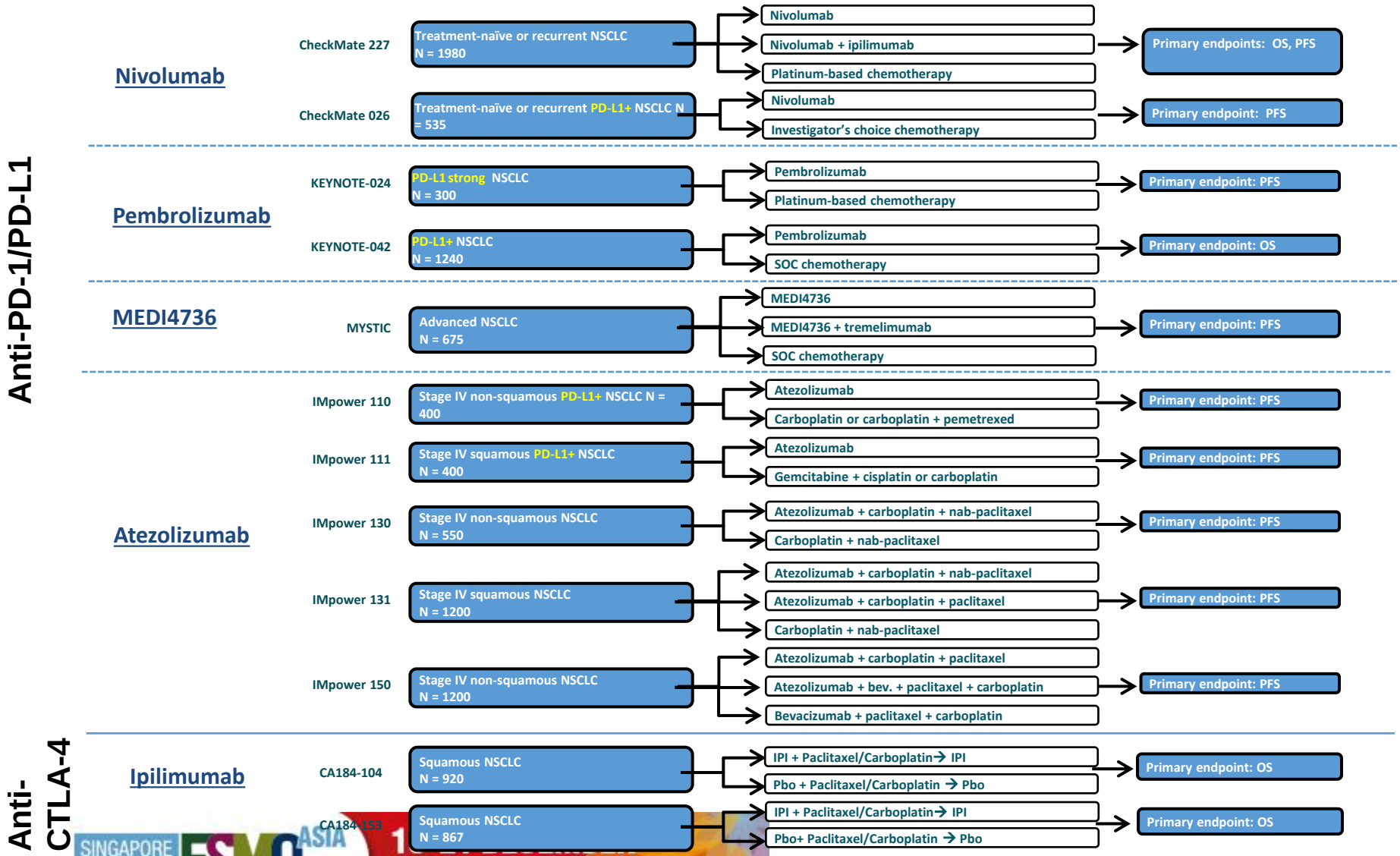


Garon E, NEJM 2015: 372: 2018-28

Some Thoughts

- How strong is the oncogenic signalling, which is characterized by PD-L1 Expression?
- How effective are the PD-1/PD-L1 antibodies?
- How reliable is the test?
- Will a monotherapy with a PD-1/PD-L1 antibody succeed platinum based chemotherapy?

Phase III trials in 1st-line advanced NSCLC (selected)



SOC=standard of care. ClinicalTrials.gov. http://www.clinicaltrials.gov/. Accessed August 2015.

Impact of PD-L1 Expression on IO combinations? Example: Nivolumab/Ipilimumab

PD-L1 expression	Nivo 1 + Ipi 1 Q3W		Nivo 1 Q2W + Ipi 1 Q6W		Nivo 3 Q2W + Ipi 1 Q12W		Nivo 3 Q2W + Ipi 1 Q6W	
	≥1% PD-L1 (n = 12)	<1% PD-L1 (n = 13)	≥1% PD-L1 (n = 21)	<1% PD-L1 (n = 7)	≥1% PD-L1 (n = 21)	<1% PD-L1 (n = 9)	≥1% PD-L1 (n = 23)	<1% PD-L1 (n = 7)
ORR, %	8	15	24	14	48	22	48	0
mPFS, mos (95% CI)	2.6 (1.6,)	7.8 (2.0,)	4.9 (2.6,)	NR (2.3,)	8.0 (3.6, 8.1)	5.3 (0.9,)	NR (3.5,)	2.4 (1.7, 2.9)
PFS rate at 24 wks, % (95% CI)	42 (15, 67)	57 (25, 80)	40 (18, 61)	NC	74 (48, 88)	39 (9, 69)	65 (42, 81)	0

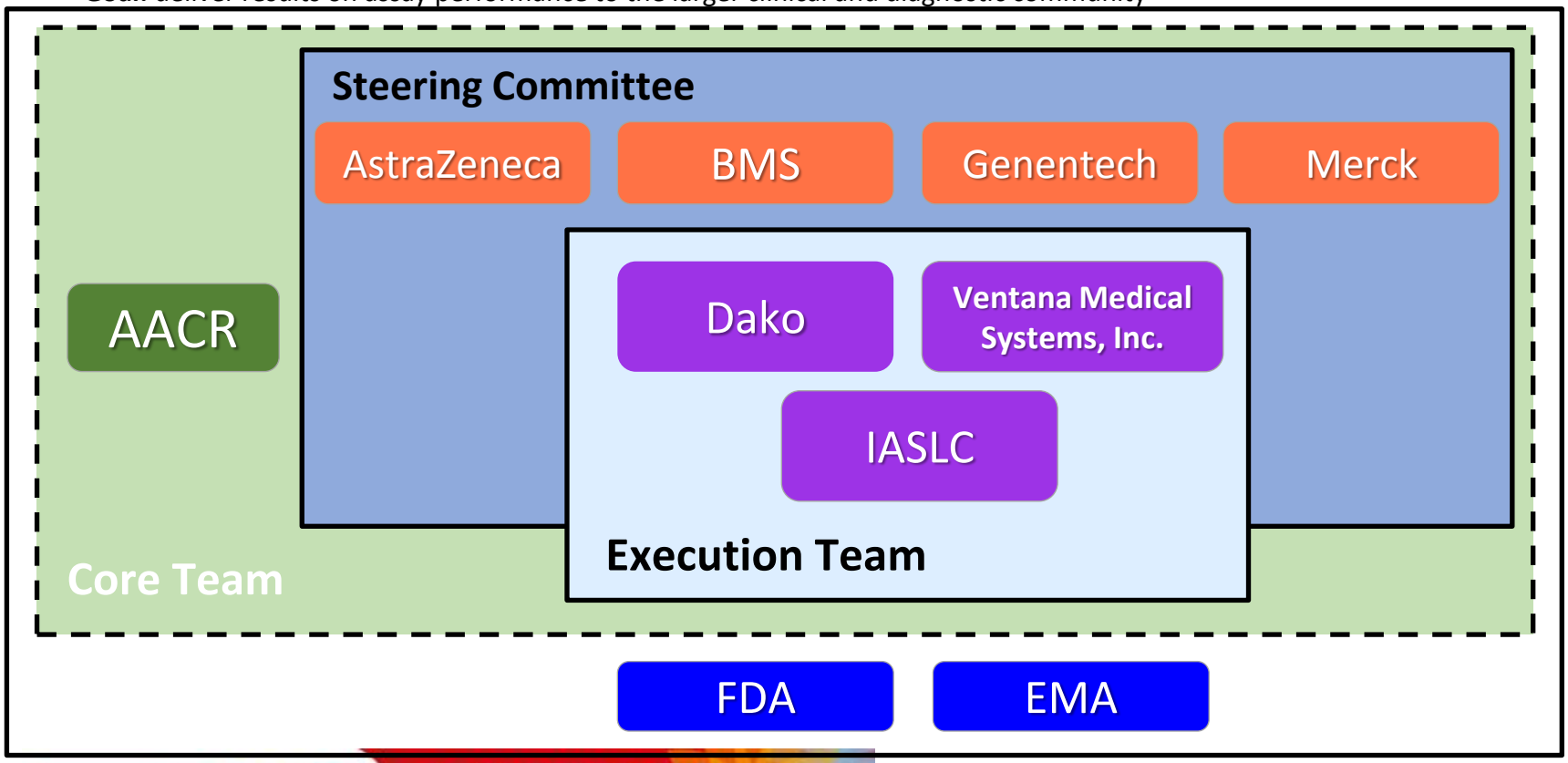
NR due to high percentage of ongoing response or insufficient number of events and/or follow-up.

Hellmann M, abstract 349, ECCO ESMO 2015

Harmonization of PD-L1 assessment desperately needed!

Blueprint Project Collaboration May Provide a practical solution

- A **collaboration** between different stakeholders
  - Evaluate and compare the analytical performance of 4 IUO assays (manufactured by Dako and Ventana) that are currently being used for PD-L1 diagnostic purposes under controlled conditions
- **Goal:** deliver results on assay performance to the larger clinical and diagnostic community



Additional markers

- PD-L2 (in combination with PD-L1)
  - Limited expression in normal tissue, high expression in tumor, endothelial and stroma cells of NSCLC
  - High concordance with PD-L1 expression
  - Associated with response to PD-1 antibody in adjustment with PD-L1 expression
- Infiltration of CD8+ cells in the „invasive margin“ of the tumor
  - Tumor response to PD-1/PD-L1 is correlated with density of preexisting CD8+ cells in the tumor

Yearly J, ECCO ESMO 2015; Tumeh PC Nature 2014

Conclusions

- Immunotherapy by checkpoint inhibition represents a fascinating new treatment option
- Development of predictive markers remain a challenge
- So far PD-L1 expression is the only practical marker, which suggests correlation with clinical efficacy
  - Further development and harmonization is urgently needed
- Other markers like mutational load, PD-L2 expression, T-cell infiltration are under exploration and development
- The goal of biomarker development has to be defined