Genomic Alterations as Guides for Resistance/Response to Immunotherapy: Looking Beyond TMB, MSI, PD-L1

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Conflicts of Interest

- Employee at Foundation Medicine
- Roche Shareholder

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Key Topics

1. Immunotherapy basics
2. Future of IO response prediction
3. Emerging biomarkers for immunotherapy sensitivity
4. Emerging biomarkers for immunotherapy resistance

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Immunotherapy Basics

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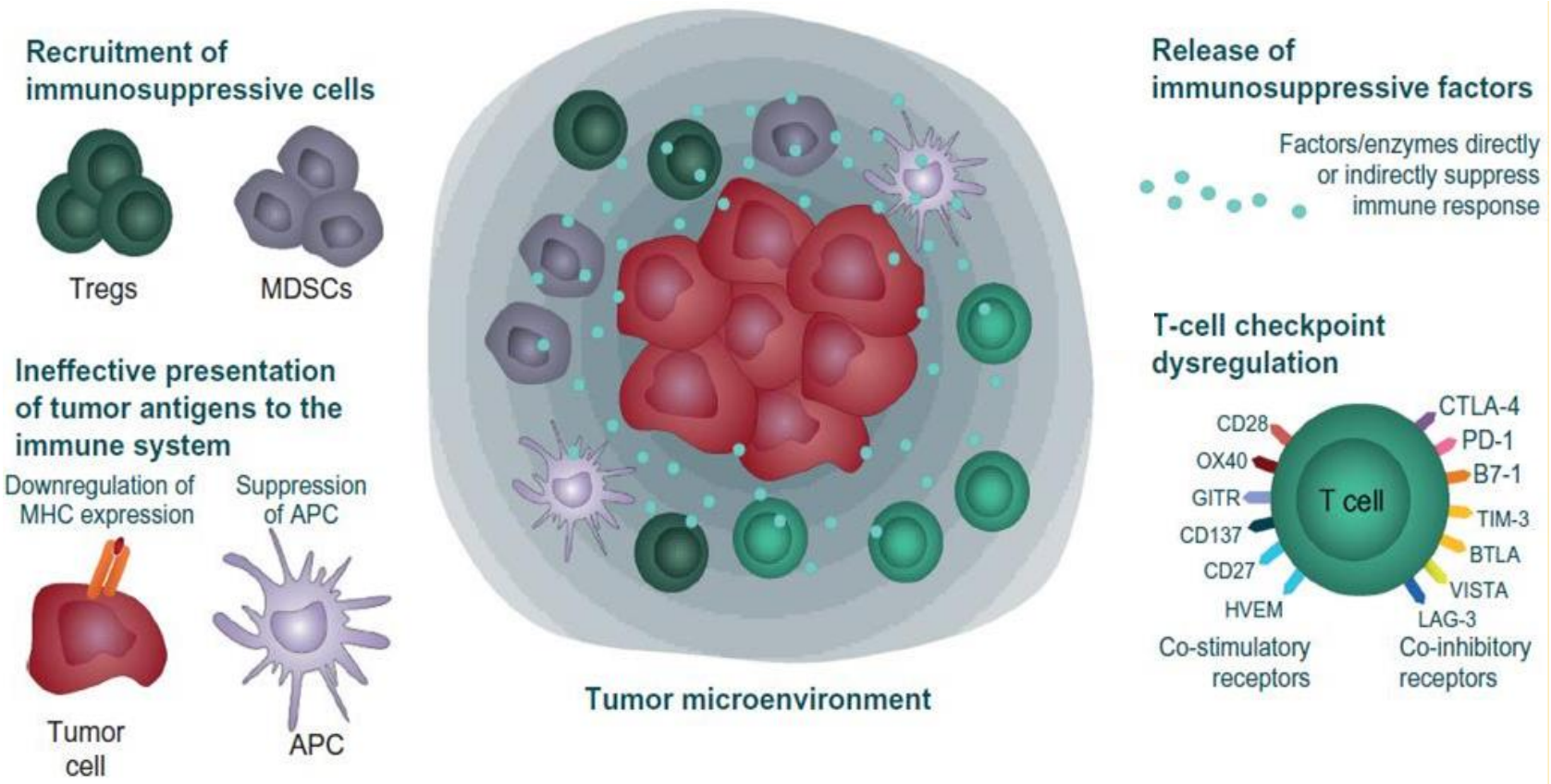
The Ability to Evade the Immune System is a Hallmark of Cancer

There are many ways tumors can avoid immune detection:



1. Davies M. *Cancer Manag Res*. 2014;6:63-75.

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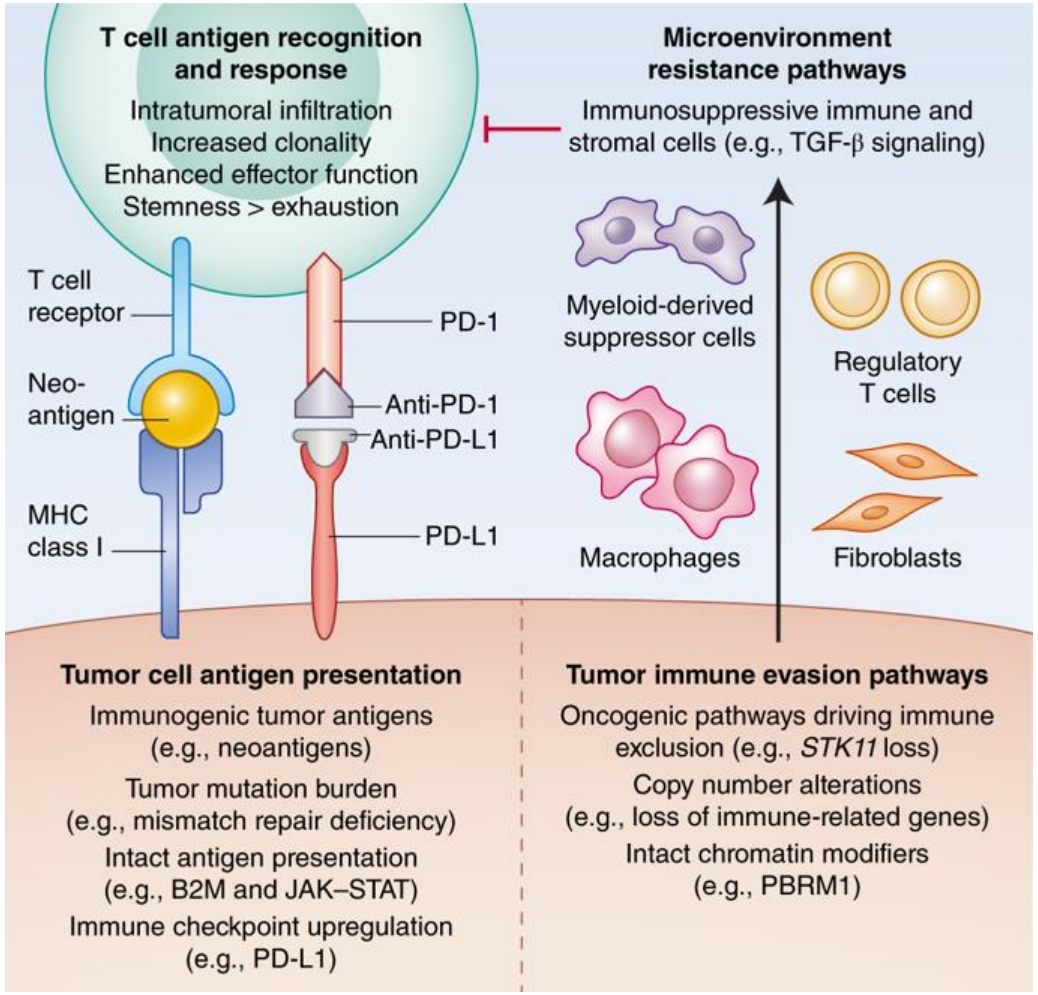
Complex Landscape for Immune Checkpoint Blockade

T-cell antigen recognition and presentation and tumor immune evasion and microenvironment

- Tumor Cells
  - Stimulatory receptors to present neoantigens
  - Inhibitory receptors put the brakes on the immune system
- Immune Cells
  - Tumor infiltrating T-cells (TILs) to secrete cytokines
- Regulatory T cells
  - Need to enhance anti-tumor activity but avoid deleterious autoimmunity
- Microenvironment resistance pathways that can suppress signaling



1. Keenan TE et al, *Nat Med*. 2019;25(3):389-402

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Future of IO Response Prediction

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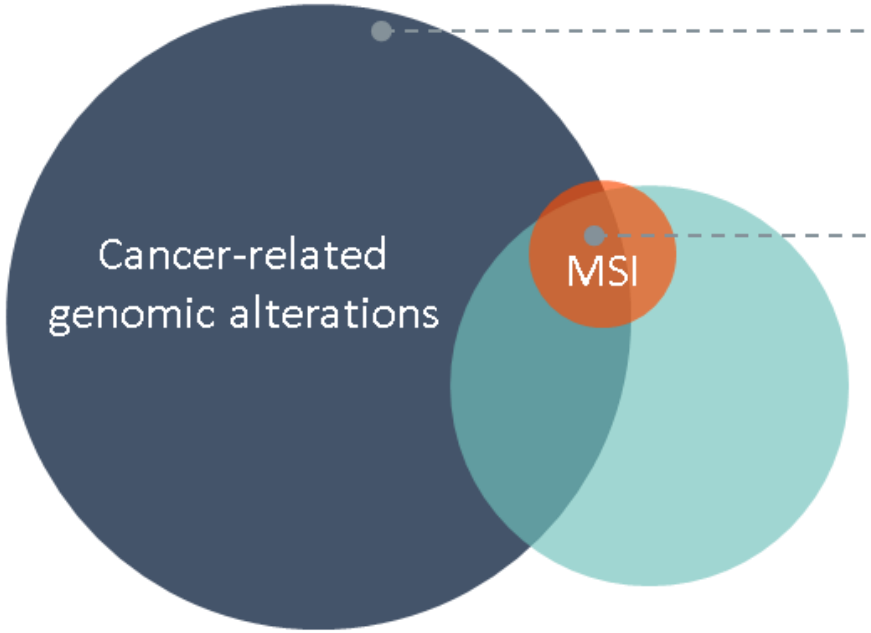
TMB and MSI measurement supplements extensive genomic information



Cancer-related genomic alterations[1]

- Base substitutions, indels, rearrangements and CNAs
- Measured using various methods, e.g. NGS, PCR, FISH and IHC

Microsatellite instability (MSI)[2-4]

- Predisposition to mutations caused by impaired MMR
- Characterised by high rates of alteration to repetitive DNA sequences
- Assessed by IHC (for MMR proteins), microsatellite PCR or CGP

Tumour mutational burden (TMB)[5]

- Quantitative measure of the total number of genomic alterations per coding area of a tumour genome
- Measured directly by WES or extrapolated from CGP

.....Specific genomic alterations may independently impact likelihood of response to checkpoint inhibitors.....

1. Frampton, G.M., et al. (2013) *Nat Biotechnol* 31:1023-31;
2. Boland, C.R. and Goel, A. (2010) *Gastroenterology* 138:2073-87;
3. Salipante, S.J., et al. (2014) *Clin Chem* 60:1192-9;
4. Foundation Medicine, Inc (2018). Available at: https://www.foundationmedicine.com/genomic-testing (Accessed November 2019);
5. Meléndez, B., et al. (2018) *Transl Lung Cancer Res* 7:661-7.

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Genomic correlates of response and resistance organized by primary location

Table 1 | Genomic correlates of response and resistance organized by primary location

Primary location	Response category	Defining characteristics or examples
T cell	Intratumoral infiltration[85,115,135–137,139]	Transcriptional signatures of cytotoxic lymphocytes infiltrating the tumor core
	Enhanced effector function[52,134,141]	Increased expression of *PRF1*, *GZMA/B*, *CD8A*, and *IFNG*
	Increased clonality[14,144,41]	Ranging from 0 to 1, with 1 indicating a monoclonal population
	Greater stemness[147,150]	Express chemokine receptor CXCR5 and transcription factor TCF7; lack TIM-3/CD39
	Reduced exhaustion[147,150]	Express co-inhibitory receptor TIM-3 and ectonucleotidase CD39; lack CXCR5/TCF7
Tumor cell (response mechanisms)	Tumor antigens[31,32,34–40,54,57,65,67]	Neoantigens, viral antigens
	Increased tumor mutation burden[9,37,48]	Mismatch repair deficiency
	Immunogenic alterations[159]	Inactivating mutations in *SERPINB3* and *SERPINB4*
	Mutational signatures[39,53,108]	Smoking, ultraviolet light, alkylating agent therapy, APOBEC
	Genomic upregulation of PD-L1 (refs. [50,92–94,97–100])	*PDL1* amplification and loss of *CDK4*, *SPOP*, and *CMTM4* and *CMTM6*
	Chromatin modifier loss[152,154,157,158]	Inactivating mutations in *PBRM1*, *ARID1A*, and *SMARCA4*
Tumor cell (resistance mechanisms)	Tumor antigens[68]	Cancer/testis antigens similar to self and less immunogenic
	Deficient antigen presentation[37,53]	Inactivating mutations in *B2M*, *HLA*, JAK/STAT, and IFN-γ response genes
	Oncogenic pathways[45,113 115,117,118,124,125,129,130,133]	Inactivating *STK11* and *PTEN* mutations, WNT/β-catenin, *EGFR* and *KRAS* mutations
	Immune evasion alterations[141]	Increased expression of *SERPINB9*
	CNAs[144,160]	High levels of copy-number loss, chromosome arm and whole-chromosome CNAs
Microenvironment	Immunosuppressive stromal cells[115,123,126,140]	Transcriptional signatures of fibroblasts, endothelial cells, and TGF-β signaling
	Immunosuppressive immune cells[136,141]	Transcriptional signatures of myeloid-derived suppressor cells and regulatory T cells

1. Keenan TE et al, *Nat Med*. 2019;25(3):389-402

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Emerging biomarkers for immunotherapy sensitivity

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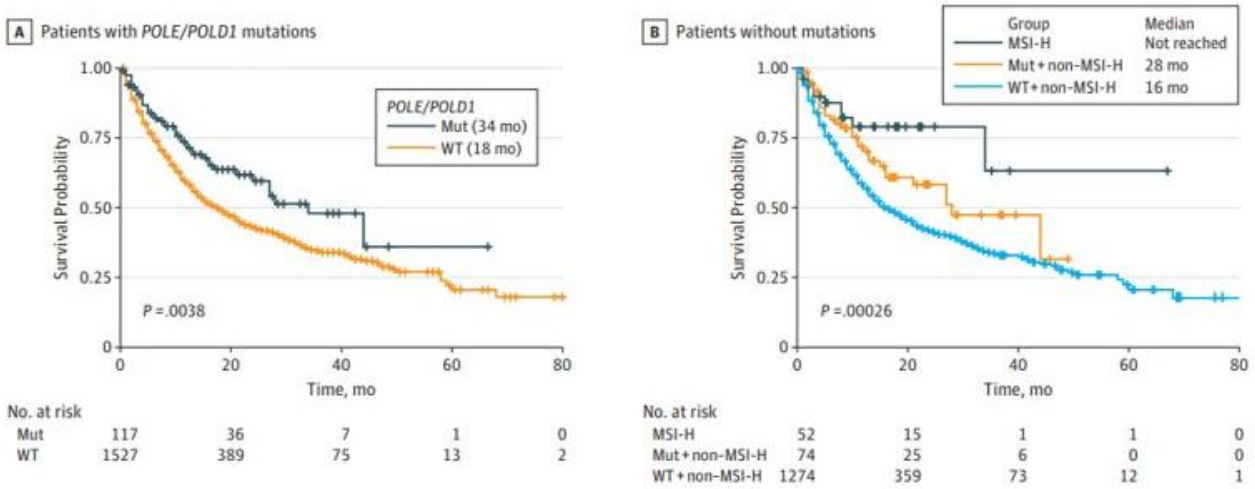
DNA damage repair pathway

DNA polymerases

- Across 47,721 patients, the mutational frequencies of POLE and POLD1 were 2.79% and 1.37%, respectively
  - nonmelanoma skin cancer having the highest levels of POLE/POLD1 mutations
- The TMB of patients with these mutations was substantially higher
- POLE/POLD1 alterations predicted response:
  - patients with either POLE or POLD1 mutations showed a significantly longer OS of 34 months vs 18 months



Overall survival of patients with POLE/POLD1 mutations vs those without or with MSI-H

1. Wang F, et al. *JAMA Oncol*. 2019;5(10):1504-1506

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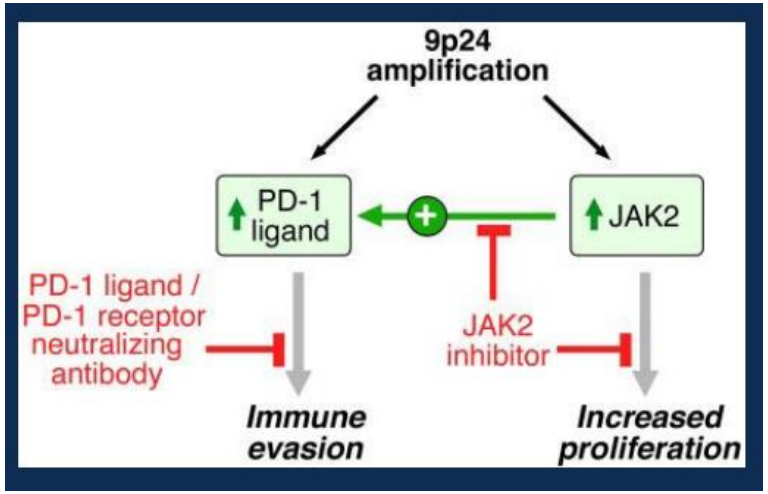
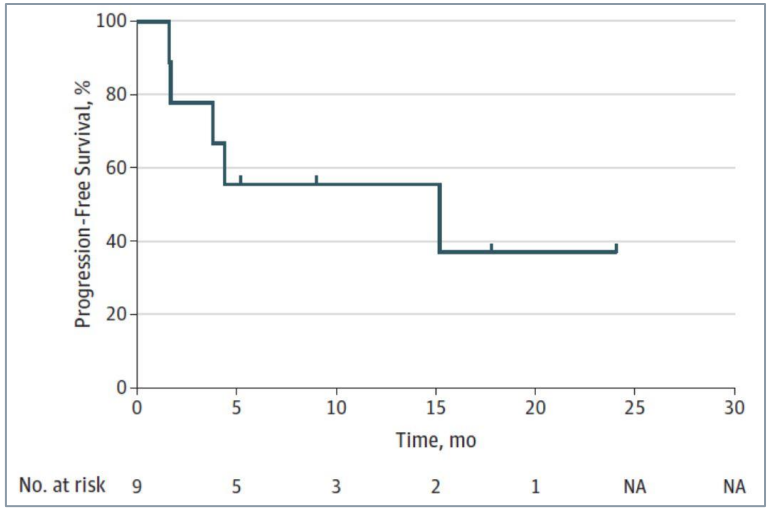
PD-L1/PD-L2 amplification as predictive biomarker for checkpoint inhibitor response

- PD-L1 (*CD274*) gene amplification identified in 843 of 118,187 samples (0.7%) from 100 different tumor types
- PD-L1-amplified tumors were most commonly associated with low to intermediate TMB
- PD-L1 amplification predicted response:
  - 66.7% of patients (6 of 9) with PD-L1 gene amplification vs 29.8% patients (45 of 151) in the overall treated cohort (P=.03)
  - Median PFS among the 9 patients was 15.2 months (range, 1.6 to ≥ 24.1 months)





Progression-free survival (PFS) in 9 patients with PD-L1 amplifications who received checkpoint inhibitor therapy

1. Goodman AM, et al. *JAMA Oncol*. 2018;4(9):1237-1244

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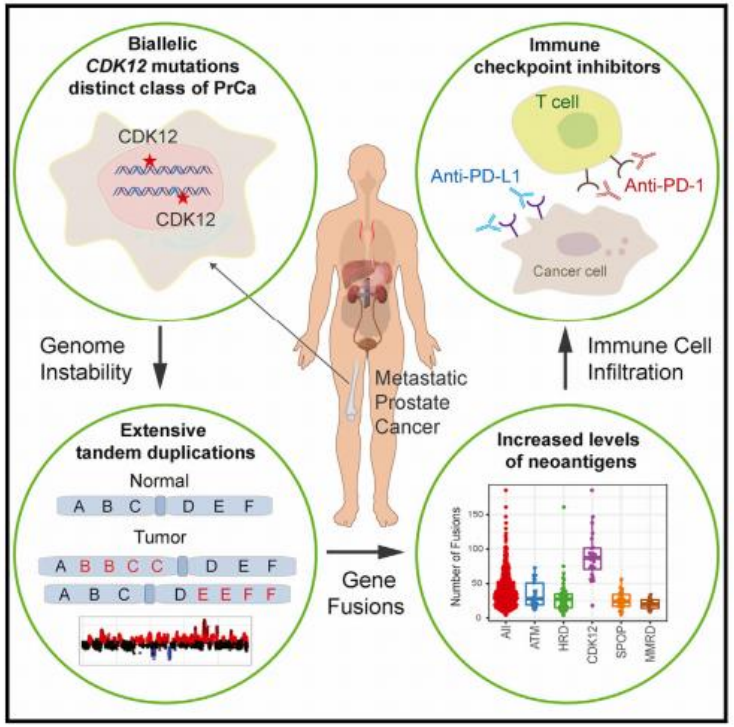
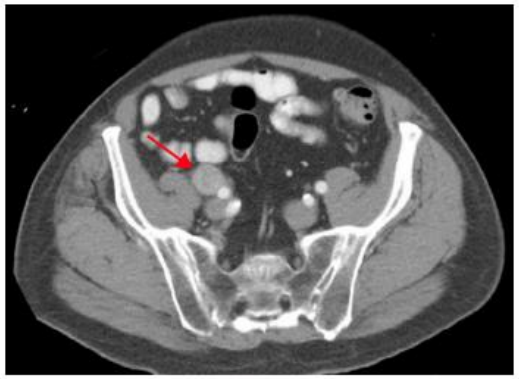
CDK12 inactivating mutations and response to ICIs

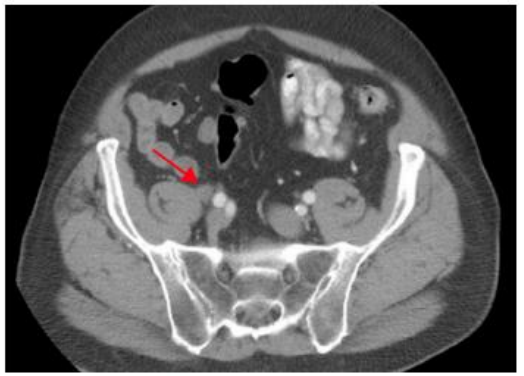
- CDK12 biallelic inactivating mutations define a distinct subtype of prostate cancer
- CDK12 loss is associated with genomic instability and focal tandem duplications
  - Across multiple cancer types, including gastric/ esophageal, ovarian, breast, and endometrial cancer, the number of focal tandem duplicates (FTDs) was significantly increased in CDK12-LOF versus CDK12 wild-type cases
- CDK12 loss leads to increased gene fusions, neoantigen burden (FTD burden), and T cell infiltration



Exceptional PSA responses have been observed (two of four patients) in men with mCRPC treated with an anti-PD-1 immune checkpoint inhibitor



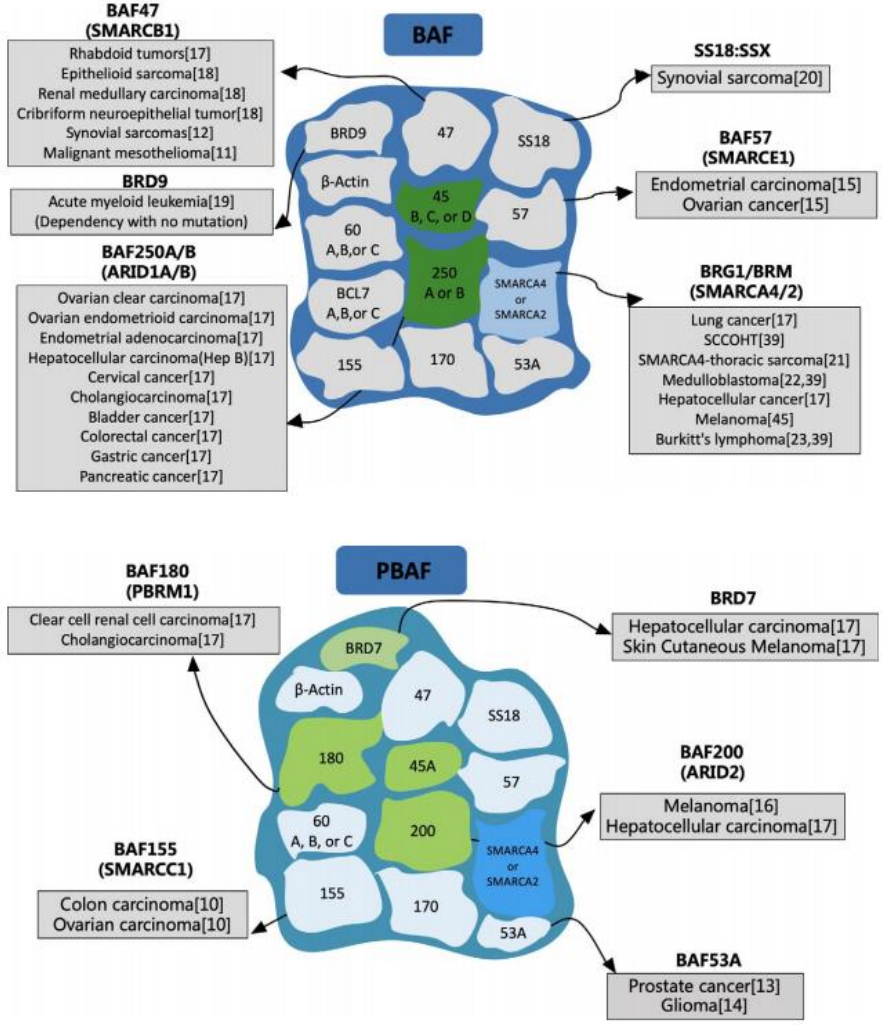
Prior to anti-PD-1 immunotherapy
Right external iliac LN, 2.4 cm, PSA 8.9 ng/mL

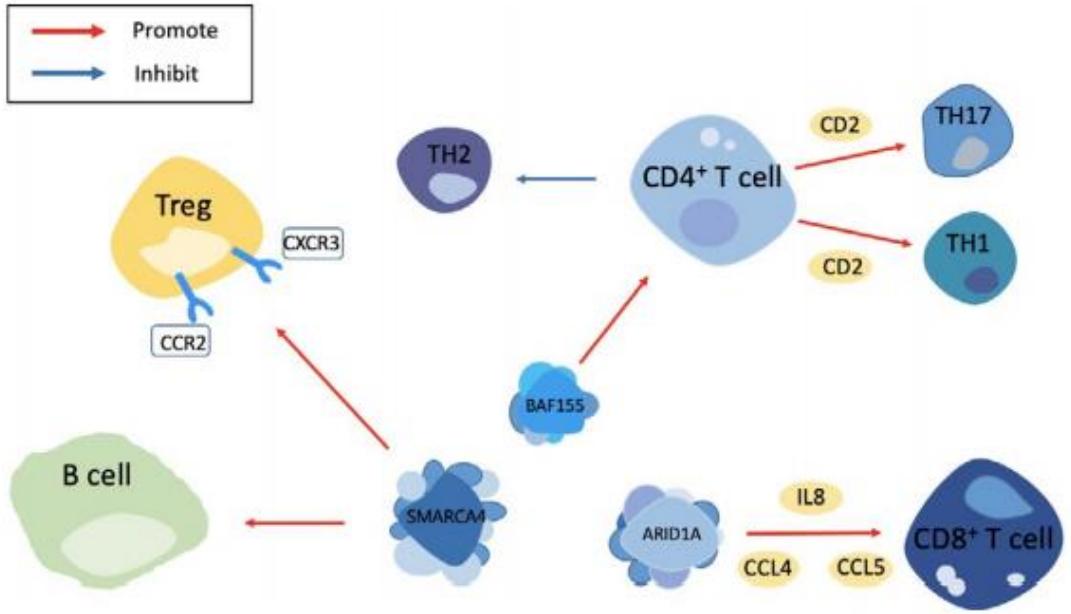


After 4 doses of anti-PD-1 immunotherapy
Right external iliac LN, 1.1 cm, PSA 0.9 ng/mL

1. Wu YM et al, *Cell.* 2018;173:1770-1782

Roles of the SWI/SNF complexes in modulating the immune system





The alteration of chromatin dynamics accounts for a potential mechanism that induces target gene expression in the case of immune cell activation

1. Hu B et al, *Cell Prolif*. 2020;53(4):e12791

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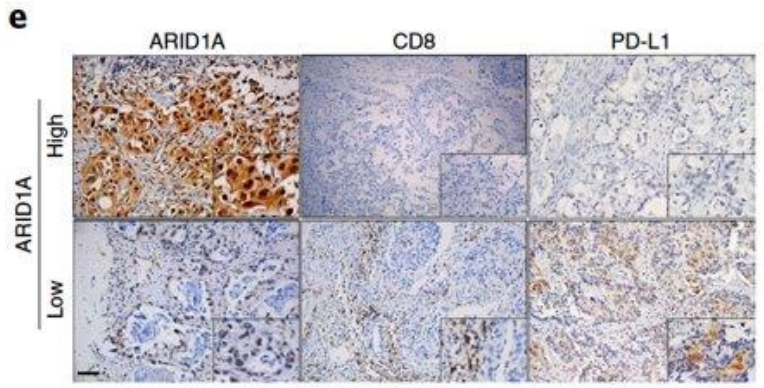
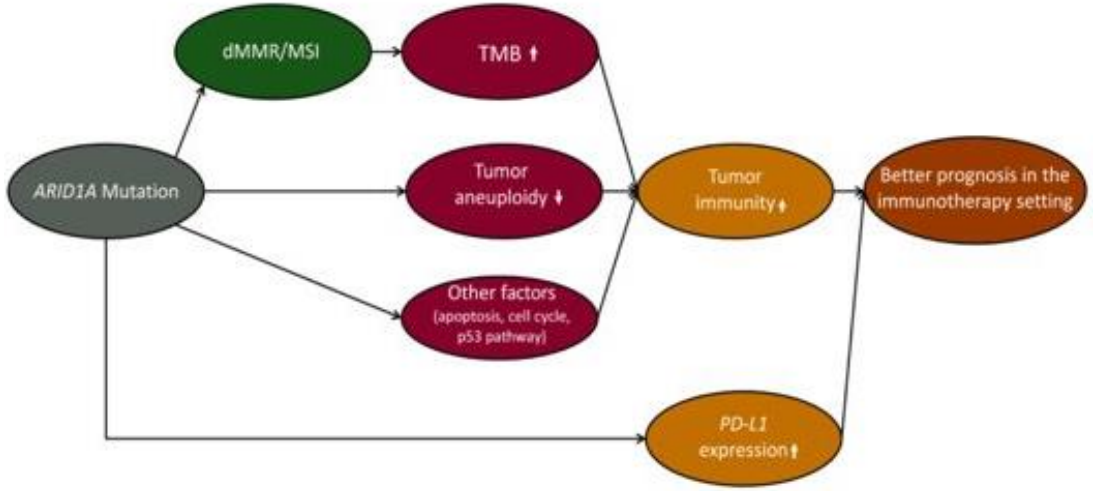
ARID1A(BAF250A)



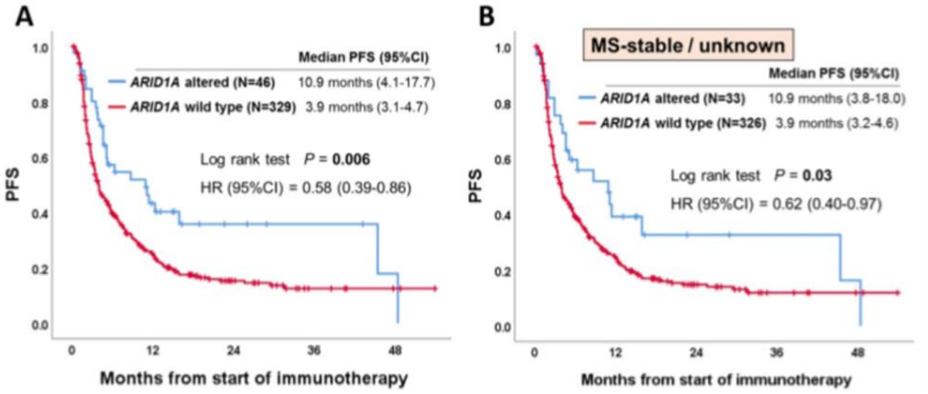
	ARID1A High	ARID1A Low
CD8⁻	5	1
CD8⁺	2	8
	(P = 0.0134)	

	ARID1A High	ARID1A Low
PD-L1⁻	6	0
PD-L1⁺	1	9
	(P = 0.0004)	

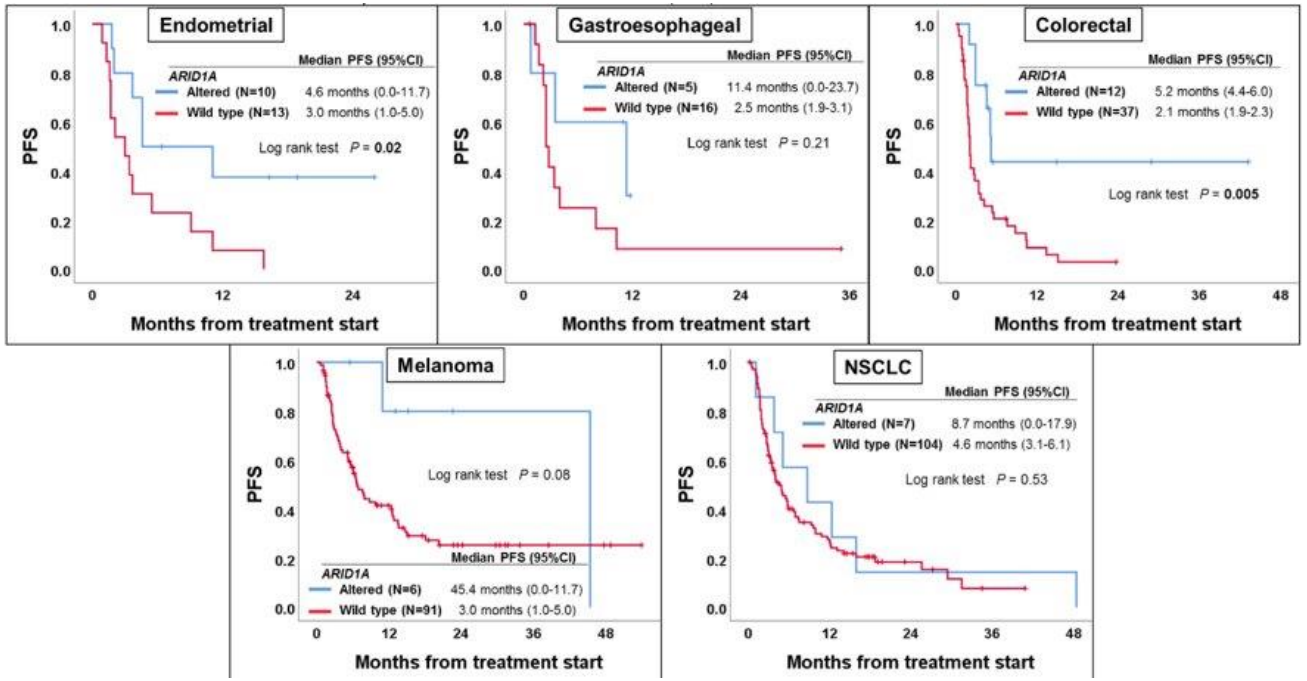


Pan-cancer analysis

All patients treated w/ anti-PD1/PDL1



All MSS patients treated w/ anti-PD1/PDL1



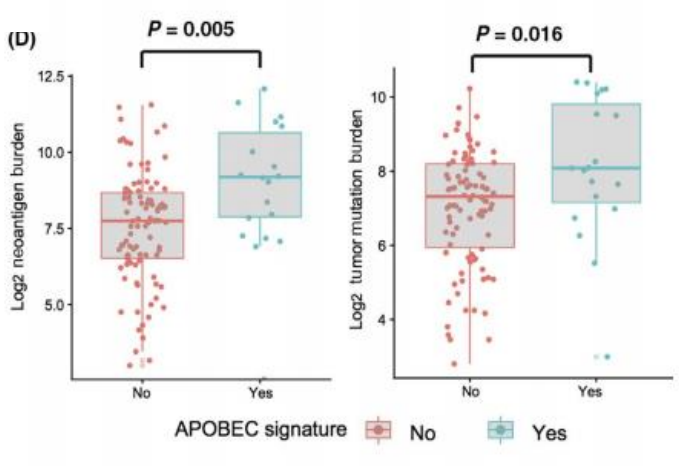
1. Okamura R et al, *J Immunother Cancer* 2020;8:e000438
2. Li L, et al. Cells. 2019;8(7):678
3. Shen J, et al. Nat Med, 556–562 (2018)

Mutational signatures

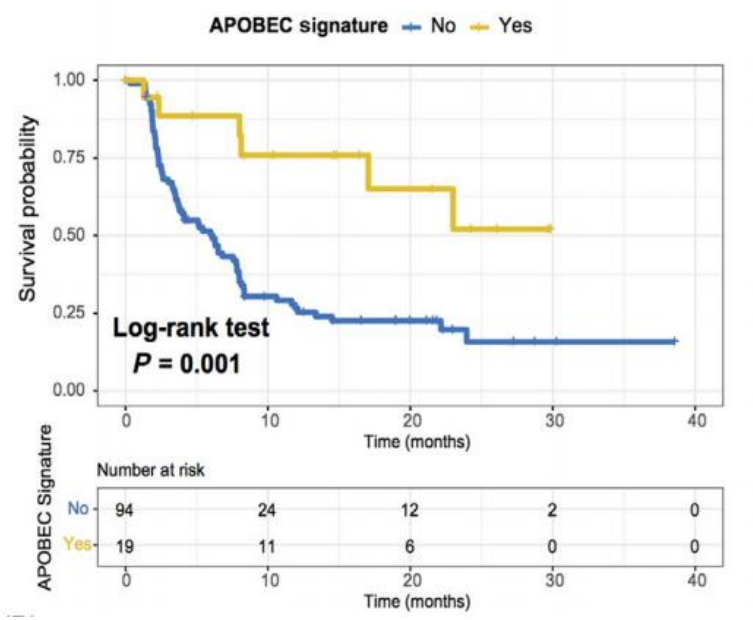
APOBEC mutational signature associated with immunotherapy benefit

B Underlying mutational process	C Relevant genes	D Predisposition syndrome	E Proposed therapy choice
Homologous Recombination Repair Deficiency	*BRCA1, BRCA2, RAD51C, PALB2*	Hereditary Breast and Ovarian Cancer Syndrome	PARP inhibition[32-34], Platinum-based chemotherapy[35-37]
Mismatch Repair Deficiency	*MLH1, MSH2, MSH6, PMS1, PMS2*	Lynch, CMMRD, BMMR-D, HNPCC	PD1-immunotherapy[48-49,52]
Nucleotide Excision Repair Deficiency	*ERCC1, ERCC2, XPC*	Xeroderma Pigmentosum	Cisplatin[63-65]
Base excision Repair Deficiency	*MUTYH,OGG1*	MAP	
	NTHL1, SMUG1	NAP	
Deficient DNA polymerase proofreading activity	*POLE, POLD1*	PPAP	PD1-immunotherapy[48-49,52]
Non-Homologous End Joining Deficiency		Nijmegen Breakage Syndrome	
APOBEC Over-activity	*APOBEC1, APOBEC3A, APOBEC3B*		Tamoxifen Resistance[70,71]

APOBEC signature is associated with increased tumor mutation burden and neoantigen burden



NSCLC



MOLECULAR INSIGHTS IN PATIENT CARE

Durable Complete Response With Immune Checkpoint Inhibitor in Breast Cancer With High Tumor Mutational Burden and APOBEC Signature

Saranya Chumsri, MD[1]; Ethan S. Sokol, PhD[2]; Aixa E. Soyano-Muller, MD[1]; Ricardo D. Parrondo, MD[1]; Gina A. Reynolds, APRN[1]; Aziza Nassar, MD[3]; and E. Aubrey Thompson, PhD[4]

ORR was 68.4% with the APOBEC signature vs 27.6% without

1. Chen H et al, *Cancer Sci*. 2019;110(8):2348-2356
2. Hoeck AV et al, *BMC Cancer* 2019;19:457

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Emerging biomarkers for immunotherapy resistance

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Antigen presentation

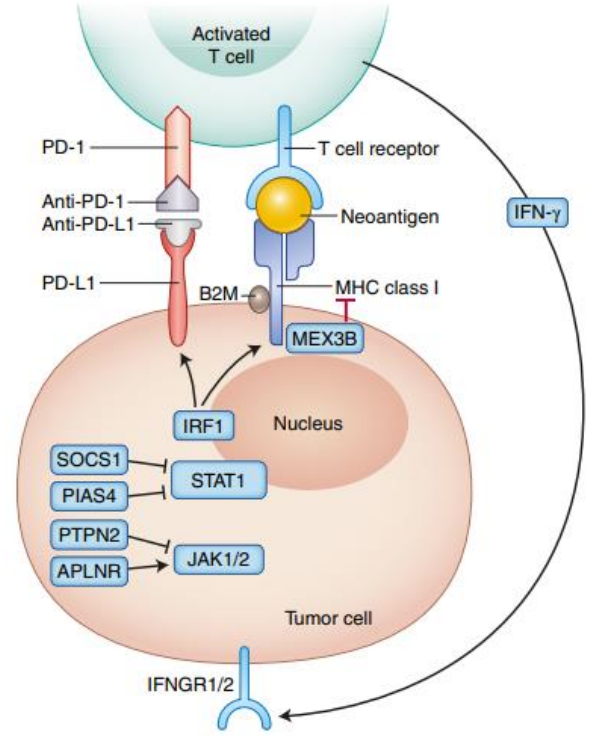
Genomic correlates of response to ICB

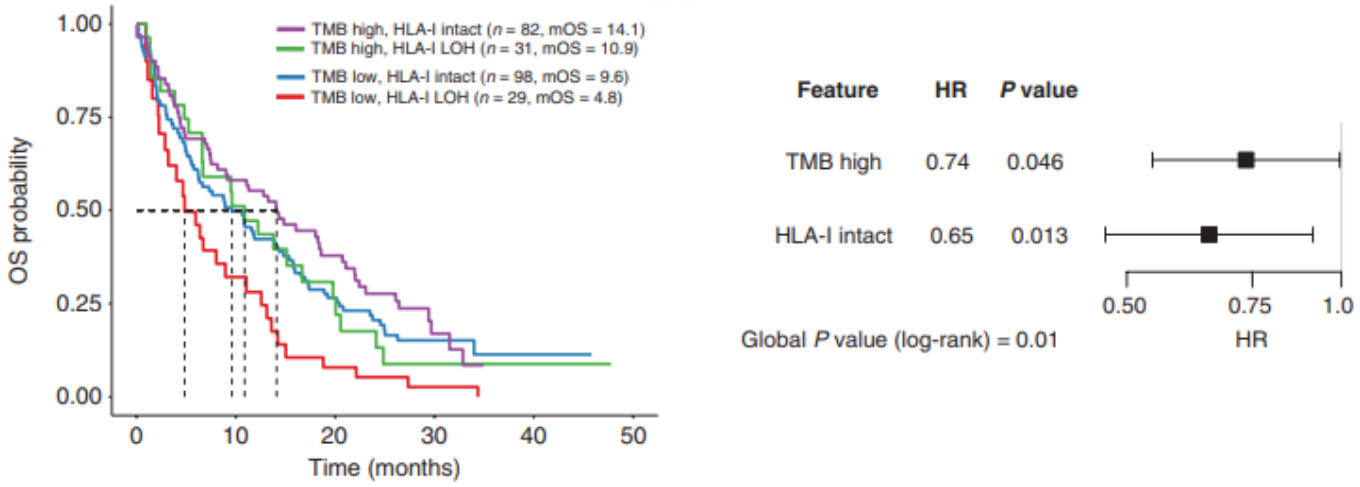
- **HLA pathway alterations:** *B2M* and *HLA-I* genes has been demonstrated to influence ICB response.

- **Interferon-γ pathway alterations**

- **JAK/STAT pathway alterations:** IFN-γ-mediated JAK/STAT signaling contributes to resistance to CTLA-4 blockade.

- **TGF-β pathway:** TGFβ promoted T cell exclusion and a "cold" TME phenotype





1. Keenan TE et al, *Nat Med*. 2019;25(3):389-402
2. Montesion M et al, *Cancer Discov*. 2021;11:282-92

STK11 (LKB1) Mutations Associated with Poor Response to ICI in NSCLC

STK11 (LKB1) mutated tumors tend to be TMB intermediate or high and PD-L1 negative

Analysis of 1,208 patients across 4 cohorts:

- Foundation Medicine dataset (n=924)
- Stand Up To Cancer (SU2C, n=174)
- Checkmate-057 (n=44)
- MD Anderson Cancer Center (n=66)

1

Patients with both *KRAS* and *STK11* mutations were mostly resistant to ICIs in the SU2C cohort:

- *KRAS/STK11* mutant ORR = 7%
- *KRAS/P53* mutant ORR = 36%
- *KRAS* mutant only ORR = 29%

2

STK11 mutations were common in NSCLC

- 16.7% in overall FMI cohort
- 25% in *KRAS*-mutated NSCLC from FMI and combined SU2C cohort

3

STK11 mutations were significantly enriched among tumors that were PD-L1 negative *and* TMB intermediate or high

1. Skoulidis F, et al. *Cancer Discov*. 2018;8(7):822-835

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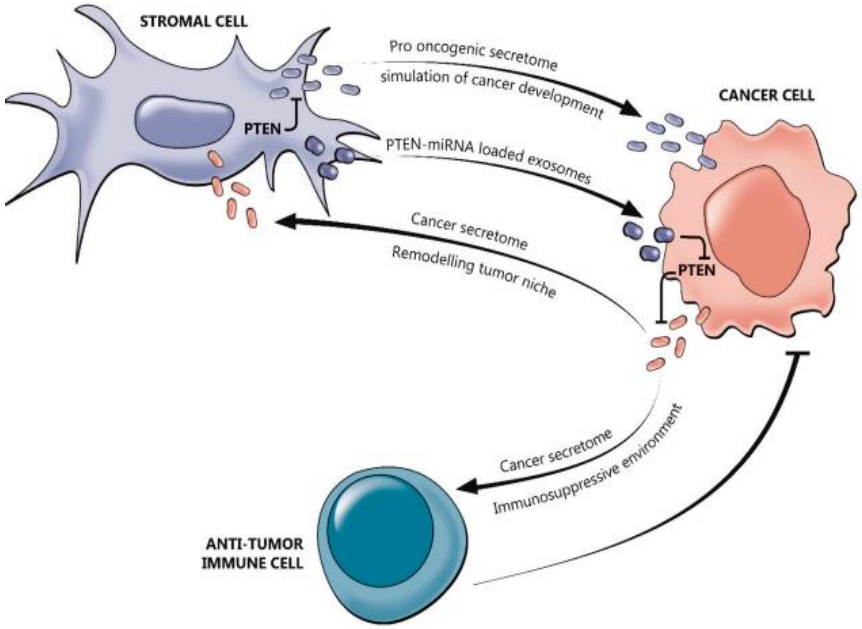
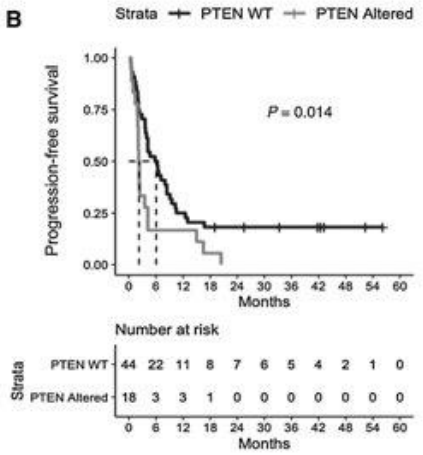
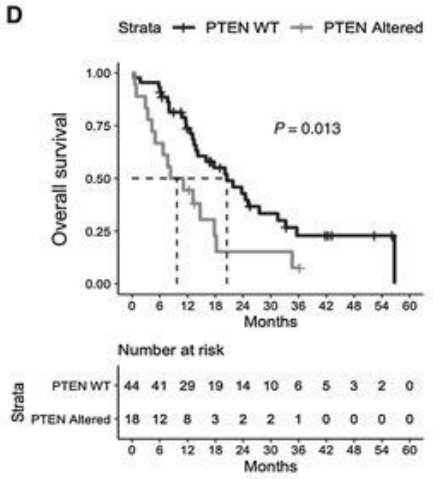
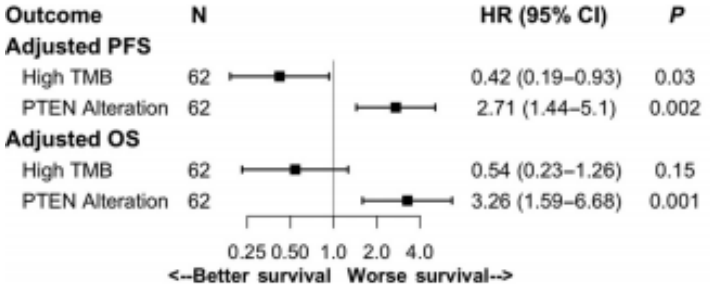
Role of PTEN in the regulation of TME



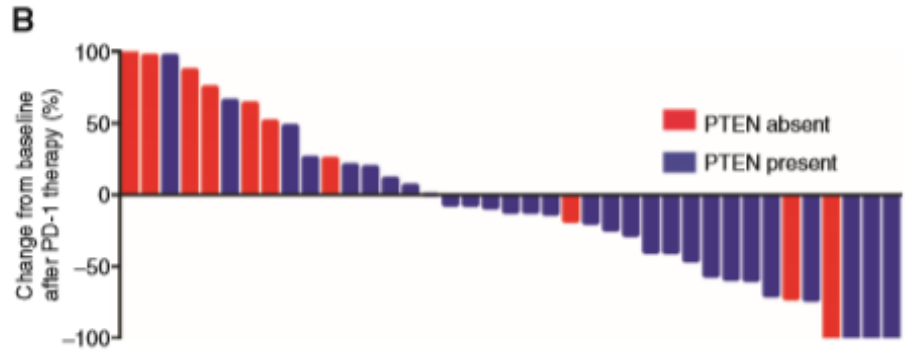
mTNBC

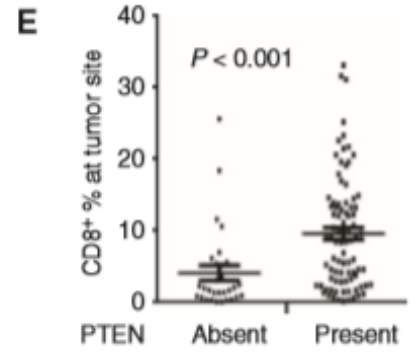






Melanoma





1. Peng et al, *Cancer Discov*. 2016;6(2):202-16
2. Barroso-Sousa R et al, *Clin Cancer Res*. 2020;26:2565-72

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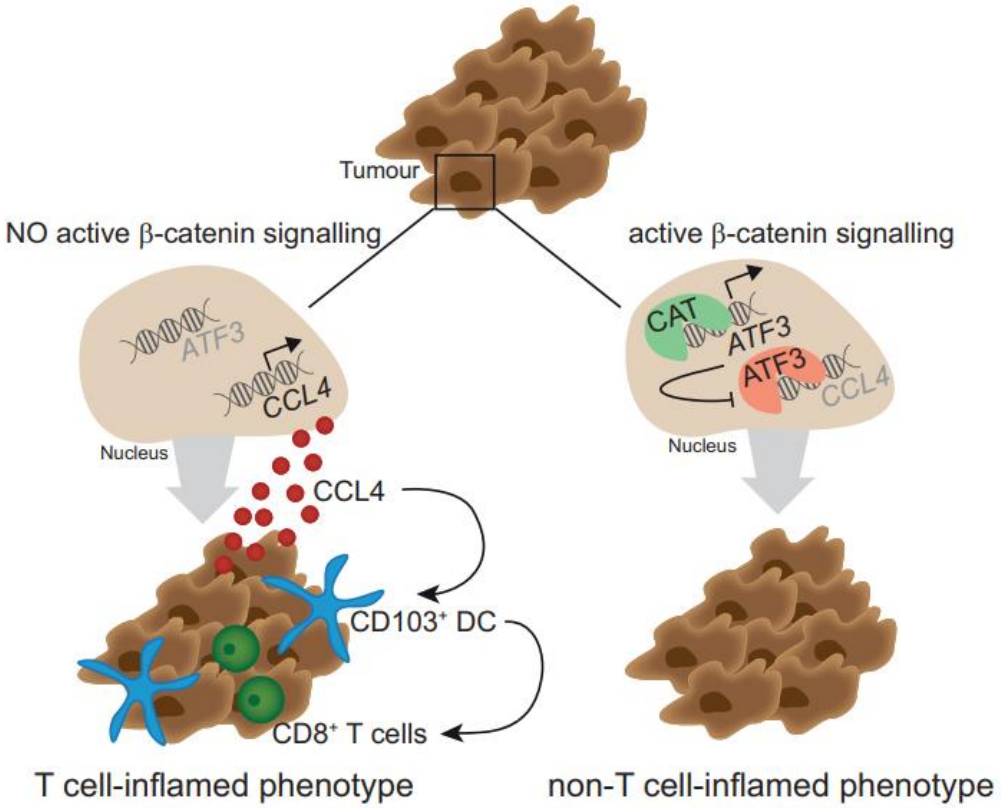
WNT/β-catenin pathway

Activating mutations in CTNNB1 (β-catenin) results in T-cell exclusion and resistance to ICIs



CCR Translations

Clinical Cancer Research

Immune Exclusion-Wnt/CTNNB1 Class Predicts Resistance to Immunotherapies in HCC

Roser Pinyol[1], Daniela Sia[2], and Josep M. Llovet[1,2,3]

Check for updates

Trujillo *et al. Journal for ImmunoTherapy of Cancer* (2019) 7:295
https://doi.org/10.1186/s40425-019-0780-0

Journal for ImmunoTherapy of Cancer

RESEARCH ARTICLE Open Access

Secondary resistance to immunotherapy associated with β-catenin pathway activation or PTEN loss in metastatic melanoma

Check for updates

JCO Precision Oncology > List of Issues > Volume 3 >

CASE REPORT

Acquired *CTNNB1* Mutation Drives Immune Checkpoint Inhibitor–Acquired Resistance in a Microsatellite Instability–High Gastroesophageal Adenocarcinoma With Brain Metastases

Check for updates

Samuel J. Klempner, Alexa B. Schrock, Siraj M. Ali, Charlotte D. Kubicky, and Matthew H. Taylor

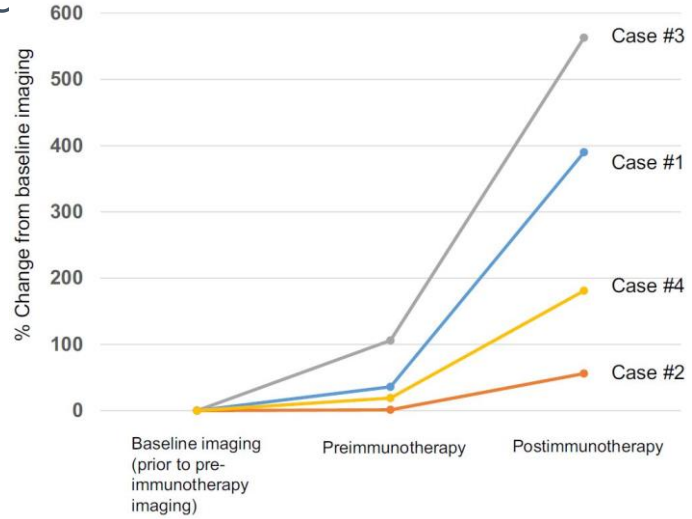
Show More

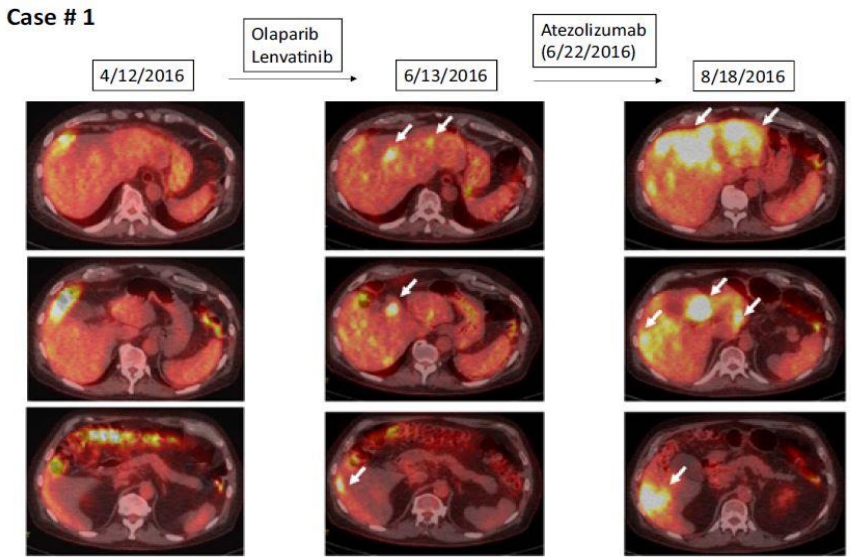
1. Spranger S et al, *Nature*. 2015;523 (7559):231-5

MDM2 Amplification Associated with Hyperprogression

MDM2 amplification has been identified in a wide variety of malignancies

- Study identified 6 patients with *MDM2* amplification and TTF <2 months after ICI treatment
- 4 patients showed marked increases in existing tumor size
- *MDM2* amplification independently correlated with TTF <2 months on multivariate analysis





Case 1: 73-year-old man with metastatic bladder cancer. After ICI therapy: 390% increase in tumor size from baseline, 7.2x increase in progression pace

1. Kato S, et al. *Clin Cancer Res*. 2017;23(15):4242-4250. TTF = time to treatment failure

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Conclusions

- Understanding genomic correlates of response and resistance to checkpoint blockade may enhance benefits for patients with cancer by elucidating biomarkers for patient stratification and resistance mechanisms for therapeutic targeting.

- The differential effects of cancer-related genes and pathways on the immune system can be leveraged for combination therapy with ICB.

- Much work remains to develop these correlates of checkpoint blockade response into reliable biomarkers that can guide treatment decisions and therapeutic development.

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Bladder urothelial (transitional cell) carcinoma

Dr.Sewanti Limaye, MD

Kokilaben Dhirubhai Ambani Hospital and Medical Research Institute, India

  M-TH-00001183

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@Sewanti Limaye

Kokilaben Dhirubhai Ambani
hospital & medical research institute

Every Life Matters

FMI Webinar
Urinary Bladder Case 1

Dr Sewanti Limaye MBBS, MD (NYU), MS (Columbia University) (USA)
Ex-Consultant Dana Farber Cancer Institute/Harvard Med School
Ex Consultant Columbia University Medical Center
Director Precision Oncology
Director Clinical and Translational Oncology Research
Consultant Medical Oncologist
Kokilaben Dhirubhai Ambani Hospital and Medical Research Center, Mumbai

Case History - Urinary Bladder

- 65 y/o lady with prior history of hypertension presented with history of recurrent hematuria of over a period of 3 months to the local center
- A CT scan done at the local center was consistent with a soft tissue mass in the posterior wall of the urinary bladder 4.1cm x 3.1cm in size leading to retrograde gross hydro-uretronephrosis
- A TURBT guided biopsy done two days later was positive for high grade invasive urothelial urothelial carcinoma with invasion into muscularis propria and areas of necrosis. No lympho-vascular invasion seen.
- No neo adjuvant chemotherapy was given at the time
- The patient was taken directly for Robot Assisted Anterior Exoneration with Extracorporeal Pitcher Pot Neobladder with bilateral tube uretero ileal anastomosis and bilateral pelvic lymph node dissection with no residual tumor seen post operatively.
- No adjuvant therapy was given at the time due to a negative PET scan and reluctance to chemotherapy
- Past medical history – Hypothyroidism
- Family history – distant positive family history

Case History - Urinary Bladder

- Around 5 months later the patient presented with chronic cough and a restaging PET scan showed a right lung mass and mediastinal lymphadenopathy which were new. A bronchoscopic biopsy was positive for metastatic urothelial carcinoma and patient was referred to local medical oncology division for chemotherapy

In a case of carcinoma urinary bladder, post neobladder formation on 22/8/2018, PET CT scan findings are suggestive of:

- ***Post neobladder formation surgery status, with absence of any lesion or abnormal metabolism in the postoperative bed. Note is made of bilateral hydroureteronephrosis (right > left).***
- ***Metabolically active soft tissue density right suprahilar mass lesion (~3.7 x 3.8 x 3.3 (CC) cm), inferiorly extending into the right hilar region, partially encasing the right main bronchus, with abrupt cutoff of the anterior segmental bronchus of right upper lobe resulting in distal collapse consolidation with other extensions, as detailed above: Likely malignant in aetiology– Bronchoscopic and histopathological evaluation is advised.***
- ***Metabolically active pleural-based soft tissue density mass lesion (~3.7 x 2.7 cm) in the lateral aspect of posterior segment of right lung upper lobe with likely infiltration in the chest wall and minimal erosion of right 2nd rib laterally : ? Metastatic.***
- ***Metabolically active mediastinal and right hilar lymph nodes : ? metastatic.***
- ***No other metabolically active disease in the regions of body surveyed.***

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Variant Details

DNA Sequence Variants

Gene	Amino Acid Change	Coding	Variant ID	Locus	Allele Frequency	Transcript	Variant Effect
JAK1	p.(=)	c.2199A>G	.	chr1:65310489	55.91%	NM_002227.3	synonymous
JAK1	p.(=)	c.1977C>T	.	chr1:65312342	54.82%	NM_002227.3	synonymous
ALK	p.(I1461V)	c.4381A>G	.	chr2:29416572	99.78%	NM_004304.4	missense (Benign)
IDH1	p.(=)	c.315C>T	.	chr2:209113192	50.93%	NM_005896.3	synonymous
FGFR3	p.(=)	c.1953G>A	.	chr4:1807894	99.74%	NM_000142.4	synonymous
PDGFRA	p.(=)	c.1701A>G	.	chr4:55141055	99.84%	NM_006206.5	synonymous
KIT	p.(=)	c.1638A>G	.	chr4:55593481	49.76%	NM_000222.2	synonymous
FGFR4	p.(P136L)	c.407C>T	.	chr5:176517797	63.41%	NM_213647.2	missense (Benign)
FGFR4	p.(=)	c.483A>G	.	chr5:176517985	34.34%	NM_213647.2	synonymous
EGFR	p.(=)	c.2361G>A	.	chr7:55249063	41.71%	NM_005228.4	synonymous
MET	p.(N375S)	c.1124A>G	.	chr7:116340262	47.65%	NM_001127500.2	missense (benign)
RET	p.(=)	c.2307G>T	.	chr10:43613843	100.00%	NM_020975.4	synonymous
JAK3	p.(P664T)	c.1990C>A	.	chr19:17945949	47.06%	NM_000215.3	missense (VUS)

RESULTS

VARIANT OF UNCERTAIN SIGNIFICANCE RELATED TO THE GIVEN PHENOTYPE WAS DETECTED

Gene (Transcript) #	Location	Variant	Zygosity	Disease (REF)	Inheritance	Classification
CHEK2 **(-)** (ENST00000382580.2)	Exon 14	c.1535T>C **(p.Val512Ala)**	Heterozygous	Increased risk of urinary bladder cancer [19-22]	–	Uncertain Significance

Somatic Oncomine focus
Germline Testing
PD-L1 by Ventana SP263

MARKER:	RESULT:
ANTI PD-L1 (CE LABELLED)	NO STAINING SEEN IN TUMOR CELLS.
	1% OF IMMUNE CELLS STAIN WITH PDL1.

COMMENTS

1. PD-L1 TESTING DONE BY VENTANA PD-L1 (SP263) ASSAY USING RABBIT ANTI-HUMAN

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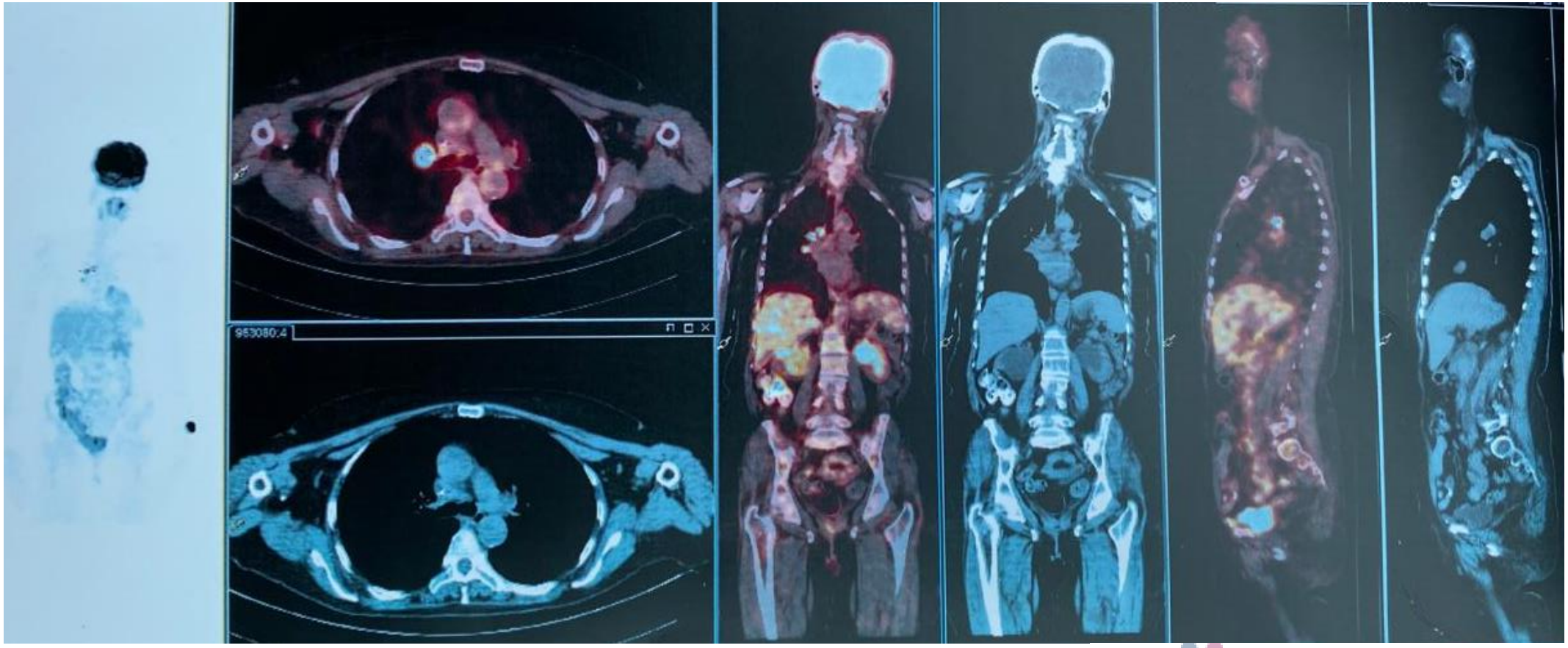
Case History - Urinary Bladder

- Received Gemcitabine and Cisplatin x 6 cycles
- Progressed on the regimen after initial response

Case History - Urinary Bladder



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Case History - Urinary Bladder

- Saw me in second opinion at this time
- Started on Nivolumab
- Progressed on IO after 7 months
- Shifted to Carboplatin Nab Paclitaxel
- Stopped due to toxicity
- Foundation one was sent

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Biomarker Findings

Tumor Mutational Burden - 14 Muts/Mb
Microsatellite status - MS-Stable

Genomic Findings

For a complete list of the genes assayed, please refer to the Appendix.

CCND1 amplification
MDM4 amplification - equivocal†
MYC amplification - equivocal†
ASXL1 G646fs*12
ATRX K1936fs*5
CREBBP S1304*
FGF19 amplification
FGF3 amplification
FGF4 amplification
IKBKE amplification - equivocal†
LYN amplification - equivocal†
PIK3C2B amplification - equivocal†
SPEN V1129I

2 Disease relevant genes with no reportable alterations: ***FGFR2, FGFR3***

PD-L1 IMMUNOHISTOCHEMISTRY (IHC) ANALYSIS (Dako 22C3 pharmDx™)
Combined Positive Score (CPS) 1

BIOMARKER FINDINGS	THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)	THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)
Tumor Mutational Burden - 14 Muts/Mb	Avelumab 1	Cemiplimab
	Pembrolizumab 1	Dostarlimab
	Atezolizumab 2A	Durvalumab
10 Trials *see p. 15*	Nivolumab 2A	Nivolumab + Ipilimumab
Microsatellite status - MS-Stable	**No therapies or clinical trials.** see Biomarker Findings section	
GENOMIC FINDINGS	**THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)**	**THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)**
***CCND1* -** amplification	none	none
7 Trials *see p. 17*		
***MDM4* -** amplification - equivocal	none	none
1 Trial *see p. 19*		
***MYC* -** amplification - equivocal	none	none
5 Trials *see p. 20*		

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Poll questions

- **What therapy should we consider next?**

  - ❖ IO+IO – Nivolumab + Ipilumumab
  - ❖ Enfortumab Vedotin
  - ❖ Targeted therapy
  - ❖ Single agent Paclitaxel
  - ❖ Clinical trials

@Sewanti Limaye

Genomic findings

FOUNDATIONONE® CDx

Biomarker Findings

Tumor Mutational Burden - 14 Muts/Mb
Microsatellite status - MS-Stable

Genomic Findings

For a complete list of the genes assayed, please refer to the Appendix.

CCND1 amplification
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FGF19 amplification
FGF3 amplification
FGF4 amplification
IKBKE amplification - equivocal†
LYN amplification - equivocal†
PIK3C2B amplification - equivocal†
SPEN V1129I

2 Disease relevant genes with no reportable alterations: *FGFR2, FGFR3*

† See About the Test in appendix for details.

FOUNDATION MEDICINE

Poll question 2

Which of the below molecular factors are considered as emerging biomarkers of checkpoint blockade response?

a) Copy number alterations

b) Chromatin remodeling

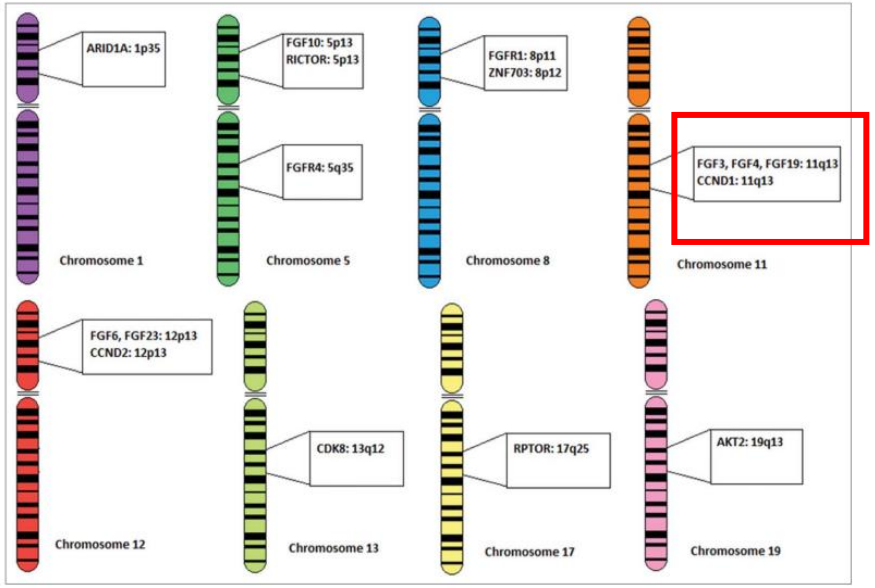
c) T-cell functionality

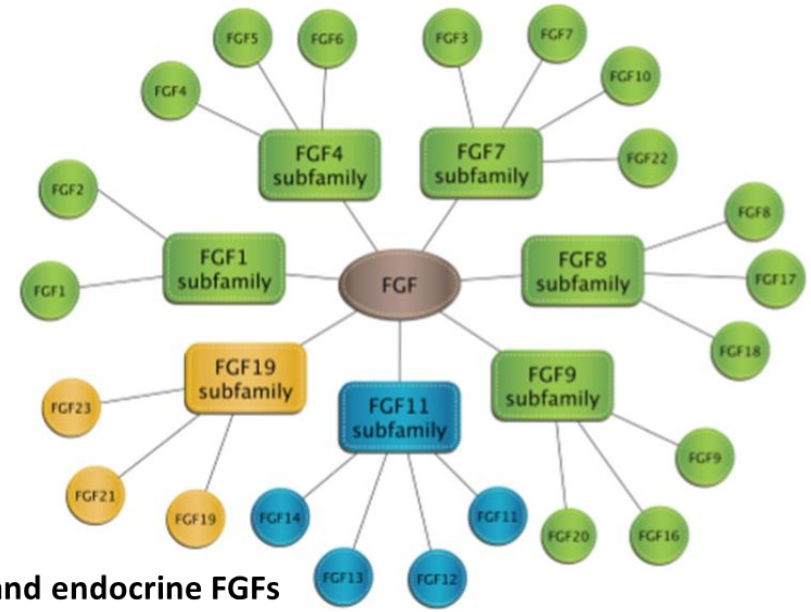
d) All the above

FOUNDATION MEDICINE

11q13 Amplicon

Relationship between FGF ligands and FGF receptors





Receptor specificity of canonical and endocrine FGFs

FGF subfamily	FGF	Cofactor	Receptor specificity
FGF1 subfamily	FGF1 FGF2		All FGFRs FGFR 1c, 3c > 2c, 1b, 4Δ
FGF4 subfamily	FGF4 FGF5 FGF6		FGFR 1c, 2c > 3c, 4Δ
FGF7 subfamily	FGF3 FGF7 FGF10 FGF22	+ Heparin or Heparan sulfate	FGFR 2b > 1b
FGF8 subfamily	FGF8 FGF17 FGF18		FGFR 3c > 4Δ > 2c > 1c >> 3b
FGF9 subfamily	FGF9 FGF16 FGF20		FGFR 3c > 2c > 1c, 3b >> 4Δ
FGF15/19 subfamily	FGF15/19 FGF21 FGF23	+βKlotho +αKlotho	FGFR 1c, 2c, 3c, 4Δ FGFR 1c, 3c FGFR 1c, 3c, 4

1. Parish A et al, *Cell Cycle*. 2015;14(13):2121-2128
2. Dolegowska K et al, *J Physiol Biochem*. 2019;75(2): 229-240
3. Ornitz DM and Itoh N, *WIREs Dev Biol.* 2015;4:215-266

FOUNDATION MEDICINE

11q13 Amplicon

Response to immunotherapy

Journal of Clinical Oncology > List of Issues > Volume 37, Issue 15_suppl >

GASTROINTESTINAL (NONCOLORECTAL) CANCER

Association of frequent amplification of chromosome 11q13 in esophageal squamous cell cancer with clinical benefit to immune check point blockade.

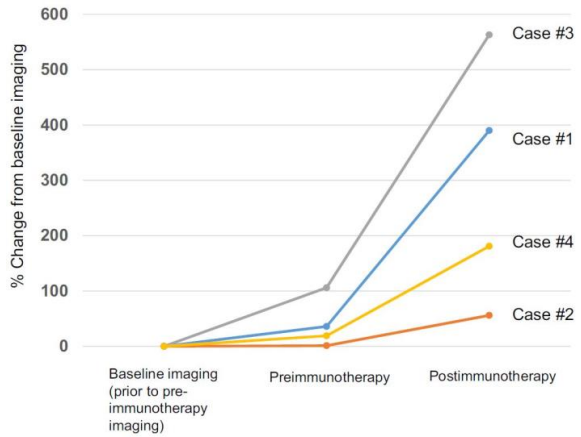
- Patients included in this analysis were part of multi-center, phase Ib/II trial (NCT02915432) evaluating the safety and activity of toripalimab
- Copy number analysis identified 24 out of 50 (48%) patients with amplifications of chromosome 11q13 region
- Patients without 11q13 amplification, had significantly better objective response rate (ORR 30.8% versus 4.2%) and progression free survival (3.7 versus 2.0 months)

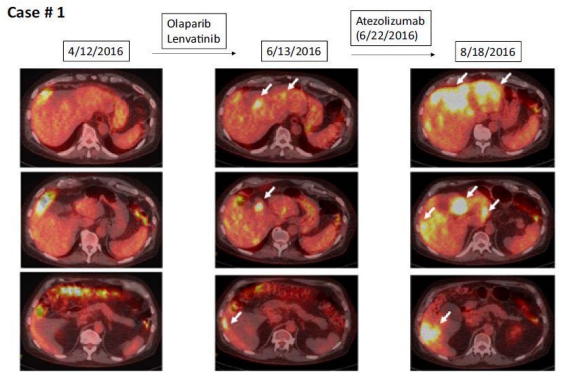
1. DOI: 10.1200/JCO.2019.37.15_suppl.4036 *Journal of Clinical Oncology* 37, no. 15_suppl (May 20, 2019) 4036-4036

FOUNDATION MEDICINE

Hyperprogressive disease

Predictors of HPD:
- *MDM2/4* amplification
- *EGFR* alteration





Case 1: 73-year-old man with metastatic bladder cancer. After ICI therapy: 390% increase in tumor size from baseline, 7.2x increase in progression pace

Journal of Clinical Oncology > List of Issues > Volume 38, Issue 15_suppl >

DEVELOPMENTAL THERAPEUTICS—IMMUNOTHERAPY

The landscape of chromosome 11q13 amplification in Chinese solid tumor patients and hyperprogressive disease (HPD) clinical example.

1140PD **Predictive biomarkers for hyper-progression (HP) in response to immune checkpoint inhibitors (ICI) – analysis of somatic alterations (SAs)**

A.K. Singavi[1], S. Menon[1], D. Kilari[1], A. Alqwasmi[1], P.S. Ritch[1], J.P. Thomas[1], A.L. Martin[1], C. Oxencis[1], S. Ali[2], B. George[1]

[1]*Hematology and Oncology, Medical College of Wisconsin, Milwaukee, WI, USA,*
[2]*Clinical Development, Foundation Medicine, Cambridge, MA, USA*

Table: 1140PD

Age - Sex	Disease	# Prior lines of chemotherapy	ICI	Time to HP (months)	NGS
65 - Male (M)	NSCLC	2	Nivolumab (N)	2	CCDN1, CDK4, FGF19, FGF4, MDM2, FGF3, FRS2
68 - M	Esophageal Adeno Ca	1	Pembrolizumab (P)	2	CCND1, EGFR, FGFR19, FGF3, FGF4,
77 - M	Esophageal SCC	3	P	3	EPHA3, MDM4, CHEK2, EP300, NOTCH1, NOTCH3, SPOP, TP53
59 - M	Lung Ca (neuroendocrine features)	1	N	2	CCND1, FGF19, FGF3, FGF4, KRAS, NFE2L2, TP53
58 F	Renal Cell Ca	2	N	1	NA

1. Kato S, et al. *Clin Cancer Res*. 2017;23(15):4242-4250. TTF = time to treatment failure
2. DOI: 10.1200/JCO.2020.38.15_suppl.e15222 *Journal of Clinical Oncology* 38, no. 15_suppl (May 25, 2020)

FOUNDATION MEDICINE

Head and neck adenoid cystic carcinoma

M-TH-00001148

FOUNDATION MEDICINE

Adenoid cystic carcinoma

Prof. Mutlu Demiray, M.D.

Medicana International Istanbul Hospital, Turkey

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Case history

- Our patient 48 years old women, Adenoid cystic carcinoma diagnosis was made in 2012 form left eye lacrimal gland origin. She was operated and then adjuvant radiotherapy was administered.
- In 2016, She felt swelling in the left eye again. Imaging studies showed that relapse. Enucleation with wide surgical margin was done due to eye invasion. Local relapse detected again in 2018. Left maxillary resection and adjuvant chemoradiotherapy (with cisplatin) was done. After chemoradiotherapy single agent cisplatin was continued four more cycles.
- Imaging studies performed 3 months after completion of chemotherapy showed multiple lung metastases and local recurrence.
- Combination chemotherapy was suggested, but she refused all chemo options.
- Her daughter is doctor (chest surgery specialist), she admitted to our clinic for second opinion.
- I sugested to her FMI CDx test

FOUNDATION MEDICINE

Poll question 1

How should the treatment be?

a) Chemotherapy

b) Lenvatinib

c) Immunotherapy

d) Local treatment options (radiofrequency ablation and/or stereotactic irradiation)

FOUNDATION MEDICINE

Genomic findings

Biomarker Findings

Microsatellite status - MS-Stable

Tumor Mutational Burden - TMB-Low (4 Muts/Mb)

Genomic Findings

For a complete list of the genes assayed, please refer to the Appendix.

NOTCH1 D2442fs*35

CTCF splice site 223+1G>A

KDM6A P1107fs*13

PD-L1 IMMUNOHISTOCHEMISTRY (IHC) ANALYSIS (Dako 22C3 pharmDx™)

Tumor Proportion Score (TPS) (%) 0

FOUNDATION MEDICINE

Poll question 2

Which of the below statement is incorrect?

a) The foundation of anti-tumor immunity rets on the generation or reactivation of cytotoxic T-cell responses

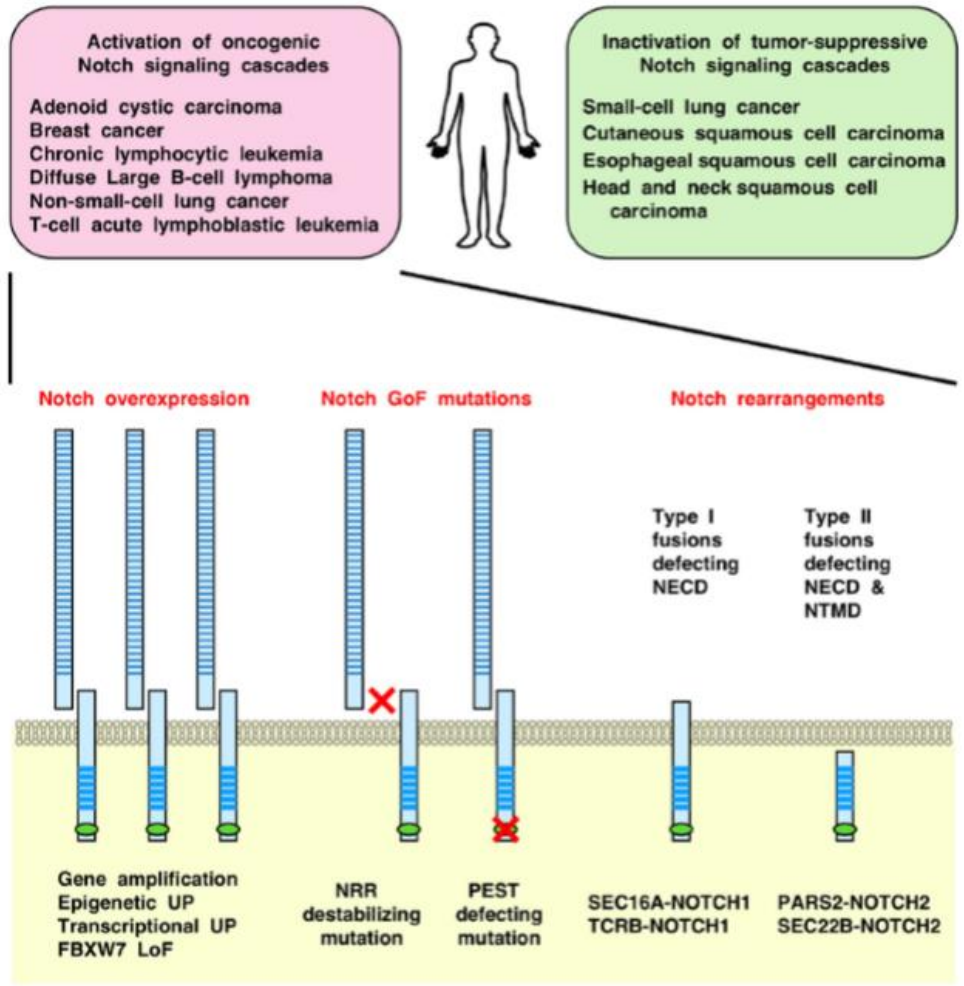
b) All neoantigens generate immunogenic peptides that can be recognized by T-cells

c) Canonical cancer pathways implicated in response and resistance to ICB

FOUNDATION MEDICINE

Notch signaling

Notch alterations have been identified in a wide variety of malignancies



1. Katoh M and Katoh M, *Int J Mol Med*. 2020;45(2):279-297

FOUNDATION MEDICINE

Effects of Notch signaling on the immune system

Response to immunotherapy

Notch pathway member	Cytokine	Main functions	Cancer type	Immune mediators	Reference
Notch1	TGFβ	Immunosuppression, anti-inflammatory, epithelial-to-mesenchymal transition, angiogenesis	–	DC, Treg	(7, 8)
Dll4	TGFβ	as above	Lung carcinoma	MDSC	(9)
Notch3, Jagged1	IL-6	as above	Breast cancer	MDSC	(10–12)
Unknown	CXCL12	Migration, proliferation, angiogenesis	Multiple myeloma	M2	(13, 14)
Unknown	CXCL12	as above	Ovarian cancer	T lymphocyte	(15, 16)
Unknown	CXCL12	as above	Hepatocellular carcinoma	Treg, M2	(6, 17)
Dll family, Jagged1/2	IL-10	as above	–	Th1	(18)
Unknown	IL-10	as above	Melanoma, lung carcinoma	TAM	(19–21)
Dll family	IL-10	Immunosuppression, anti-inflammatory	–	DC, Th1	(22, 23)
Jagged1/2, Notch1	IL-4	as above	–	Th2, DC	(24–26)
Dll4	IL-4	Immunosuppression	–	TAM	(27)
Dll4	IL-17	as above	–	γδT cell	(28)
Unknown	IL-17	as above	Oral cancer	CD4+ T, Th17	(29)
Notch1, Jagged2	CCL5	Proliferation, invasion, metastasis	Breast cancer	TAM M2	(30)
Jagged1	IL-1β, CCL2	Pro-inflammatory, proliferation	Breast cancer	TAM	(31, 32)
Notch1	CCL2	Proliferation	Lung carcinoma	Mo-MDSC macrophage	(33)
Jagged1	IFN-γ	Killing immunological functions	–	DC, T cell	(34)
Jagged2	IFN-γ	as above	Lymphoma	NK	(35)
Notch1, Notch2	IFN-γ	as above	–	CD4+ T, CD4+ Th1, CD8+ T	(36–38)
Dll1	VEGF	Angiogenesis, immunosuppression	Lung carcinoma	T cell	(39)

1. Colombo M et al, *Front Immunol*. 2018;9:1823

FOUNDATION MEDICINE

Functional consequence matters

Response to immunotherapy

Cancer Letters 434 (2018) 144–151

Contents lists available at ScienceDirect

Cancer Letters

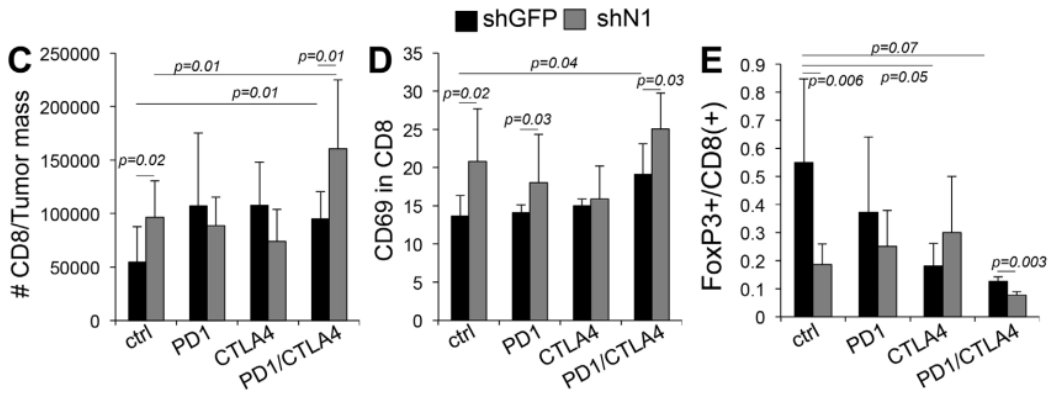
journal homepage: www.elsevier.com/locate/canlet

Original Articles

Inhibiting Notch1 enhances immunotherapy efficacy in melanoma by preventing Notch1 dependent immune suppressive properties

Hong Qiu[a], Patrick M. Zmina[c], Alex Y. Huang[b], David Askew[b], Barbara Bedogni[a,c,*]

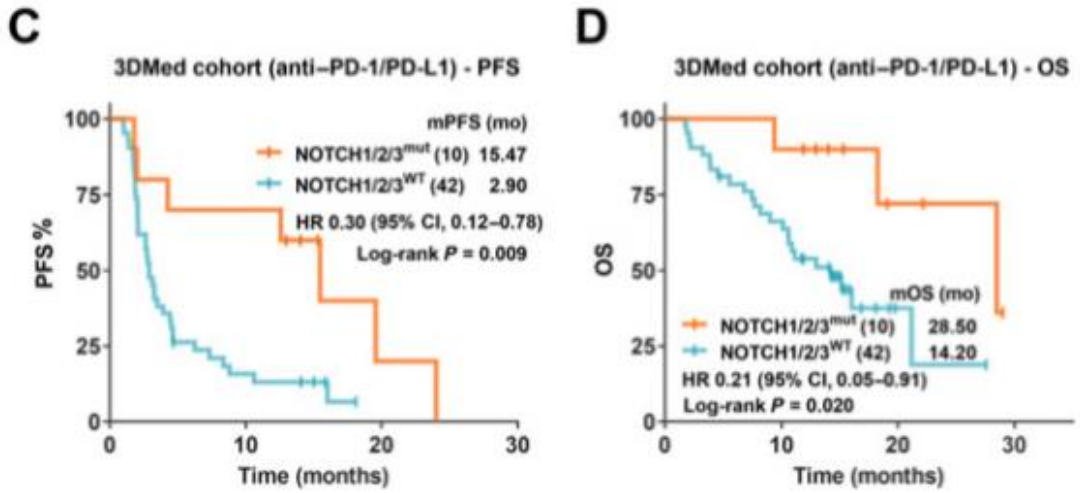
[a] Department of Biochemistry, Case Western Reserve University, Cleveland, OH 44106, United States
[b] Department of Pediatrics, Case Western Reserve University, Cleveland, OH 44106, United States
[c] Department of Dermatology, Miller School of Medicine, Miami, FL 33136, United States



CLINICAL CANCER RESEARCH | PRECISION MEDICINE AND IMAGING

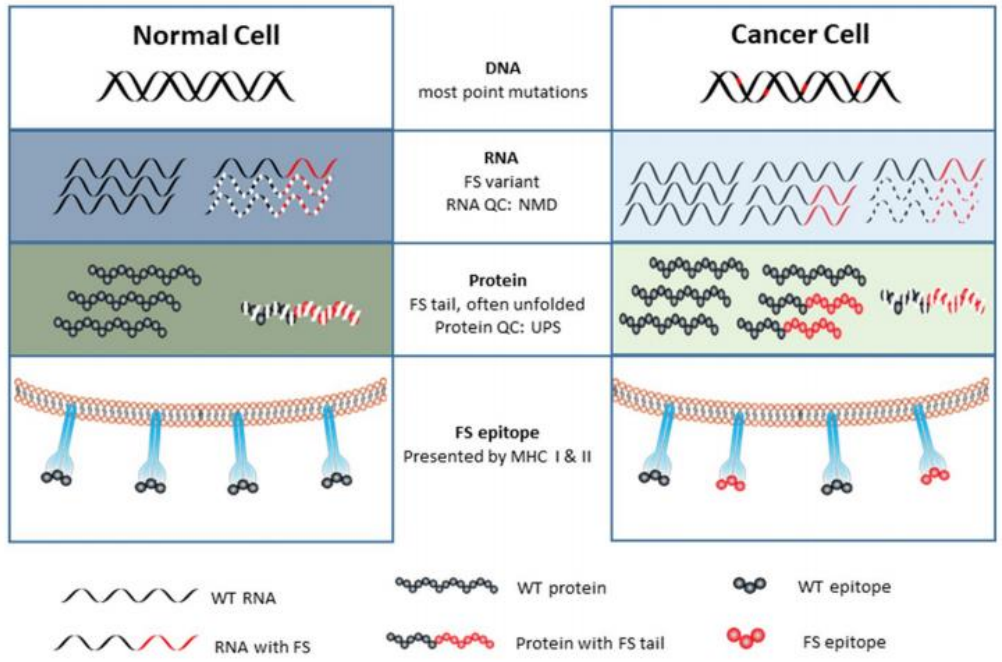
Identification of Deleterious *NOTCH* Mutation as Novel Predictor to Efficacious Immunotherapy in NSCLC

Kai Zhang[1], Xiaohua Hong[1], Zhengbo Song[2], Yu Xu[3], Chengcheng Li[3], Guoqiang Wang[3], Yuzi Zhang[3], Xiaochen Zhao[3], Zhengyi Zhao[3], Jing Zhao[3], Mengli Huang[3], Depei Huang[3], Chuang Qi[3], Chan Gao[3], Shangli Cai[3], Feifei Gu[1], Yue Hu[1], Chunwei Xu[4], Wenxian Wang[5], Zhenkun Lou[6], Yong Zhang[7], and Li Liu[1]



FOUNDATION MEDICINE

Frameshift indels generate highly immunogenic tumor neoantigens



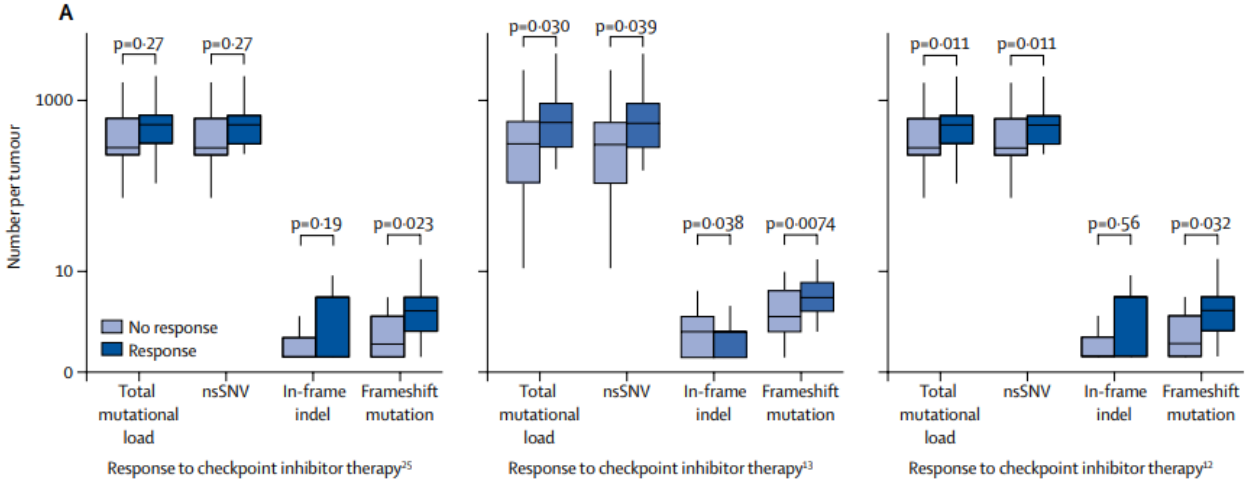
Frameshift indels could generate around three times more high-affinity neoantigen binders than SNV

	Mutations (n)	Neoantigens (n)*	Mutant-specific neoantigens (n)†	Neoantigens per mutation	Mutant-specific neoantigens per mutation
nsSNVs	335 594	214 882	75 224	0·64	0·22
fs-indels	19 849	39 768	39 608	2·00	2·00
Enrichment	..	..	..	3·13	8·94

nsSNVs=non-synonymous single nucleotide variants. fs-indels=frameshift insertions and deletions. *Strong binders (<50 nM affinity). †Wild-type allele non-strong binding (>50 nM affinity).

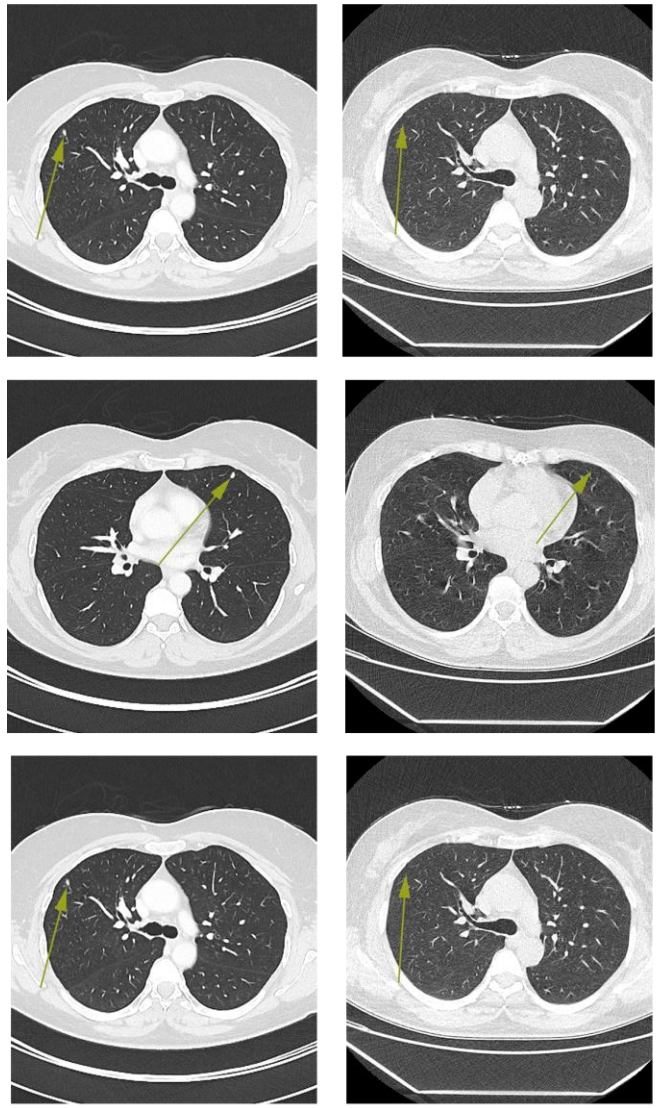
Table: **Neoantigens per variant class**

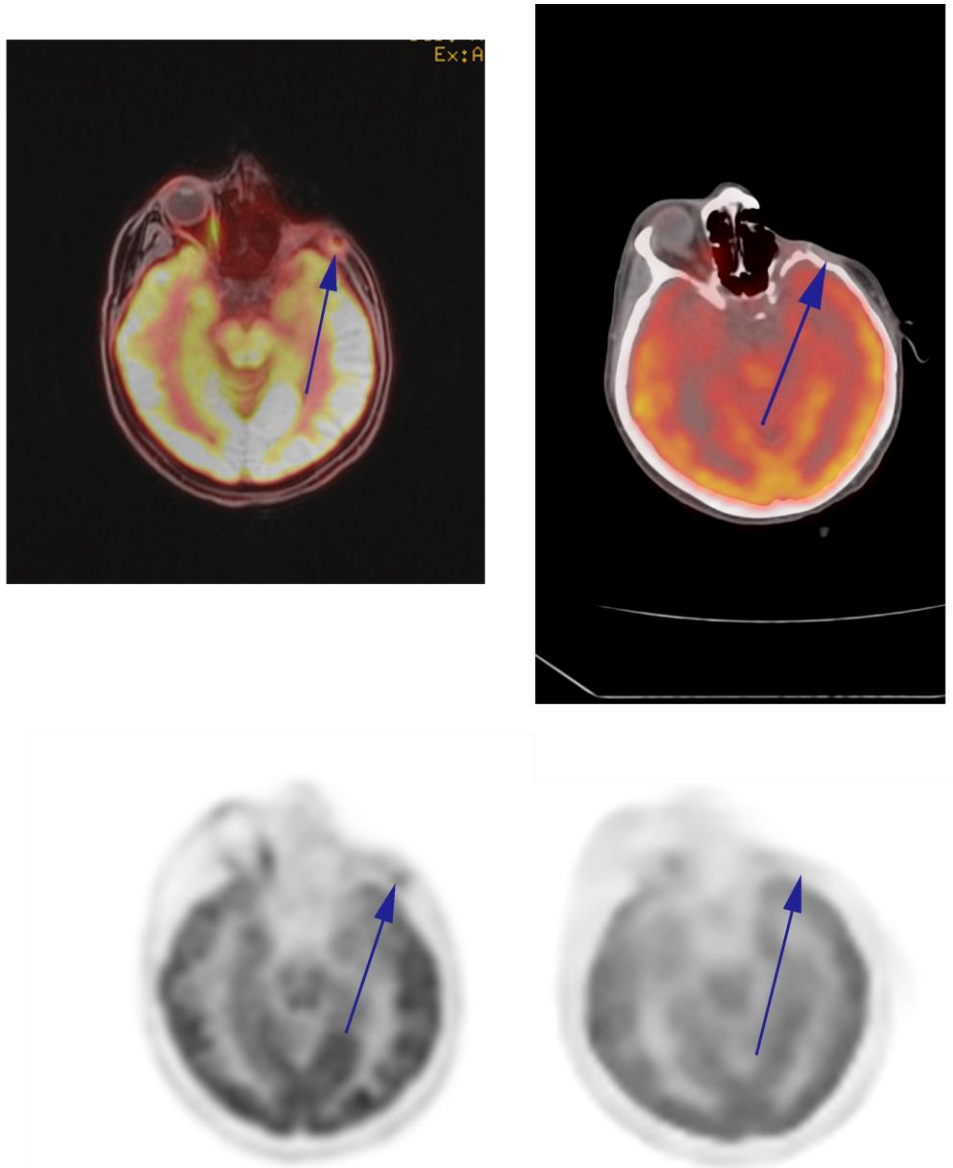
Frameshift indel mutations were significantly associated with anti-PD-1 response



1. Shen L et al, *Sci Rep*. 2019;9(1):14184
2. Turajlic S et al, *Lancet Oncol*. 2017;18:1009-21

Patient’s outcome





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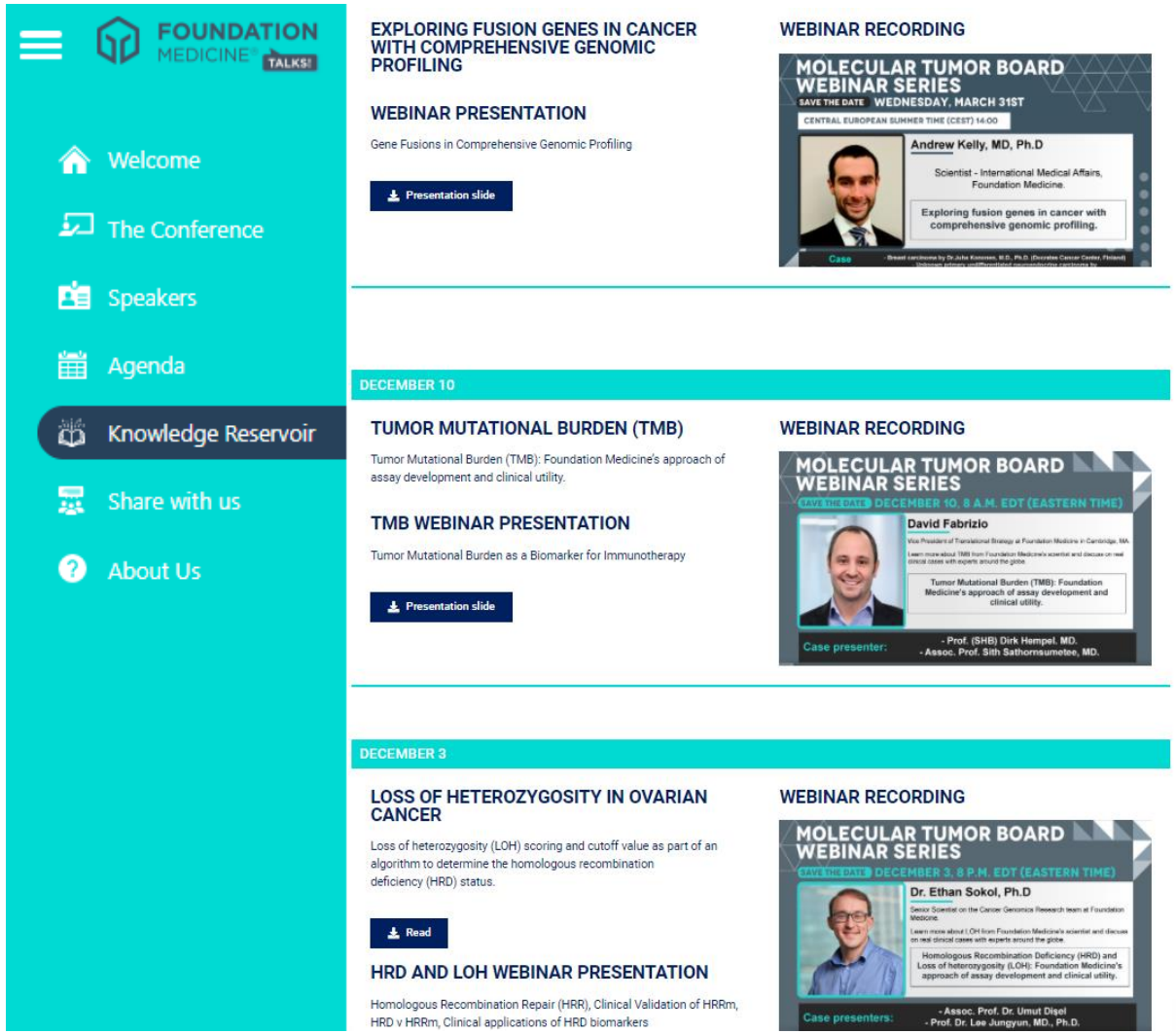
Playback VDOs and presentation slides



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