

Post-ASCO 2017

Molecular oncopathology

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Disclosure

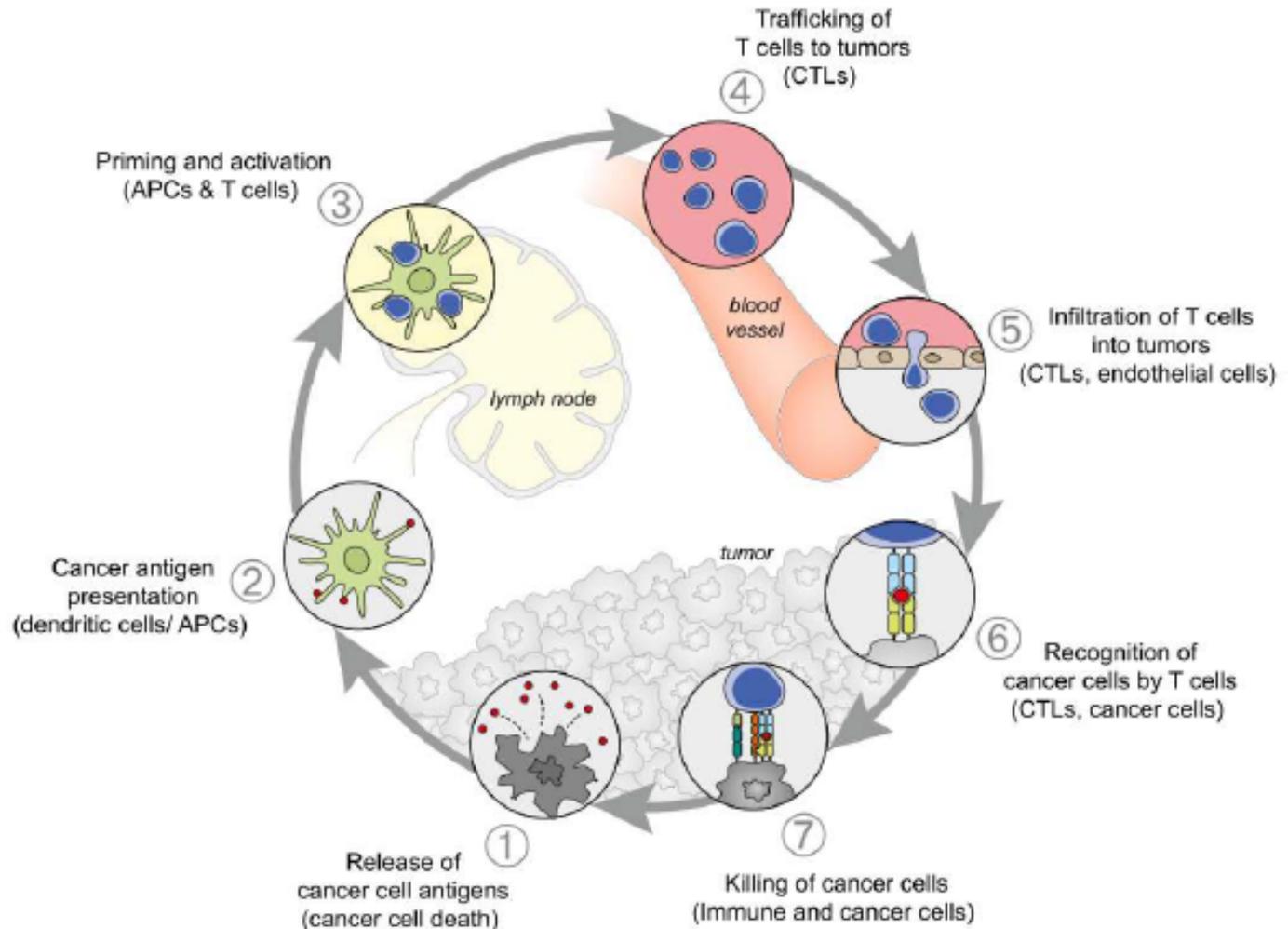
(potentiële) belangenverstrengeling	
Voor bijeenkomst mogelijk relevante relaties met bedrijven	MSD, Roche, BMS, Pfizer, Novartis
<ul style="list-style-type: none">• Sponsoring of onderzoeksgeld• Honorarium of andere (financiële) vergoeding• Aandeelhouder• Andere relatie, namelijk ...	<ul style="list-style-type: none">• KWF, Novartis, Pfizer, BMS• MUMC+• Geen• Geen

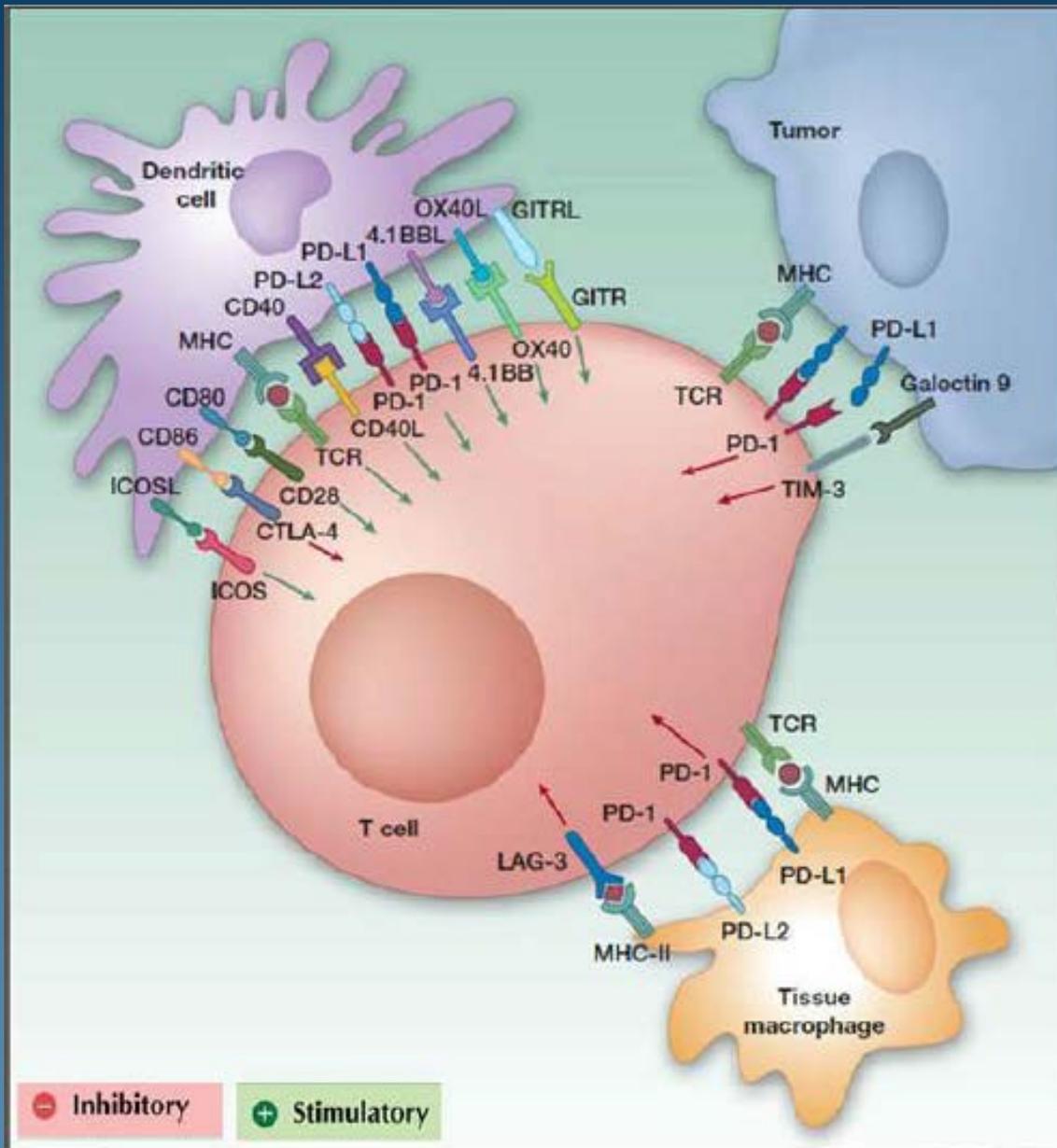
Overview

- Immunotherapy:
 - Drugs and applications
 - NSCLC
 - PD-L1 immunohistochemistry
 - Other biomarkers for prognosis/prediction
 - Resistance to immunotherapy
- Biomarkers and targeted therapy for NSCLC
- Molecular Tumor Board

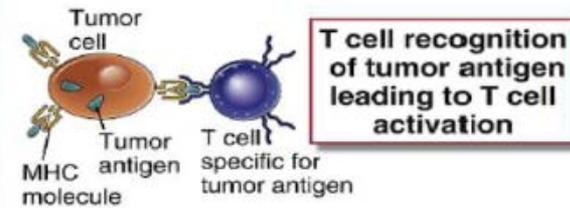


The Cancer-Immunity Cycle is Central to Immune Surveillance and Defence

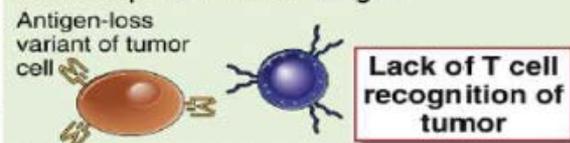




Anti-tumor immunity

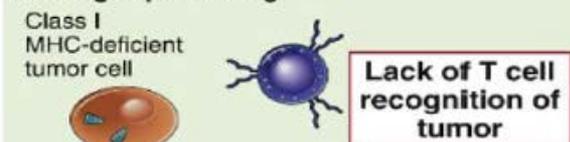


Failure to produce tumor antigen

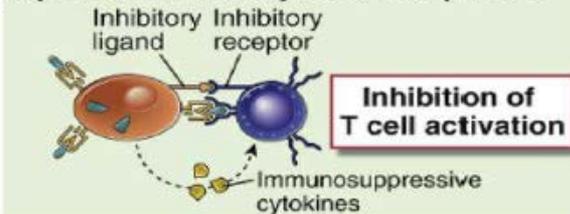


Immune evasion by tumors

Mutations in MHC genes or genes needed for antigen processing

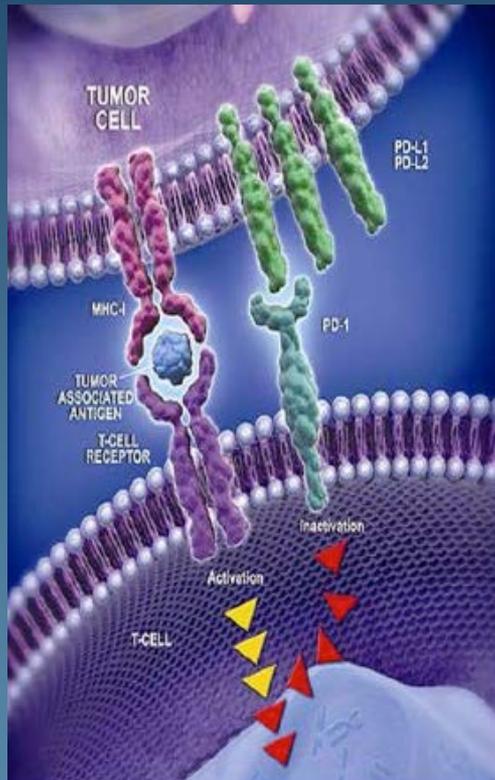


Secretion of immunosuppressive proteins or expression of inhibitory cell surface proteins



Abbas, Lichtman, Pilai: Cellular and Molecular Immunology, 8th ed

PD-1/PD-L1 immunotherapy: state of the art 2015!



Pembrolizumab

Atezolizumab

7 Companies Developing PD-1 checkpoint inhibitors!

Anti-PD-1

Nivolumab	Bristol-Myers Squibb	IgG4	Phase 3
MK-3475	Merck & Co	IgG4 (humanized)	Phase 3
Pidilizumab	CureTech	IgG1 (humanized)	Phase 2
AMP-224	AstraZeneca/	PD-1/B7 Fc fusion protein	Phase I
AMP-514	Medimmune	IgG	Phase I
	Novartis (CoStim)	IgG	Phase I

Anti-PD-L1

MPDL3280A	Genentech/Roche	IgG1	Phase 3
MEDI-4736	AstraZeneca/	IgG1	Phase 2
MSB0010718C	EMD Serono (Merck KGa)	IgG	Phase 1

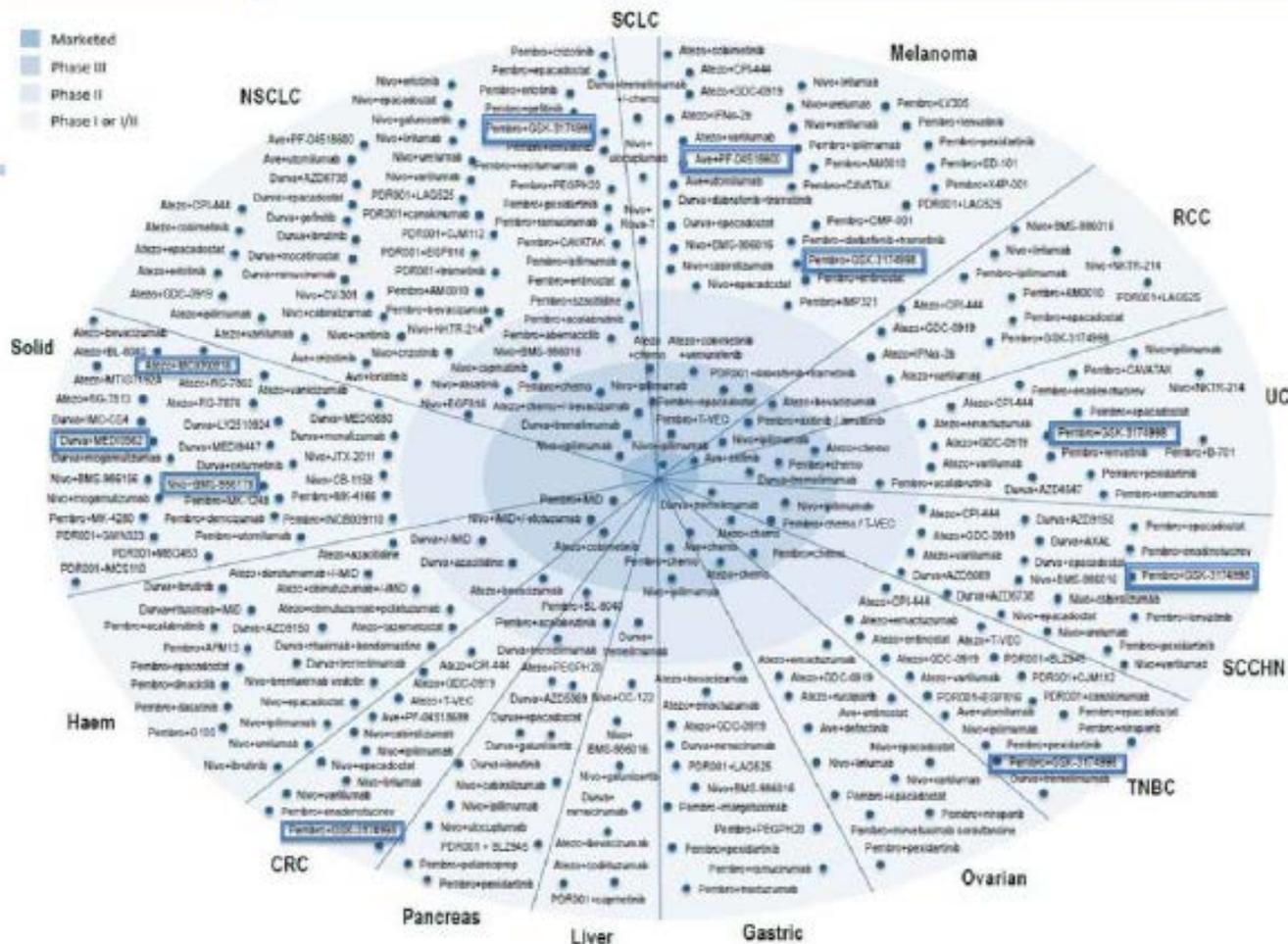
www.ClinicalTrials.gov accessed may 12th, 2014



NSCLC

H. Groen, 03-06-2015

Immunotherapy combinations currently in clinical trials



New applications anti-PD1/PDL1 immunotherapy (FDA)

Nivolumab (PDL1 *complementary*: Dako 28-8)

FULL PRESCRIBING INFORMATION: CONTENTS*

1 INDICATIONS AND USAGE

- 1.1 Unresectable or Metastatic Melanoma
- 1.2 Metastatic Non-Small Cell Lung Cancer
- 1.3 Renal Cell Carcinoma
- 1.4 Classical Hodgkin Lymphoma
- 1.5 Squamous Cell Carcinoma of the Head and Neck
- 1.6 Urothelial Carcinoma

Atezoluzimab
PDL1 *complementary*
(Ventana SP142)

1 INDICATIONS AND USAGE

- 1.1 Melanoma
- 1.2 Non-Small Cell Lung Cancer
- 1.3 Head and Neck Cancer
- 1.4 Classical Hodgkin Lymphoma
- 1.5 Urothelial Carcinoma
- 1.6 Microsatellite Instability-High Cancer

INDICATIONS AND USAGE

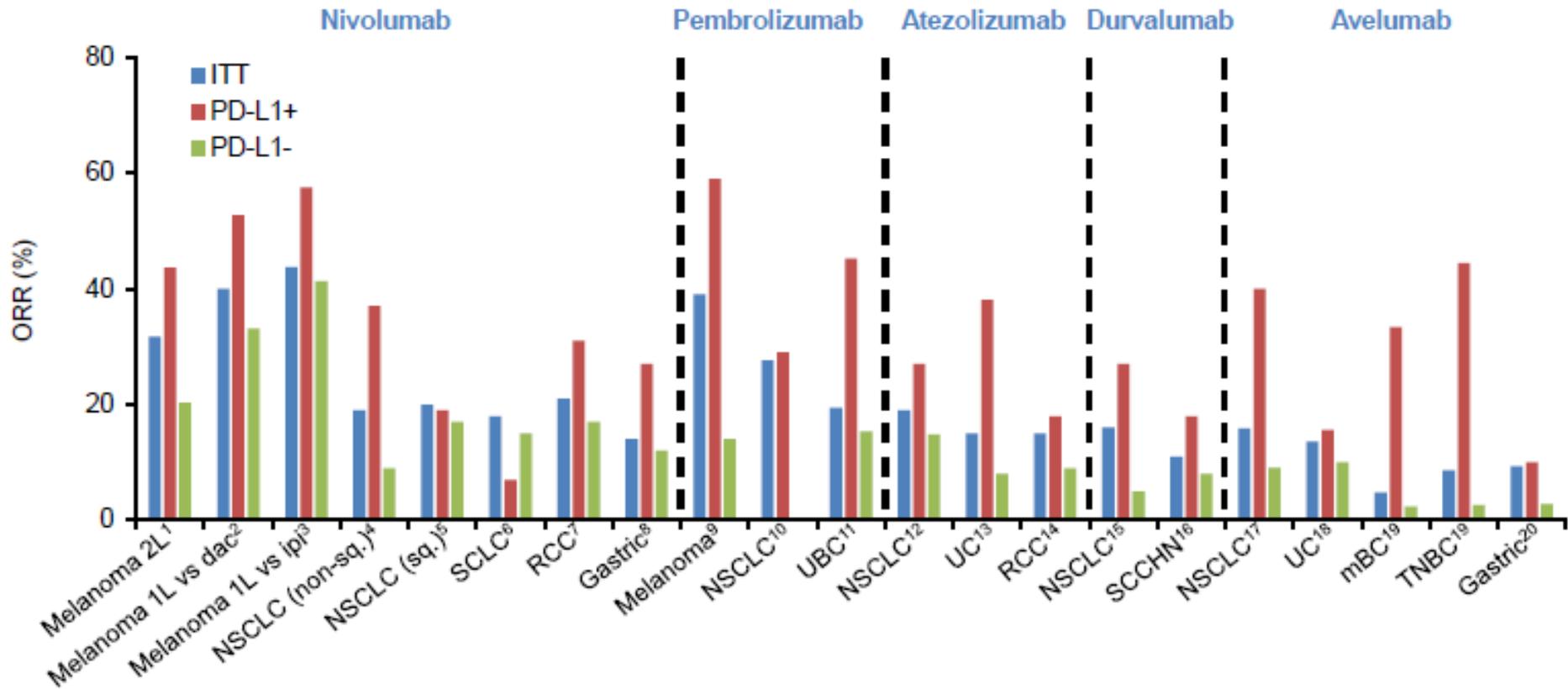
TECENTRIQ is a programmed death-ligand 1 (PD-L1) blocking antibody indicated for the treatment of patients with locally advanced or metastatic urothelial carcinoma who:

- Have disease progression during or following platinum-containing chemotherapy (1)
- Have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy (1)

This indication is approved under accelerated approval based on tumor response rate and duration of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials. (1, 14)

Pembroluzimab (PDL1 *companion*: Dako 22C3)

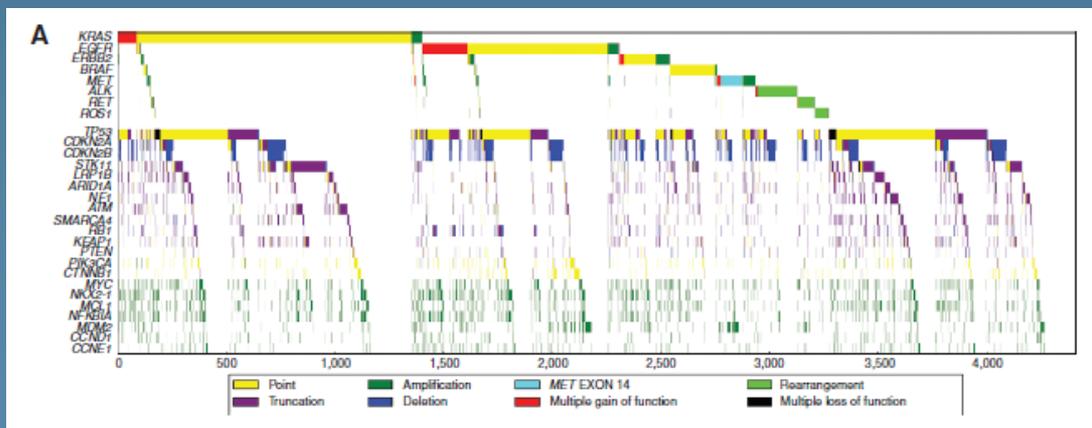
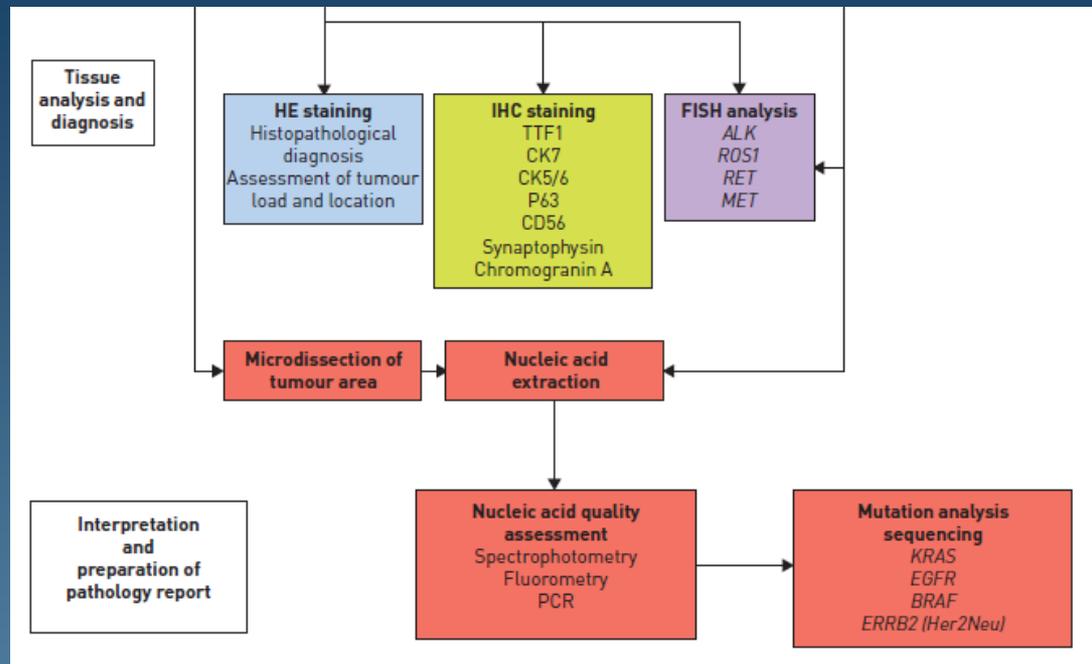
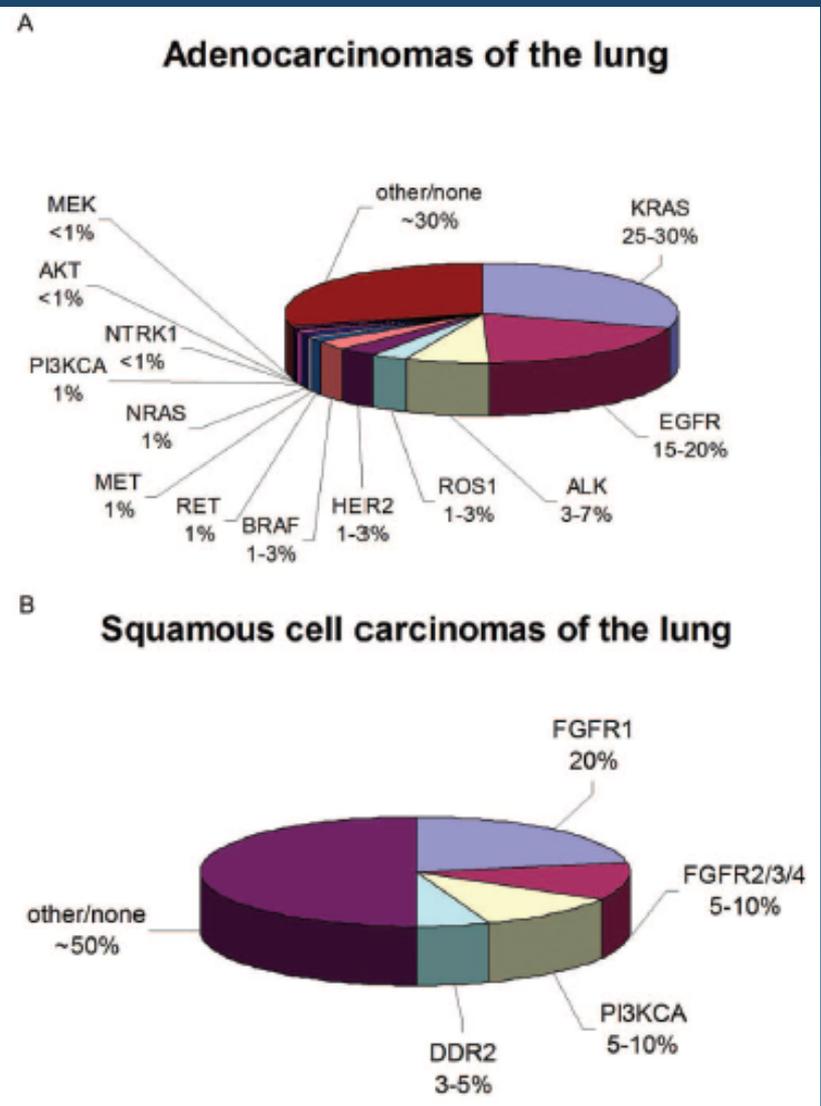
Response Rate by PD-L1 Status



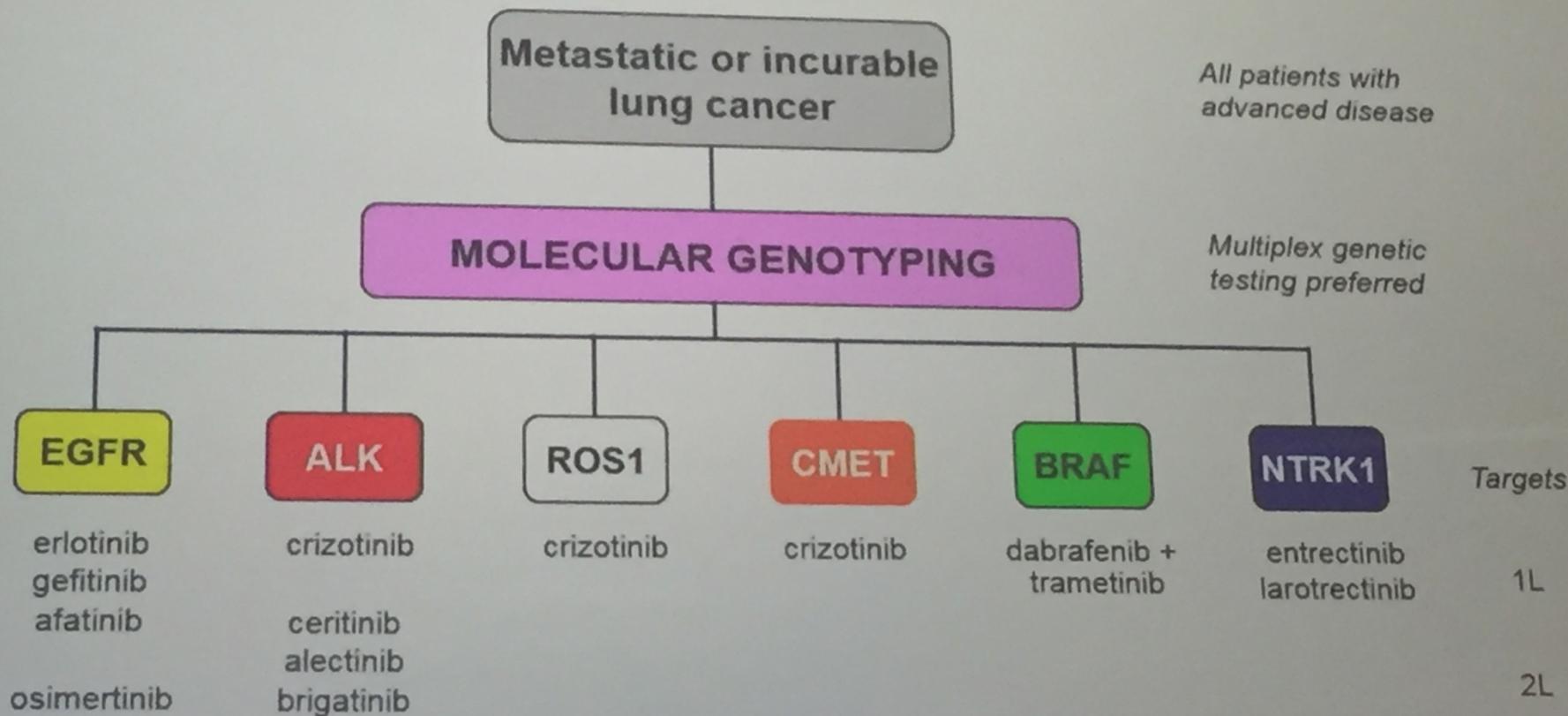
Non-sq.=non-squamous; sq.=squamous

1. Weber et al. Lancet 2015;
2. Robert et al. Lancet 2015;
3. Larkin et al. N Engl J Med 2015;
4. Borghaei et al. N Engl J Med 2015
5. Brahmer et al. N Engl J Med 2015;
6. Antonia et al. ASCO 2015;
7. Motzer et al. J Clin Oncol 2015;
8. Le et al. ASCO GI 2016
9. Kefford et al. ASCO 2014;
10. Garon et al. N Engl J Med 2015;
11. Plimack et al. ASCO 2015;
12. Vansteenkiste et al. ECC 2015
13. Rosenberg et al. Lancet 2016;
14. McDermott et al. J Clin Oncol 2015;
15. Rizvi et al. ASCO 2015;
16. Segal et al. ASCO 2015
17. Gulley et al. ASCO 2015;
18. Apolo et al. ASCO GU 2016;
19. Dirix et al. SABCS 2015;
20. Chung et al. ASCO GI 2016

Molecular diagnostics NSCLC



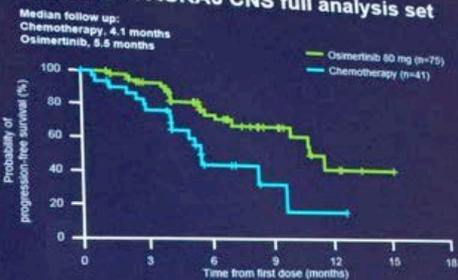
Matching Targeted Therapies to Targets in NSCLC



Changes in management stage IV NSCLC with EGFR and ALK mutations

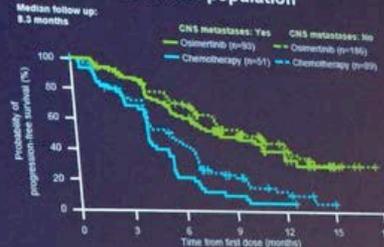
- Osimertinib has high CNS activity (35-50% brain metas at diagn/progr)
- Dacomitinib is a new active first line EGFR mutant treatment option
- Alectinib is the new first-line ALK + treatment
- Lorlatinib is highly active in relapsed ALK + NSCLC for brain metastasis

CNS PFS in AURA3 CNS full analysis set



Median CNS PFS, months	n	CNS PFS	HR (95% CI)
Osimertinib 80 mg	75	11.7	0.32 (0.15, 0.69), p=0.004
Chemotherapy	41	5.6	

PFS in AURA3 overall population



Median PFS, months	CNS metastases: Yes			CNS metastases: No		
	n	PFS	HR (95% CI)	n	PFS	HR (95% CI)
Osimertinib 80 mg	93	8.5	0.32 (0.21, 0.49), p<0.001	186	10.8	0.40 (0.25, 0.63), p<0.001
Chemotherapy	51	4.2		69	5.4	

Population: CNS full analysis set: patients with at least one measurable and/or non-measurable CNS metastases on baseline brain scan by BCR
 Data cut-off: April 15, 2016
 *Censored patients only; **Only includes progression events that occurred within 15 weeks of the last available assessment; †Estimated by Kaplan-Meier technique

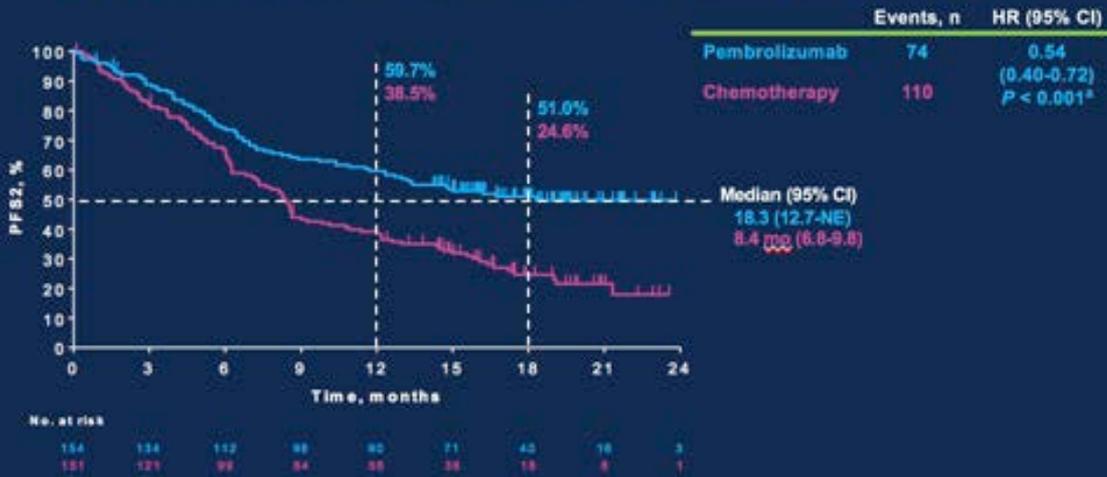
J-ALEX vs ALEX vs ASCEND 4: efficacy

Trial	J-ALEX	ALEX	J-ALEX	ALEX	ASCEND4
Drug	crizotinib	crizotinib	alectinib	alectinib	ceritinib
n	104	151	103	152	189
Median PFS	10.2	10.4	NR (>21)	25.7	16.6
PFS HR (95% CI)			0.34** (0.17-0.71)	0.50** (0.36-0.70)	0.55* (0.42-0.73)
ORR (%)	79	76	92	83	73
Median PFS BM+	10.2	7	NR (>21)	NR (>27)	10.7
Median PFS BM-	10.0	15	20.3	NR (>27)	26.3
Intracranial ORR(%)	-	50	-	81	73

Shaw ASCO (2017); Hida Lancet (2017); Kim JCO (2016); Hida JCO (2016); Shaw ASCO (2017); NR, not reached; * vs chemotherapy; ** vs crizotinib

Immunotherapy in lung cancer

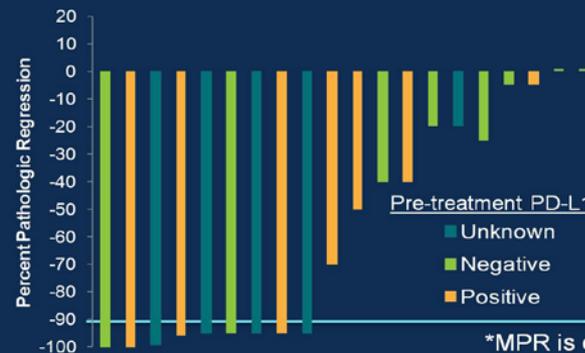
First-line pembrolizumab study updated



Immunotherapy should be given frontline in patients with advanced NSCLC with PD-L1 => 50% with negative mutation profile.

Immunotherapy in early stage NSCLC:
 neoadjuvant nivolumab :
 MPR: 43%
 no correlation PDI-L1 ≥ 1%

Pathologic responses to neoadjuvant nivolumab



•Major Pathologic Response (MPR) was seen in 9/21 cases [43% (95% CI 24-63%)]

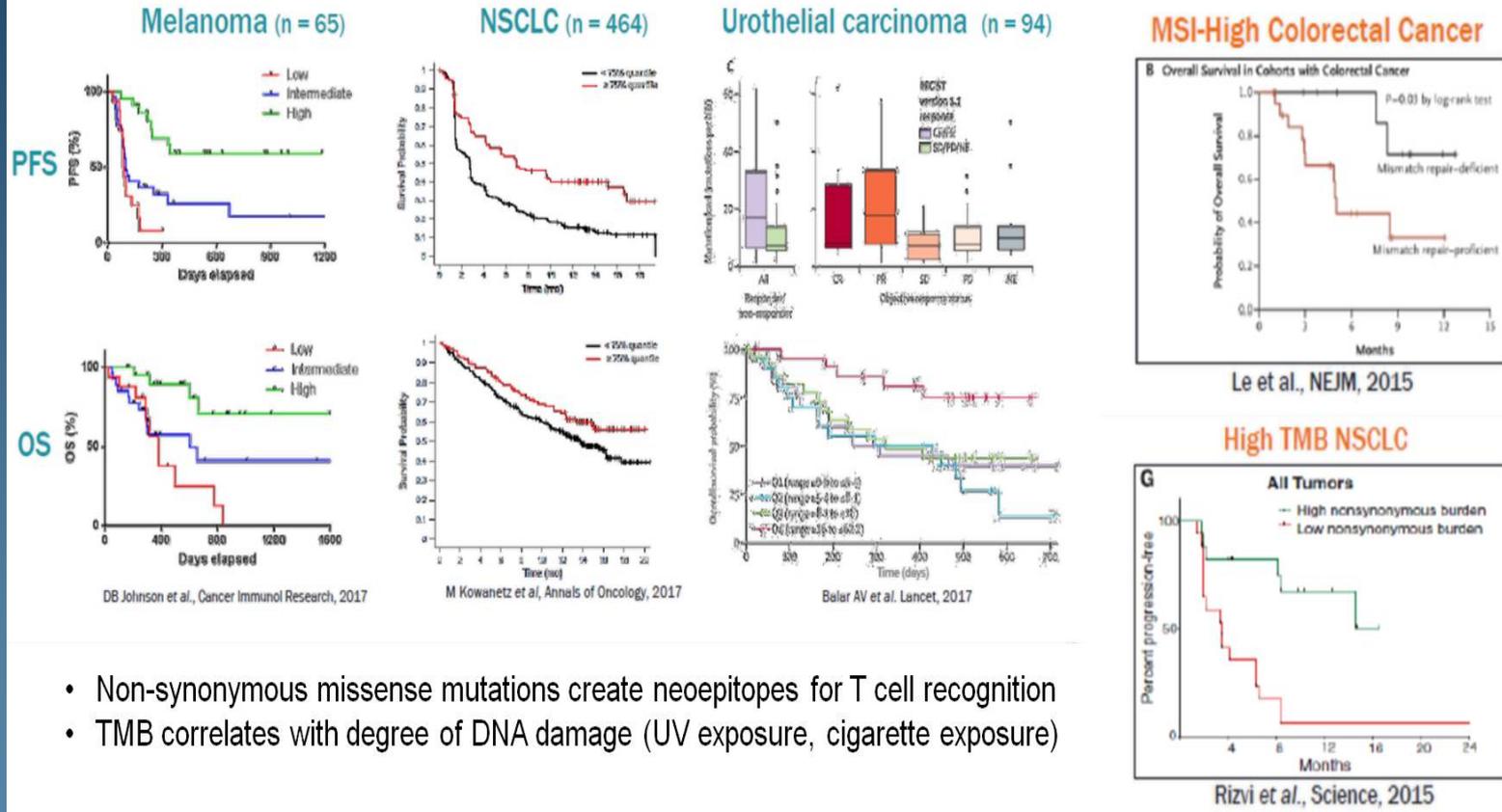
•Pre-treatment PD-L1 positivity [≥1% membranous staining (Dako 28-8)] did not correlate with MPR

*MPR is defined as ≤10% viable tumor cells (Pataer, et al. JTO, 2012. Hellmann, et al. Lancet Oncol 2014.)

1st line PD1 inhibitors +/- CT

Study	Phase	n	RR (%)	PFS (months)	OS (months)	AEs \geq grade 3 (%)
KEYNOTE 024 [15] Pembrolizumab vs. CT	III	305 PD-L1 \geq 50% ^a	44.8 vs. 27.8 $P <$ 0.001	10.3 vs. 6.0 HR, 0.50; $P <$ 0.001	HR, 0.60; $P =$ 0.005 1-year OS: 70% vs. 54%	26.6 vs. 53.3
CheckMate 026 [31] Nivolumab vs. CT	III	423 PD-L1 \geq 5% ^b	26.1 vs. 33.5	4.2 vs. 5.9 HR, 1.15; $P =$ 0.251	HR, 1.02 14.4 vs. 13.2 months	17.6 vs. 50.6
KEYNOTE 021 [39] Pembrolizumab + CT vs. CT	II	123	55 vs. 29 $P =$ 0.0016	13.0 vs. 8.9 HR, 0.53; $P =$ 0.0102	HR, 0.90; $P =$ 0.39	39 vs. 26
CheckMate 012 [42]	I	38	47	8.1	Not calculated	37
Nivolumab + Ipilimumab/12 weeks until disease progression or toxicity		40	38	3.9		33
Nivolumab + Ipilimumab/6 weeks until disease progression or toxicity						

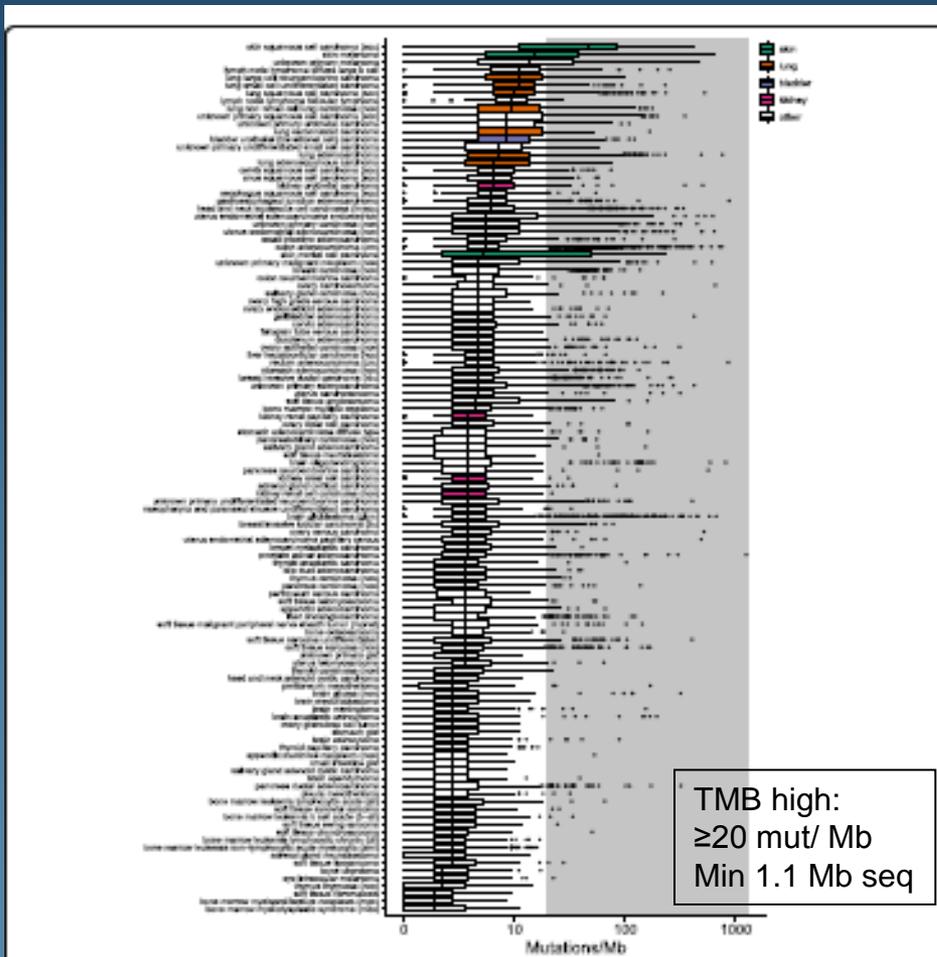
TMB is predictive of benefit to IO



- Non-synonymous missense mutations create neopeptides for T cell recognition
- TMB correlates with degree of DNA damage (UV exposure, cigarette exposure)

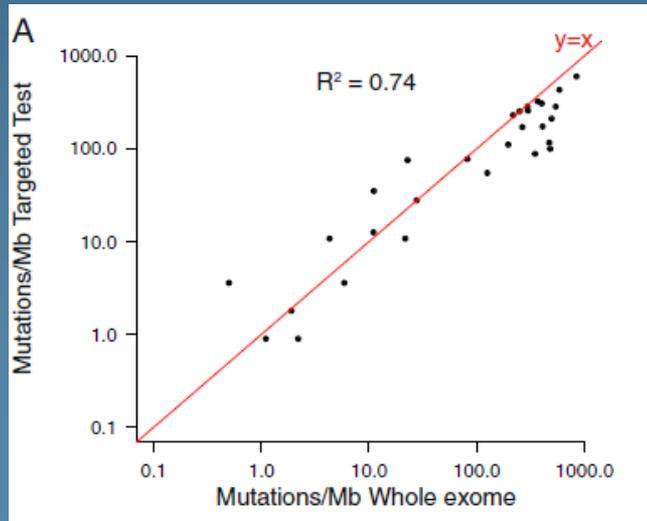
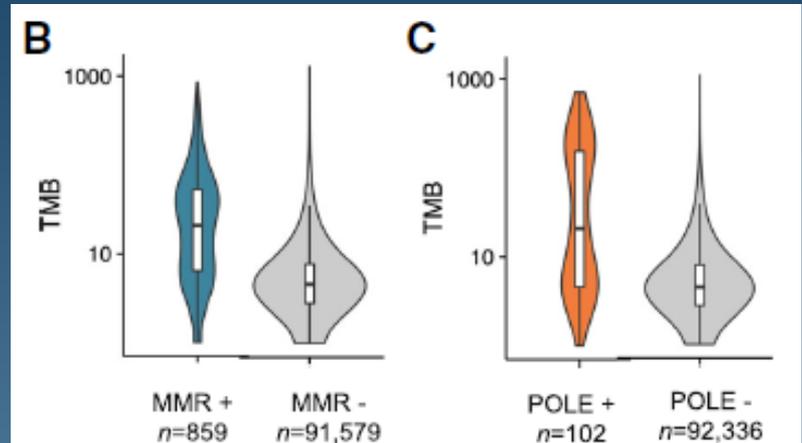
Stephen P. #SY40-02 AACR 2017

How is Tumor Mutational Burden defined?

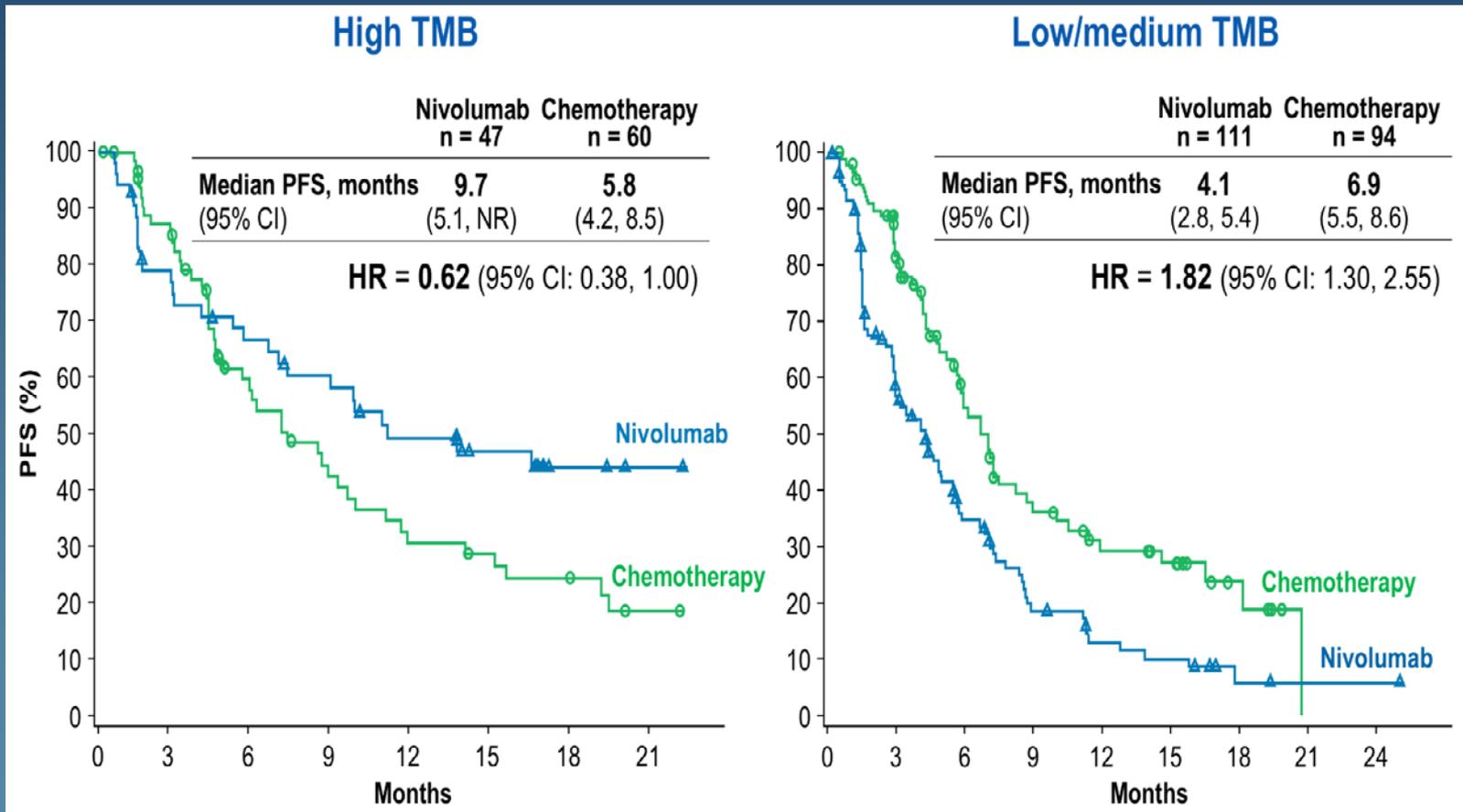


TMB high:
 ≥20 mut/ Mb
 Min 1.1 Mb seq

Fig. 2 The landscape of tumor mutation burden, for all disease types, with greater than 100 samples, the median mutation burden is plotted for each disease type. The left and right edges of the boxes correspond to the 25th and 75th percentiles. Whiskers extend to the highest value that is within 1.5 x IQR of the hinge, where IQR is the inter-quartile range, or distance between the first and third quartiles. Points beyond this are plotted individually. Tumor types of interest are shown in color, as follows: skin, green; lung, orange; bladder, red; kidney, purple; other, pink; white. The area above 20 mutations/Mb, which we have designated as high TMB, is colored in grey.

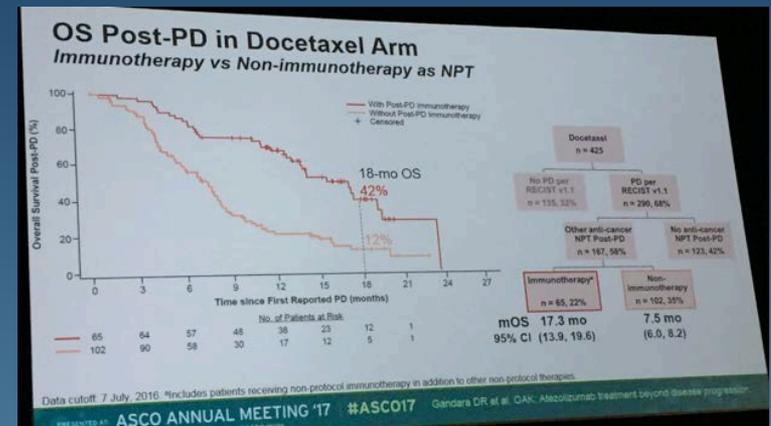
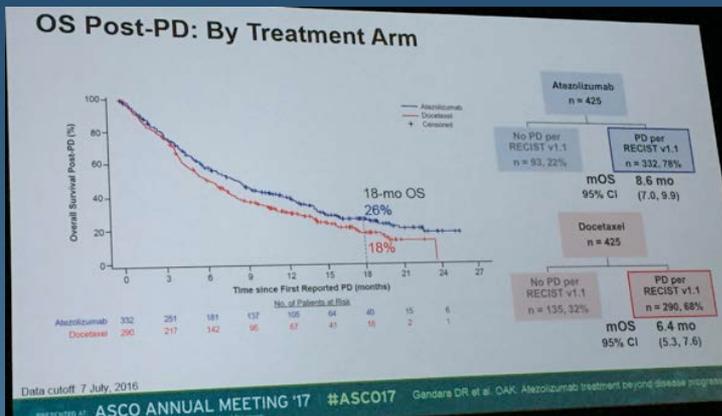


Tumor mutation burden and response in first-line negative nivolumab study



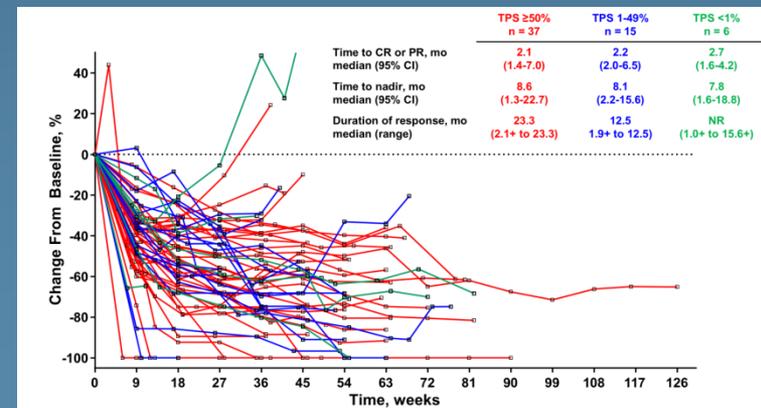
Does immunotherapy select chemosensitive tumor?
Modification tumor microenvironment?

- Continuation of immunotherapy beyond PD is beneficial in an **selected** population of advanced NSCLC who have **clinical benefit**.
- Stable disease in 30 – 70% but they are short lasting!



Gandara et al., randomized ph II OAK study

- Immune response occurs usually within 6-8 weeks; patients do improve clinically.
- Nivolumab + ipilumimab promising in Ph I/II studies malignant mesothelioma and SCLC



Immunotherapy after failure of targeted therapy may be an option in selected cases (if PD-L1 positive).

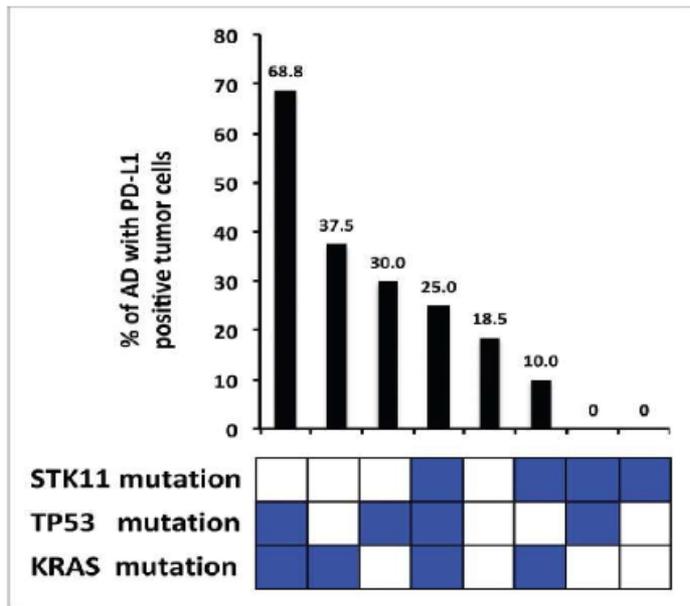
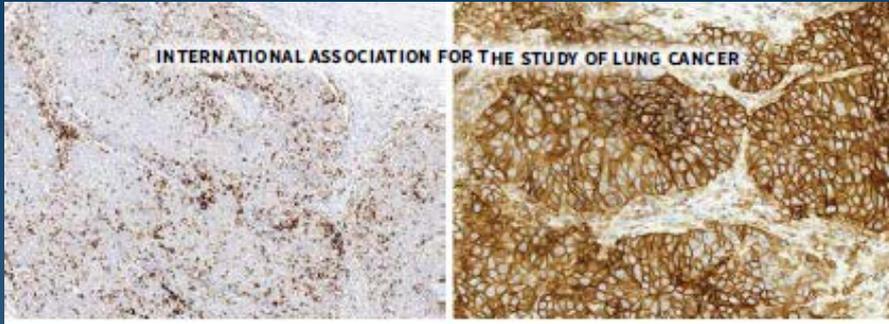


Figure 4. Percentage of PD-L1 expression in adenocarcinomas and mutational status of significantly associated genes. The combination of *TP53* mutation, *KRAS* mutation and *STK11* wildtype is associated with the highest percentage of PD-L1 expression in adenocarcinoma tumor cells. Conversely, *STK11* mutations in the absence of *TP53* and *KRAS* mutations are associated with the lowest percentage.

ASCO 2017: (Ben Creelan)

- Idem
- EGFR+: no
- ALK+: no
- MET ex14 skip: no
- High TMB: yes



IASLC ATLAS OF PD-L1 IMMUNOHISTOCHEMISTRY TESTING IN LUNG CANCER



EDITED BY
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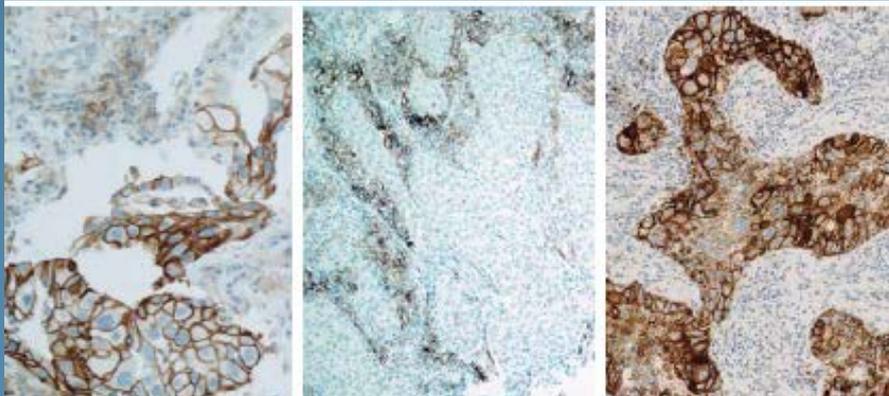


Table 1. Five-Assay Comparison*

Assay Antibody PD-L1 Clone	Staining Platform	Immunotherapy Drug	Clinical Cut-off(s) for PD-L1 Expression	FDA Designation
28-8	Dako Link 48	Nivolumab (Bristol-Myers Squibb)	≥ 1%, ≥ 5%	Complementary device
22C3	Dako Link 48	Pembrolizumab (Merck)	≥ 1%, ≥ 50%	Companion device
SP142	Ventana Benchmark or Ultra	Atezolizumab (Genentech/Roche)	Tumor cells ≥ 1%, ≥ 5%, ≥ 50% Immune cells ≥ 1%, ≥ 5%, ≥ 10% by area	Complementary device
SP263	Ventana Benchmark or Ultra	Durvalumab (AstraZeneca/MedImmune)	≥ 25%	No designation
73-10	Dako Link 48	Avolumab (Pfizer/Merck Serono)	≥ 1%, ≥ 50%, ≥ 80%	In development

* Details for each platform, are products of Ventana

Table 1. Recommended Preanalytic Conditions for Immunohistochemistry (IHC)

Parameter	Recommendation
Cold ischemia time	Fewer than 30 minutes if possible, not exceeding 1 hour
Fixative	10% neutral buffered formalin
Time of fixation (biopsy)	6 to 48 hours
Time of fixation (resection)	24 to 48 hours
Preparation	Paraffin-embedded sections, cut at a thickness of 3 to 5 μm
Specimen storage	Tissue blocks
Storage time for blocks	Fewer than 3 years for PD-L1 IHC
Storage conditions for blocks	Prevented from light, heat, and humidity
Storage time for cut sections	Fewer than 2 months, particularly for testing with SP263 antibody
Decalcification	EDTA, if necessary

PD-L1 = programmed cell death-ligand 1.

PD-L1 IHC variation

Different PD-L1 clones on consecutive sections of one tumor

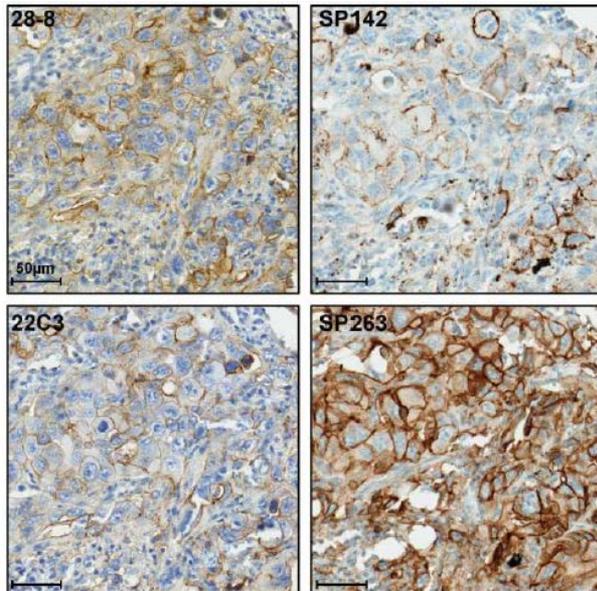
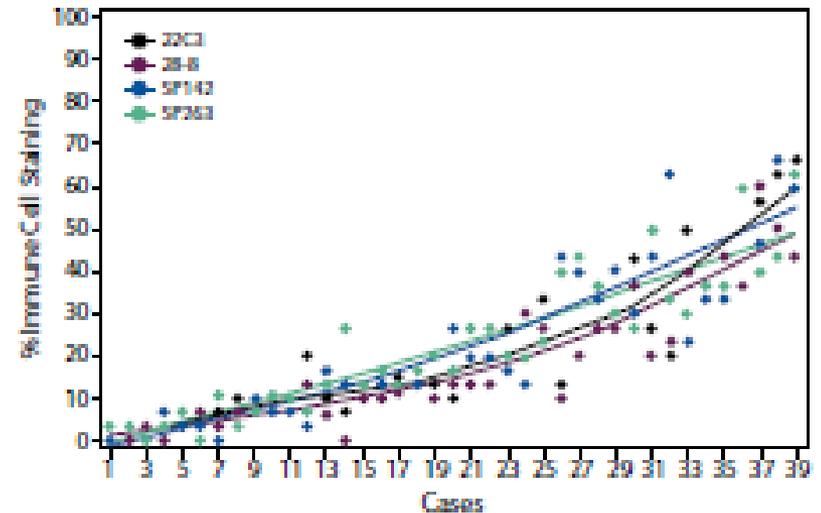
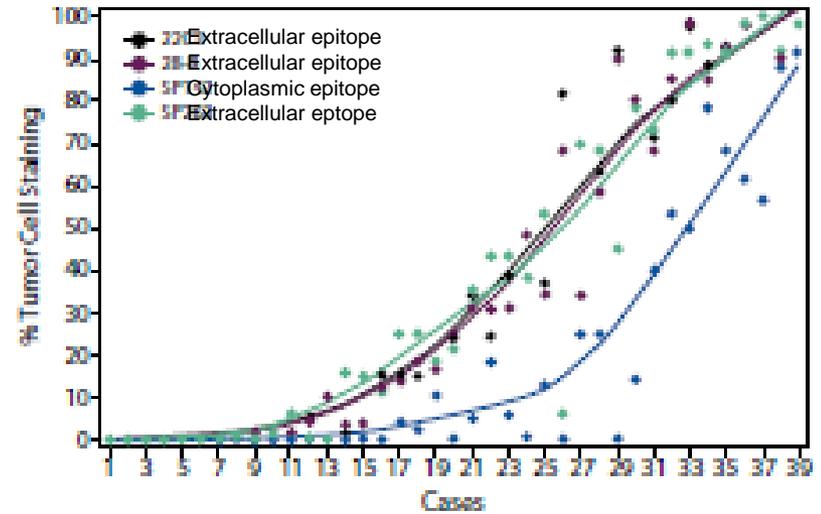
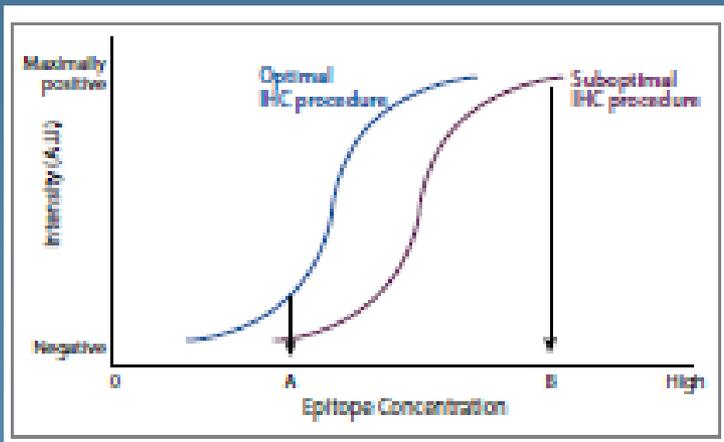


Figure 1 Staining patterns of clinical trial assays for PD-L1 immunohistochemistry. Example micrograph of four clinical trial assays for PD-L1 immunohistochemistry; matched regions on consecutive slides stained with the indicated assays. The case was scored PD-L1 positive (score 5, $\geq 50\%$) by all assays.



IASLC Atlas PD-L1 2017
 Scheel et al., Modern Pathol 2016
 Hirsch et al., JTO 2017



Schats et al., ASCO 2017 Histogenex

PD-L1 (Q9NZQ7); [expected MW isoform 1 = 33 kDa; isoform 2 = 20 kDa]

1 18 26 56 58 113115 121 125 142

MRIFAVFIFMITYWHLLNAFTVTVPKDLVYVEYGSNMETIECKFPVEKQLDLAALIVYWEEMEDKNIIQFVHGEEEDLVQSSYRQRARLLKDKLSLGNAAALQIDVYKLDQAGVYRCMISYGGADYKRITVYKVNAPYNKINQRIL

143 238 259 290

VVDVPTSEHELTCQAEQPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKRGRMMDVKKCGIQDTNSKKQSDTHLEET

PD-L1 Ab clone	Linear epitope mapping : concentration primary ab (µg/ml)	Immunogen [adapted from *datasheet; ** patent]	Epitope location
28-8	1 ; 10 ; 100	Purified recombinant human PD-L1 containing the extracellular domain of huPD-L1 (Phe19-Thr239). ²	Intracellular
SP142	0.78	Synthetic peptide derived from the C-terminus of human PD-L1 protein.* (aa 279-290; SKKQSDTHLEET).**	Intracellular & extracellular
SP263	1.61	Human PD-L1 (aa 272-290; CGIQDTNSKKQSDTHLEET).**	Intracellular & extracellular
E1L3N	1 ; 10	Synthetic peptide corresponding to residues carboxy terminus of human PD-L1 protein.*	Intracellular
22C3	Not tested	Human extracellular domain of PD-L1 (Phe19-Thr239) fused to a human IgG1 fragment.**	Extracellular; 156-178 & 196-206

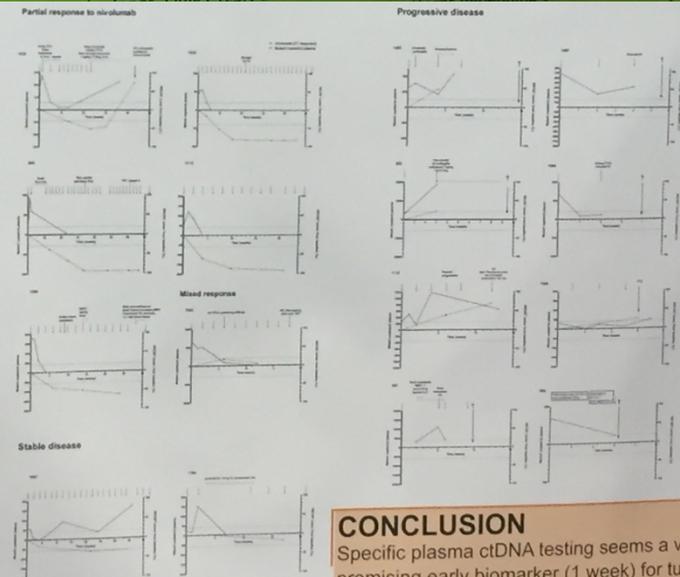
Signal peptide
Extracellular domain
Helical domain
Intracellular domain
PD-1 binding Site ?
Absent in isoform 2

* Only E1L3N demonstrated a clear linear epitope. Blasting each of the ... depending on PD-L1 conformation

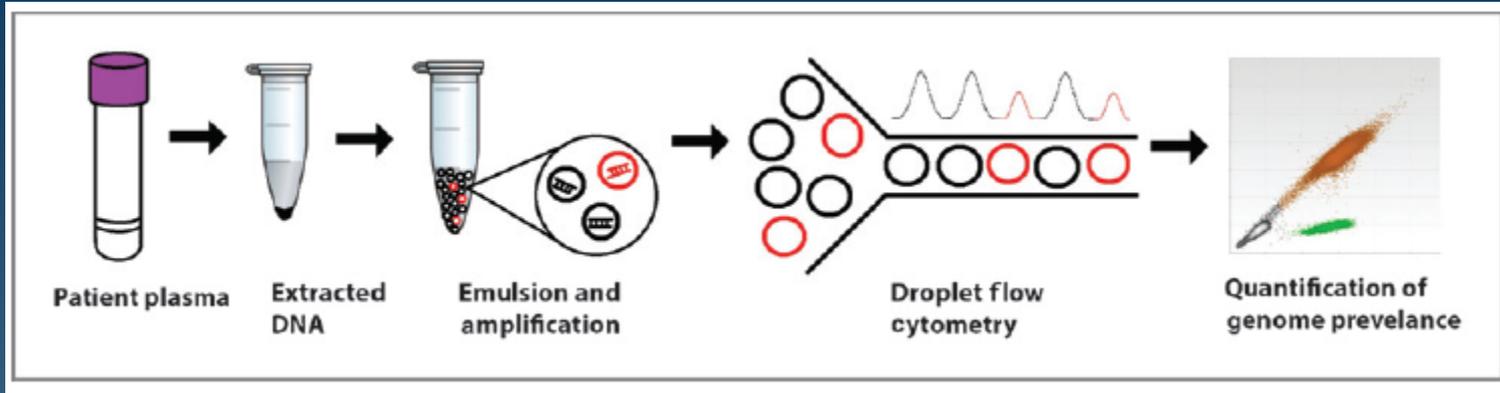
Hilterman et al., ASCO 2017

Table 1. patient characteristics

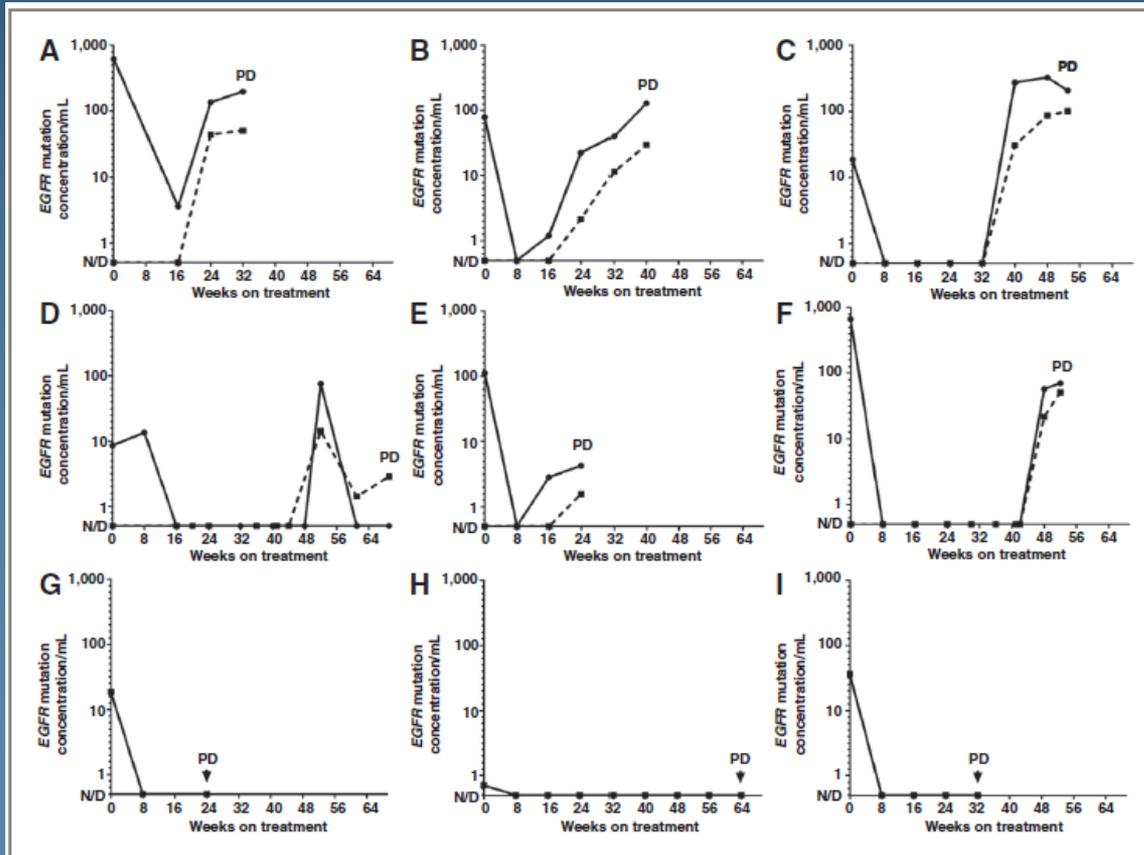
	Sex	Age	KRAS mutation (plasma)	Cycles of Nivolumab	Responder (RECIST)	ECOG PS	PFS (weeks)	OS (weeks)
Patient 1 (980)	M	59	c.34G>T, p.G12C	34+	R	0	NR	NR
Patient 2 (1032)	F	59	c.35G>A, p.G12D	9	R	0	42	NR
Patient 3 (1035)	M	68	c.34G>T, p.G12C	33+	R	1	NR	NR
Patient 4 (1067)	F	64	c.34G>T, p.G12C	3	NonR	1	4	5
Patient 5 (1083)	F	60	c.34G>T, p.G12C	2	NonR	0	7	14
Patient 6 (1096)	F	53	c.35G>A, p.G12D	24+	R	1	NR	NR
Patient 7 (952)	F	50	c.38G>A, p.G13D	1	NonR	1	3	8
Patient 8 (1091)	M	69	c.35G>A, p.G12D	9	M	1	18	NR
Patient 9 (1066)	M	71	c.34G>T, p.G12C	2	NonR	1	4	4
Patient 10 (986)	F	59	c.35G>T, p.G12V	1	NonR	1	1	1
Patient 11 (987)	F	53	c.34G>A, p.G12S	3	NonR	0	5	13
Patient 12 (1116)	M	68	c.34G>T, p.G12C	5	NonR	0	4	NR
Patient 13 (1112)	F	29	c.34G>T, p.G12C	19+	R	1	NR	NR
Patient 14 (1097)	F	55	c.35G>T, p.G12V	24+	SD	0	NR	NR
Patient 15 (1166)	F	66	c.35G>T, p.G12V	5+	SD	1	NR	NR
Patient 16 (7008)	M	70	c.35G>T, p.G12V	3	NonR	1	4	NR



CONCLUSION
Specific plasma ctDNA testing seems a very promising early biomarker (1 week) for tumor response to PD-1 therapy.



ddPCR

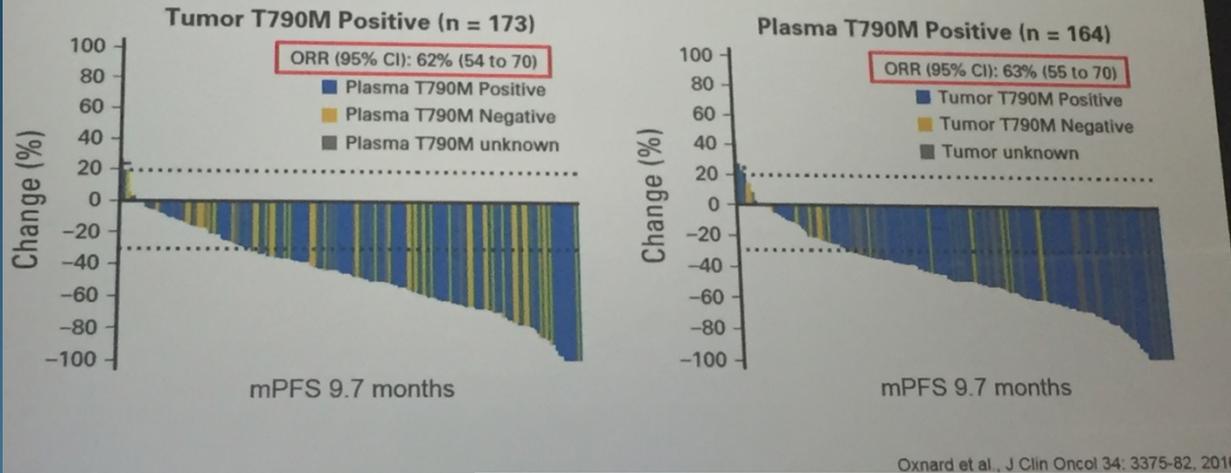


- Lung cancer
- EGFR mutated
- Liquid biopsies
- Blood cf DNA
- ddPCR
- EGFR T790M resistance
- Disease monitoring

Oxnard et al., CCR 2014

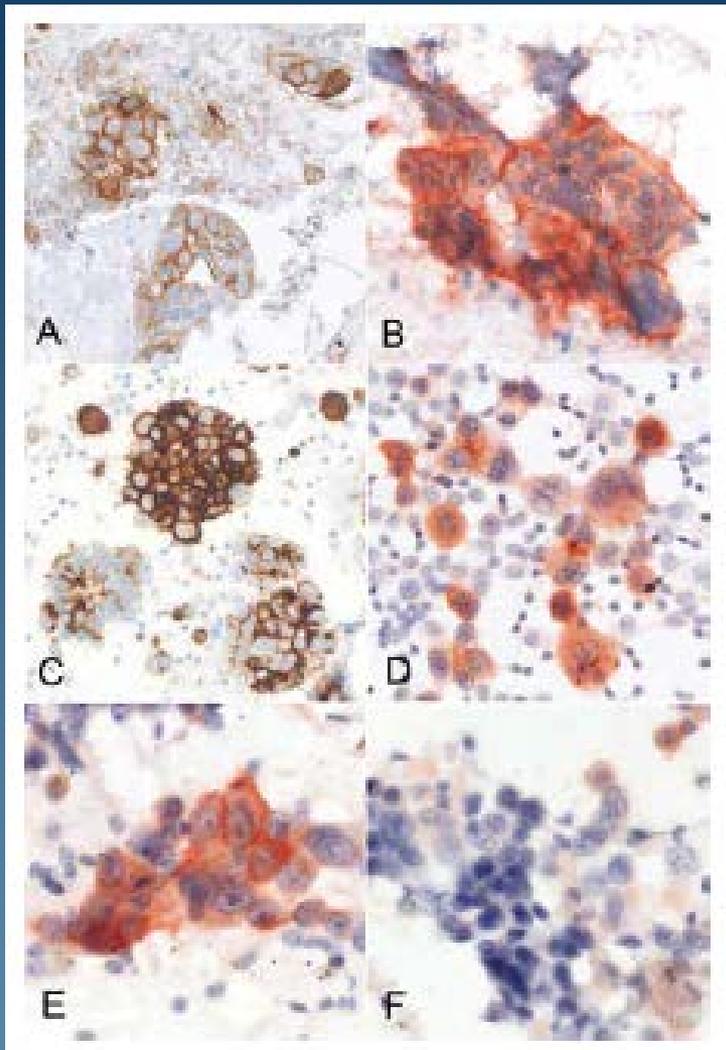
Momenteel n=20
in validatiefase

Clinical Outcomes in Patients Identified as T790M-Positive by Tumor or Plasma Testing



Summary

- Liquid biopsies have entered clinical practice for NSCLC
- At diagnosis of advanced NSCLC, tissue genotyping remains the gold standard
- Plasma genotyping can be used upfront or in the resistant setting
 - Cobas EGFR Mutation Test v2 is a blood-based assay approved by the FDA as a companion diagnostic for EGFR TKIs
 - **Negative plasma assays require tumor genotyping**



- [Appl Immunohistochem Mol Morphol.](#) 2017 May 25. doi: 10.1097/PAI.00000000000000540. [Epub ahead of print]
- **Paired Comparison of PD-L1 Expression on Cytologic and Histologic Specimens From Malignancies in the Lung Assessed With PD-L1 IHC 28-8pharmDx and PD-L1 IHC 22C3pharmDx.**
- [Skov BG¹](#), [Skov T.](#)

Pitfalls of using PDL1 immunohistochemistry as a biomarker test for anti-PD1–PDL1 therapy

- Focal programmed cell death 1 ligand 1 (PDL1) expression in some tumours may be missed in small biopsy specimens, such as needle biopsies
- PDL1 expression among multiple tumour lesions from individual patients can vary over time and by anatomical site
- PDL1 expression in tumour biopsies collected months or years earlier might not accurately reflect PDL1 status at the time of treatment initiation; therapies given after biopsy but before administration of programmed cell death protein 1 (PD1) pathway blockade (radiation therapy, chemotherapy or kinase inhibitors) may alter PDL1 expression
- PDL1 epitopes detected by some antibodies are potentially unstable with prolonged specimen fixation or inadequate tissue handling before fixation (see NCI guidelines for tissue handling)
- Antibodies used for PDL1 detection have different affinities and specificities
- PDL1 protein expression can be membranous and/or cytoplasmic; however, only membranous PDL1 is functionally relevant, by contacting PD1+ T cells
- PDL1 can be expressed by multiple cell types within the tumour microenvironment, which poses challenges for scoring and interpretation

- PD-L1 testing evaluation is encouraging with respect to better and simpler, reliable routine application in pathology
- Since PD-L1 alone is not the optimal biomarker for PD-1 therapies, considerable effort is and should be spent in examining (combination with) others such as tumor-infiltrating immune cells, mutational load, gene signatures
- In the future, it is likely that it will be an optimal *combination* of biomarkers to be used to determine, with a sufficient degree of certainty, whether a particular patient will benefit from anti-PD-1/PD-L1 therapy and future immune checkpoint blocking drugs to come

The Tumor Immunity Continuum

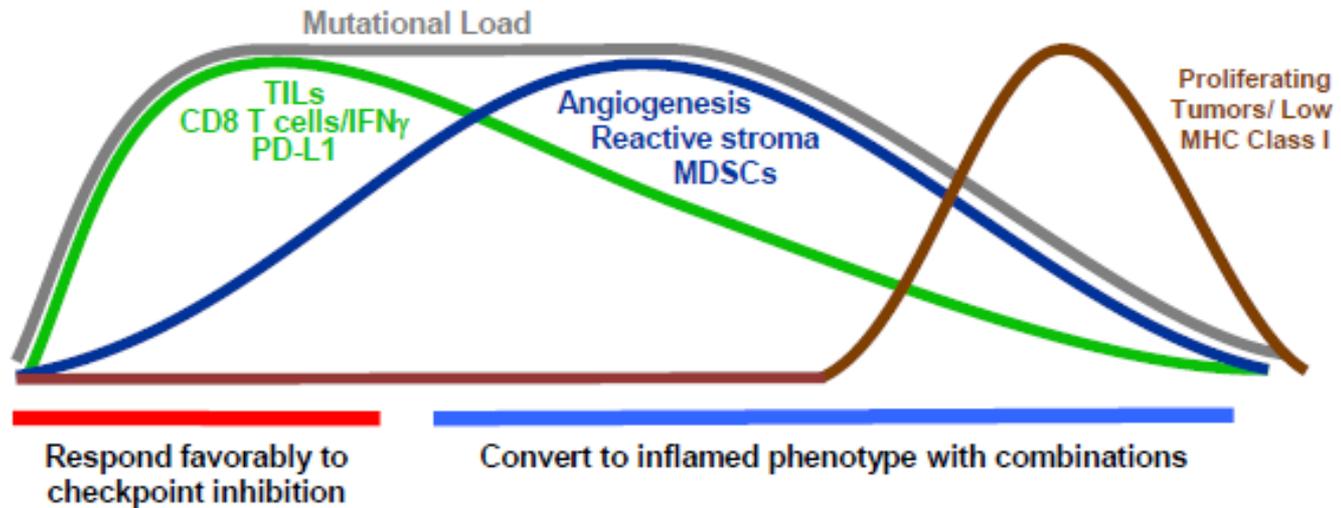
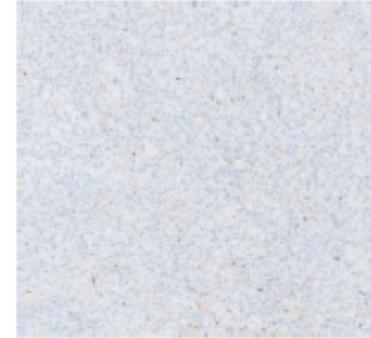
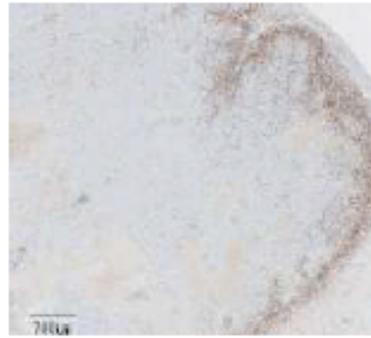
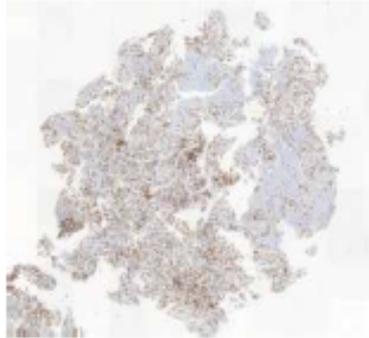
Inflamed

Non-inflamed

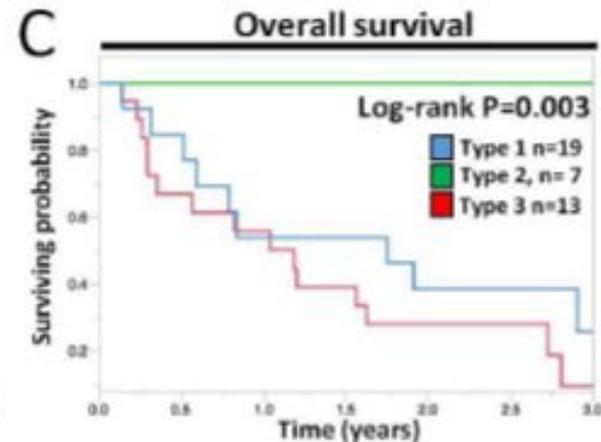
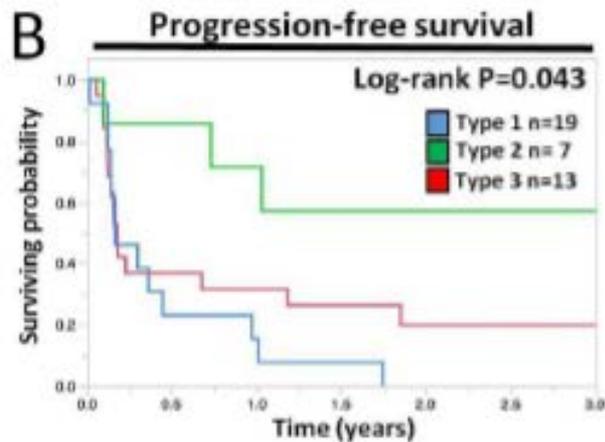
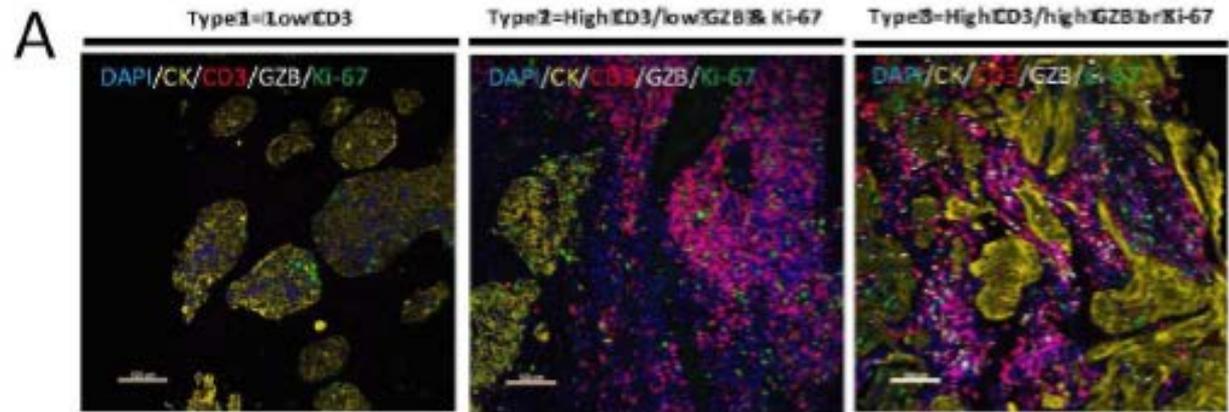
Pre-existing immunity

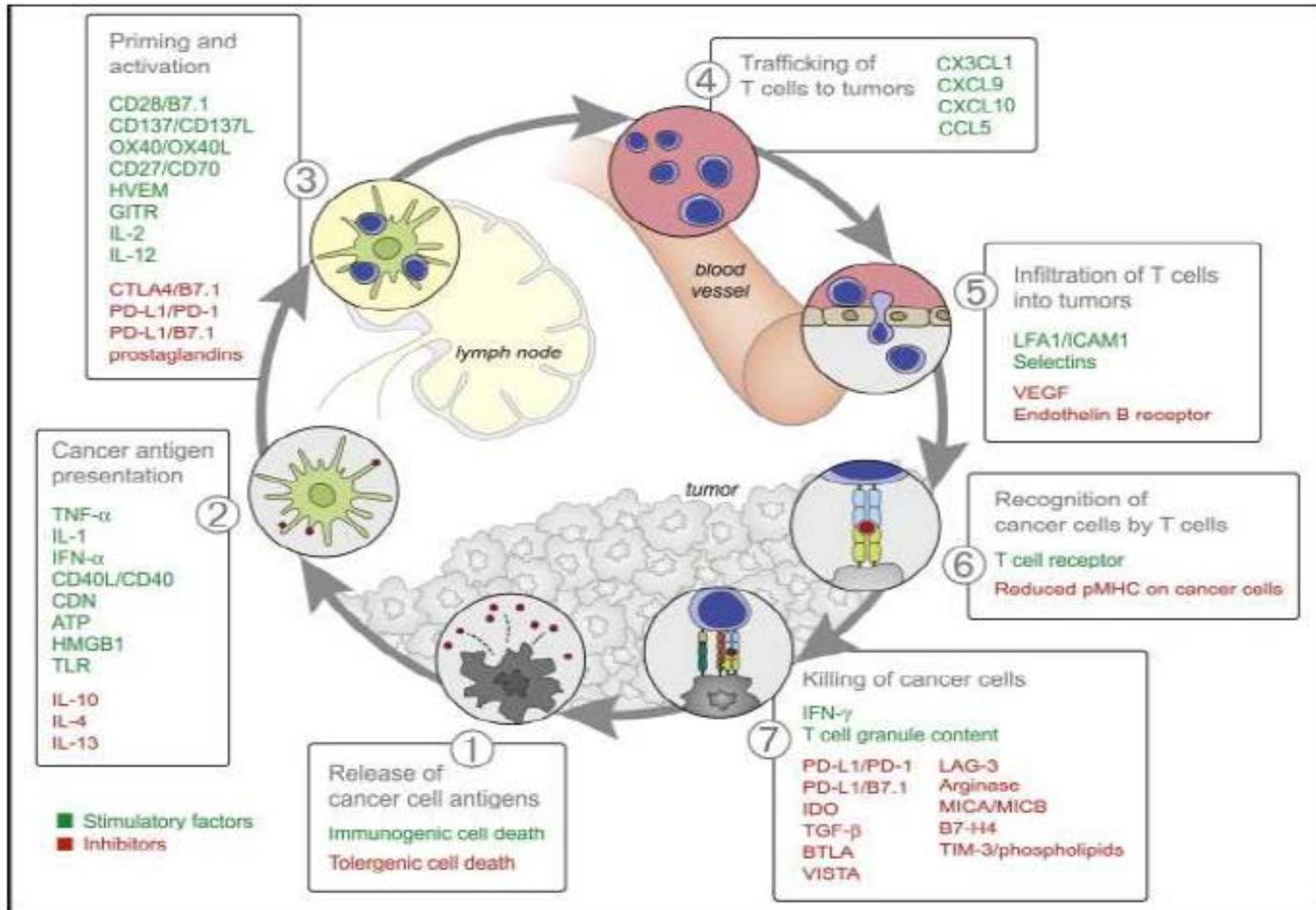
Excluded infiltrate

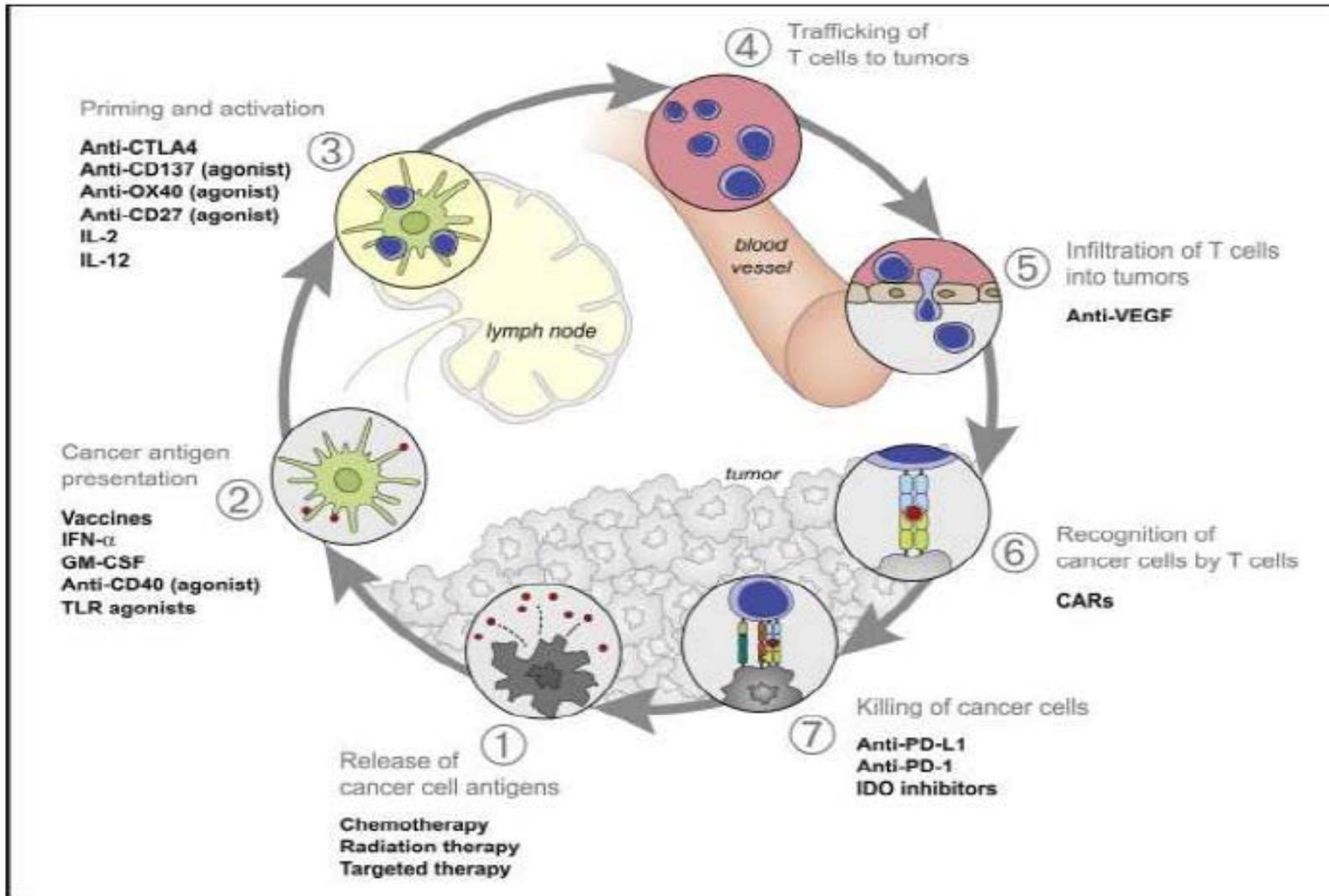
Immunologically ignorant



TIL subtype can identify susceptible tumors for immunotherapy

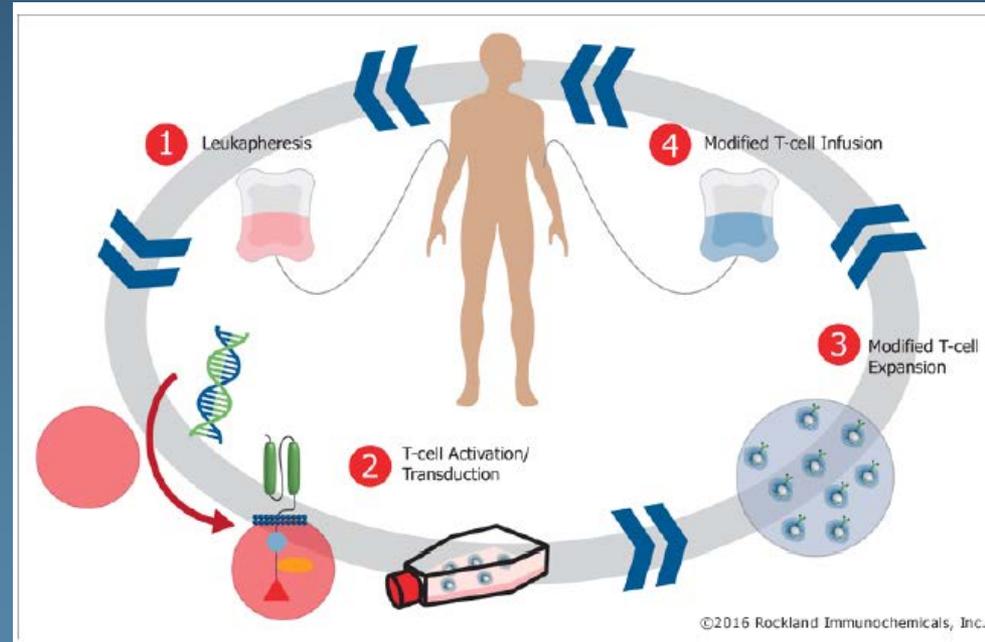
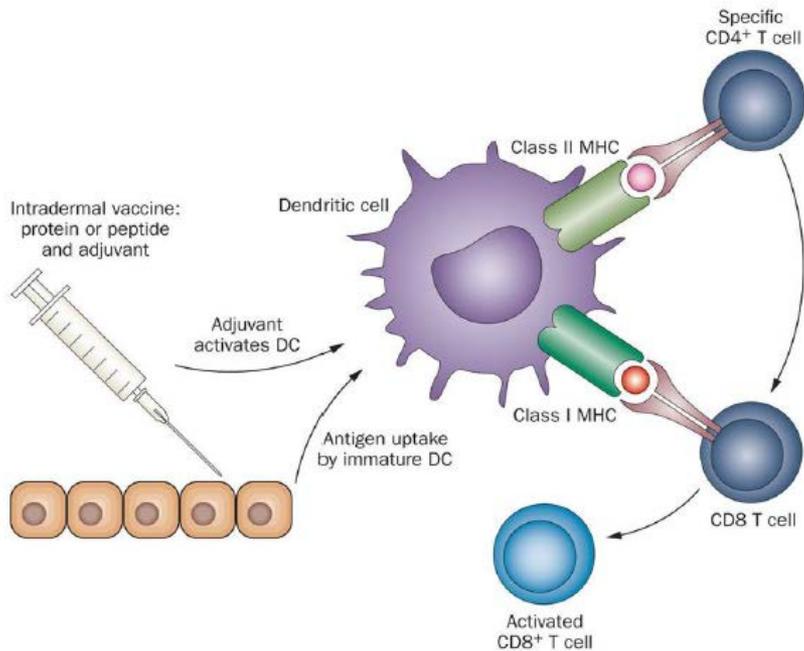






Cancer vaccines and Chimeric Antigen Receptor (CAR) T-cell therapy

Cancer vaccines



Abstracts? Sessions?

Immune monitoring beyond PD-L1 status

Monitoring strategy	Immunologically-unresponsive tumor	Immunologically-responsive tumor
Whole exome sequencing	Low mutational burden	High mutational burden
Gene signature/patterns	↓ activation signature	↑ activation signature
Epigenetic modification	↑ Treg/CD3 ratio ↓ CD3 cells	↓ Treg/CD3 ratio ↑ CD3 cells
Protein microarray	Poor general antibody response	Robust general antibody response
B/T-cell receptor repertoire	Low CD3 count Low clonality	High CD3 count High clonality
Flow/Mass cytometry *	↓ effector cells ↓ Teff/Treg ratio PD-1^{high} MDSC+	↑ effector cells ↑ Teff/Treg ratio PD-1^{low} MDSC-
Multicolor IHC	↓ effector cells, ↑ suppressor cells low PD-L1 on tumor and tumor infiltrating immune cells	↑ effector cells ↓ suppressor cells high PD-L1 on tumor and tumor infiltrating immune cell
Therapeutic strategy	Vaccination, ablation, radiotherapy, chemotherapy, oncolytic therapy, adaptive cellular therapy first	Immune checkpoint blockade therapies and other immunotherapies first
Legend		

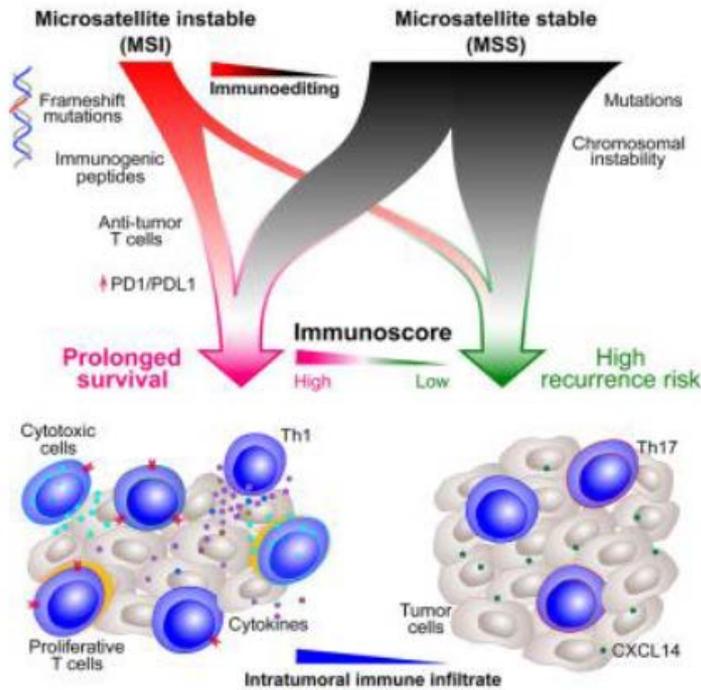
* Proliferating subset as assessed by Ki67⁺ fraction

Yuan et al, J Immunother Ca, 2016

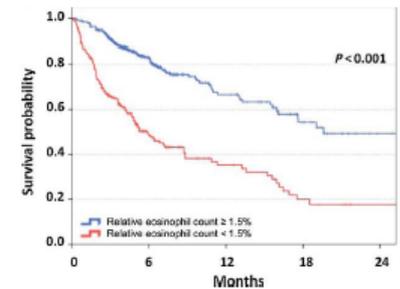
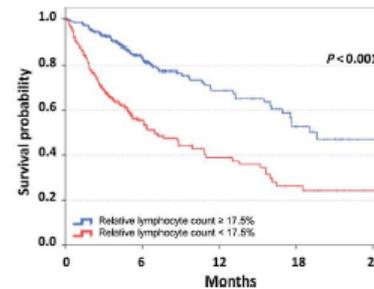
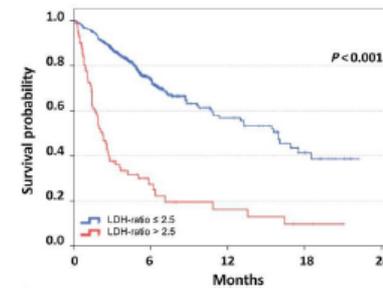
Microbiome as a biomarker for checkpoint inhibition

- Microbiota composition enriched in *Bacteroides philym* associated with decreased immune colitis in patients treated with anti-CTLA-4¹
- Significant differences in diversity and composition of the gut microbiome were noted in responders (R) vs non-responders (NR) to anti-PD-1, with a higher diversity of bacteria in R vs NR ($p = 0.03$). There was a higher abundance of Clostridiales in R and of Bacteroidales in NR. Diversity ($p = 0.009$; HR = 7.67) and abundance of specific bacteria in R ($p = 0.007$; HR = 3.88) was associated with improved PFS²
- 1. Dubin, K et al Nat Commun 2016 2. Wargo, J et al ASCO 2017

Integration of analyses?



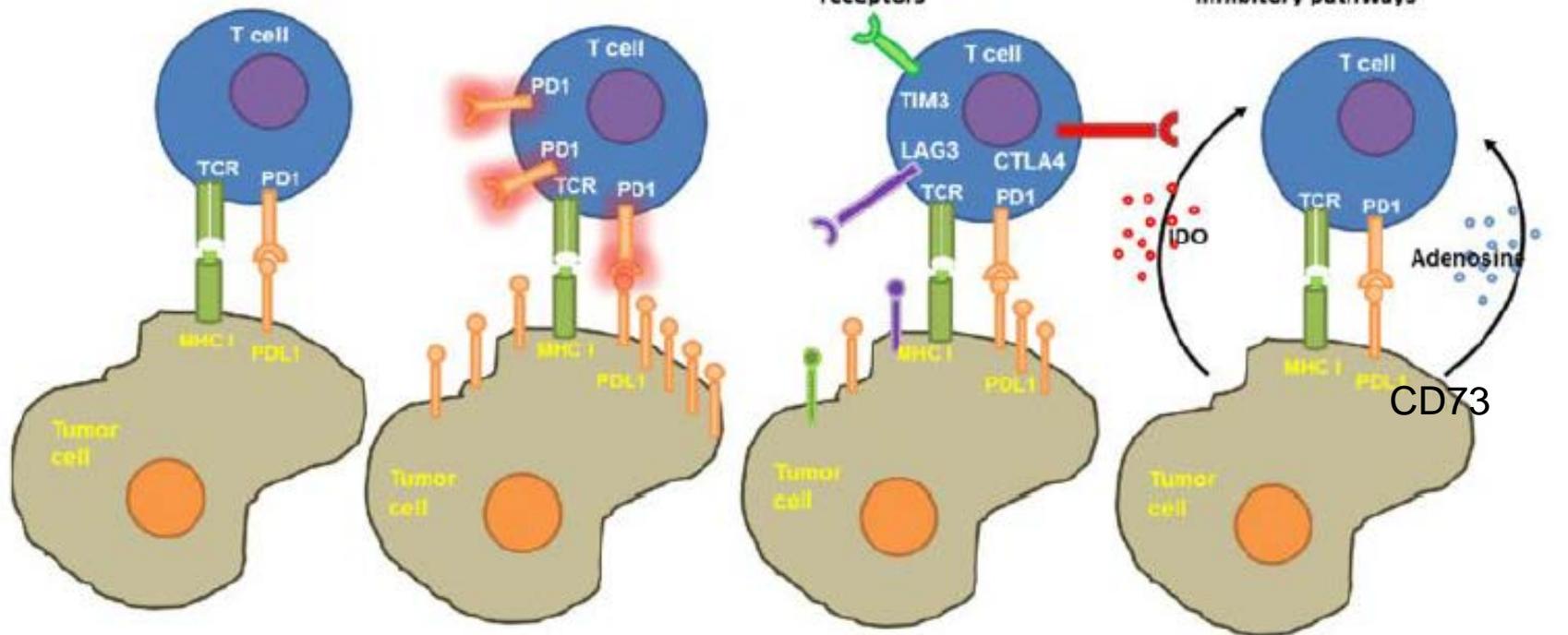
Serological predictors for response to pembro in metastatic melanoma



Resistance to immunotherapy

Anti-PD1/PDL1-sensitive

Anti-PD1/PDL1-resistant



Optimizing Access to Personalised Cancer Therapy in the Netherlands; from Tissue to Therapy

ZONMW
PATH project

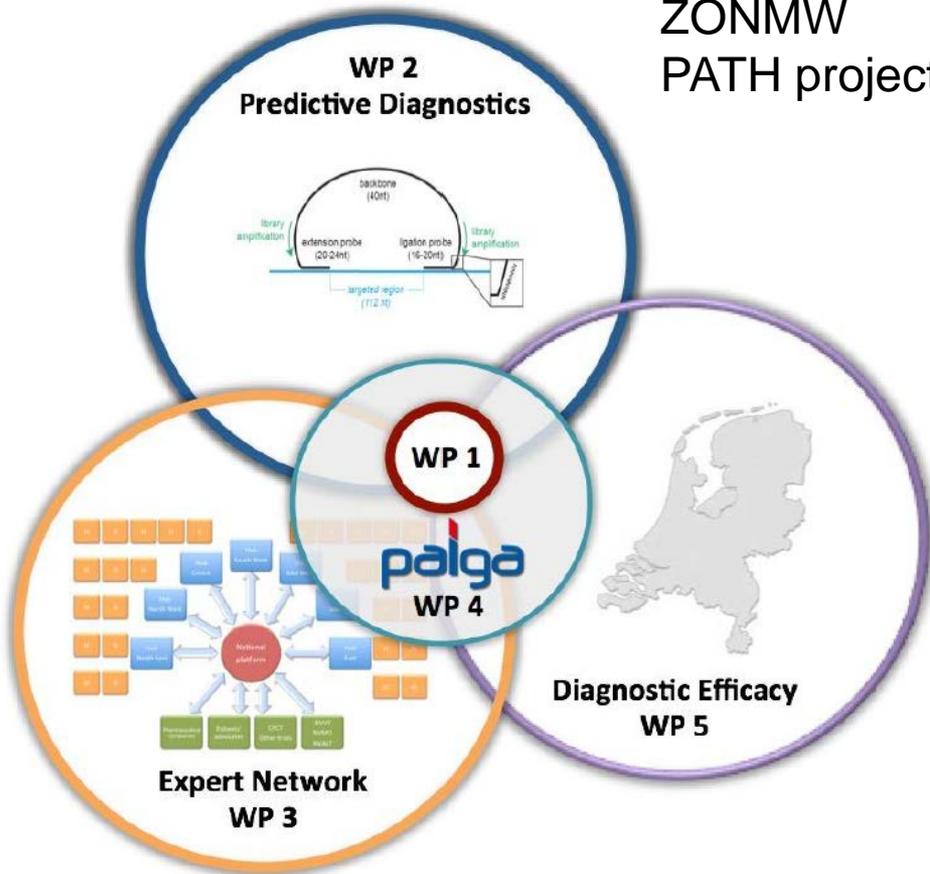


Figure 1. The Three-Tiered Approach of the Project

Table 1. Provisional Design of the PATH Gene-Panel

Predictive gene ¹	Aberrations ¹	Predictive gene ¹	Aberrations ¹
<i>AKT1</i>	SNVs, CNVs	KRAS	SNVs, CNVs
<i>AKT2</i>	CNVs	<i>IDH1</i>	SNVs
<i>AKT3</i>	CNVs	<i>IDH2</i>	SNVs
ALK	SNVs, Fusion-transcript	<i>JAK2</i>	SNVs
<i>ARAF</i>	SNVs, CNVs	<i>MAP2K1 (MEK1)</i>	SNVs
BRAF	SNVs, CNVs	<i>MDM2</i>	CNVs
<i>CCND1</i>	CNVs	<i>MET</i>	SNVs, CNVs
<i>CCND3</i>	CNVs	<i>MTOR</i>	SNVs, CNVs
<i>CDK4</i>	CNVs	NRAS	SNVs
<i>CDK6</i>	CNVs	<i>NTRK1</i>	Fusion-transcript
<i>CDKN2A (p16)</i>	SNVs, CNVs	PDGFRA	SNVs
<i>DDR2</i>	SNVs	<i>PIK3CA</i>	SNVs, CNVs
EGFR	SNVs	<i>PTEN</i>	SNVs, CNVs
ERBB2 (Her2)	SNVs, CNVs	<i>RET</i>	SNVs, Fusion-transcript
<i>FGFR1</i>	SNVs, CNVs	ROS1	SNVs, Fusion-transcript
<i>FGFR2</i>	SNVs, CNVs	<i>SMO</i>	CNVs, SNVs
<i>FGFR3</i>	SNVs, CNVs, fusion-transcript	<i>SRC</i>	CNVs, SNVs
<i>HRAS</i>	SNVs, CNVs	<i>TP53</i>	CNVs, SNVs
KIT	SNVs		

1. Predictive genetic biomarkers that are currently associated with approved targeted agents for treatment of solid tumours in the Netherlands (in bold) and predictive biomarkers that are currently under investigation in clinical trials (phase 2-4) or expected to start in 2016 for solid tumours in the Netherlands.

Note: the BRCA1 and BRCA2 genes are predictive biomarkers for olaparib treatment in ovarian cancer. For ethical considerations these genes will not be included in the PATH panel.

Molecular Tumor Board MUMC+ (MTBM) per 1-1-2017

•Doel van de MTBM

- Advies geven over behandeling individuele patiënten nav tumor-specifieke moleculaire veranderingen.

•Het advies kan betrekking hebben op bijvoorbeeld:

- Interpretatie en uitleg van het moleculair resultaat.
- Ongewone of onbekende moleculaire bevindingen waarvan de optimale behandeling niet eenduidig is.
- Nieuwe mutatie-gerichte medicatie en/of behandeling in studieverband in het MUMC+ of elders in Nederland.
- Interpretatie van mutatie-heterogeniteit (bv mutatie is slechts in een deel van de tumor aanwezig).
- Mutaties met ontwikkeling van resistentie op moleculaire therapie.
- Mogelijkheden voor immuuntherapie mbt PD-L1 expressie of alternatieve biomarkers (MSI, tumormutatie load).
- Bespreken van onverwachte resultaten van andere technieken: FISH, immunohistochemie, NGS etc.
- Testen op circulerend cel-vrij tumor DNA in bloed (of andere vochten) bij te weinig weefselmateriaal.

•Samenstelling:

- Wordt op aankomende OncoZon longziekten vergadering nader bepaald

•Bijeenkomst:

- Elke 1^e en 3^e maandag van de maand 15.45u voorafgaand aan het MDO.
- Aanvragen met complete moleculaire en aanvullende klinische gegevens vrijdag 12.45u (als ma MTBM)

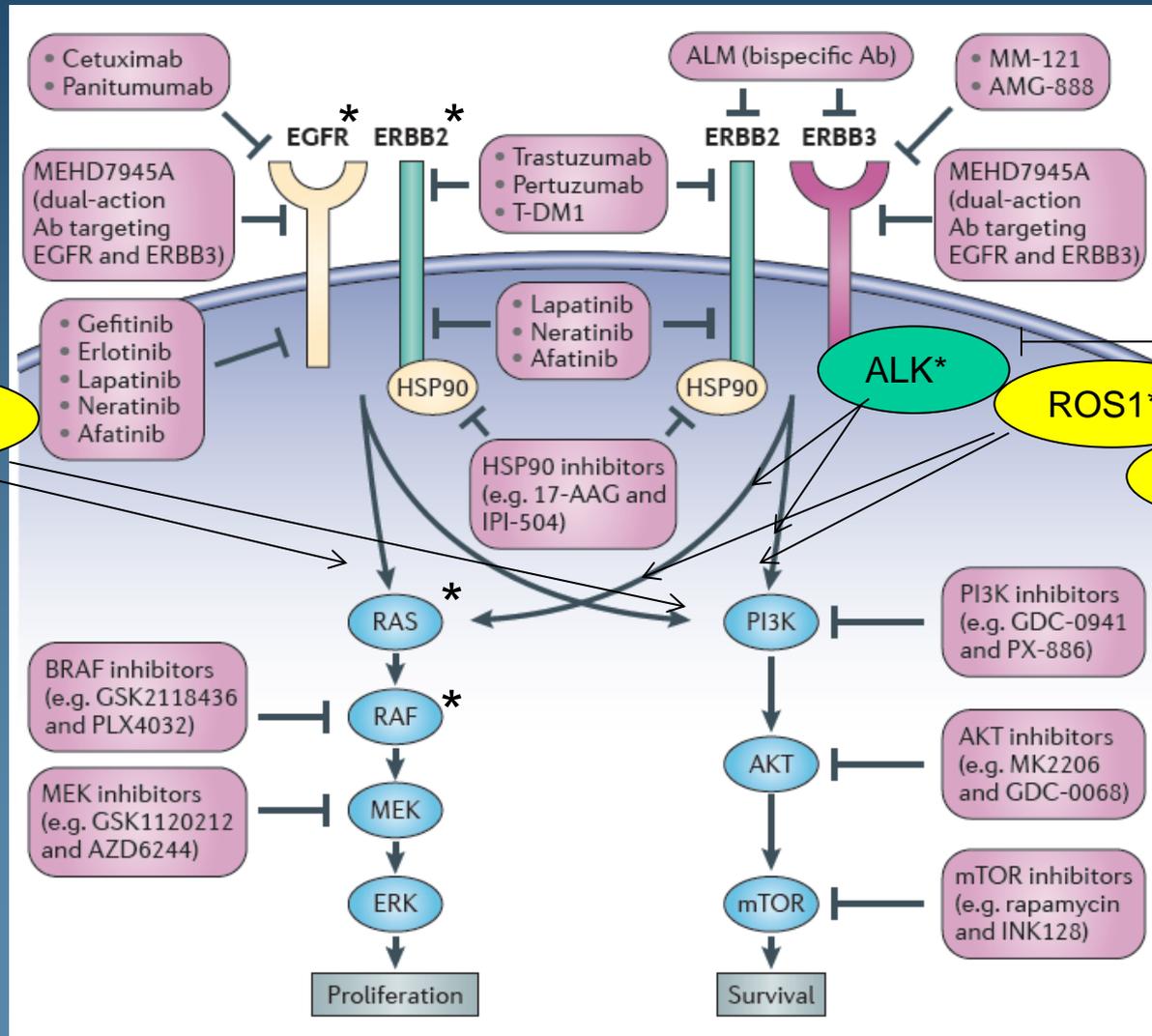
Bereidheid tot uitbreiding naar meerdere indicaties!

Future perspectives:

Don't fear change!!



Molecular pathways altered in many cancer subtypes



carbozantinib

RET*

Crizotinib

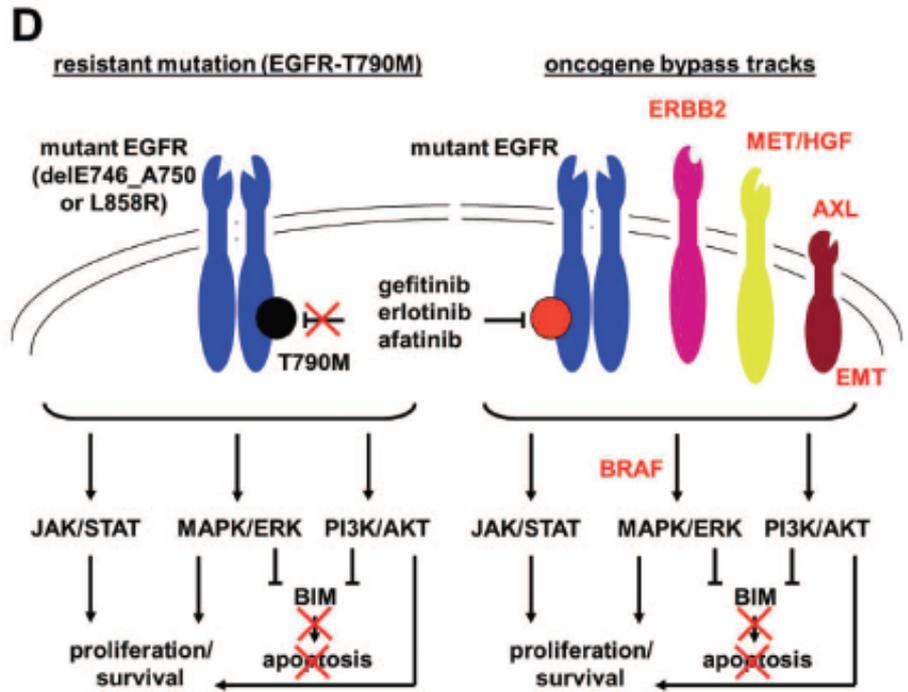
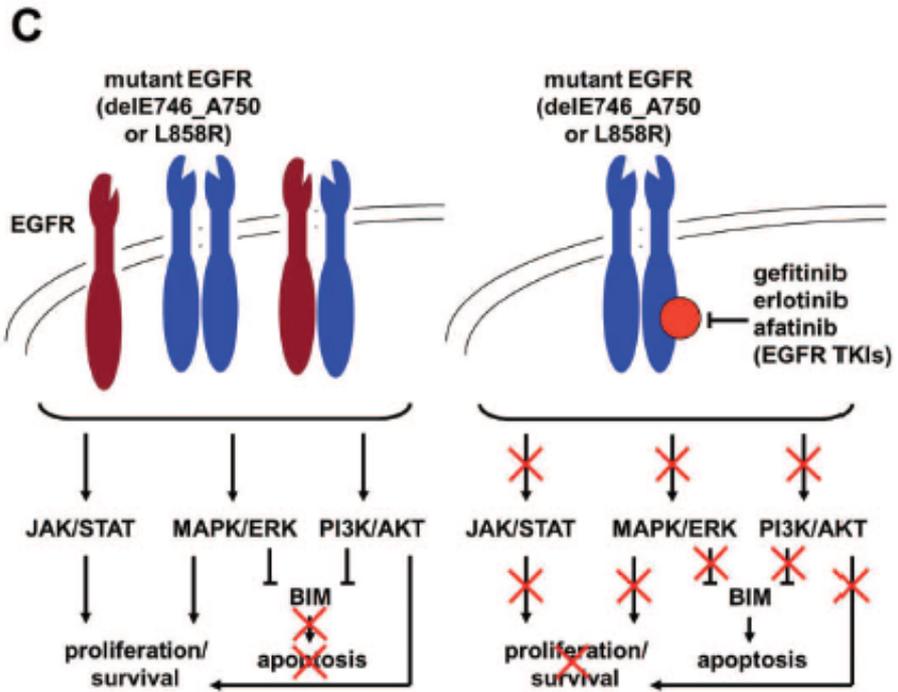
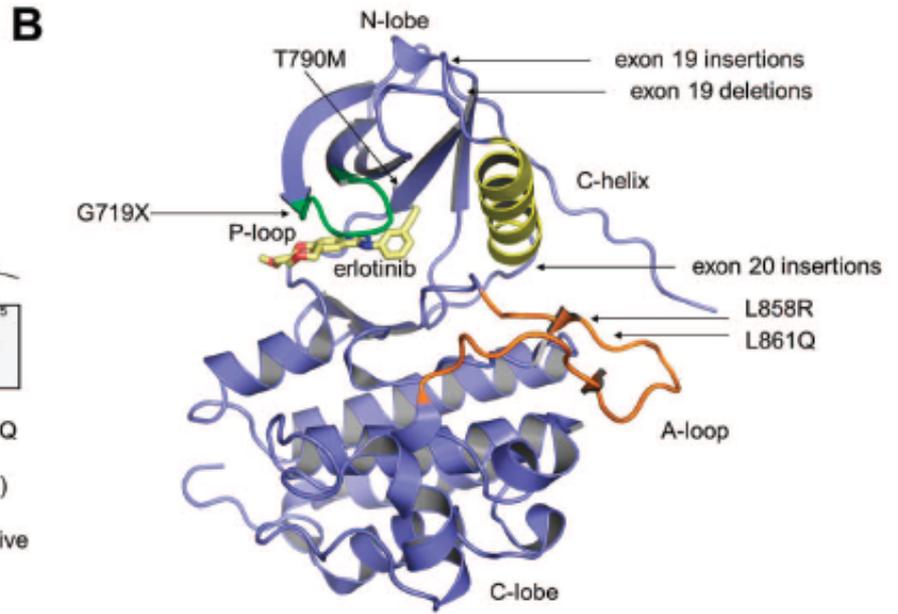
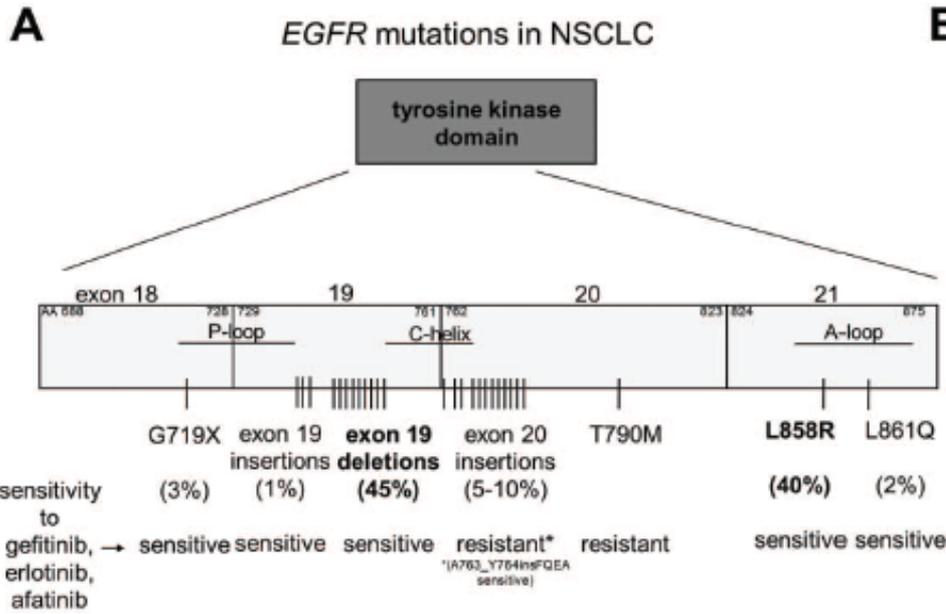
ROS1*

MET*

MAPK pathway

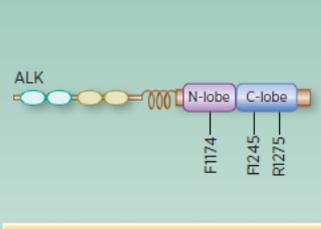
PI3K/AKT pathway

* Adenocarc





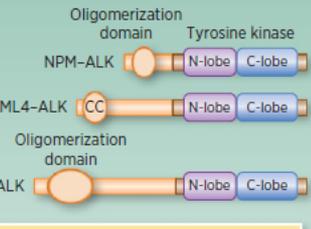
A Point mutation (In neuroblastoma)



Identified mutation in ALK (neuroblastoma)

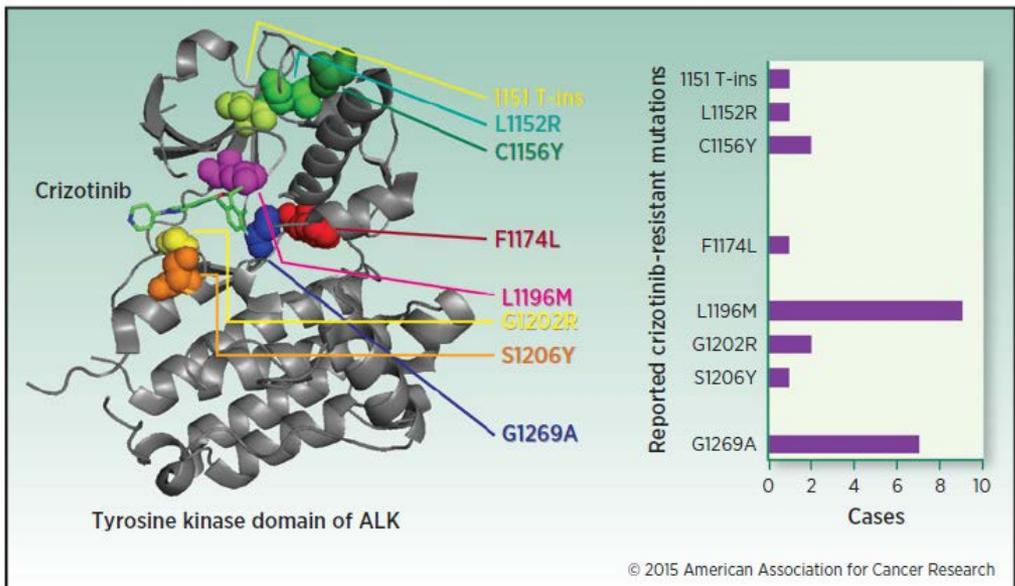
- R1060H (germline)
- T1151M
- M1166R
- I1170N/S
- I1171N
- F1174C/I/L/S/V
- I1183T (germline)
- L1196M
- A1200V
- L1204F (germline)
- L1240V
- F1245C/I/L/V
- D1270G (inactivating mutant)
- R1275L/Q (germline)
- Y1278S
- G1286R
- T1343I

B ALK fusion gene



Identified ALK fusion gene

- NPM-ALK
- EML4-ALK
- ATIC-ALK
- C2orf44-ALK
- CARS-ALK
- CLTC-ALK
- CLTCL1-ALK
- FN1-ALK
- HIP1-ALK
- KIF5B-ALK
- KLC1-ALK
- LMNA-ALK
- MSN-ALK
- PPFIBP1-ALK
- PRKARIA-ALK
- RANBP2-ALK
- SEC31A-ALK
- SQSTM1-ALK
- STRN-ALK
- TFG-ALK
- TPM3-ALK
- TPM4-ALK
- VCL-ALK



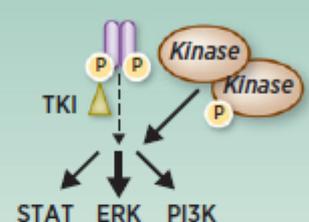
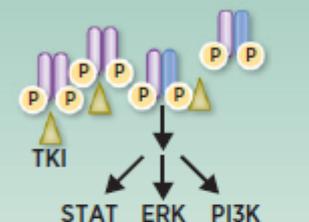
© 2015 American Association for Cancer Research

Target alteration

Bypass tracks

Other mechanisms

Mutant and/or amplified TK

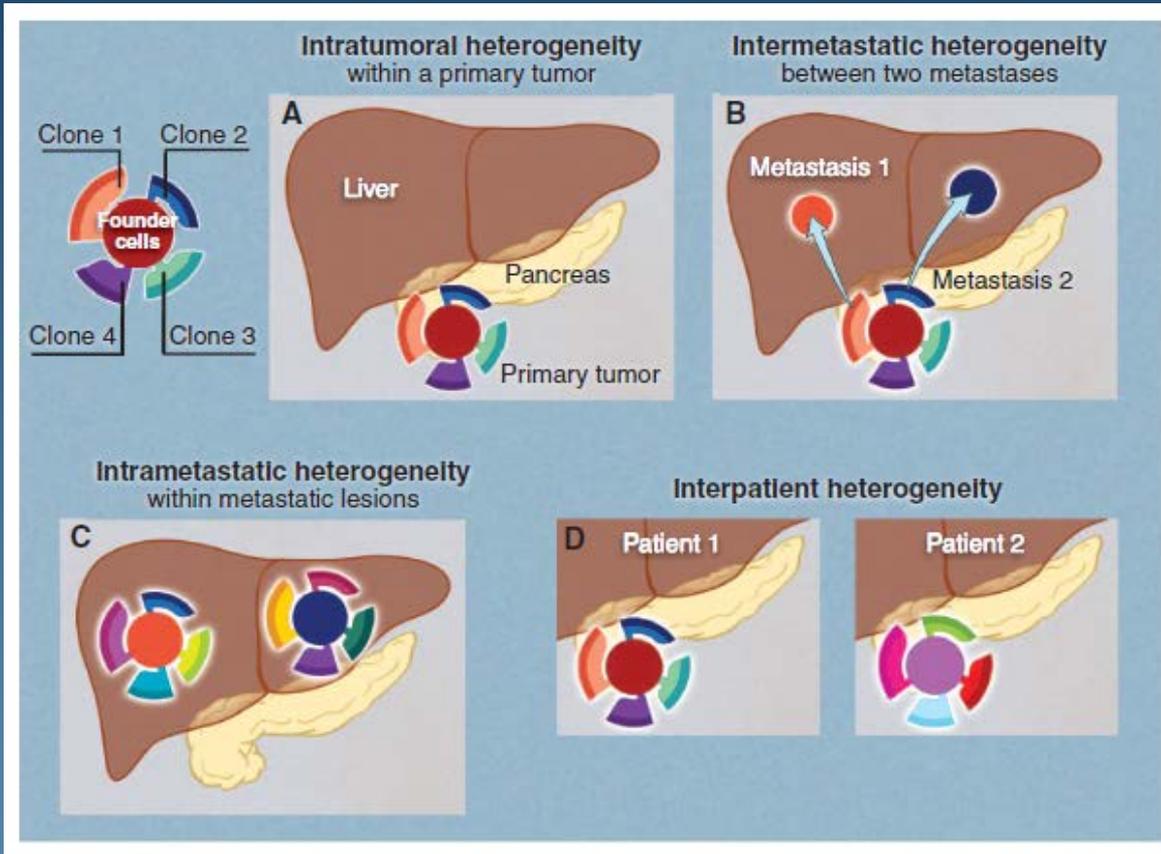


ALK resistance

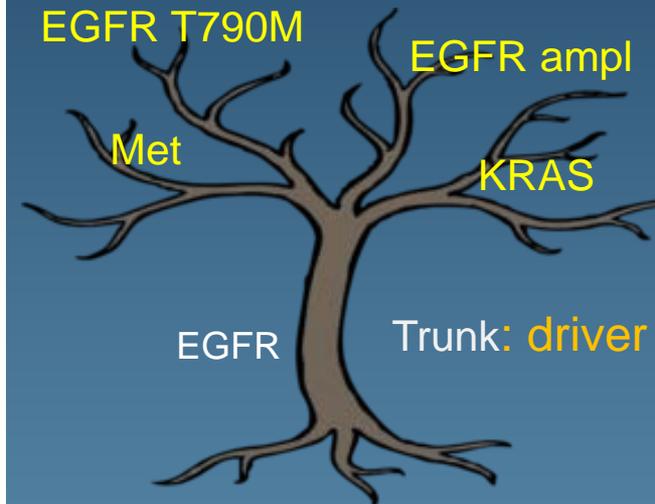
Katayama et al, CCR 2015

Crizotinib resistance	L1196M, G1269A, C1156Y, G1202R, I1151T ins, F1174L, L1152R	EGFR, cKIT with SCF, IGF1R, Src	EMT (MED12, cell line) BIM? Microenvironment? CNS penetration
Alectinib resistance	I1171T/N/S, G1202R	cMET amplification Ligand activation (EGFR or cMET activation)	
Ceritinib resistance	F1174C/V, G1202R	MEK-activating mutation	

Tumor heterogeneity



Branches:
Genetic diversity
Subclonal drivers



Vogelstein et al. 2013
Gerlinger, Swanton 2014

Drivers can be subclonal: missed through a single biopsy
Combinations of subclonal drivers distinct from patient to patient
Driving force for polyclonal drug resistance and metastasis