Importance of NGS testing for STK11 and KEAP1 mutation and impact on treatment selection

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Cancer Institute

Disclosures

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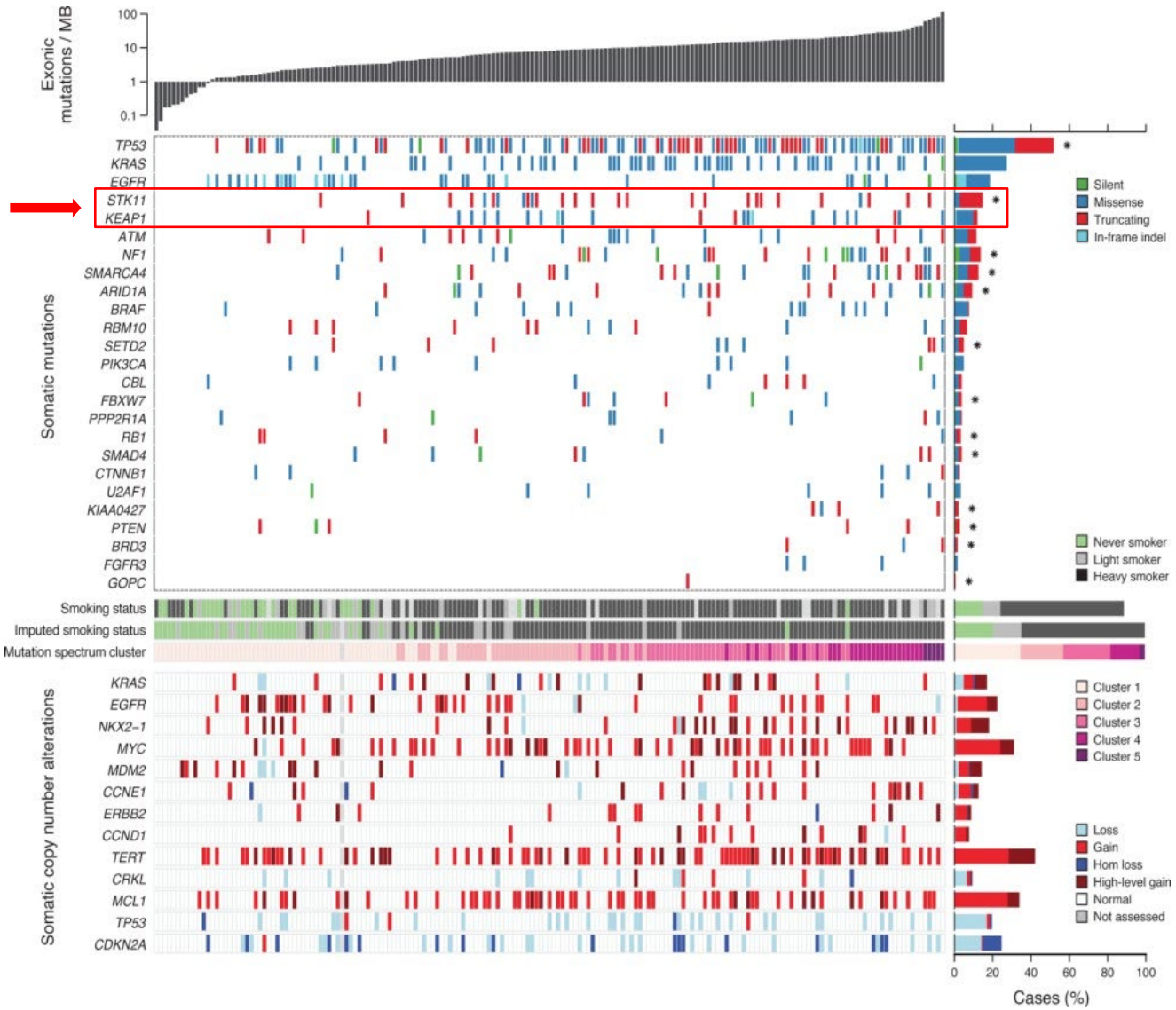
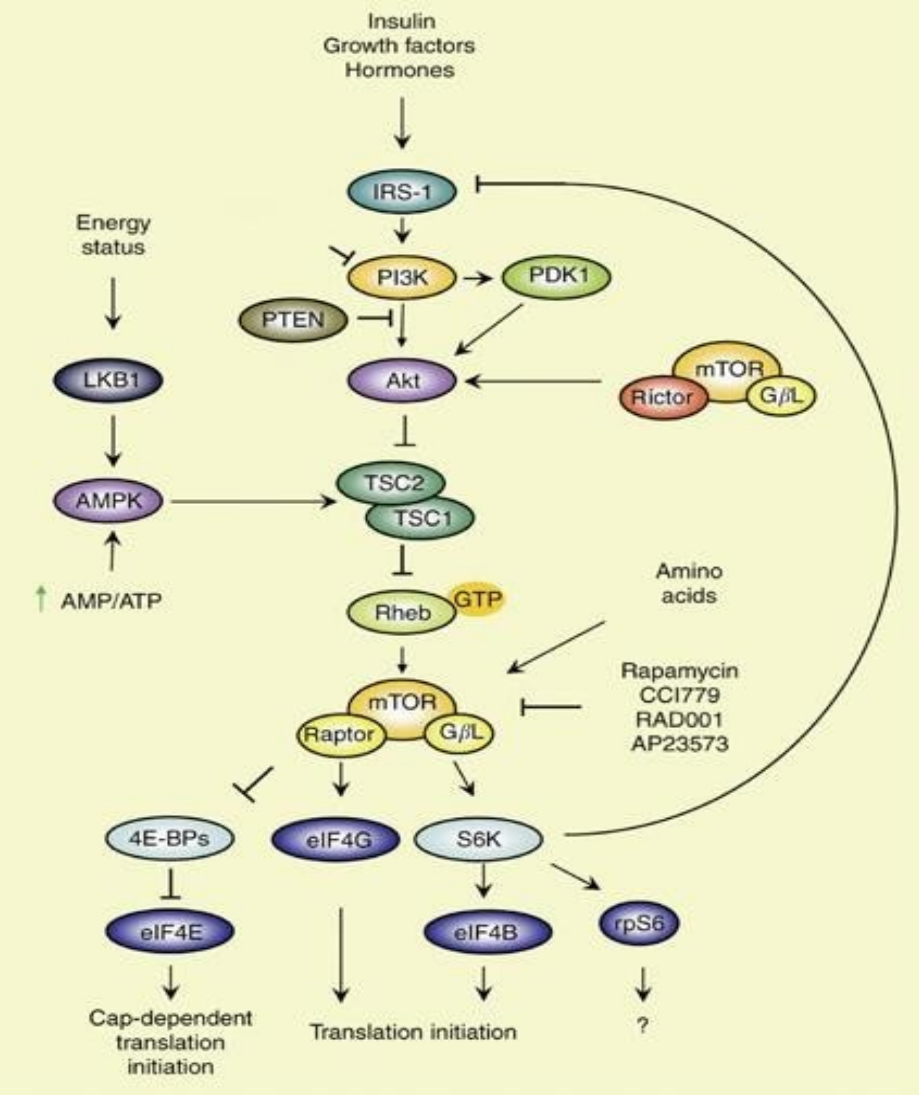
Lung cancer is genomically heterogeneous



- **STK11 and KEAP1 are the 2nd and 3rd most common TSG mutations** in lung adenocarcinoma (30%), also found in adenosquamous and squamous (KEAP1) carcinoma
- More frequently coexist with ***KRAS*** and ***TP53*** mutations, and **high TMB** but is rarely found with *EGFR* mutations
- More frequently found in **smokers**
- More frequently in **Caucasians** compared to Asian lung cancer patients

Imielinski M. Cell 2012; Ding L Nature 2008; Koivunen JP. Br J Cancer 2008; Sanchez-Cespedes et al. Cancer Research 2002

LKB1 (Liver Kinase B1)/STK11 (Serine-Threonine Kinase 11)

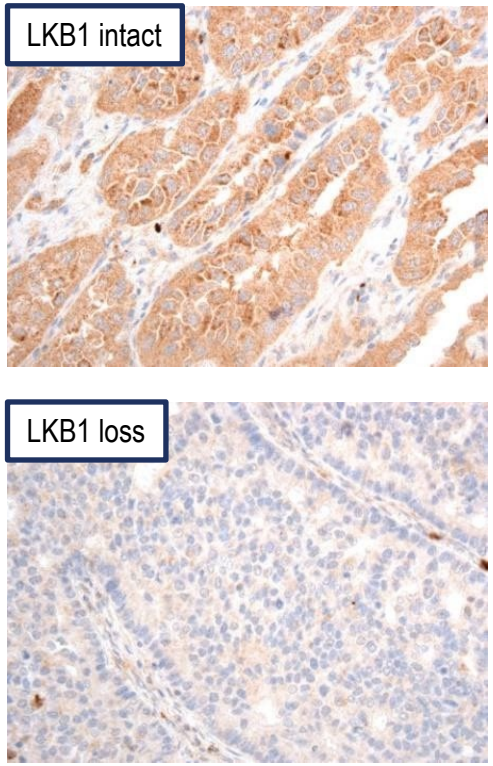


- Tumor suppressor gene
  - Control of cell polarity, growth and proliferation based on cell energetic levels
- Negative prognostic factor
  - Related with more aggressive phenotype and worse survival
- Potential predictive marker
  - Resistance to ICI therapy
  - Sensitivity to mTORi
- No validated functionality test in the clinic!
  - Complexity of mutations

Shackelford. Nat Rev Cancer 2009

STK11/LKB1 diagnostic test in the clinic is challenging

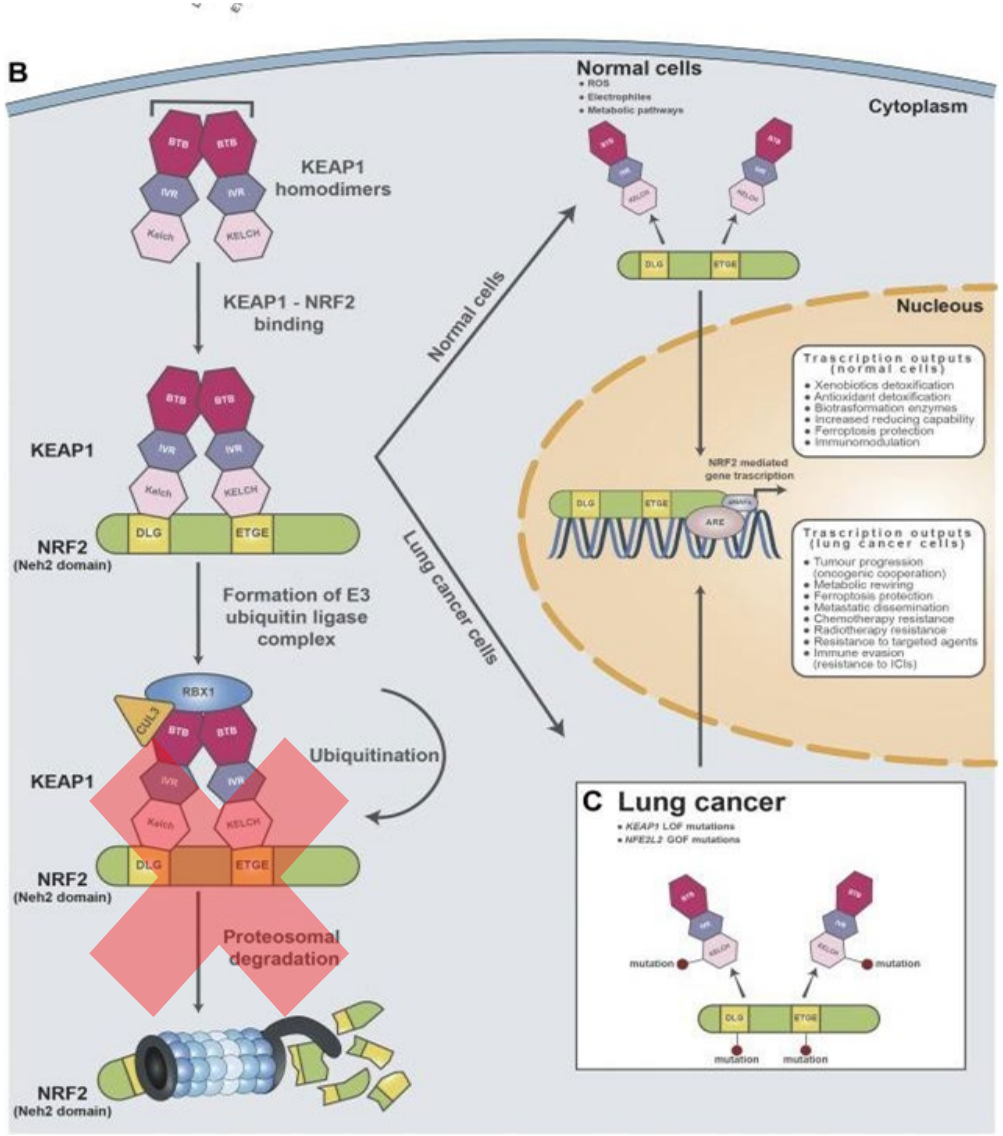
Method	Pros	Cons
NGS	Comprehensive, information on the type of mutation	Cost, no activity assesment, false positive or negative results (e.g., promoter hypermethylation)
IHC	Rapid, cost efficient, mutation agnostic	Equivocal o false negative results, no information on the type of mutation



LKB1 (clone Ley37D/G6) IHC 400x

Chen L. J Thorac Oncol 2016; Calles et al. Clin Cancer Res. 2015;
Gill R. Oncogene 2011; Sanchez-Cespedes M. Oncogene 2007

KEAP1-NRF2 axis is crucial for cellular homeostasis, enabling cells to tolerate oxidative and metabolic stress, and xenobiotics



1. *KEAP1* mutations lead to the release and constitutive activation of NRF2, promoting cellular resistance to oxidative stress, proliferation, and metabolic reprogramming
2. *KEAP1* mutations are scattered throughout the whole gene length, and approximately one-third of them are **stop-gain variants**. **LOH** and **epigenetic silencing** have also been described.

Scalera, S. J Thorac Oncol 2022

KEAP1/Nrf2 diagnostic test in the clinic is challenging

Method	Pros	Cons
NGS	Comprehensive, information on the type of mutation	Cost, no activity assesment, false positive or negative results (e.g., promoter hypermethylation)
IHC	Rapid, cost efficient, mutation agnostic	KEAP1 protein is often still expressed even when mutated, making IHC a poor surrogate.
RNA-seq, RT-qPCR (NQO1, GCLC etc.)	Rapid, mutation agnostic	Not clinically available and not validated

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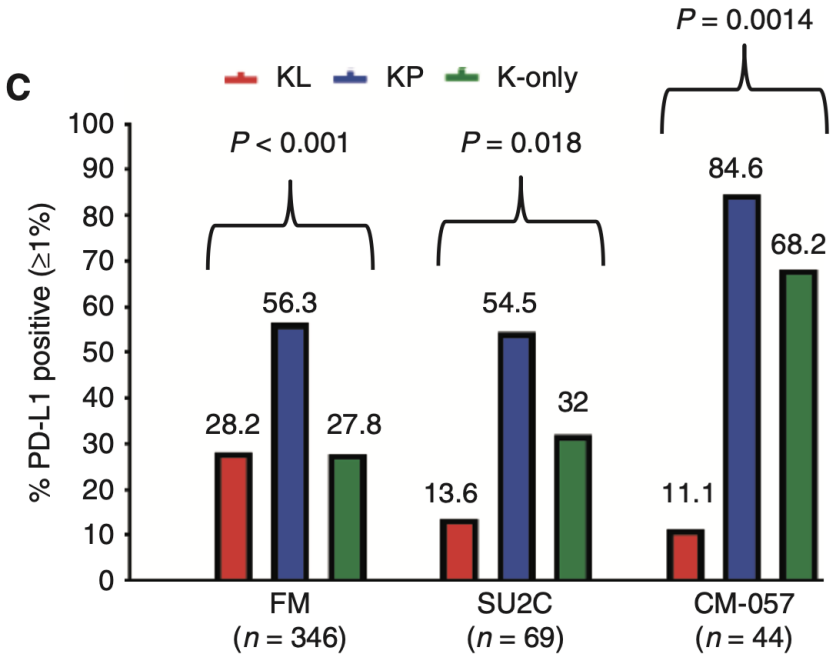
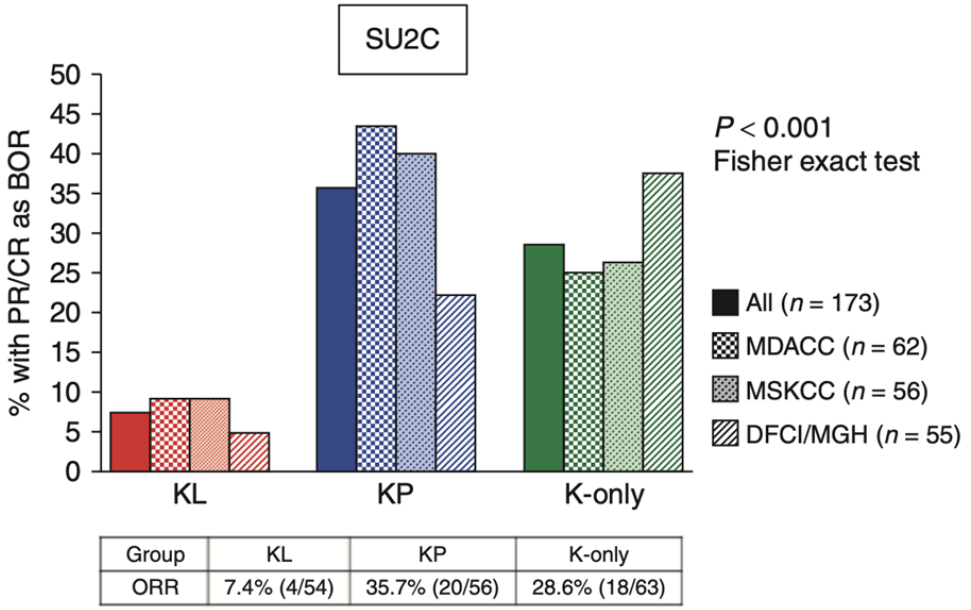
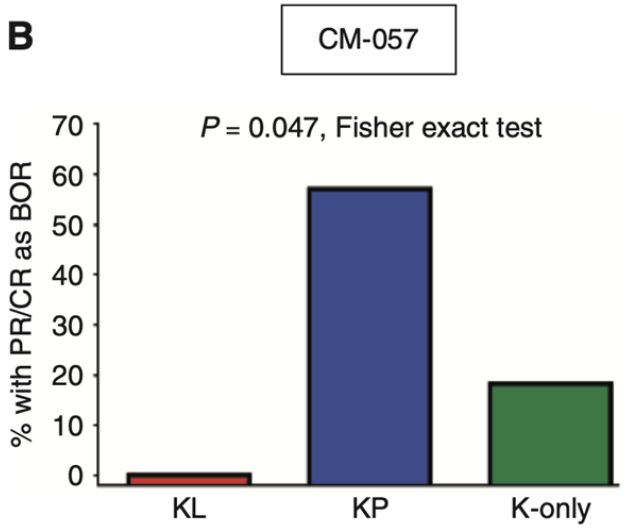
STK11 drives primary resistance to PD-(L)1 blockade





Group	KL	KP	K-only
ORR	7.4% (4/54)	35.7% (20/56)	28.6% (18/63)



Group	KL	KP	K-only
ORR	0% (0/6)	57.1% (4/7)	18.2% (2/11)

Skoulidis et al. Cancer Discovery 2018

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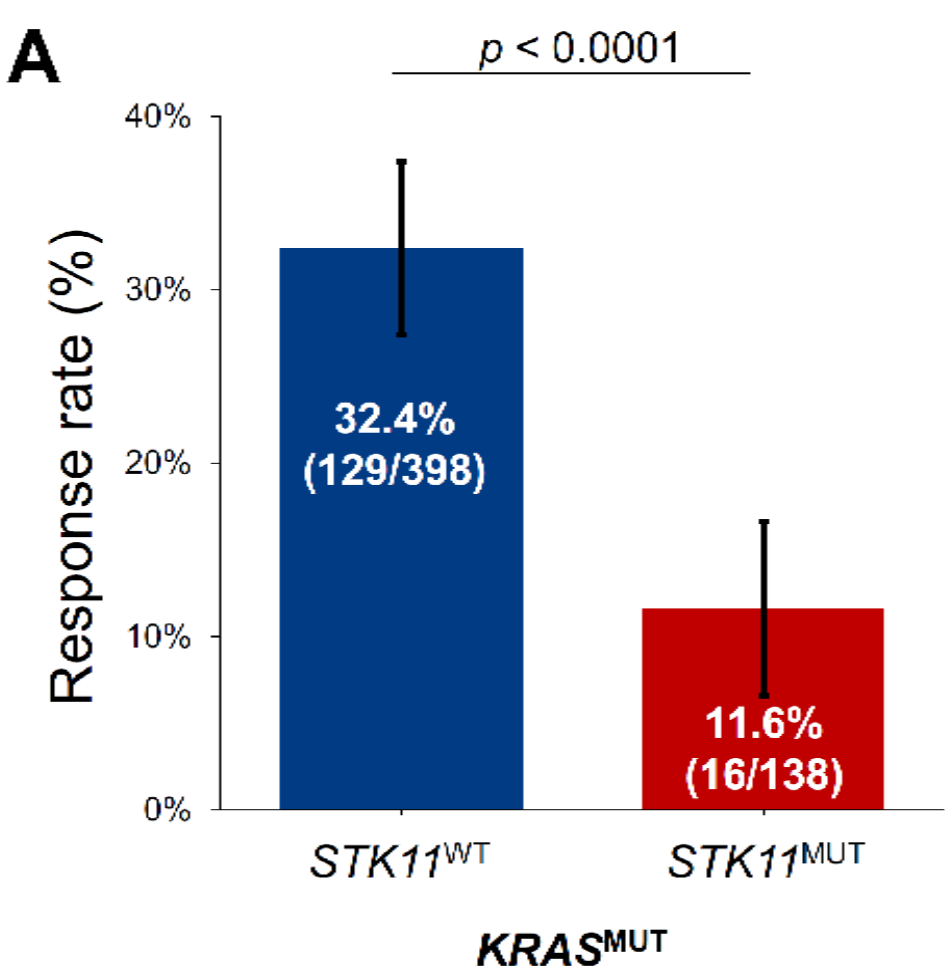
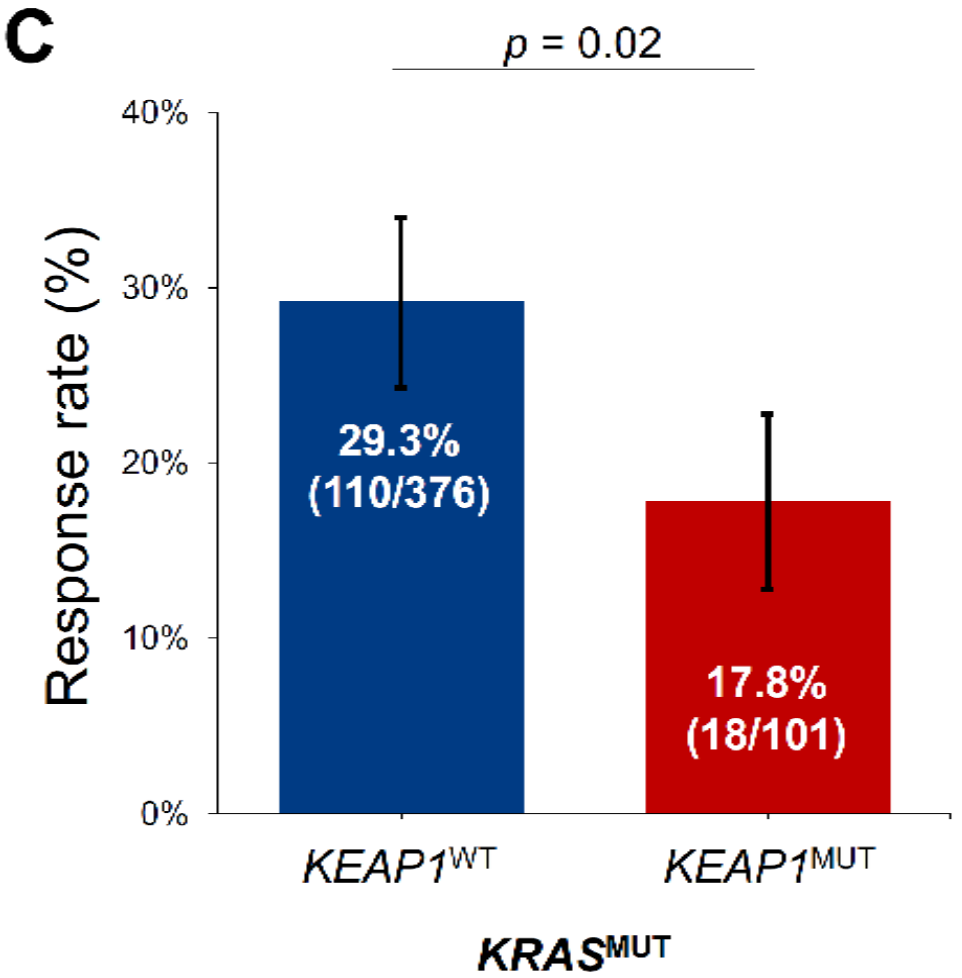
Impact of *KEAP1/STK11* mutations on PD-(L)1 monotherapy efficacy in NSCLC





Ricciuti B et al. J Thoracic Oncol 2022

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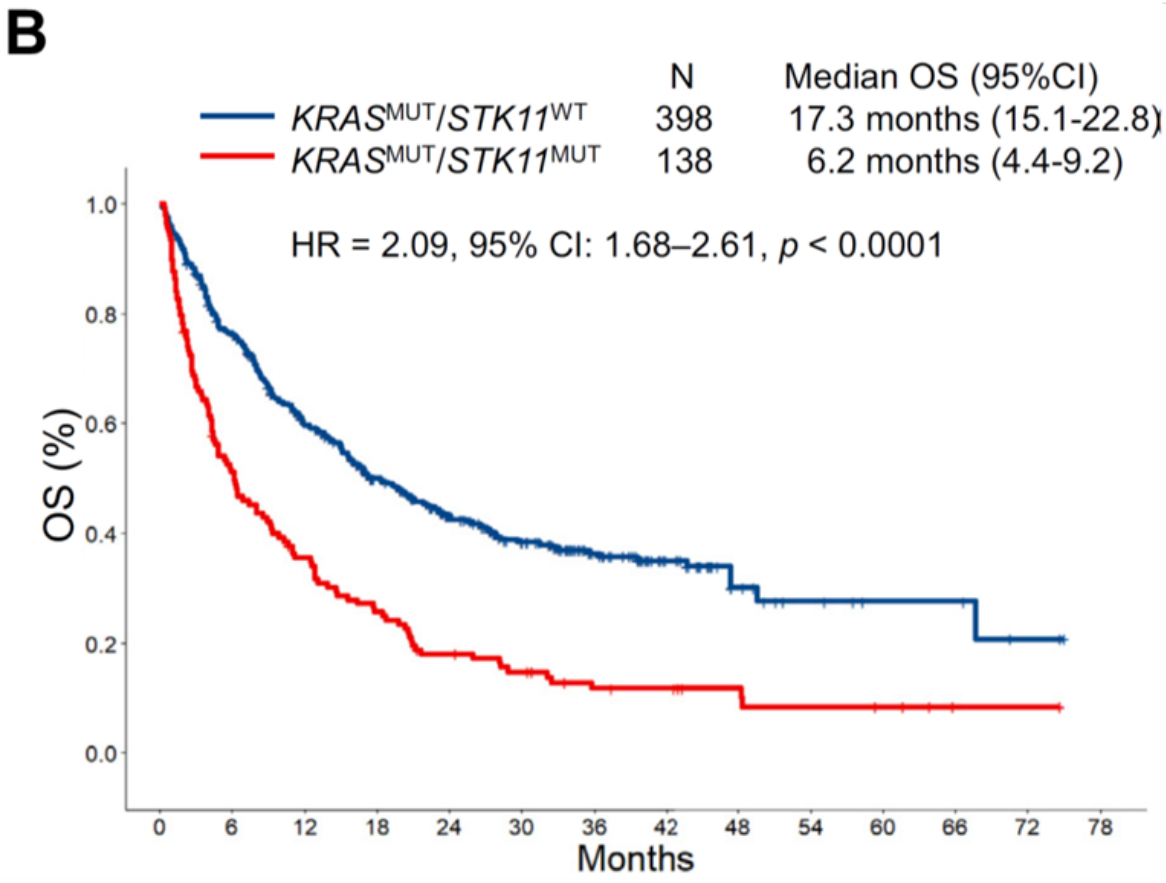
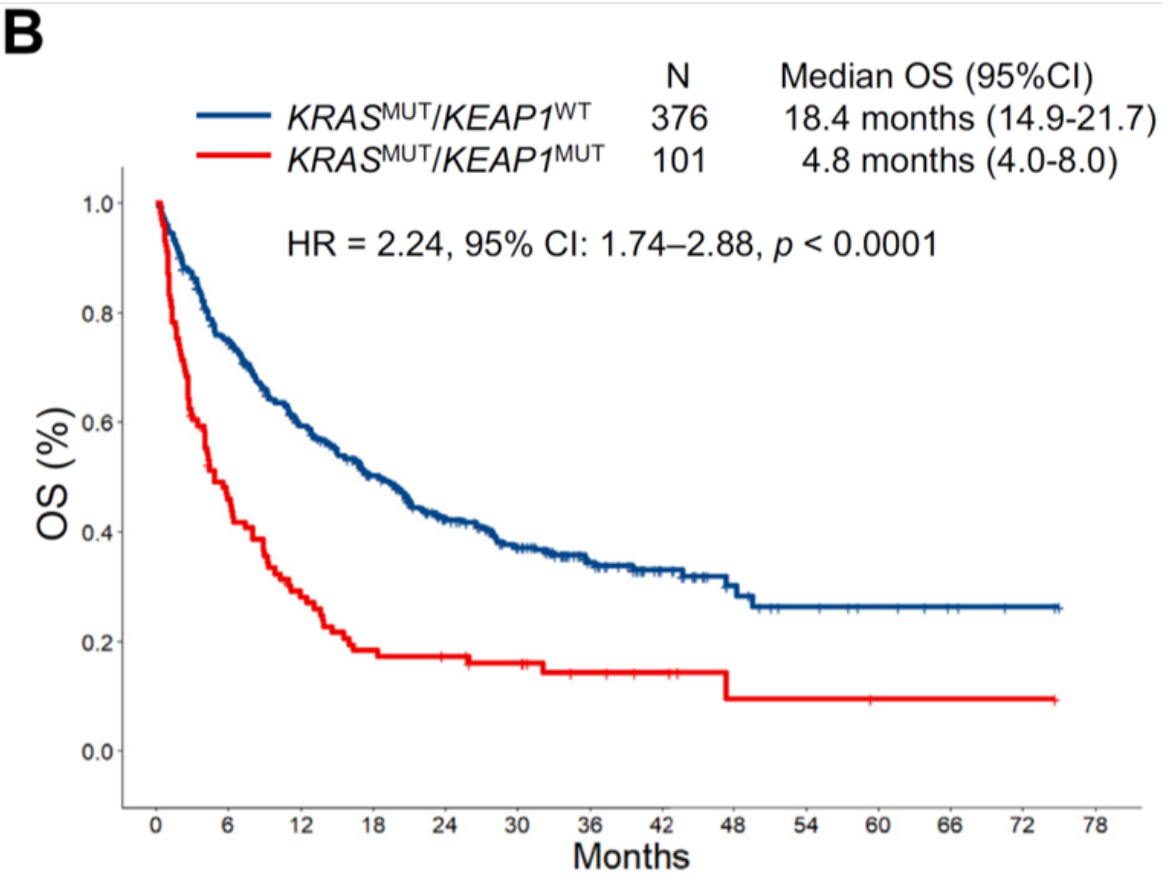
Impact of *KEAP1/STK11* mutations on PD-(L)1 monotherapy efficacy in NSCLC





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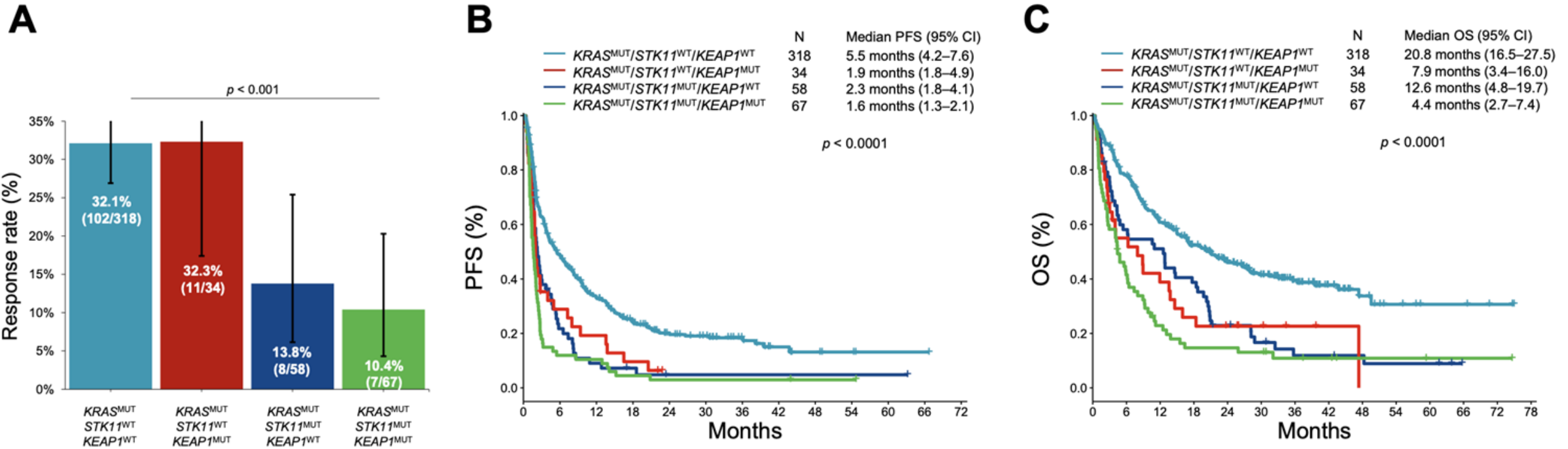
Triple mutant are very resistant to PD-(L)1 monotherapy



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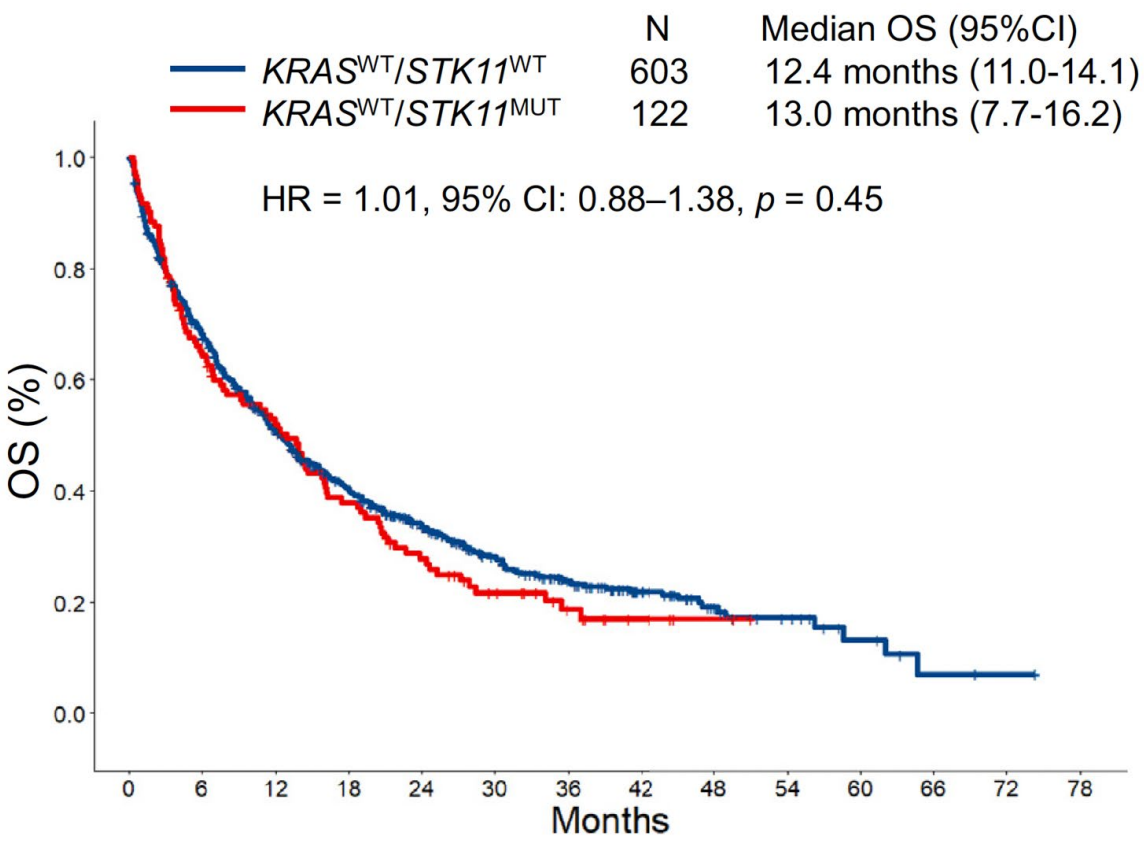
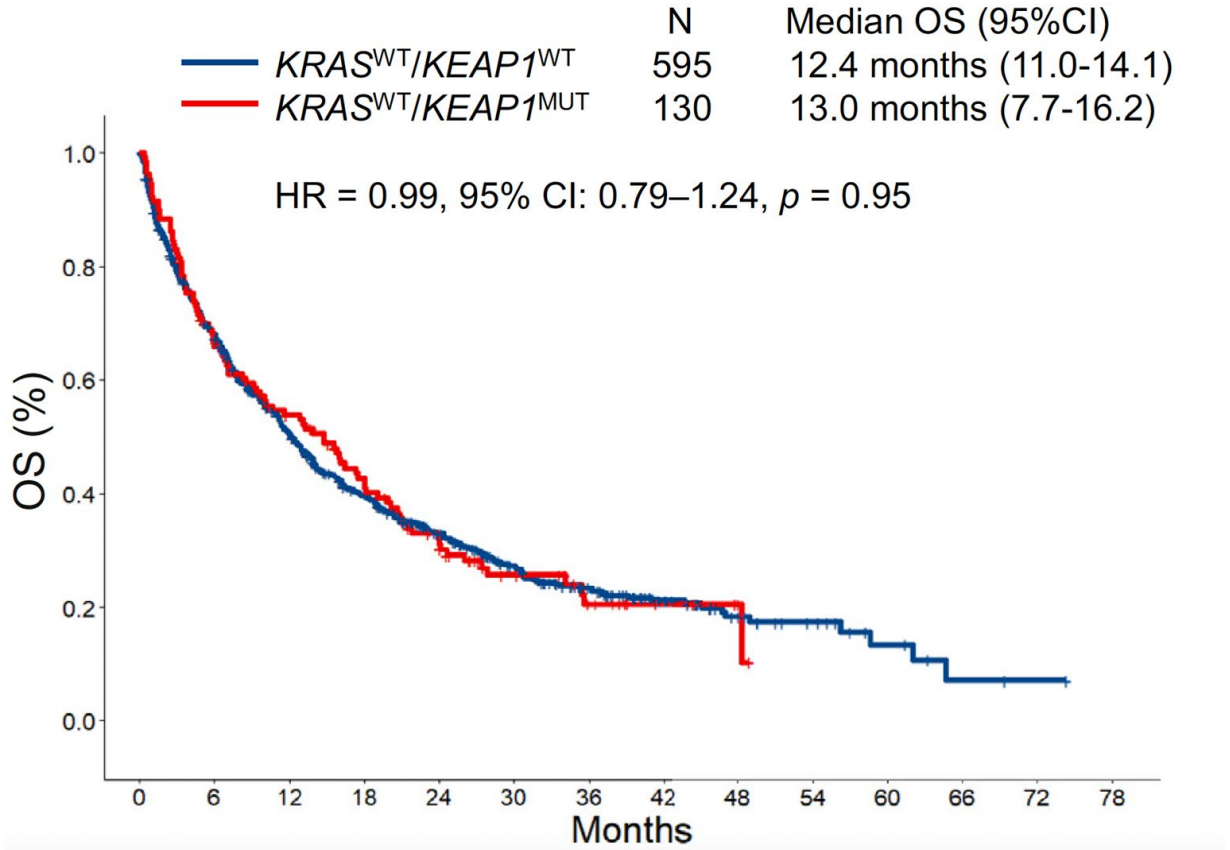
STK11/KEAP1 mutations are not associated with ICI efficacy in KRAS WT NSCLC





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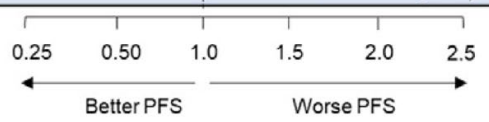
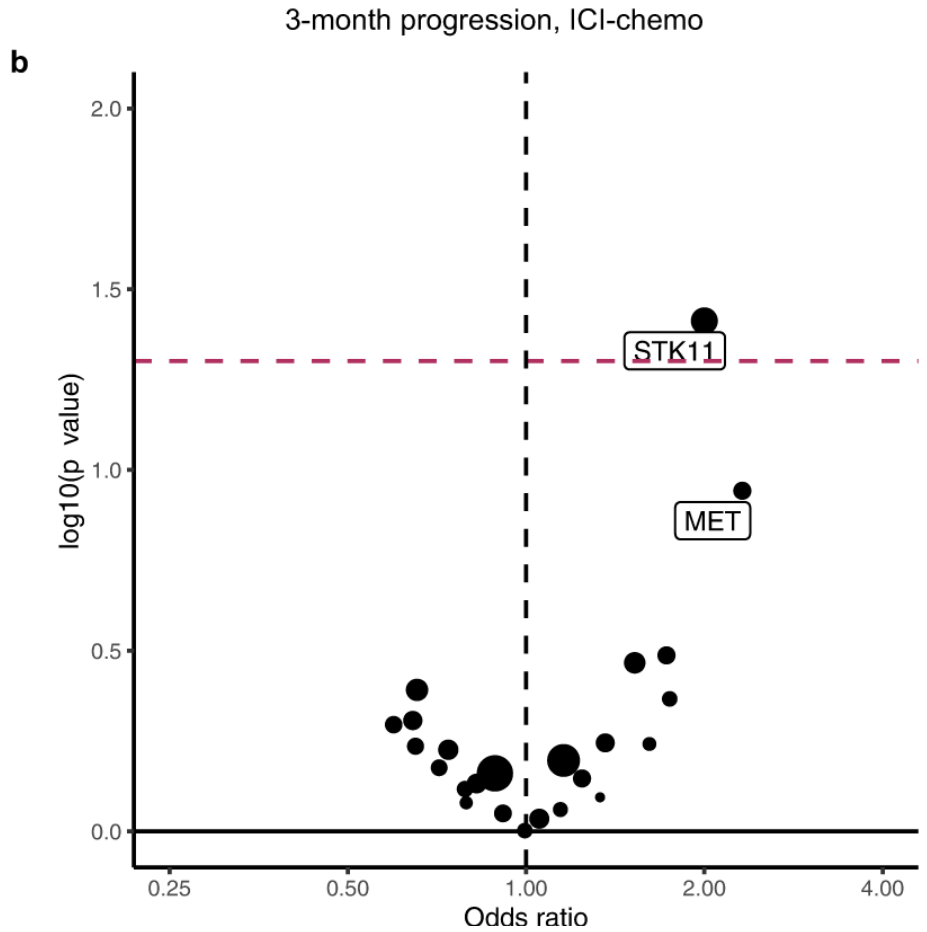
Impact of *KEAP1/STK11* mutations on chemo-immunotherapy efficacy in NSCLC

Forest-plot for progression-free survival (PFS)

Mutation status	N	HR (95%, CI) PFS	*P*-value
KRAS^{WT}	560	reference	
KRAS^{MUT}	351	1.10 (0.95-1.28)	0.21
TP53^{WT}	370	reference	
TP53^{MUT}	412	0.83 (0.71-0.97)	0.020
STK11^{WT}	516	reference	
STK11^{MUT}	191	1.46 (1.22-1.76)	<0.001
KEAP1^{WT}	518	reference	
KEAP1^{MUT}	189	1.53 (1.28-1.84)	<0.001
SMARCA4^{WT}	593	reference	
SMARCA4^{MUT}	114	1.62 (1.30-2.02)	<0.001
KRAS^{WT} *TP53*^{WT}	182	reference	
KRAS^{WT} *TP53*^{MUT}	287	0.88 (0.71-1.08)	0.21
KRAS^{WT} *STK11*^{WT}	322	reference	
KRAS^{WT} *STK11*^{MUT}	99	1.19 (0.93-1.54)	0.16
KRAS^{WT} *KEAP1*^{WT}	327	reference	
KRAS^{WT} *KEAP1*^{MUT}	104	1.37 (1.07-1.75)	0.01
KRAS^{WT} *SMARCA4*^{WT}	361	reference	
KRAS^{WT} *SMARCA4*^{MUT}	70	1.36 (1.03-1.81)	<0.001
KRAS^{MUT} *TP53*^{WT}	188	reference	
KRAS^{MUT} *TP53*^{MUT}	125	0.75 (0.58-0.97)	0.027
KRAS^{MUT} *STK11*^{WT}	184	reference	
KRAS^{MUT} *STK11*^{MUT}	92	1.92 (1.46-2.53)	< 0.001
KRAS^{MUT} *KEAP1*^{WT}	191	reference	
KRAS^{MUT} *KEAP1*^{MUT}	85	1.82 (1.38-2.41)	<0.001
KRAS^{MUT} *SMARCA4*^{WT}	232	reference	
KRAS^{MUT} *SMARCA4*^{MUT}	44	2.39 (1.67-3.42)	< 0.001





Alessi J, Ricciuti B, et al. J Thoracic Oncol 2023, Vokes N, et al. Nat Comm, 2023

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CTLA4 blockade for STK11 and KEAP1 mutant NSCLC

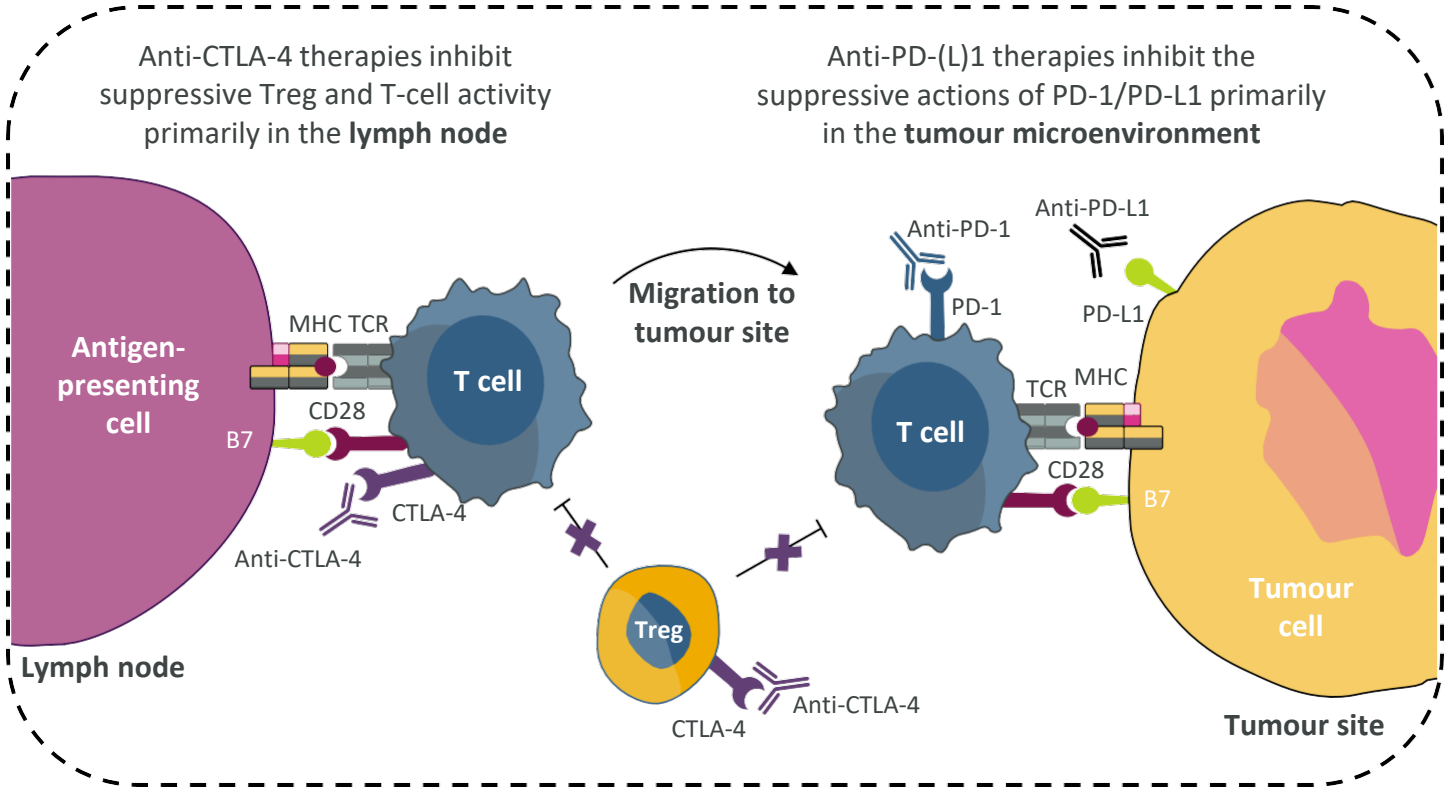
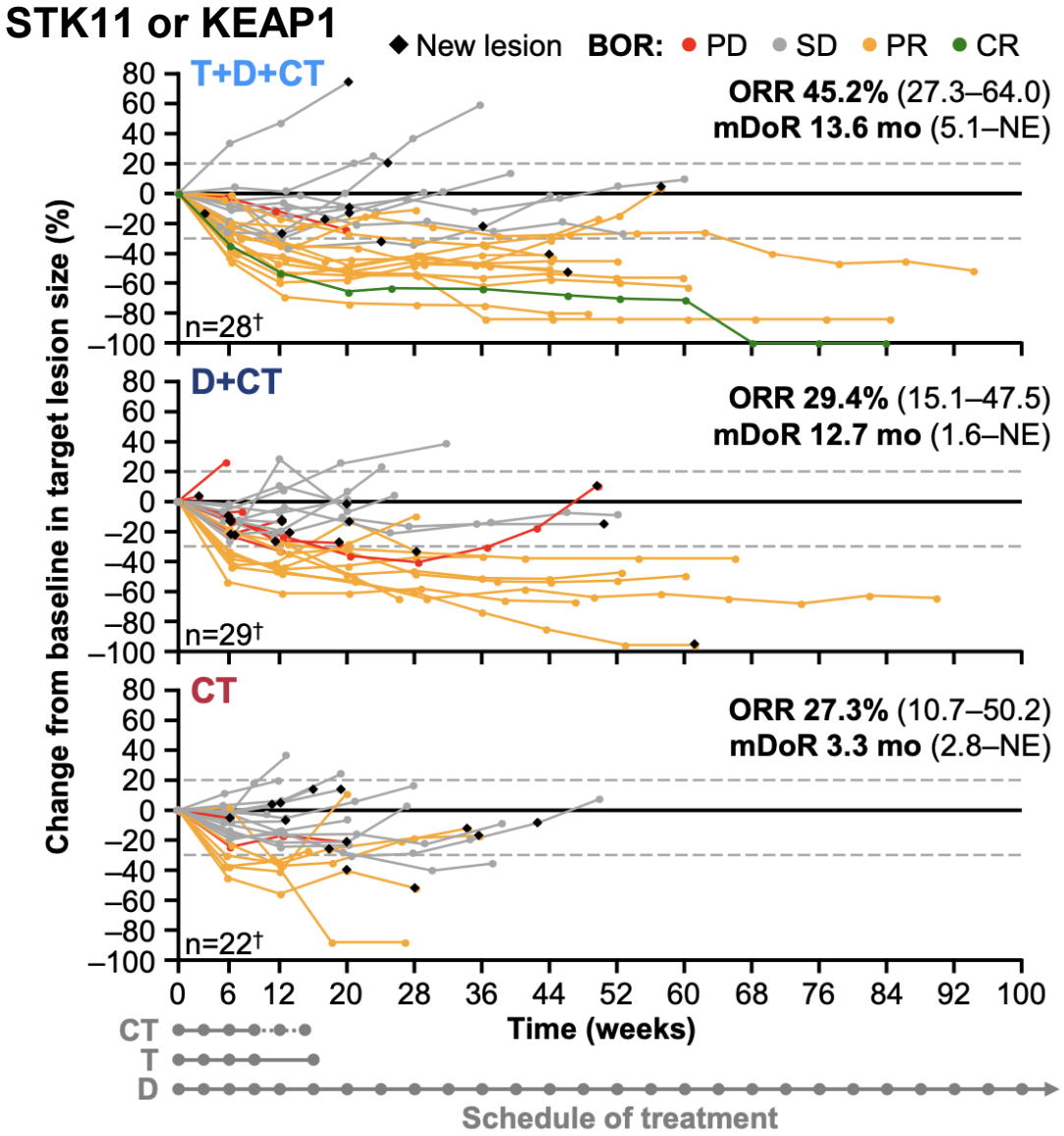


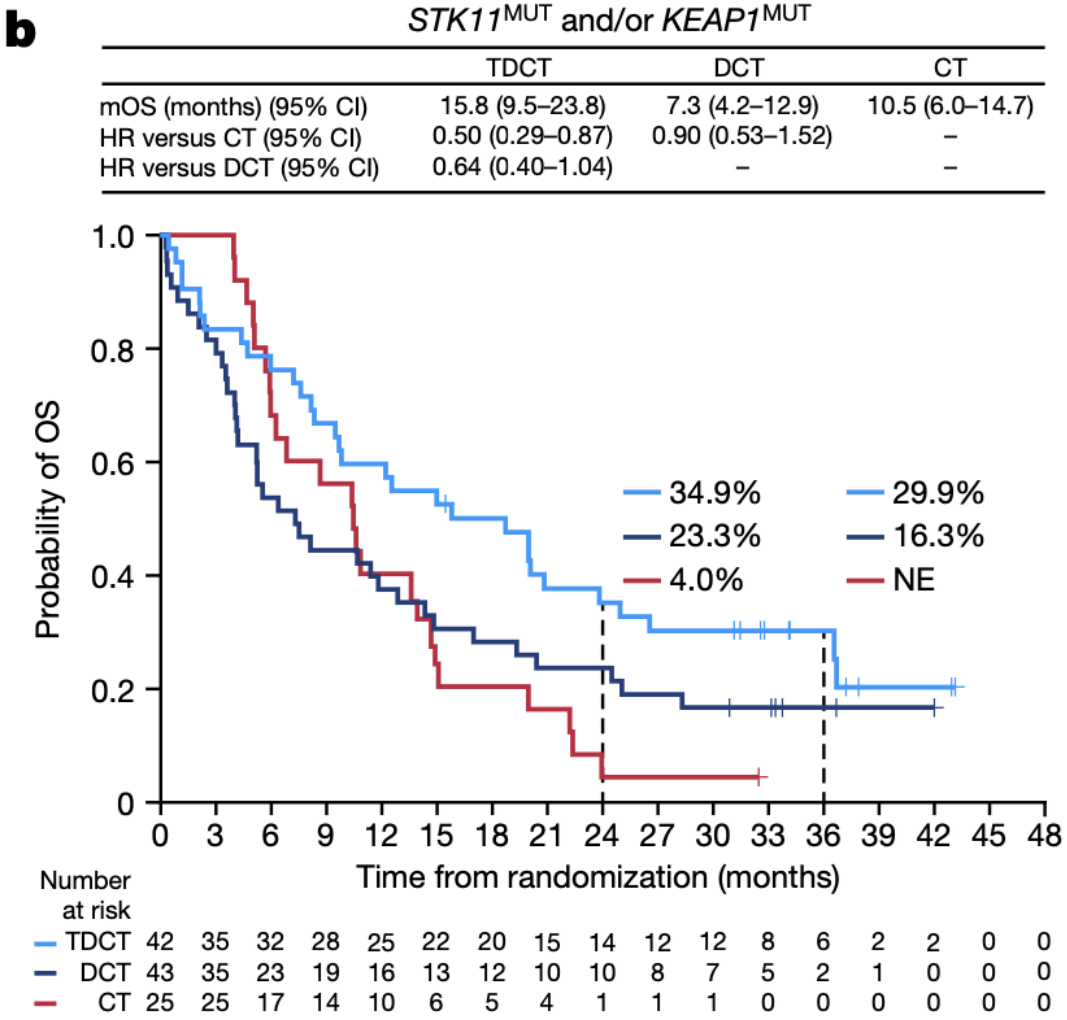
Image adapted from Buchbinder EI & Desai A. *Am J Clin Oncol* 2016;39:98–106[1]

CD28, cluster of differentiation 28; CTLA-4, cytotoxic T-lymphocyte-associated antigen-4; MHC, major histocompatibility complex; PD-1, programmed cell death 1; PD-()L1, programmed cell death-(ligand) 1; TCR, T-cell receptor; Treg, regulatory T cells

1. Buchbinder EI & Desai A. *Am J Clin Oncol* 2016;39:98–106; 2. Khan SK *et al. Clin Immunol* 2011;138:85–96; 3. Stewart R *et al. Cancer Immunol Res* 2015;3:1052–62

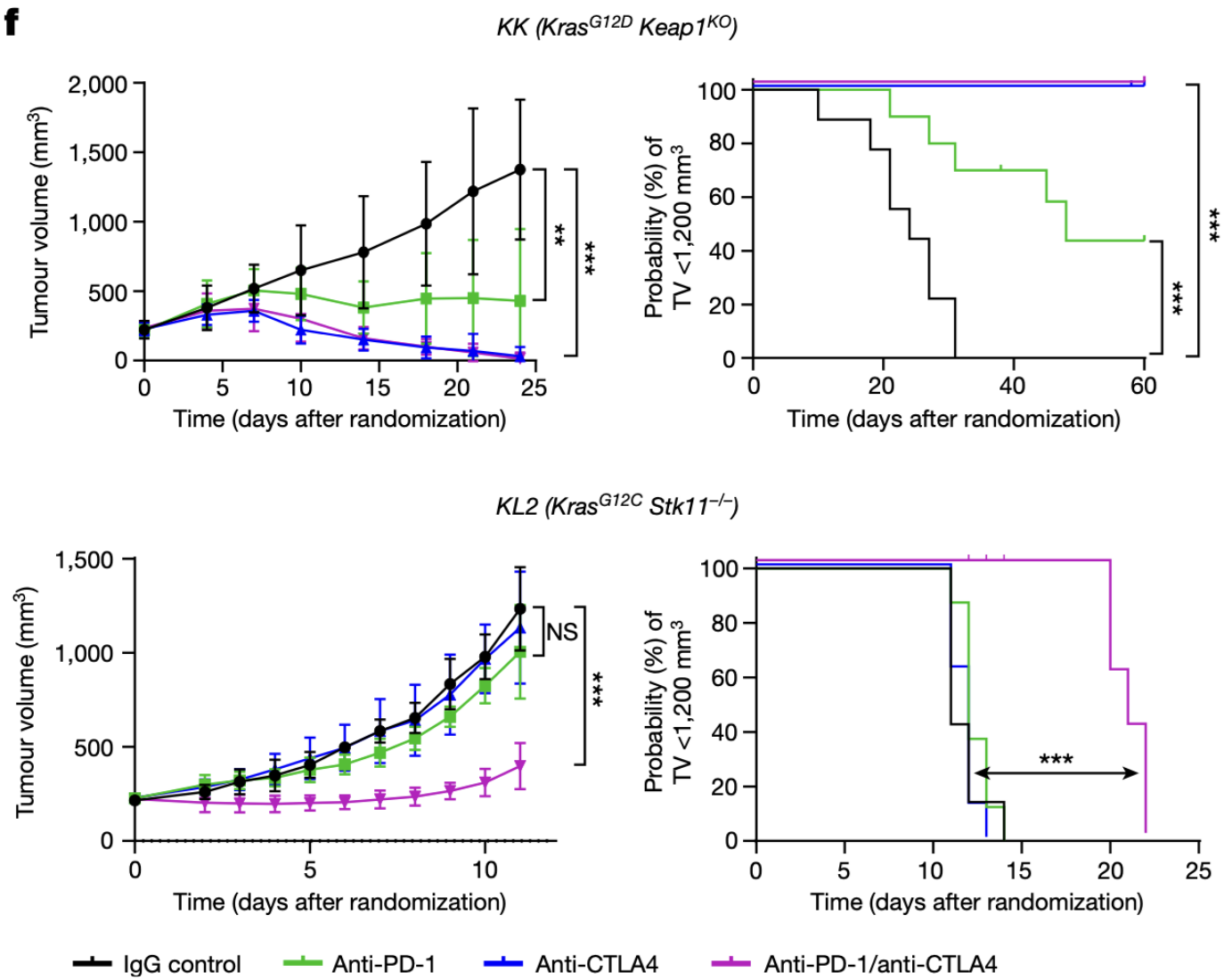
CTLA4 blockade may reduce KEAP1/STK11-related resistance to PD-(L)1 inhibitors





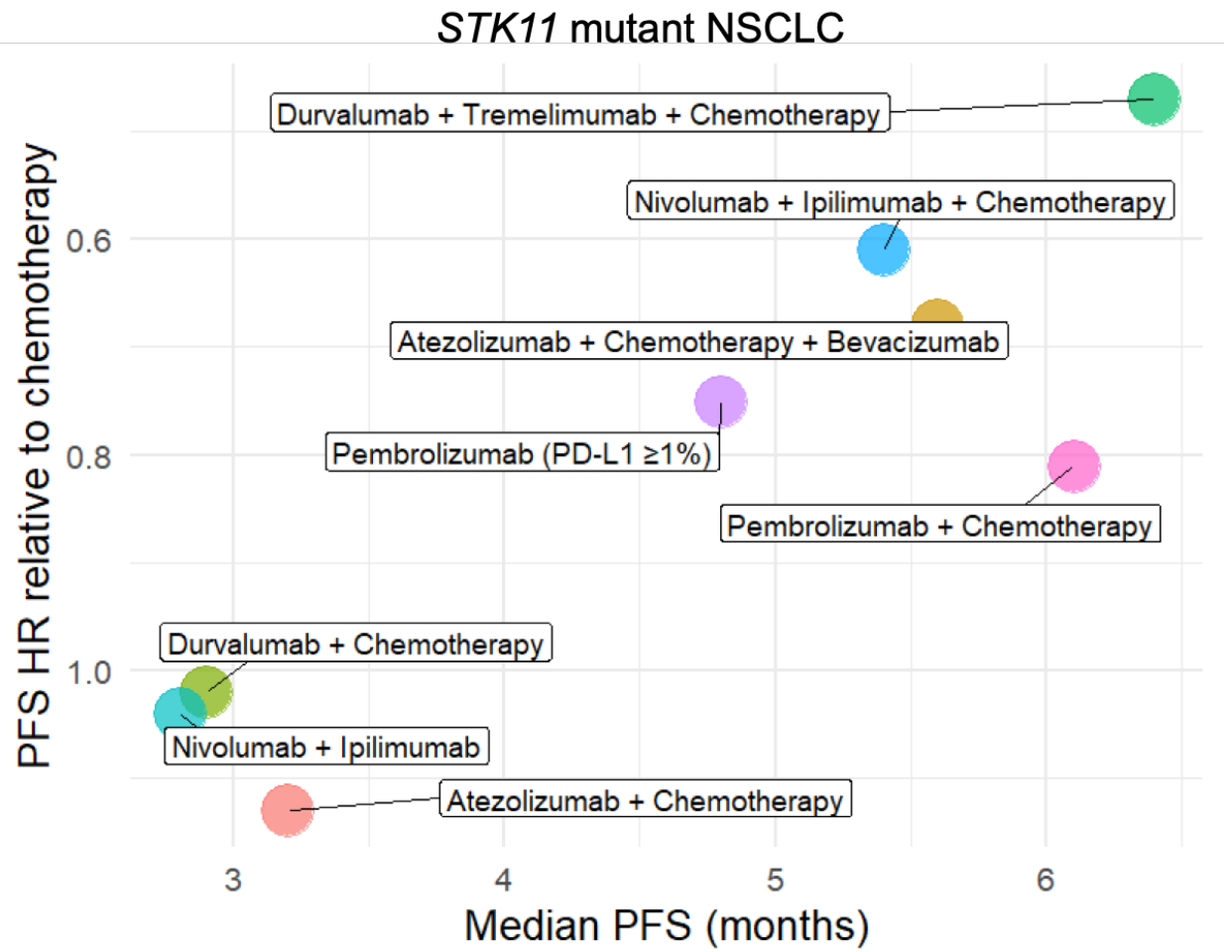
Skoulidis F et al. Nature 2024

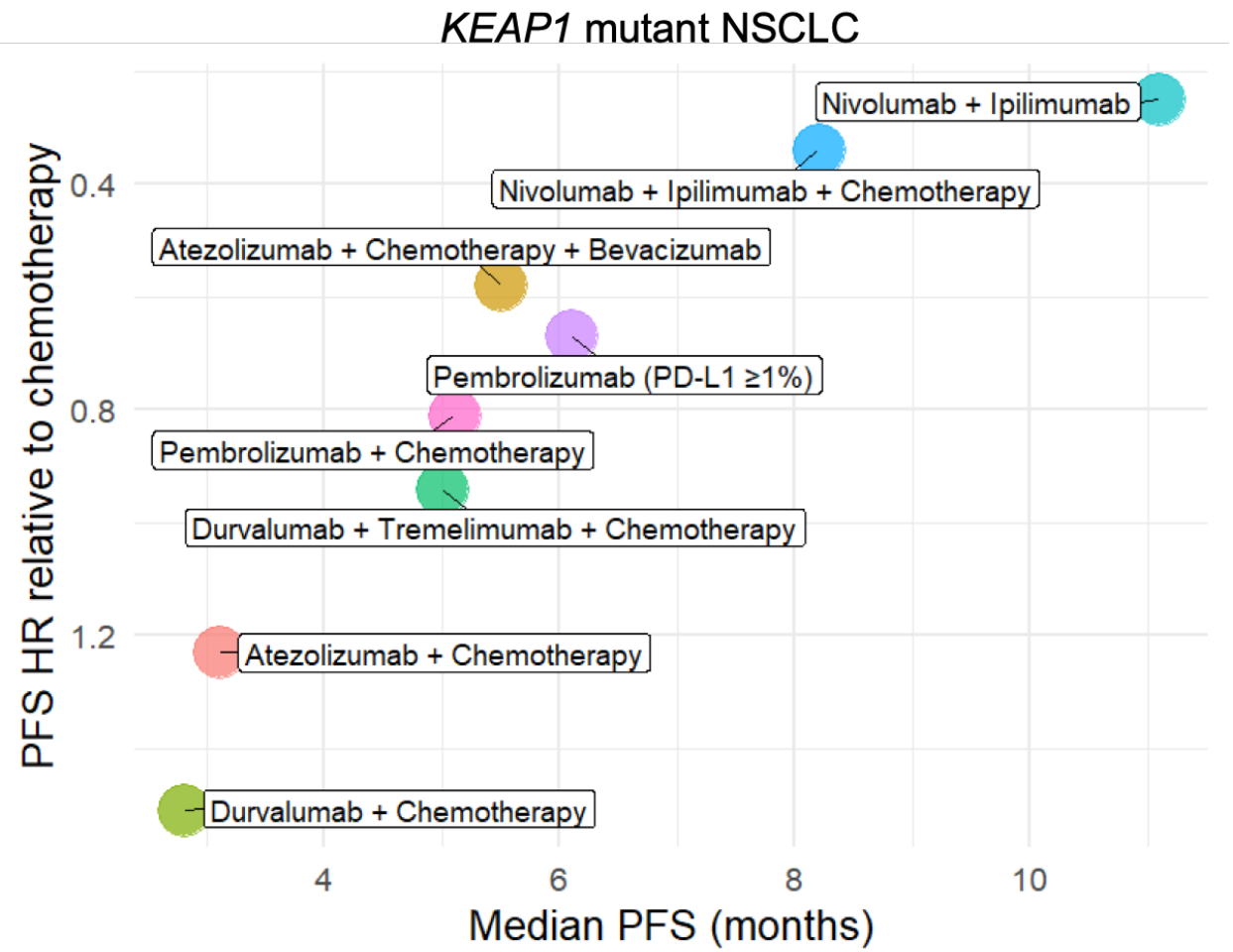
CTLA4 blockade reduces KEAP1/STK11-related resistance to PD-(L)1 inhibitors in syngenic models in vivo



Skoulidis F et al. Nature 2024

Optimal treatment strategy for co-mutations?





Ricciuti B & Garassino M, J Thoracic Oncol, 2024

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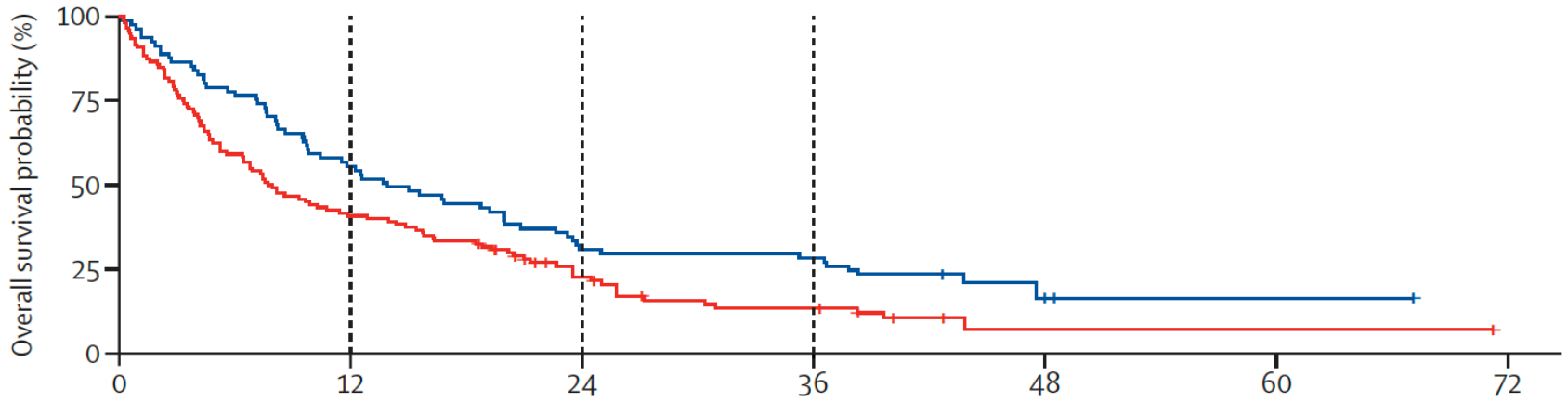
Pooled analysis of 6 randomized clinical trials reporting outcomes in STK11 mutant NSCLC

A *STK11* mutated

	Reconstructed events/patients	Median overall survival, months (95% CI)	1-year overall survival, rate (95% CI)	2-year overall survival, rate (95% CI)	3-year overall survival, rate (95% CI)
Dual CTLA-4 and PD-L1 or PD-1 blockade	65/81	13·9 (9·8–20·8)	55·6% (45·7–67·5)	30·9% (22·3–42·8)	28·4% (20·1–40·1)
Single PD-L1 or PD-1 blockade	102/120	7·8 (6·4–12·9)	40·8% (32·9–50·7)	22·7% (16·1–32·0)	13·4% (8·1–22·2)

HR 0·67; 95% CI 0·49–0·91; p=0·012



Number at risk (censored)							
Dual CTLA-4 and PD-L1 or PD-1 blockade	81 (0)	45 (0)	25 (0)	23 (0)	7 (9)	4 (12)	0 (16)
Single PD-L1 or PD-1 blockade	120 (0)	49 (0)	21 (8)	11 (10)	2 (16)	2 (16)	0 (18)

Di Federico…and Ricciuti B, Lancet Oncology 2025

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Pooled analysis of 6 randomized clinical trials reporting outcomes in PD-L1 negative vs positive NSCLC

A PD-L1 TPS <1%

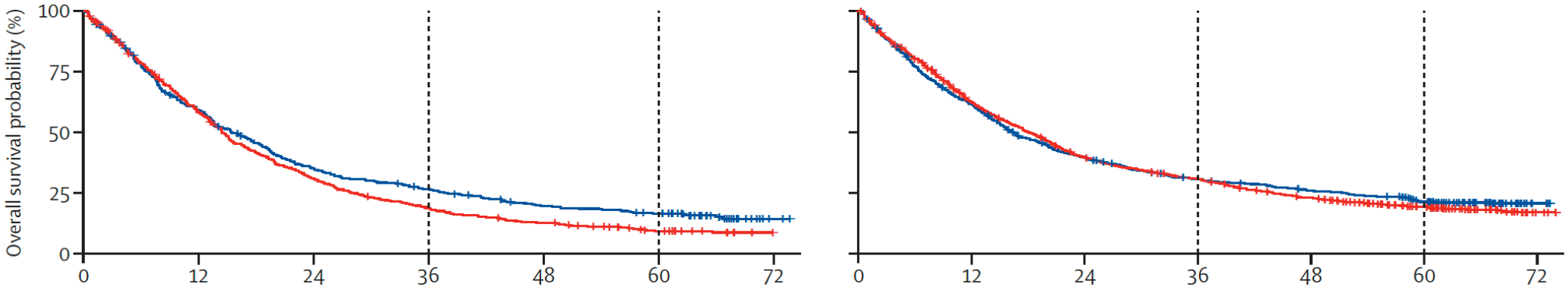
	Reconstructed events/patients	Median overall survival, months (95% CI)	3-year overall survival, rate (95% CI)	5-year overall survival, rate (95% CI)
Dual CTLA-4 and PD-L1 or PD-1 blockade	367/447	15·5 (13·6–18·5)	26·8% (22·9–31·3)	16·6% (13·4–20·6)
Single PD-L1 or PD-1 blockade	452/512	14·5 (13·4–15·9)	18·7% (15·5–22·4)	9·3% (7·0–12·3)

HR 0·85; 95% CI 0·74-0·98; p=0·021

B PD-L1 TPS ≥1%

	Reconstructed events/patients	Median overall survival, months (95% CI)	3-year overall survival, rate (95% CI)	5-year overall survival, rate (95% CI)
Dual CTLA-4 and PD-L1 or PD-1 blockade	629/813	16·5 (15·0–18·9)	30·8% (27·8–34·2)	21·5% (18·8–24·6)
Single PD-L1 or PD-1 blockade	844/1056	18·1 (16·6–19·8)	30·9% (28·2–33·8)	19·3% (17·0–21·9)

HR 0·97; 95% CI 0·88–1·08; p=0·60



Number at risk (censored)														
Dual CTLA-4 and PD-L1 or PD-1 blockade	447 (0)	260 (7)	152 (10)	114 (12)	81 (16)	61 (23)	3 (77)	813 (0)	496 (7)	317 (12)	239 (19)	199 (23)	155 (32)	6 (178)
Single PD-L1 or PD-1 blockade	512 (0)	292 (11)	155 (12)	92 (13)	62 (14)	36 (25)	1 (59)	1056 (0)	646 (17)	406 (21)	313 (25)	230 (31)	160 (66)	5 (207)

Di Federico…and Ricciuti B, Lancet Oncology 2025

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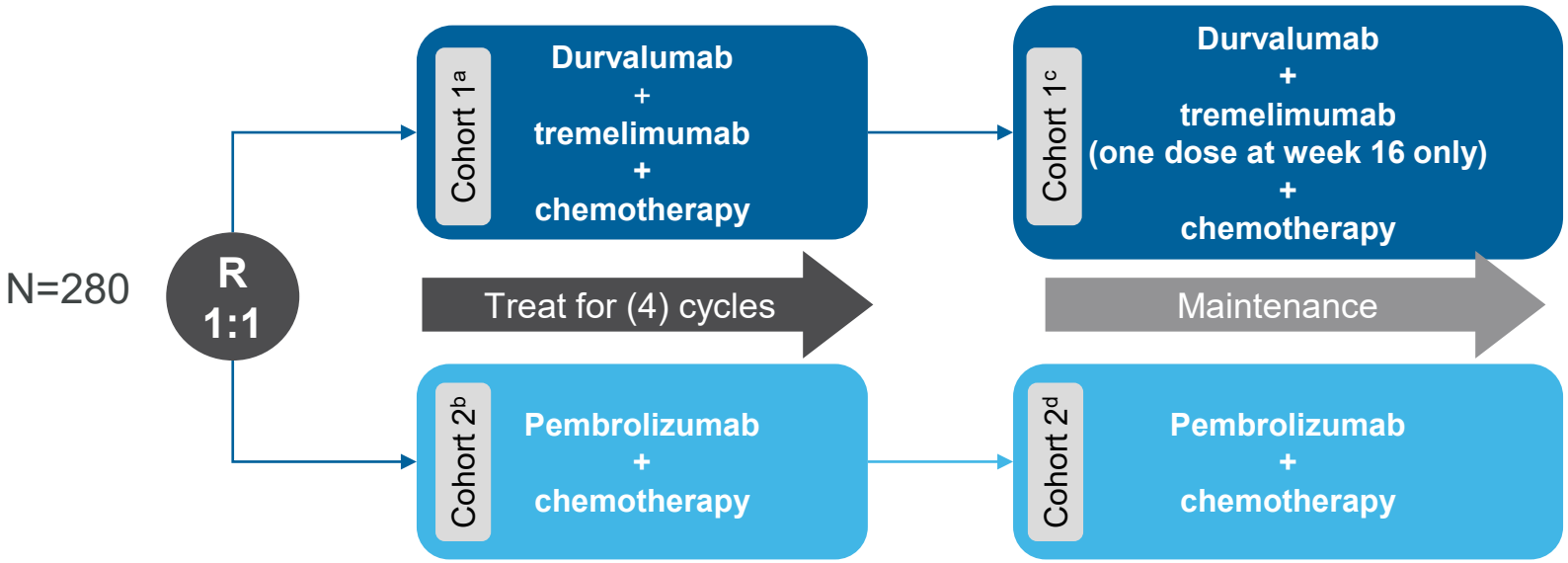
TRITON: randomized phase III clinical trial comparing POSEIDON vs KN189 in STK11/KEAP1 mutant NSCLC



Patient population

- Metastatic nonsquamous NSCLC
- No prior systemic treatment for metastatic disease
- **KRAS, STK11, and/or KEAP1 alterations**
- No EGFR or ALK alterations
- ECOG PS 0 or 1
- Mandatory tissue requirement

Primary endpoints

- OS

Key secondary endpoints

- PFS
- ORR, DoR
- Safety/Tolerability

Exploratory endpoint

- OS and PFS in patients with KRAS or KEAP1

Post Analysis

- PD-L1 expression
- Comutations
- Smoking history

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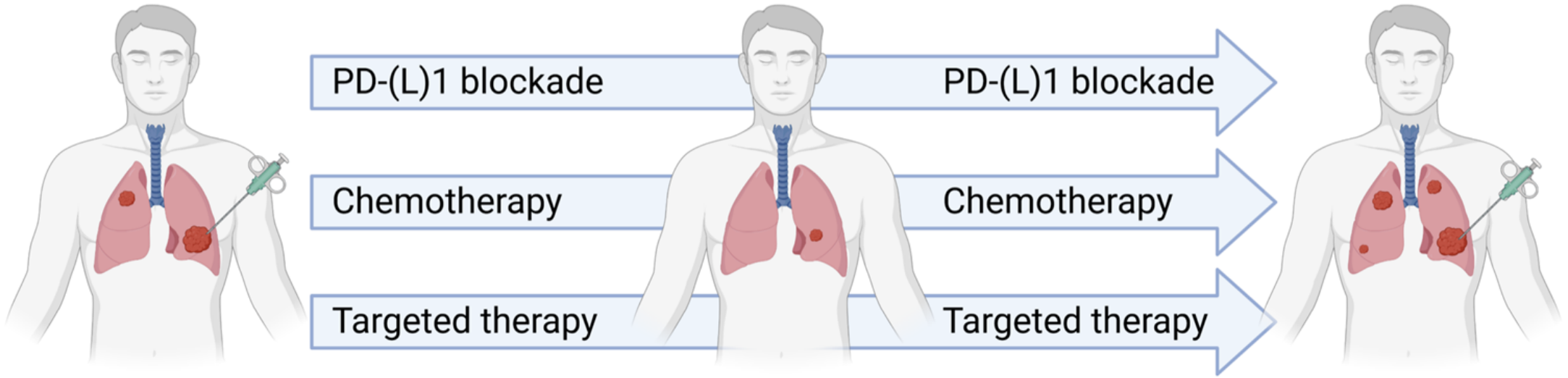
Understanding of acquired resistance to ICI in NSCLC: DFCI



Analysis	PD-(L)1 blockade (N=82)	Chemotherapy (N=32)	Targeted therapy (N=89)
• Tumor genomic profiling	N=79	N=30	N=89
• Digital pathology	N=16	N=5	N=11
• Multiplexed immunofluorescence	N=6	N=0	N=0
• HLA class I immunohistochemistry	N=8	N=7	N=9

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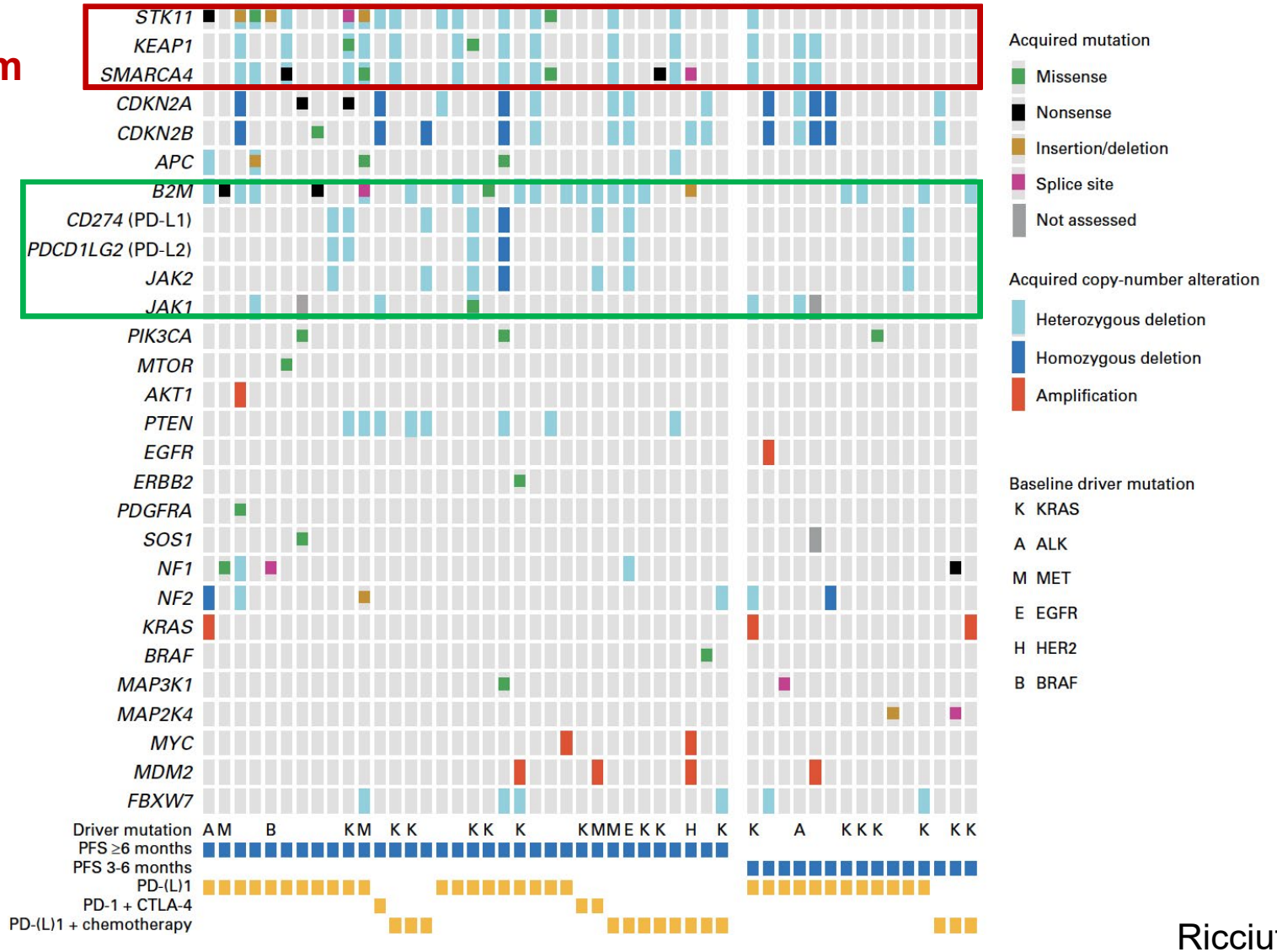
Acquired mutations and copy number changes after ICI

TSG
Immune metabolism

Immune checkpoint
Antigen presentation



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Are we testing for STK11 and KEAP1?

	Assay	Company	STK11	KRAS	KEAP1	Sample type
Decentralized (in-house) assays	TruSight Oncology 500[1]	Illumina	+	+	+	FFPE
	TruSight Oncology 500 High-Throughput[1]	Illumina	+	+	+	FFPE
	TruSight Oncology 500 ctDNA assay v2[2]	Illumina	+	+	+	ctDNA
	TruSight Tumor 170[3]	Illumina	+	+	-	FFPE
	AmpliSeq Focus[4]	Illumina	-	+	-	FFPE
	AmpliSeq Comprehensive Panel[5]	Illumina	+	+	-	FFPE
	Oncomine™ Focus Assay[6]	ThermoFisher	-	+	-	FFPE
	Oncomine™ Comprehensive Assay[7]	Thermo Fisher	+	+	-	FFPE
	Oncomine™ Precision Assay[8]	Thermo Fisher	-	+	-	FFPE
	Oncomine™ Dx Target Test[9]	Thermo Fisher	-	+	-	FFPE
	Archer™ FUSIONPlex™ Lung v2 Panel[10]	Integrated DNA Technologies	-	+	-	FFPE
	AmoyDx® HANDLE Classic NGS Panel[11]	Amoy Diagnostics	+	+	+	FFPE
Centralized (outsourced)	Guardant360® CDx[12]	Guardant Health	+	+	-	ctDNA
	FoundationOne® CDx[13]	Foundation Medicine	+	+	+	FFPE
	FoundationOne® Liquid CDx[14]	Foundation Medicine	+	+	+	ctDNA
	Caris	Caris Life	+	+	+	FFPE
	TEMPUS	TEMPUS	+	+	+	FFPE

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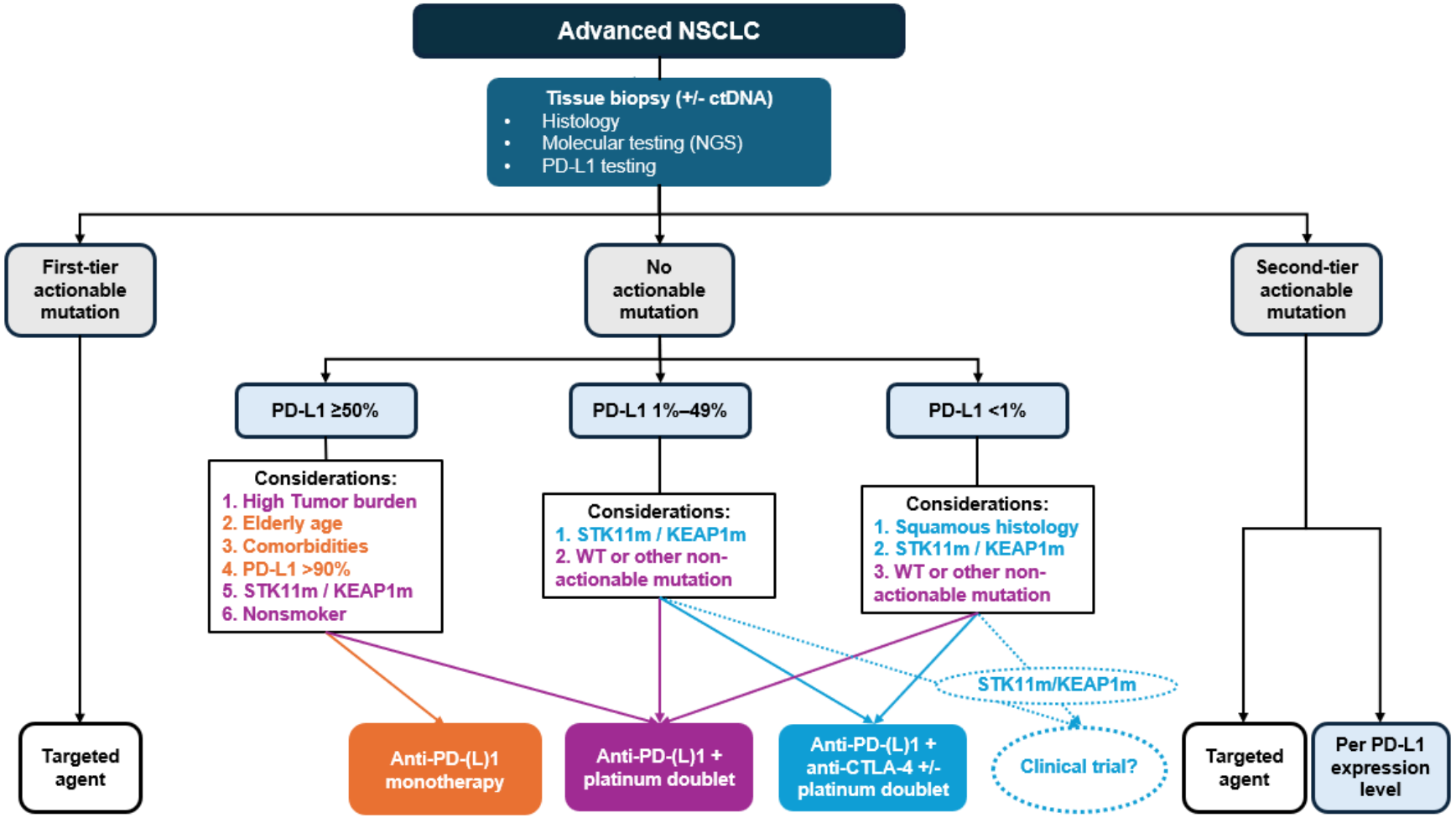
Current guidelines and pathways do not include STK11 and KEAP1



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Take home messages

- STK11 and KEAP1 are tumor suppressor genes involved in maintaining metabolic homeostasis under normal conditions. In non–small cell lung cancer, their inactivation contributes to tumor initiation, progression, and metastasis.
- Mutations in both STK11 and KEAP1 occur more commonly in smokers and carry distinct clinical implications depending on concurrent alterations in genes such as TP53 and KRAS.
- These mutations confer resistance to immune checkpoint inhibitors and are associated with an immune-cold or immune-depleted tumor microenvironment.
- Therapeutic strategies to overcome the immunosuppressive phenotype of STK11- and KEAP1-mutant NSCLC are currently under investigation and dual PD-(L)1 blockade + CTLA-4 inhibition has the potential to abrogate resistance.

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