Biomarkers in mRCC: too hot, too cold, or just right?

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Canadian Kidney Cancer Forum, April 12th 2019

McMaster University HEALTH SCIENCES

Juravinski Cancer Centre HAMILTON HEALTH SCIENCES

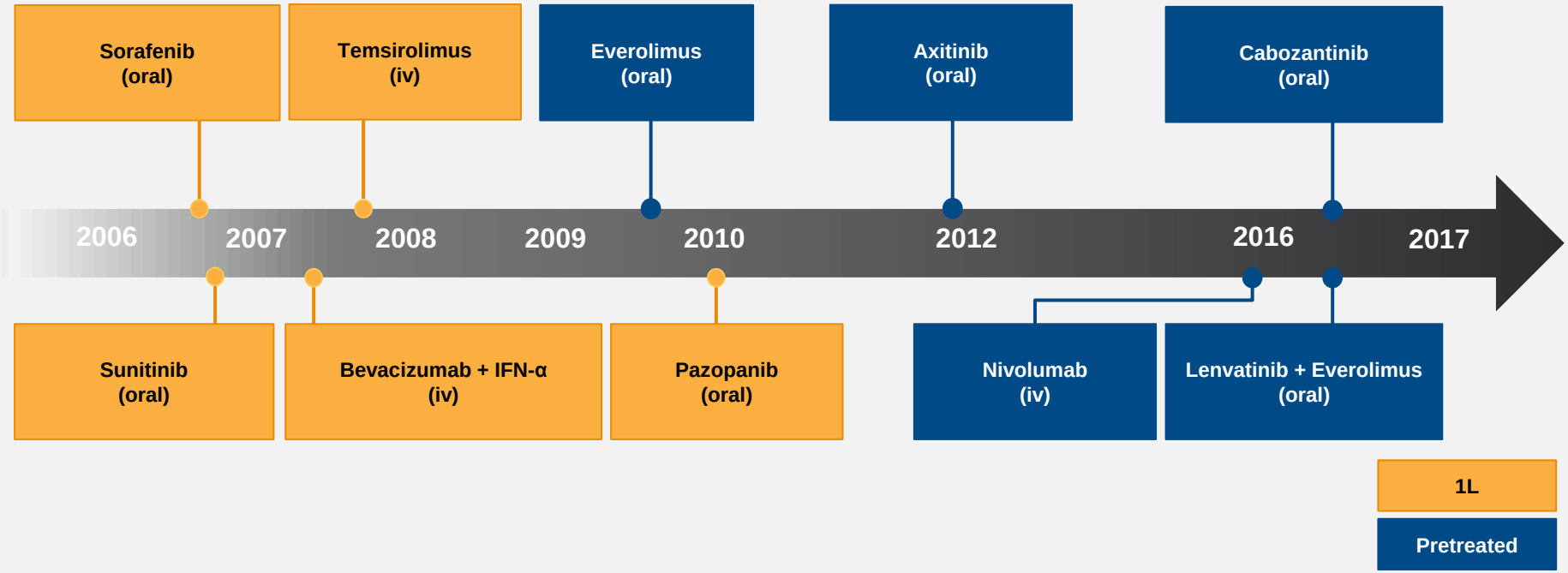




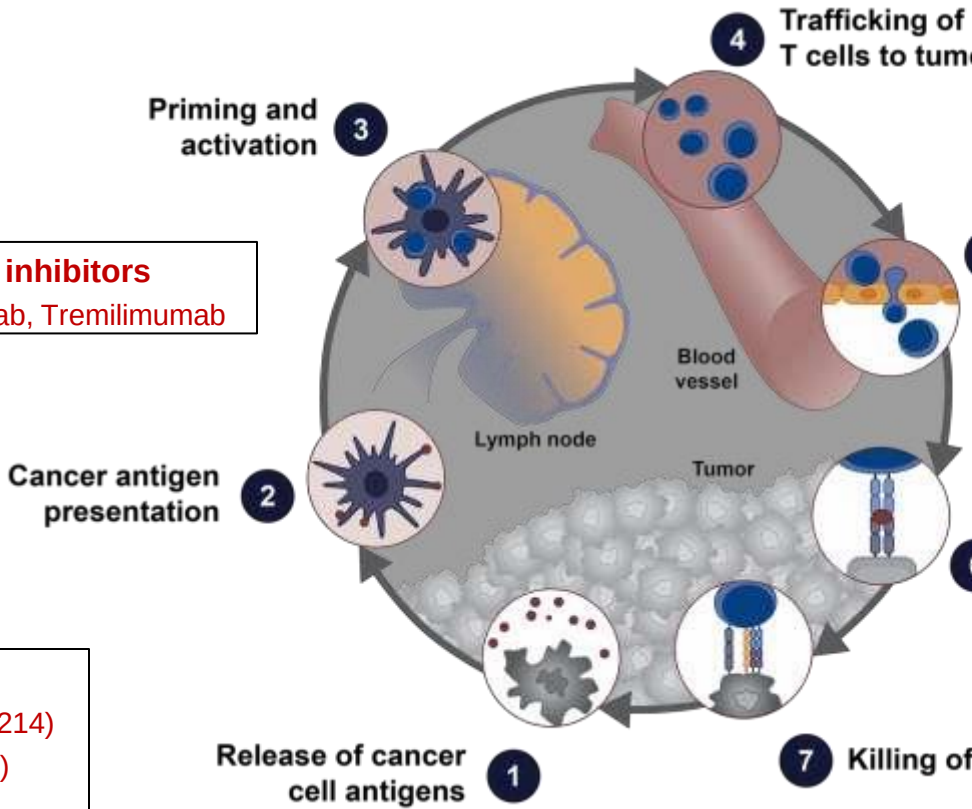

Disclosures

- Honoraria/consulting:
  - BMS, Eisai, Ipsen, Janssen, Merck, Pfizer, Roche, TerSera

Timeline circa 2016



Synergistic strategies with immunotherapy



VEGF- and VEGF-R directed
Axitinib, Cabozantinib, Lenvatinib
Bevacizumab

CTLA-4 inhibitors
Ipilimumab, Tremilimumab

PD-1/PDL-1 inhibitors
Nivolumab, Pembrolizumab
Atezolizumab, Avelumab, Durvalumab

Other
CD-122 (NKTR-214)
TLR (NKTR-262)
PEG IL-10

1. Chen DS, Mellman I. *Immunity*. 2013;39:1-10.

Combination strategies with IO backbone

	I-O + TKI			I-O + I-O	I-O + VEGF mAb	PD-1/PD-L1 monothreapy		TKI monotherapy
TRIAL	**Pembrolizumab + Axitinib[1]**	**Pembrolizumab + Lenvatinib[2]**	**Avelumab + Axitinib[3]**	**Nivolumab + Ipilimumab[4,5]**	**Atezolizumab + Bevacizumab[6]**	***Pembrolizumab[7]***	***Atezolizumab[8]***	***Sunitinib[6]***
Phase	III	IB/II	III	III	III	II	II	III
N	432	30	442	425 *(Int/Poor risk)*	454	110	103	461
Prior therapy?	No	Yes	No	No	No	No	No	No
ORR	**59% (OS HR 0.53)**	**63%**	**51%**	**42% (OS HR 0.66)**	**37%**	**38%**	**25%**	**33%**

[1]Rini et al. *N Engl J Med* 2019; [2]NCT02501096, Lee et al. ESMO 2017; [3]Motzer et al. *N Engl J Med* 2019; [4,5]NCT02231749 Escudier et al. ESMO 2017, Motzer et al. SITC 2017; [6]NCT01984242 Motzer et al. ASCO GU 2018; [7] NCT02853344 McDermott et al, ASCO 2018; [8]NCT01984242 Atkins et al, ASCO 2017.

Courtesy: AK Lalani, Z. Bakouny, T Choueiri

SOME UNANSWERED CLINICAL QUESTIONS…

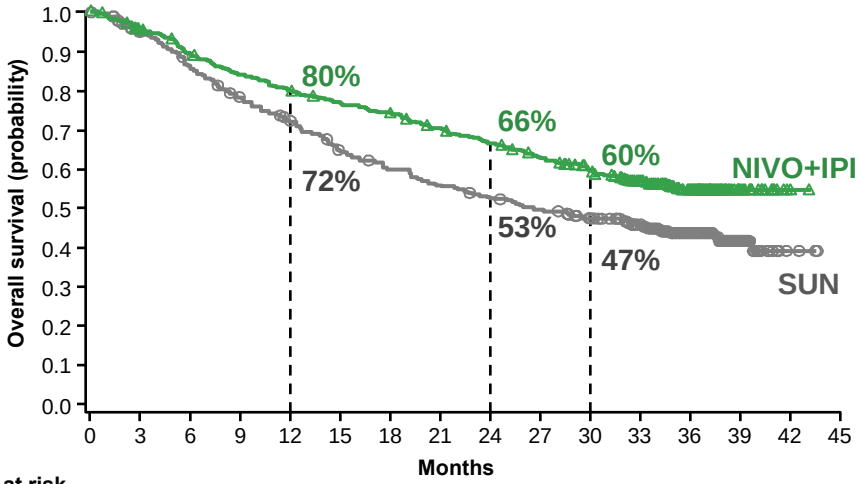
Overall Survival: by IMDC Risk

Intermediate/poor risk

Median OS, months (95% CI)

NIVO+IPI	NR (35.6–NE)
SUN	26.6 (22.1–33.4)

HR (95% CI), 0.66 (0.54–0.80)
P < 0.0001



No. at risk

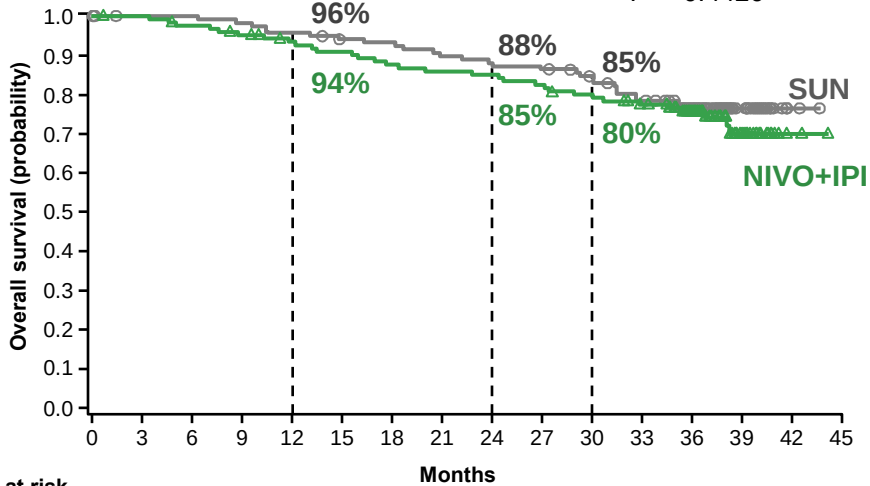
NIVO+IPI	425	399	372	348	332	317	306	287	270	253	233	183	90	34	2	0
SUN	422	388	353	318	290	257	236	220	207	194	179	144	75	29	3	0

Favorable risk

Median OS, months (95% CI)

NIVO+IPI	NR (NE)
SUN	NR (NE)

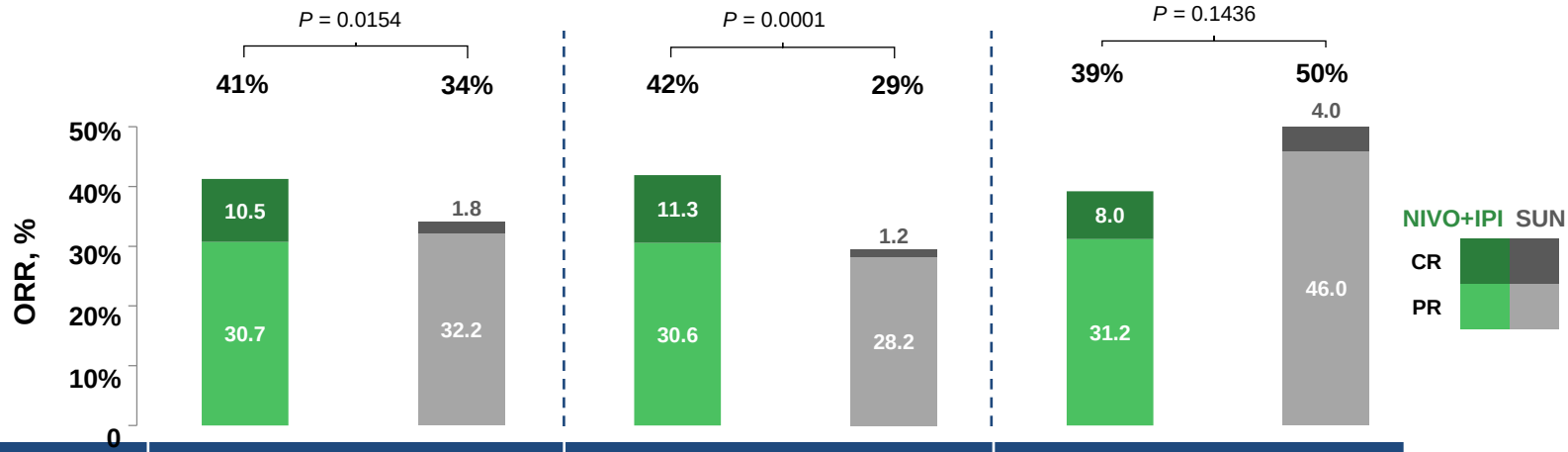
HR (95% CI), 1.22 (0.73–2.04)
P = 0.4426



No. at risk

NIVO+IPI	125	124	120	116	111	108	104	102	101	98	94	88	71	24	2	0
SUN	124	119	119	117	114	110	109	105	103	101	96	88	70	26	2	0

Investigator-Assessed Response per RECIST v1.1



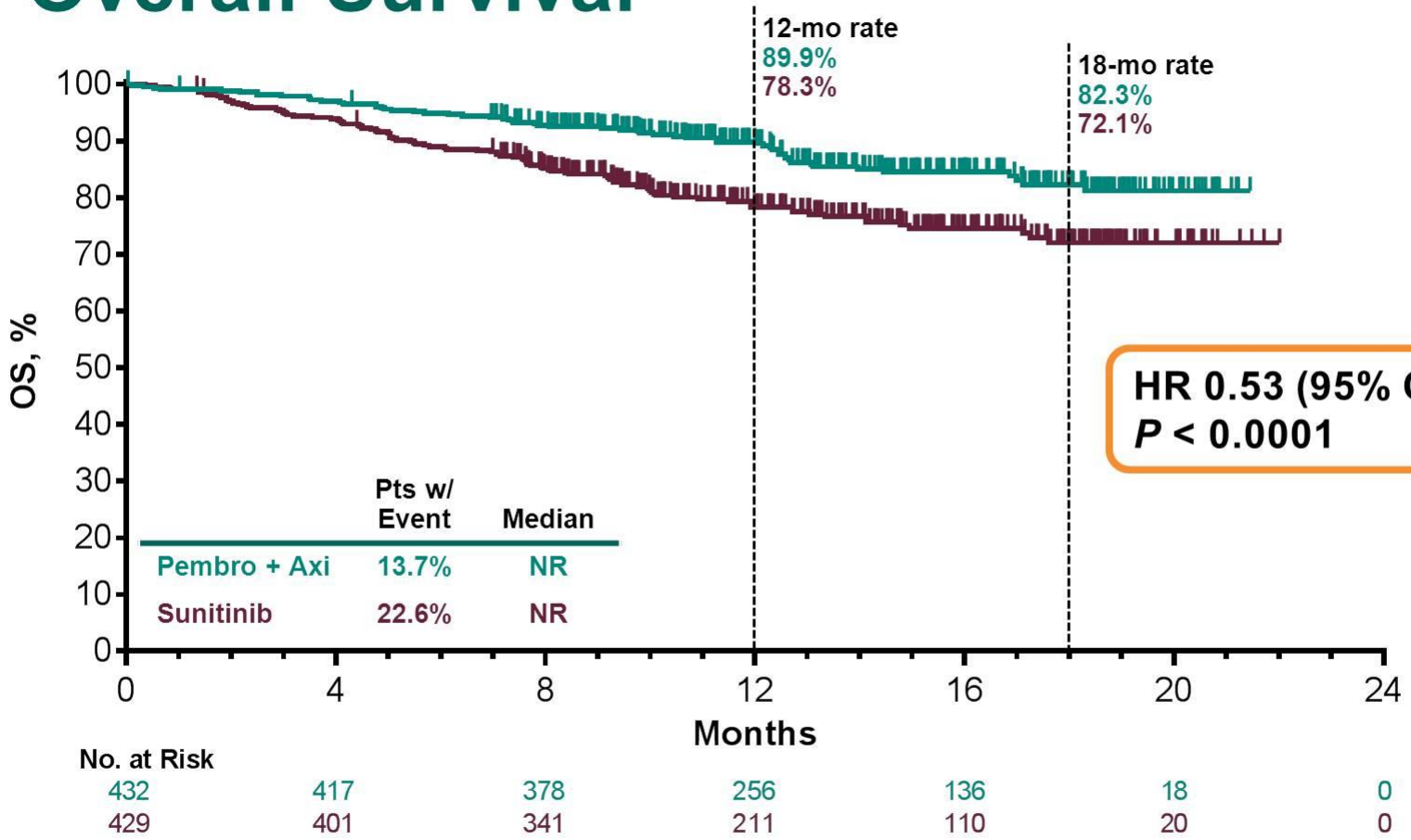
	ITT population		Intermediate/poor risk[1]		Favorable risk	
	NIVO+IPI N = 550	SUN N = 546	NIVO+IPI N = 425	SUN N = 422	NIVO+IPI N = 125	SUN N = 124
DOR ≥18 months, %	53	39	52	28	57	60
Ongoing CR, n/N (%)	51/58 (88)	6/10	42/48 (88)	4/5	9/10	2/5

- Among ITT patients, 185 (34%) versus 114 (21%) achieved ≥50% best tumor burden reduction with NIVO+IPI versus SUN

CR, complete response; DOR, duration of response; PR partial response.

1. Rini BI, et al. Poster presentation at the European Society for Medical Oncology (ESMO) Congress; October 19–23, 2018; Munich, Germany. Poster 875P.

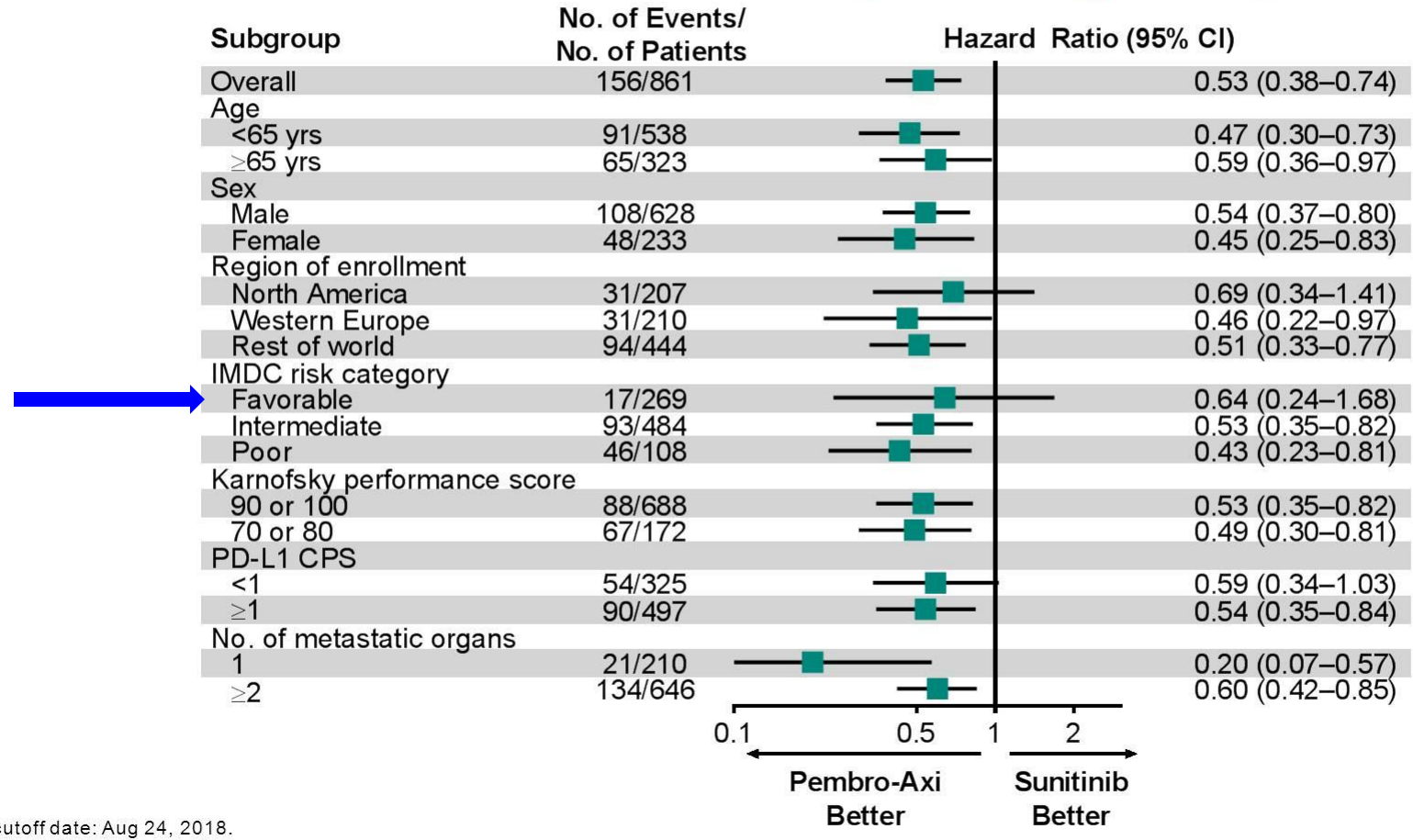
Overall Survival



HR 0.53 (95% CI 0.38-0.74)
***P* < 0.0001**

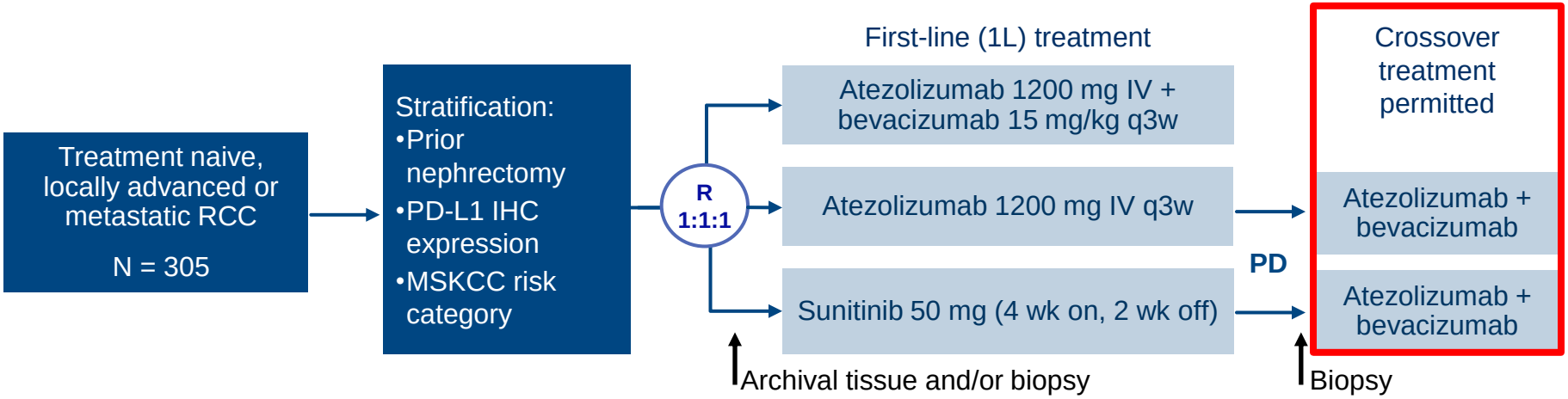
Data cutoff date: Aug 24, 2018.

Overall Survival in Key Subgroups



Data cutoff date: Aug 24, 2018.

IMMOTION150 (PHASE II) TRIAL DESIGN

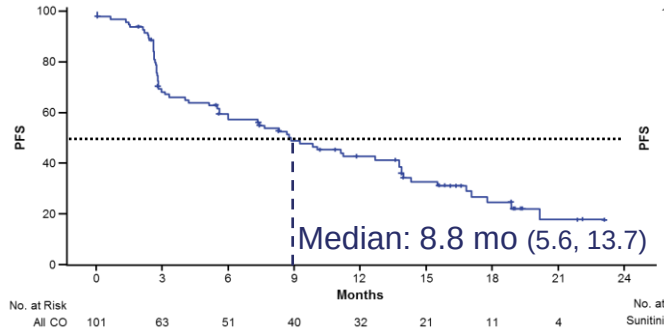


- After progression on atezolizumab or sunitinib, crossover to atezolizumab + bevacizumab was allowed

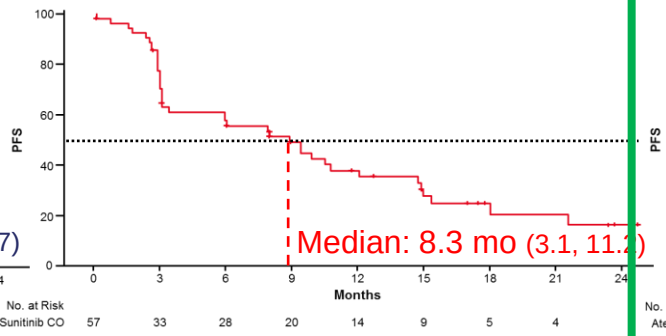
Atkins et al, ASCO 2017

CROSSOVER PROGRESSION-FREE SURVIVAL

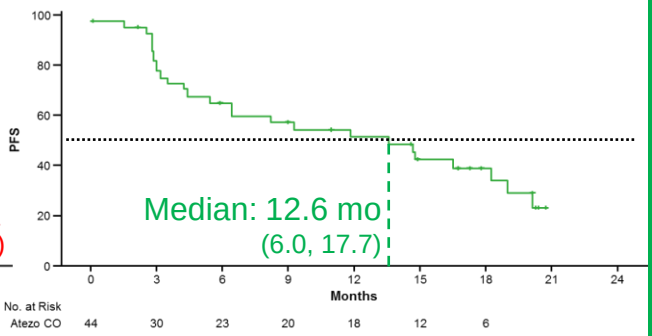
All Crossover



Crossover Post Sunitinib



Crossover Post Atezolizumab (n=44)



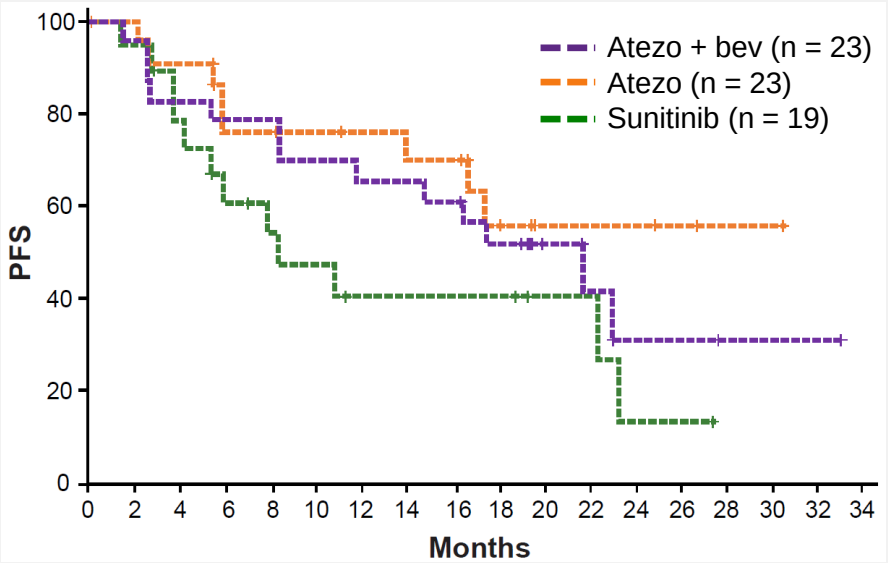
ORR: 24%

PFS measured by investigator.
Clinical cutoff date, Oct 17, 2016. Median duration of follow-up: All crossover, 12.7 mo.

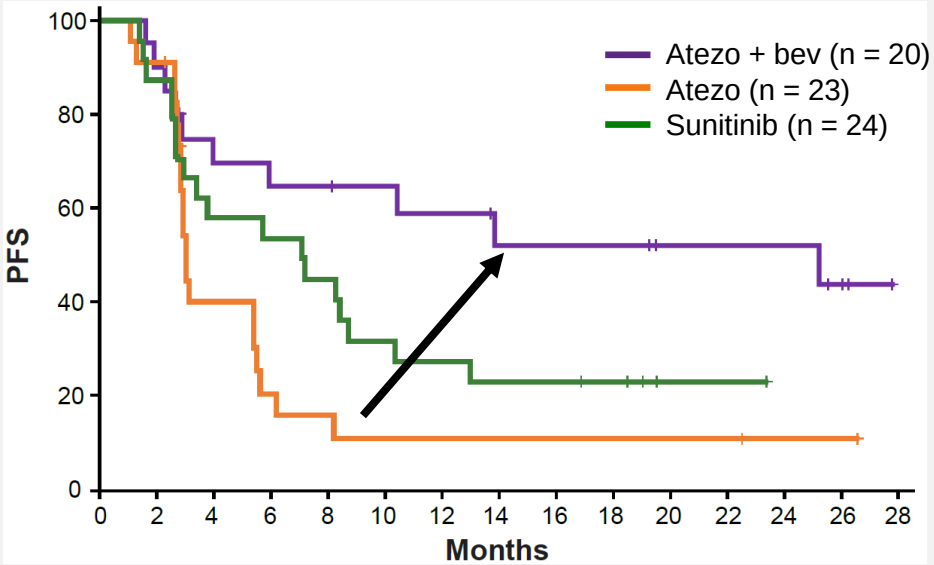
Atkins et al, ASCO 2017

ADDITION OF BEVACIZUMAB TO ATEZOLIZUMAB IN 1L WAS ASSOCIATED WITH IMPROVED BENEFIT IN T-EFFECTOR^{HIGH} MYELOID INFLAMMATION^{HIGH} SUBGROUP

T-effector^{High} Myeloid Inflammation^{Low}



T-effector^{High} Myeloid Inflammation^{High}



PFS measured by independent review facility.
T-effector gene signature: *CD8A, EOMES, PRF1, IFNG, CD274*.
High, ≥ median expression; low, < median expression. McDermott, AACR 2017.

Atkins et al, ASCO 2017

CAN BIOMARKERS HELP?

Current approach to biomarkers in mRCC

- **In situ markers**
  - Protein expression by IHC (e.g. **PD-L1)**
  - Challenges
- **Genomics/Transcriptomics:**
  - Overview
  - Integrative genomics
  - Immune signatures
- **Blood-based biomarkers:**
  - Neutrophils/Lymphocytes Ratio (NLR)
  - Metabolomics
- **Ongoing and future efforts**

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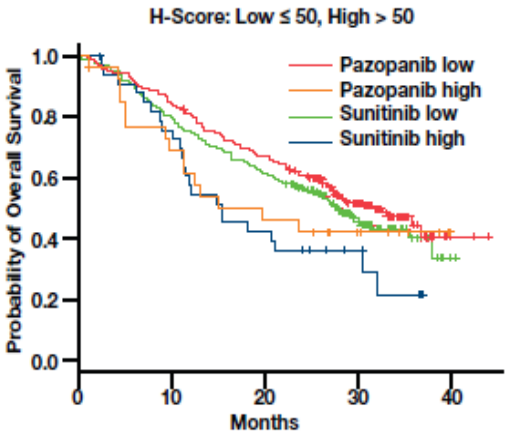
- **Ongoing and future efforts**

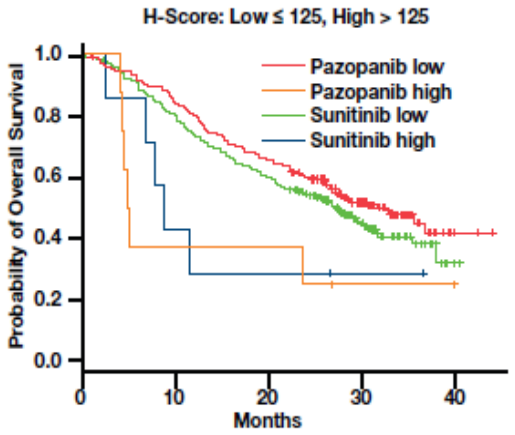
PD-L1 Expression by H-Score in COMPARZ Trial (n=453)

HS: 50



Group (n)	Median OS, months (95% CI)
Pazopanib low (194)	31.6 (26.7, NR)
Pazopanib high (27)	19.7 (9.8, NR)
Sunitinib low (198)	27.7 (23.7, 34.5)
Sunitinib high (34)	15.3 (11.2, 30.5)
P = 0.046	

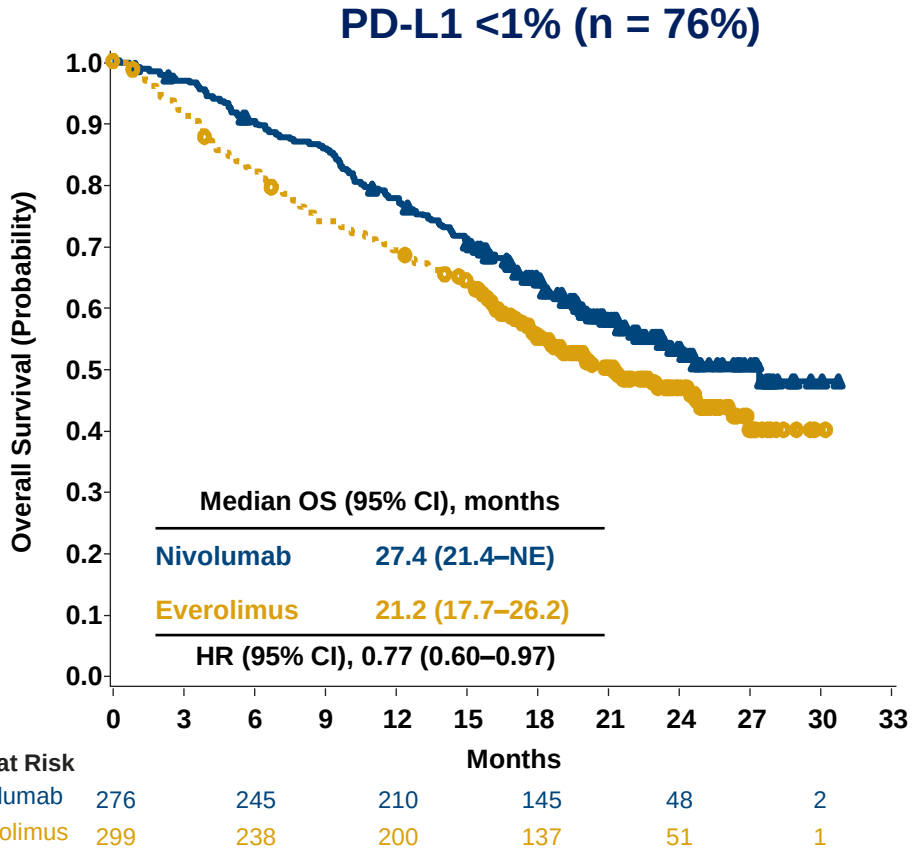
HS: 125

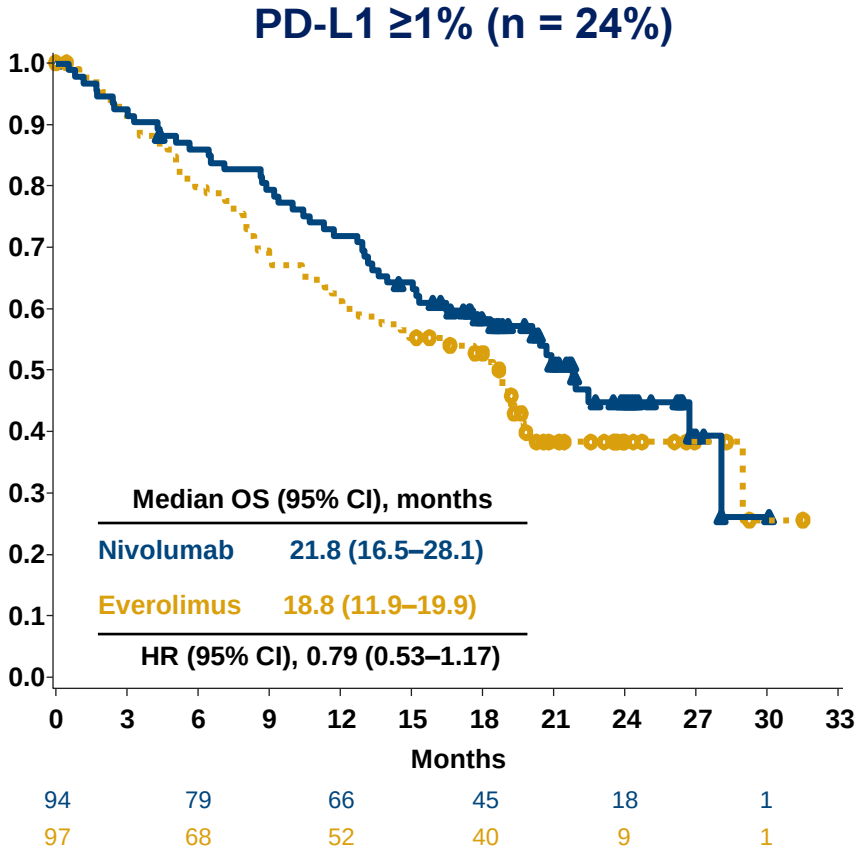


Group (n)	Median OS, months (95% CI)
Pazopanib low (213)	31.6 (26.5, NR)
Pazopanib high (8)	5.1 (4.2, NR)
Sunitinib low (225)	27.4 (21.4, 30.5)
Sunitinib high (7)	8.9 (2.6, NR)
P = 0.017	

Choueiri and Signoretti, Clin Cancer Res 2015

OS by PD-L1 expression in CheckMate 025





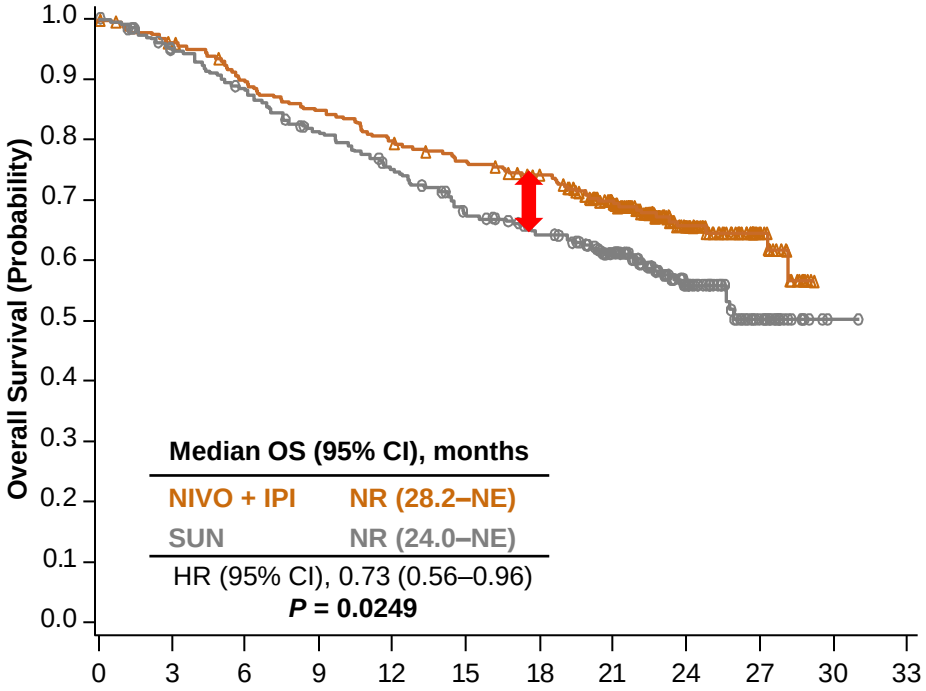
No. at Risk												
Nivolumab	276	245	210	145	48	2	94	79	66	45	18	1
Everolimus	299	238	200	137	51	1	97	68	52	40	9	1

1. Motzer RJ et al. *N Engl J Med* 2015;373:1803–13.

NE, not estimable

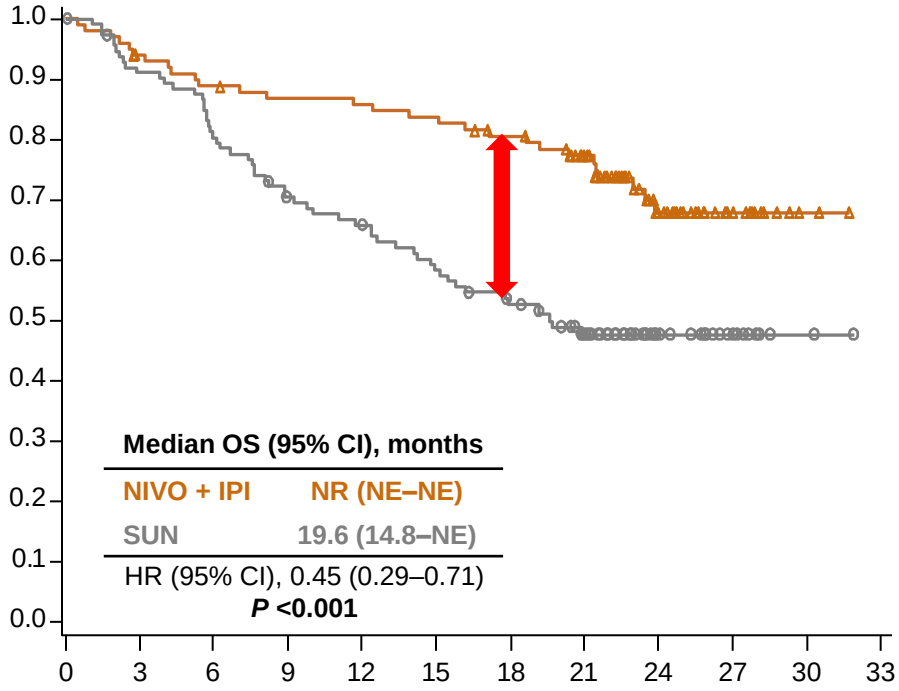
OS by tumor PD-L1 expression: IMDC intermediate/poor risk

PD-L1 <1% (n = 562)



No. at Risk	Months					
NIVO + IPI	284	251	223	200	76	0
SUN	278	239	198	157	61	1

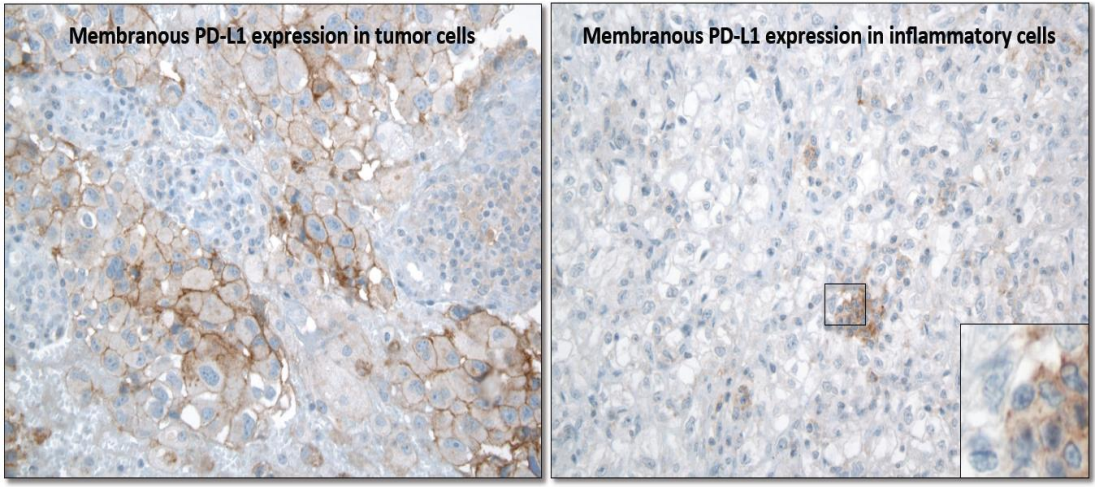
PD-L1 ≥1% (n = 214)



Months					
100	87	83	76	33	2
114	90	72	55	21	2

Motzer et al SITC 2017

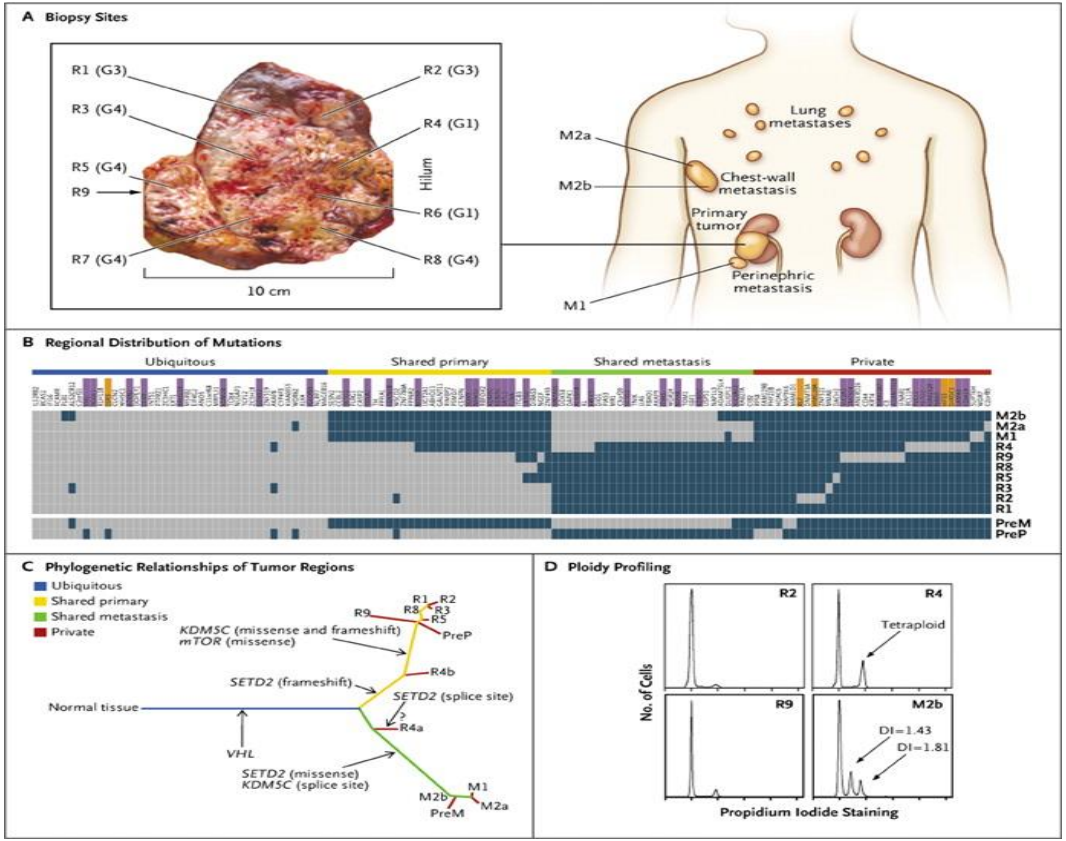
Cellular localization of biomarker at the protein level



PD-L1 expression in **tumor cells** or **immune cells** might have different predictive value in ccRCC patients treated with PD-1/PD-L1 blockade

Motzer et al, NEJM 2015
McDermott, et al JCO 2016

Tumor heterogeneity in ccRCC: challenge to genomic & non-genomic personalized-medicine



Gerlinger M et al. N Engl J Med 2012

PD-L1 testing across mRCC trials

	Atezolizumab + Bevacizumab[1]	Avelumab + Axitinib[2]	Pembrolizumab + Axitinib[3]	Pembrolizumab + Levantinib[4]	Sunitinib vs Pazopanib[6]	Nivolumab + Ipilimumab vs Sunitnib[8,9]
PHASE	3	3	3	1b/2	3	3
PD-L1 IHC Antibody clone	SP 142 Ventana (rabbit clone)	SP 263 Ventana (rabbit clone)	22C3 PharmDx (mouse clone)	22C3 Agilent (mouse clone)	5H1 Medtox (mouse clone)	28-8 Dako (rabbit clone)
LOCATION: Tumor cell (TC) or Immune cell (IC)	IC	IC	TC	TC/IC	TC	TC
CUT OFF FOR POSITIVITY	≥ 1%	≥ 1%	CPS ≥ 1%	CPS ≥ 1%	H-score > 0	≥ 1%

[1]NCT01984242, Motzer et al. ASCO-GU 2018; [2]NCT02493751, Choueiri et al. ASCO 2017; [3]NCT02133742, Atkins et al. ASCO GU 2018;;[4]NCT02501096, Lee et al. ESMO 2017; [5]NCT02178722, Lara et al. ASCO 2017; [6]Choueiri et al. *Clin Cancer Res* 2015;21(5):1071-7; [7]Motzer RJ et al. *N Engl J Med* 2015;373:1803–13; [8,9]NCT02231749 Escudier et al. ESMO 2017, Motzer et al. SITC 2017

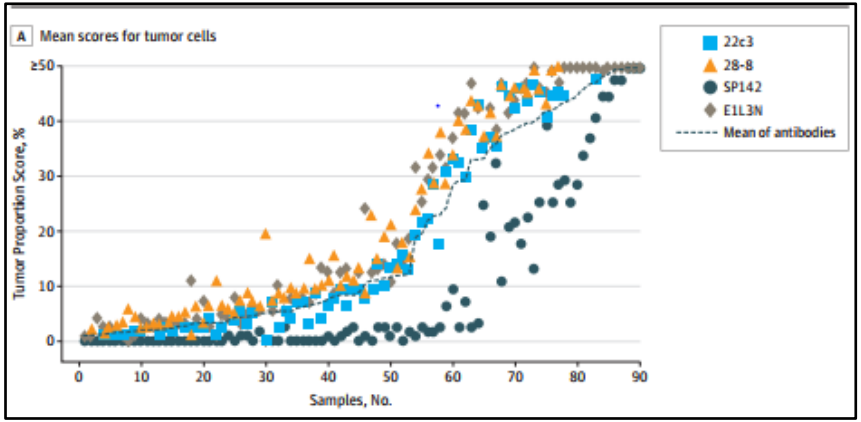
Courtesy: AK Lalani, T Choueiri

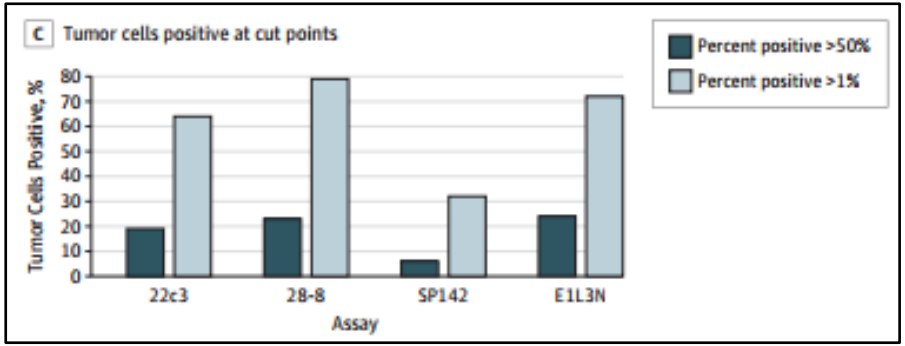
Concordance of PD-L1 assays can vary!

JAMA Oncology | Original Investigation

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

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





Rimm et al. *JAMA Oncology* 2017

www.impactjournals.com/oncotarget/ Oncotarget, 2017, Vol. 8, (No. 61), pp: 103428-103436

Research Paper

Differential expression of c-Met between primary and metastatic sites in clear-cell renal cell carcinoma and its association with PD-L1 expression

Aly-Khan A. Lalani[1], Kathryn P. Gray[2], Laurence Albiges[3], Marcella Callea[4], Jean-Christophe Pignon[5], Soumitro Pal[6], Mamta Gupta[7], Rupal S. Bhatt[8], David F. McDermott[8], Michael B. Atkins[9], G.F. Vande Woude[10], Lauren C. Harshman[1], Toni K. Choueiri[1,*] and Sabina Signoretti[5,*]

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[9]Georgetown Lombardi Comprehensive Cancer Center, Washington D.C., USA

[10]Van Andel Research Institute, Grand Rapids, MA, USA

[*]Co-senior authors contributed equally to this work

Methods

- **c-Met expression:** evaluated by IHC (anti-Met monoclonal antibody; MET4 Ab, VARI) and calculated by a combined score (CS, 0-300) as:*intensity of c-Met staining (0-3) x % of positive cells (0-100)*

- **PD-L1 expression:** previously assessed by IHC[1], and PD-L1+ was defined as PD-L1> 0% positive cells or PD-L1- otherwise

- c-Met expression (average c-Met CS) between paired primary and metastatic samples were compared using Wilcoxon signed-rank test. Associations of c-Met expression with PD-L1 expression (+/-), Fuhrman nuclear grade (FNG), T-stage, were assessed with Wilcoxon rank-sum tests

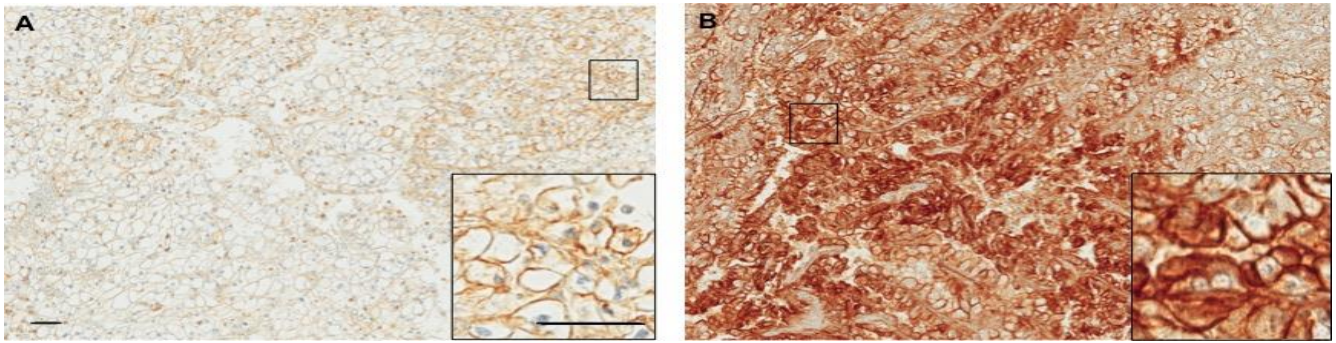
Characteristics	Total n = 45 Patients, n (%)
Gender	
Male	29 (64)
Female	16 (36)
Median age at primary surgery, y (range)	58 (49-62)
T stage	
T1	3 (7)
T2	14 (31)
T3	21 (47)
T4	4 (9)
Tx	3 (7)
N stage	
N0	12 (27)
N1	13 (29)
Nx	20 (44)
Fuhrman nuclear grade (FNG)	
III	32 (71)
IV	13 (29)
Number of metastatic sites analyzed per case	
1	35 (78)
2	6 (13)
3-6	4 (9)

[1]Callea M, Albiges L, Gupta M et al. *Cancer Immunol Res* 2015 Oct;3(10):1158-64

➢ c-MET expression is higher in metastatic sites than in primary tumors

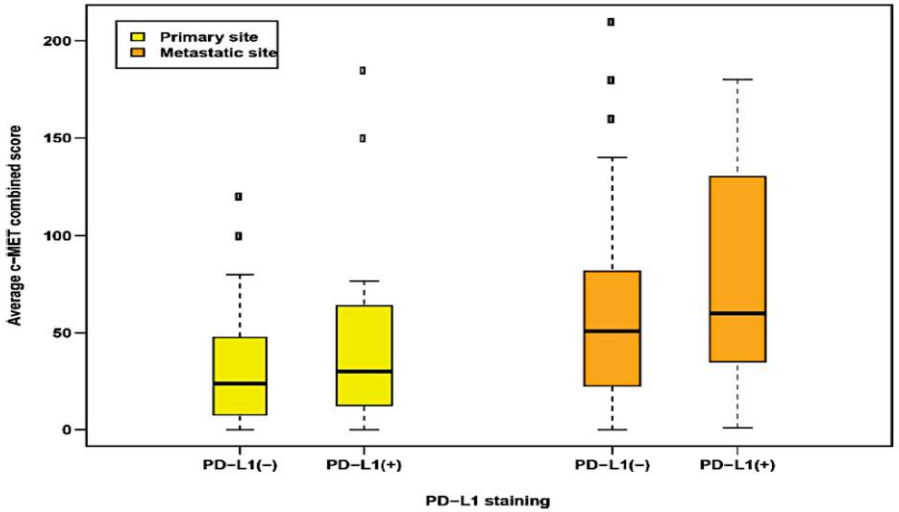
		Primary site	Metastatic site	p-value*
Average c-MET combined score	Median (IQR)	**28** (10,55)	**55** (30,83)	0.0003
Highest c-MET combined score	Median (IQR)	**30** (20,80)	**60** (30,130)	0.02

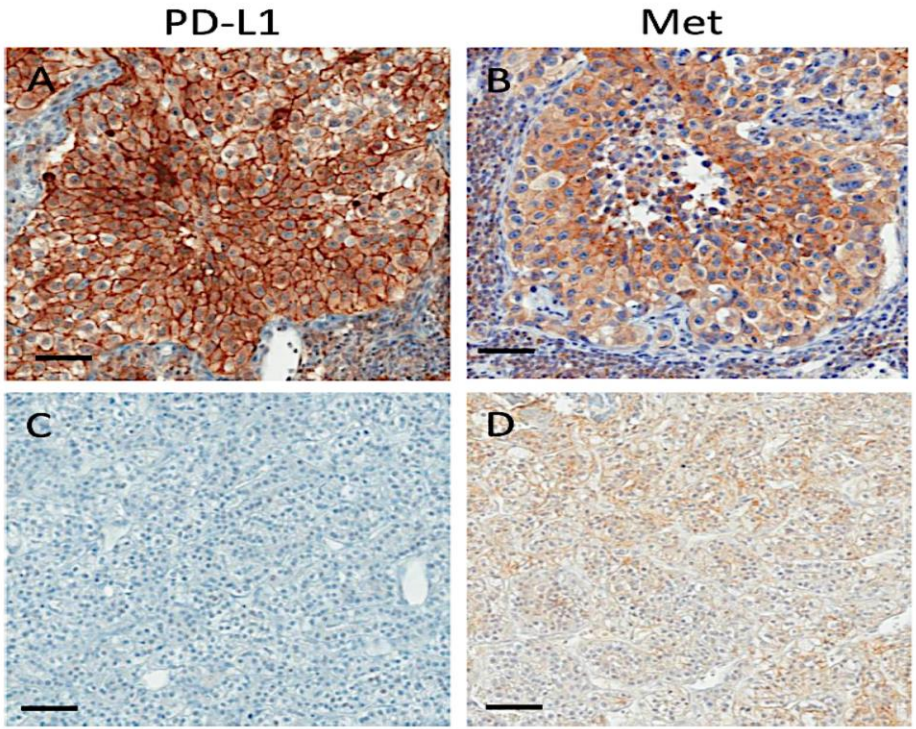
*based on Wilcoxon signed rank to test p-value for continuous c-MET



Representative images of a primary ccRCC (A) and its corresponding metastasis (B) immunostained for Met. A, weak (1+) membranous staining is observed in a subset of tumor cells. B, intense (3+) membranous staining is observed in a large fraction of tumor cells. Insets show higher magnifications of the selected areas. Scale bars: 50 µm.

➢ c-MET expression was numerically-greater in PD-L1+ vs. PD-L1- tumors





LEFT: Distributions of c-Met expression according to PD-L1 staining positivity (PD-L1+, > 0% positive cells vs. PD-L1-, = 0% positive cells) based on tumor sample sites.

RIGHT: Representative images of 2 ccRCC metastases immunostained for PD-L1 (A,C) and Met (B,D).

PD-L1 positive metastasis (A) with intense (3+) membranous Met staining (B).
PD-L1 negative metastasis (C) with weak (1+) membranous Met staining (D).

Conclusions

- In our cohort of metastatic ccRCC patients, c-Met expression is higher in metastatic sites compared to paired primary samples
- Higher c-Met expression in metastases compared to paired primary tumors suggests that testing for biomarkers of response to c-Met inhibitors should be conducted in metastatic sites
- Although the observation of higher c-Met expression in PD-L1+ tumors requires further investigation, it supports exploring these targets in combination trials
  - Evaluating this in METEOR and CABOSUN trials

Considerations of PD-L1 expression and interpretation

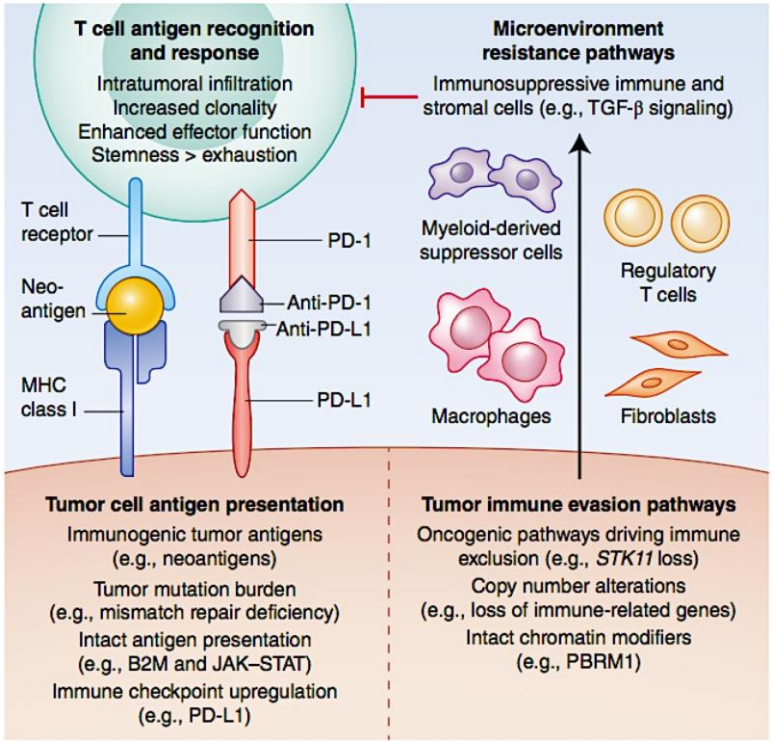
Variable	Potential Impact on PD-L1 Expression
Age of tumor tissue specimen	Decrease with older sections; epitope degradation/masking
Primary vs Metastases	Discordant expression between primary and metastases
Low vs. high-grade areas	Higher expression in higher grade tumors
Method of collection	Excisional/resection samples have higher number of tumor cells – may impact potential for detection of PD-L1+ cells
Type of antibody used	Mouse clone 5H1, Rabbit clone 28-8, Roche mAb…
Cutoffs /reporting methods	1%, 5%, 50%, H-score...
Localization	Tumor membrane, immune cells or both
The Reader	Pathologist (with inter-variability), automated system
The Therapy	Single agent (nivo) vs. combination (nivo+ipi, nivo+VEGF inh)…or another non-immunotherapy drug

c/o Signoretti and Choueiri

Current approach to biomarkers in mRCC

- **In situ markers**
  - Protein expression by IHC (e.g. **PD-L1)**
  - Challenges
- **Genomics/Transcriptomics:**
  - Overview
  - Integrative genomics
  - Immune signatures
- **Blood-based biomarkers:**
  - Neutrophils/Lymphocytes Ratio (NLR)
  - Metabolomics
- **Ongoing and future efforts**

Framework for genomic correlates of response to ICB



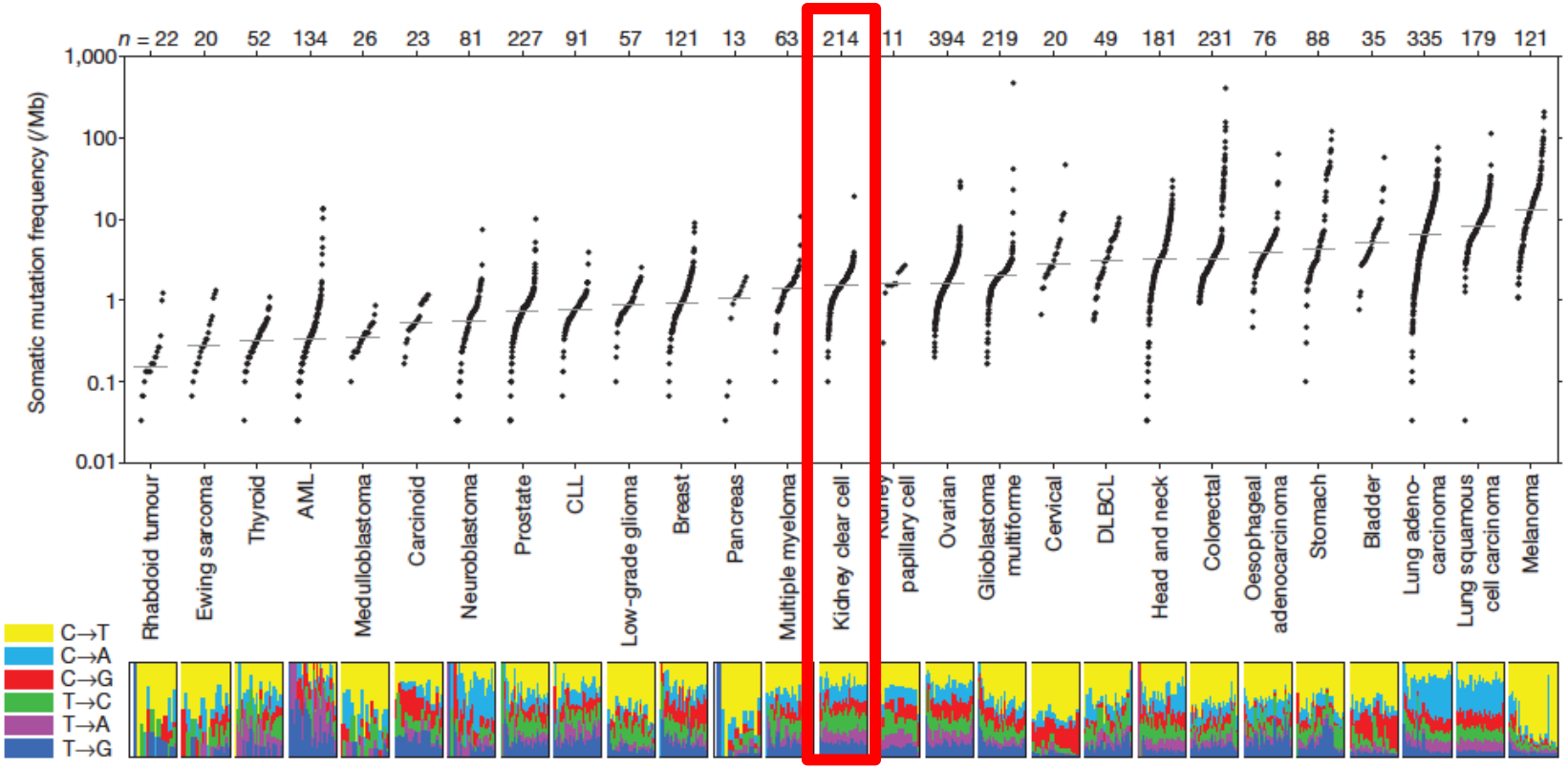
TE Keenan, KP Burke, EM Van Allen. *Nat Med* 2019

Genomic correlates of response and resistance

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

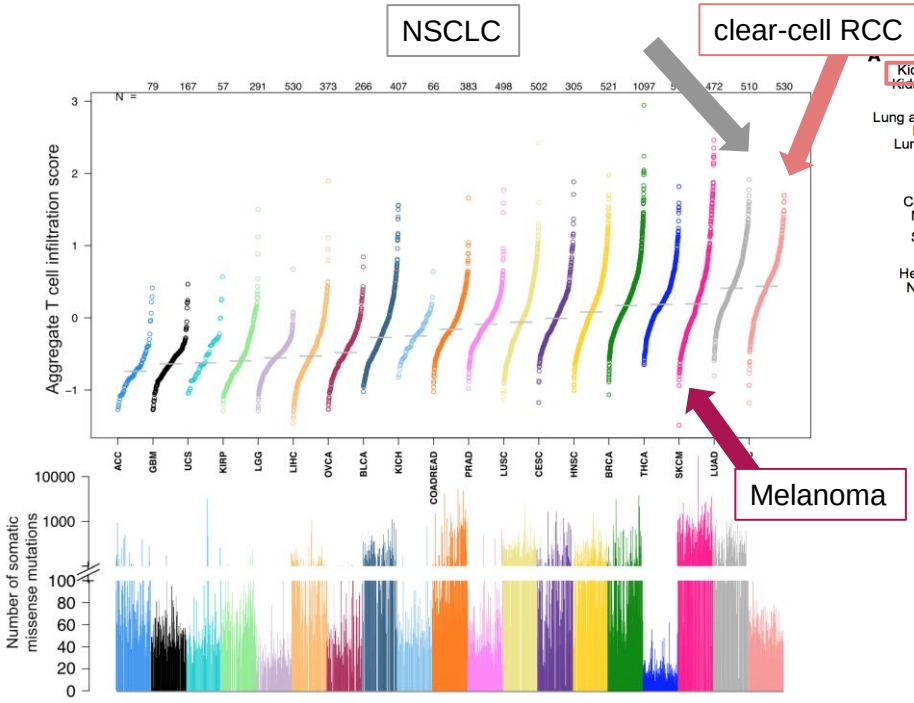
TE Keenan, KP Burke, EM Van Allen. *Nat Med* 2019

Genomic mediators of response to cancer immunotherapy?

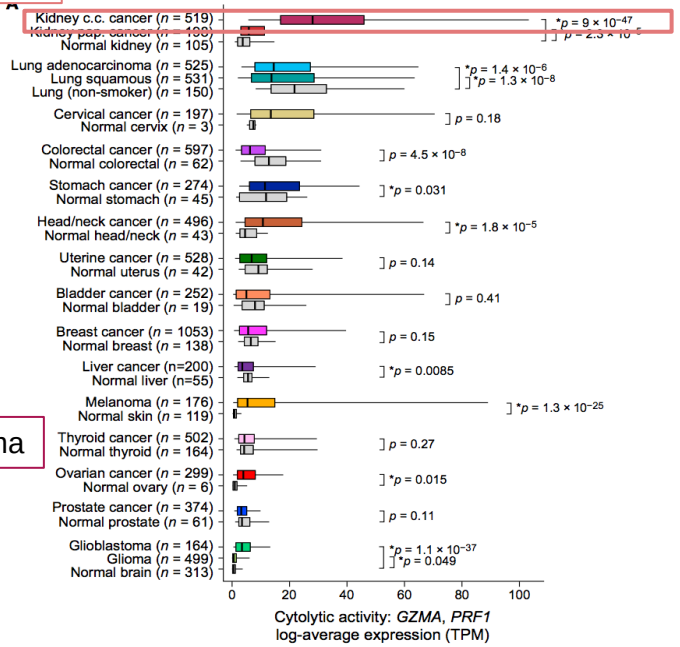


Lawrence et al *Nature* 2013

Clear-cell RCC is distinguished by **relatively low mutational burdens**, but ranks among the highest in **tumor cytolytic activity** and **T cell infiltration signatures**

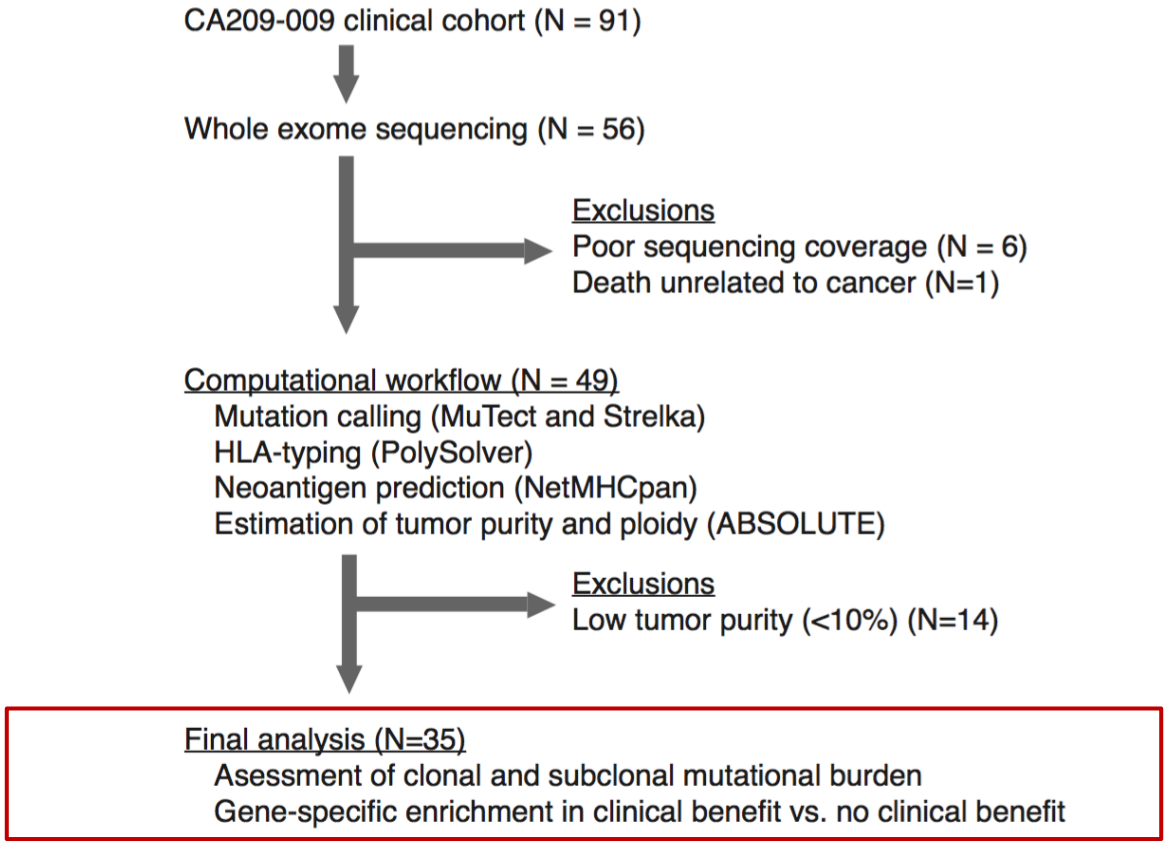


Senbabaoglu et al. *Genome Biology* 2016



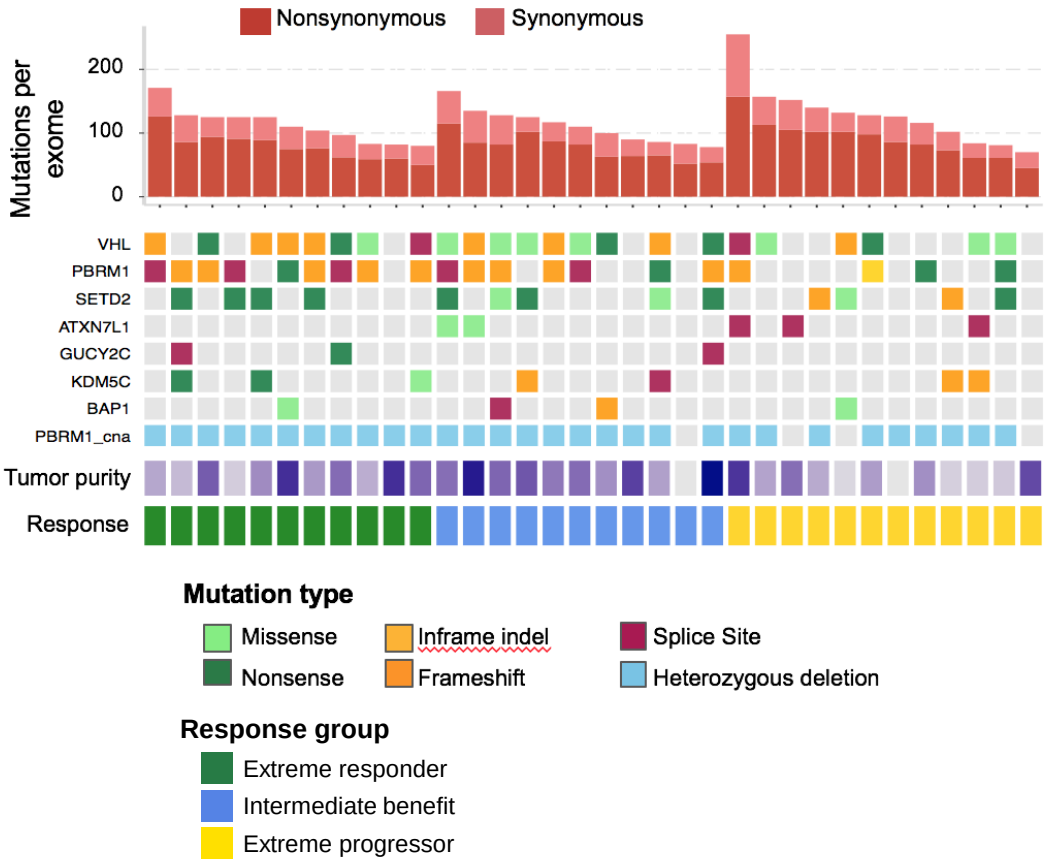
Rooney et al. *Cell* 2015

Using WES in pre-ICB treatment samples to explore genomic predictors of response: discovery cohort, nivolumab clinical trial



Miao et al, *Science* 2018

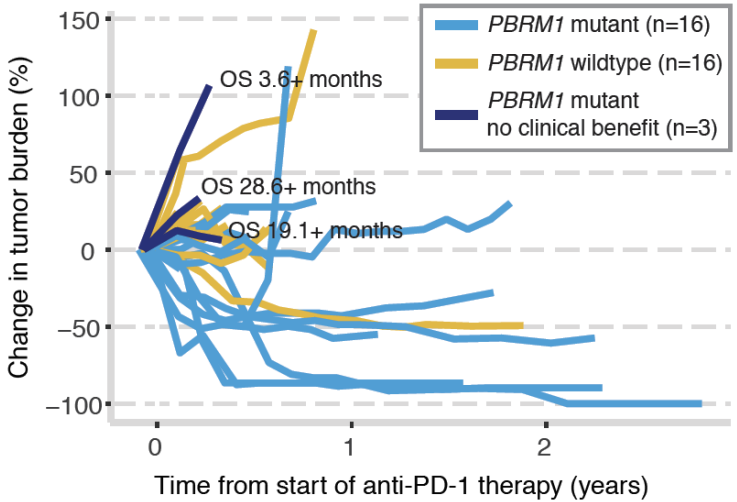
While mutation load did not predict response, truncating mutations in *PBRM1* gene were enriched in responders



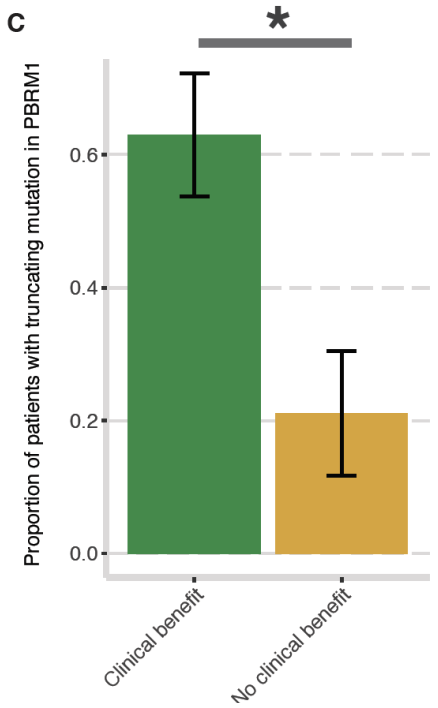
no difference in mutation or neoantigen load between response groups

Miao et al, *Science* 2018

Clinical genomics and immunotherapy in ccRCC



RCC discovery clinical trial cohort (N=35)
PBRM1 p < 0.01

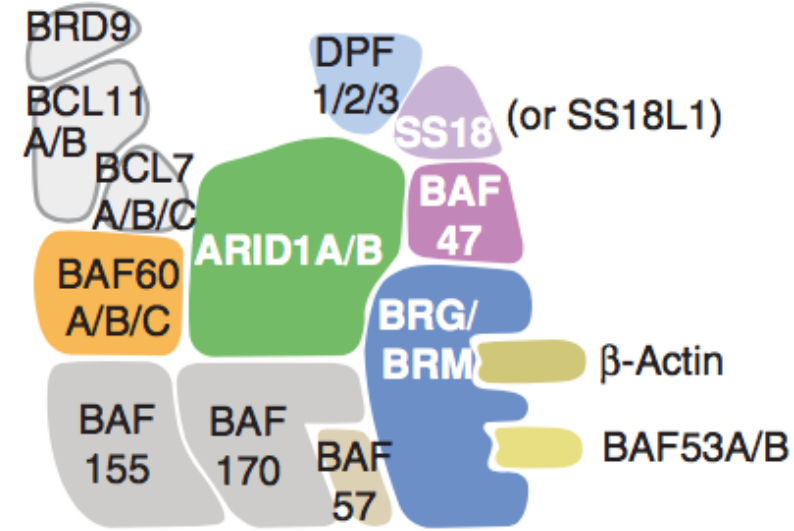


RCC validation cohort (N=63)
PBRM1 p < 0.01

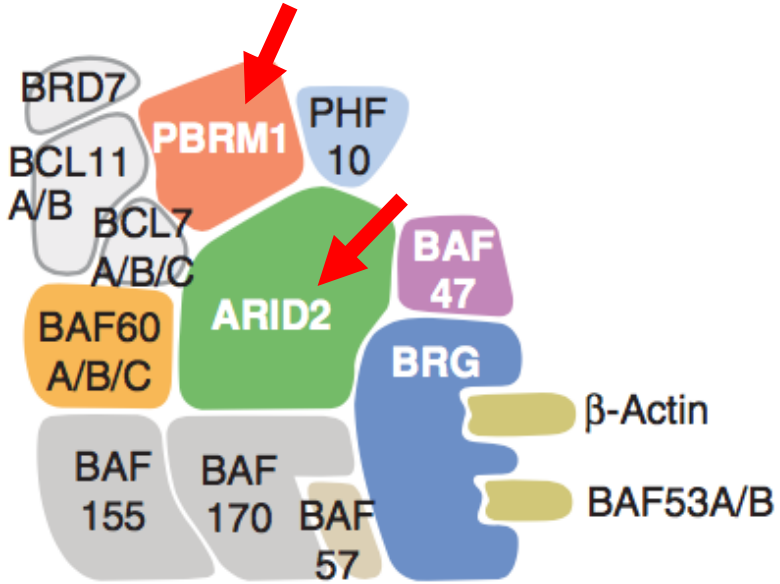
Collaboration with the **Van Allen lab**

Miao et al, *Science* 2018

A role for SWI/SNF in cancer immunotherapy?



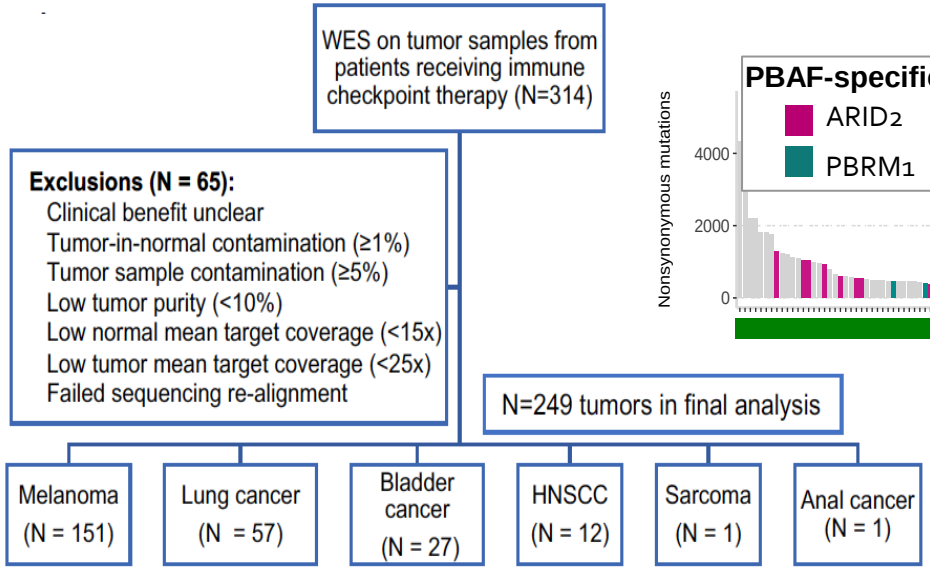
BAF complexes
Homo sapiens, Mus musculus

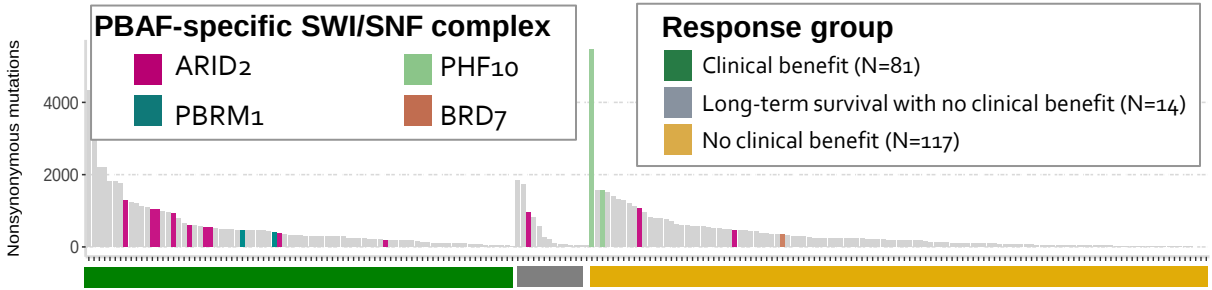


PBAF complexes
Homo sapiens, Mus musculus

Hodges, Kirkland, and Crabtree *Cold Spring Harb Perspect Med* 2016

Genomics and immunotherapy meta-analysis

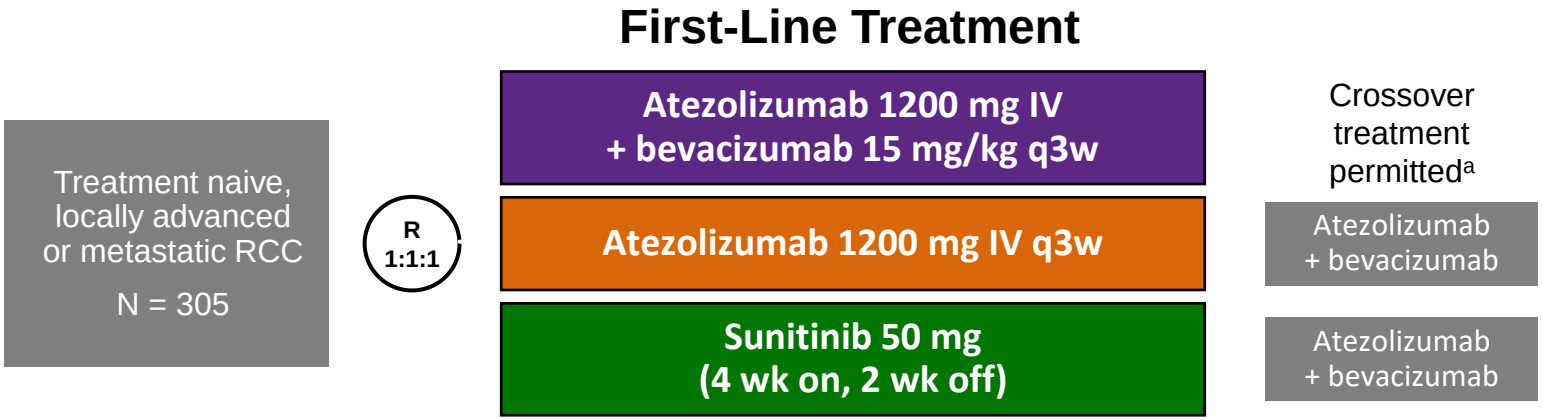




***ARID2** truncating alterations significantly associated with response ($p < 0.01$)*

Van Allen lab, DFCI

IMmotion150 (Phase II) Trial Design

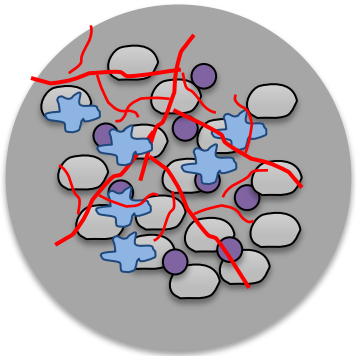


- IMmotion150 was designed to be hypothesis generating and inform the Phase III study IMmotion151
- Coprimary endpoints were PFS (RECIST v1.1 by IRF) in ITT patients and patients with ≥ 1% of IC expressing PD-L1
- **Exploratory endpoints included interrogation of the association between outcome and TME gene signatures**

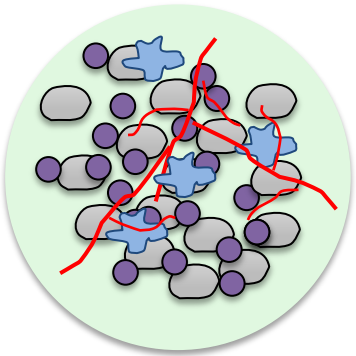
McDermott, *JCO* 2016; McDermott, ASCO GU 2017

McDermott D, et al. IMmotion150 biomarkers: AACR 2017

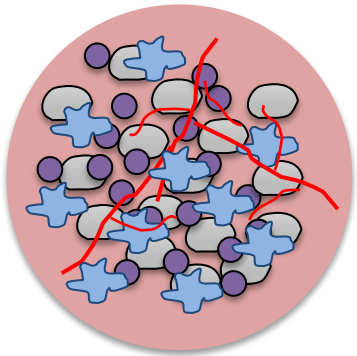
Molecular Correlates of Differential Response to Atezolizumab ± Bevacizumab vs Sunitinib in mRCC



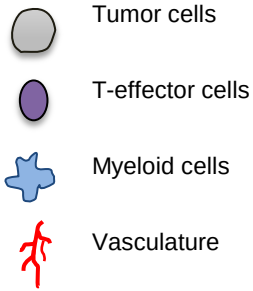
Angiogenic



T-effector^{High}
Myeloid Inflammation^{Low}



T-effector^{High}
Myeloid Inflammation^{High}
Immune Suppressed



Clinical Activity

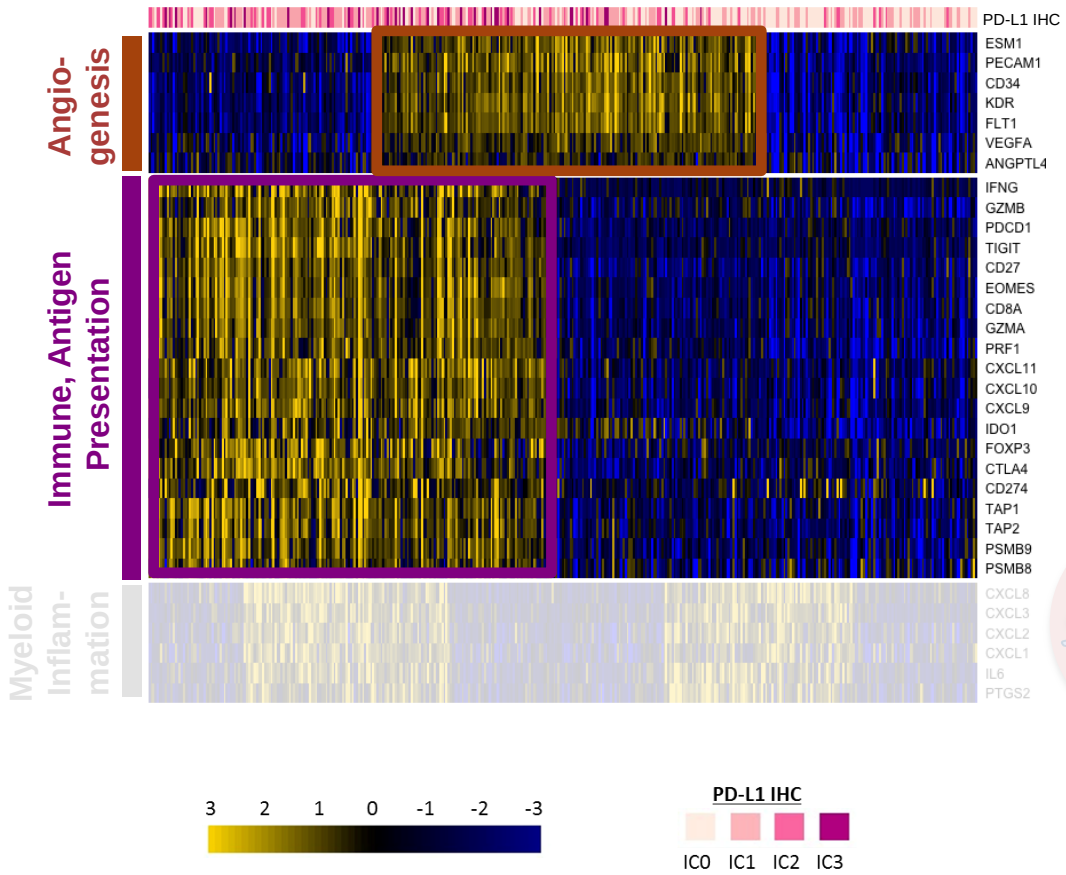
Sunitinib

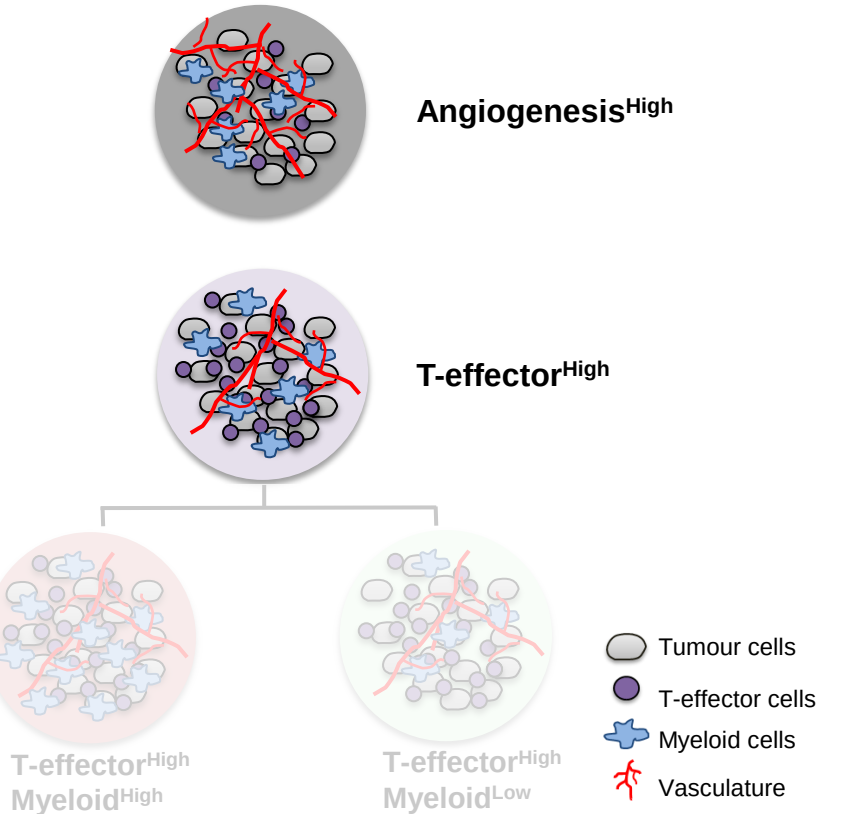
Atezolizumab

Atezolizumab + Bevacizumab

McDermott D, et al. IMmotion150 biomarkers: AACR 2017

IMmotion151: Transcriptome Map Confirms Biological Subgroups Identified in IMmotion150





Rini B, et al. IMmotion151 Biomarkers. ESMO 2018 [abstract LBA31]. http://bit.ly/2yaVgyI

Summary

- IMmotion151 validated Angiogenesis and T-effector gene signatures identified in **IMmotion150**
  - **Atezolizumab + bevacizumab** improved PFS vs sunitinib in **T-effector^{High} and Angiogenesis^{Low} tumours**
  - Within the **sunitinib arm, patients with an Angiogenesis^{High} gene signature** showed improved PFS vs the Angiogenesis^{Low} subgroup
- MSKCC **favourable-risk patients** are characterised by a predominant **Angiogenesis^{High} gene signature**
- **Sarcomatoid RCC** is characterised by an **Angiogenesis^{Low} gene signature, a T-effector^{High} gene signature** / higher PD-L1 expression and enhanced clinical benefit with atezolizumab + bevacizumab

Rini B, et al. IMmotion151 Biomarkers. ESMO 2018 [abstract LBA31]. http://bit.ly/2yaVgyI

Current approach to biomarkers in mRCC

- **In situ markers**
  - Protein expression by IHC (e.g. **PD-L1)**
  - Challenges
- **Genomics/Transcriptomics:**
  - Overview
  - Integrative genomics
  - Immune signatures
- **Blood-based biomarkers:**
  - Neutrophils/Lymphocytes Ratio (NLR)
  - Metabolomics
- **Ongoing and future efforts**

Lalani *et al. Journal for ImmunoTherapy of Cancer* (2018) 6:5
DOI 10.1186/s40425-018-0315-0

Journal for ImmunoTherapy of Cancer

RESEARCH ARTICLE Open Access

Change in Neutrophil-to-lymphocyte ratio (NLR) in response to immune checkpoint blockade for metastatic renal cell carcinoma

Aly-Khan A. Lalani[1], Wanling Xie[2], Dylan J. Martini[1,3], John A. Steinharter[1], Craig K. Norton[1], Katherine M. Krajewski[4], Audrey Duquette[1], Dominick Bossé[1], Joaquim Bellmunt[1], Eliezer M. Van Allen[1,5], Bradley A. McGregor[1], Chad J. Creighton[6], Lauren C. Harshman[1] and Toni K. Choueiri[1*]

- **Objective:** To investigate the utility of NLR at baseline and during therapy in metastatic RCC patients treated with PD-1/PD-L1 immunotherapy (IO)
- **Methods:**
  - 142 patients from Dana-Farber Cancer Institute (Boston, MA) receiving IO-based therapies were included.
  - NLR was examined at baseline and 6 (±2) weeks later.
  - Landmark analysis at 6 weeks was conducted to explore the prognostic value of relative NLR change on overall survival (OS), progression-free survival (PFS), and objective response rate (ORR) using Cox or logistic regression models, adjusted for line of therapy, number of IMDC risk factors, histology and baseline NLR.

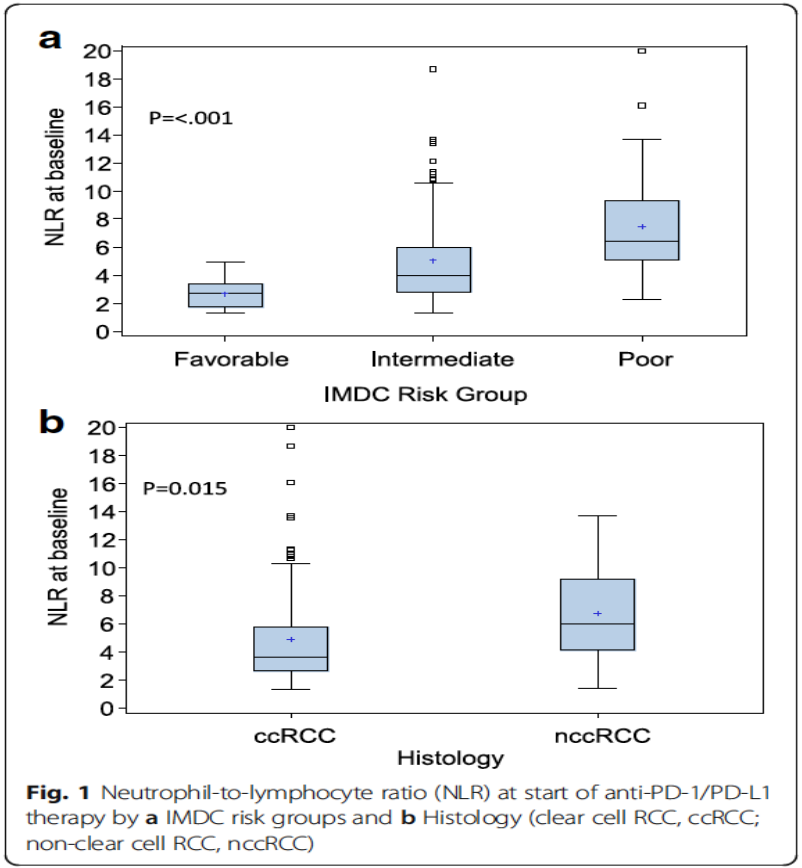


Fig. 1 Neutrophil-to-lymphocyte ratio (NLR) at start of anti-PD-1/PD-L1 therapy by **a** IMDC risk groups and **b** Histology (clear cell RCC, ccRCC; non-clear cell RCC, nccRCC)

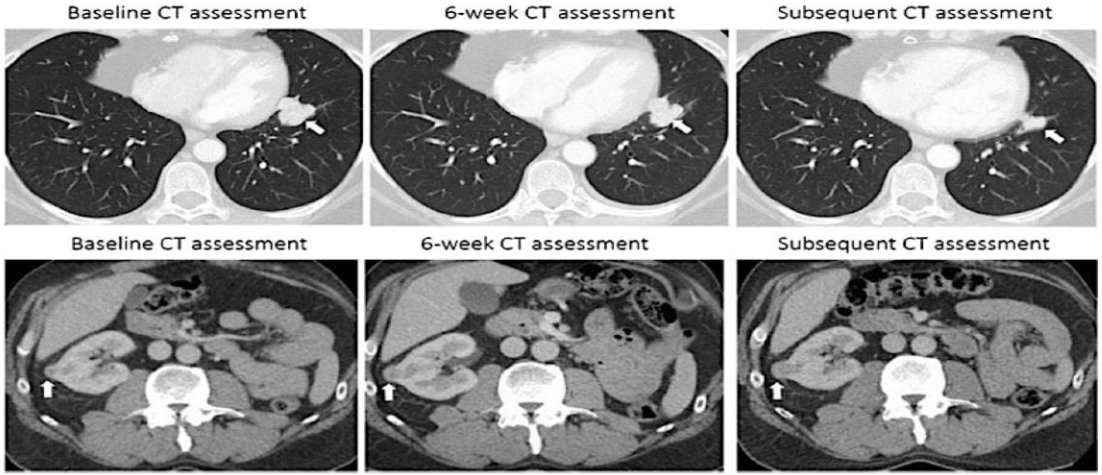
- Baseline NLR levels were significantly higher in the poor IMDC risk group ($p < 0.001$)
- Patients with Non-clear cell histology had elevated NLR compared ($p=0.015$)
- No significant association of baseline NLR with other patient characteristics such as age, gender, smoker status, and line of therapy (p-values >0.15)

Table 2 Association of NLR at baseline, at 6-weeks, and change at week 6 (±2 weeks) with treatment outcomes in multivariable Cox and Logistic regression models

	ORR (CR + PR)			PFS			OS		
	Total N/ N response	Adjusted-OR[b]	*p*-value	Total N/ N event	Adjusted-HR[b]	*p*-value	Total N/ N event	Adjusted-HR[b]	*p*-value
Continuous Ln(NLR) [baseline]	142/44	0.49 (0.22–1.09)	0.081	142/96	1.80 (1.14–2.86)	**0.012**	142/51	1.70 (0.99–2.94)	0.056
Continuous Ln(NLR) [6-weeks][a]	134/44	0.22 (0.10–0.52)	**0.001**	117/72	3.61 (2.21–5.88)	**<0.001**	134/46	2.51 (1.71–3.69)	**<0.001**
NLR-change [6-weeks][a]									
Decrease ≥25%	28/12	1.52 (0.49–4.68)	0.112	27/13	0.55 (0.26–1.18)	**<0.001**	28/6	0.33 (0.12–0.88)	**0.004**
No change	58/21	1.00 (reference)		53/30	1.00 (reference)		58/18	1.00 (reference)	
Increase ≥25%	48/11	0.45 (0.18–1.16)		37/29	2.60 (1.53–4.39)		48/22	1.57 (0.83–2.99)	

- Higher 6 week NLR was **independently associated** with a lower ORR, shorter PFS and OS
- A higher baseline NLR trended toward lower ORR, shorter PFS, and shorter OS
  - Nearly identical to HR seen in a study of NLR in mRCC treated with VEGF-TT (Templeton et al., *Eur Urol* 2016)
- Relative NLR change from baseline to 6 weeks was an **independent prognostic factor** for PFS and OS ($p < 0.001$ and $p = 0.004$).
  - A decrease ≥25% = associated with an improved PFS, and significantly better OS
  - By contrast, an NLR increase by ≥25% = associated with significantly worse PFS and OS
  - An NLR increase by ≥25% was associated with poorer PFS and OS, regardless of baseline NLR levels

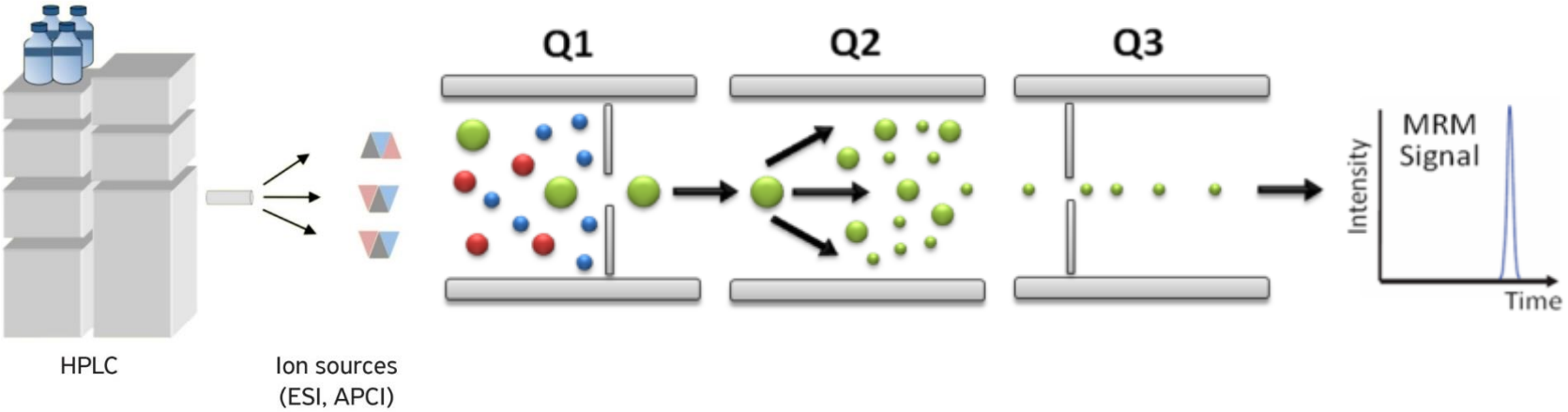
Clinical applicability: CT scans at baseline, 6-week, and next assessment



- First patient (upper panels) had SD on 6-week scan with a ***34% decrease*** in NLR from baseline → subsequently displayed PR on next assessment
- Second patient (lower panels) had SD on 6-week scan with a ***113% increase*** in NLR from baseline → subsequently displayed PD on next assessment

Cellular metabolism has a role in cancer: analysis of serum metabolomics

One method/instrument: Liquid chromatography-mass spectrometry (LC-MS) / triple quadrupole

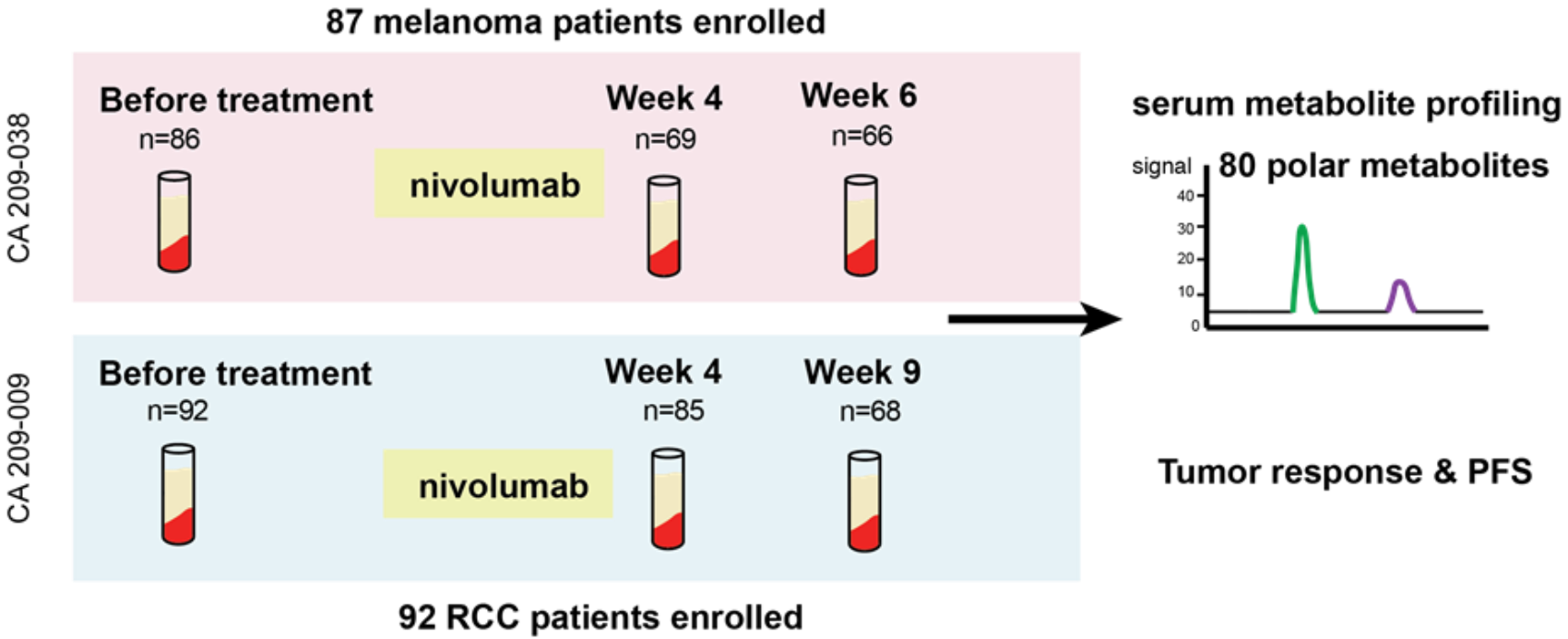


1) Chromatography 2) Ionization 3) Metabolite Selection 4) Fragmentation 5) Fragment Selection 6) Detection

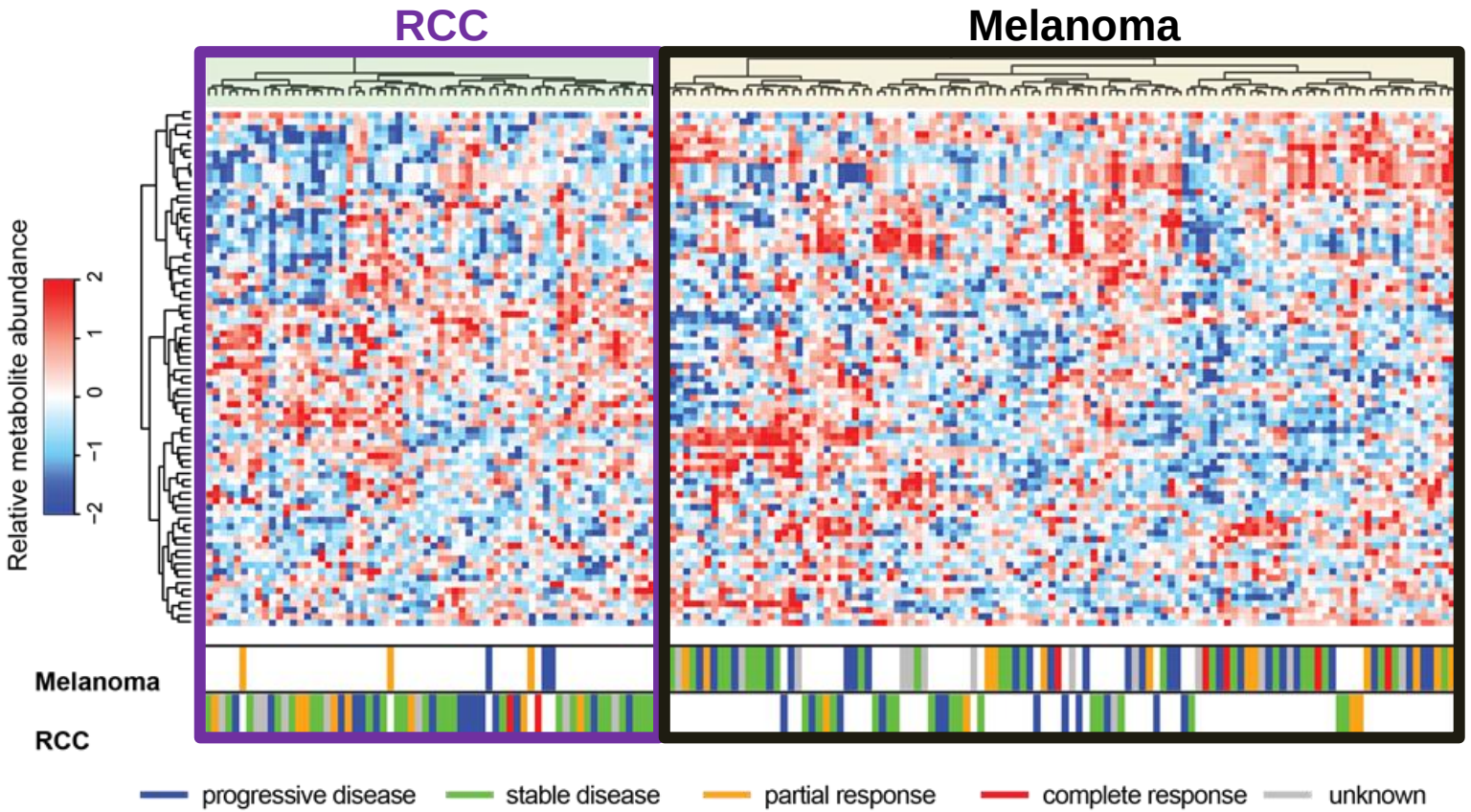
HPLC: High performance liquid chromatography; ESI: Electro-spray ionization; APCI: Atmospheric pressure chemical ionization; MRM: Multiple reaction monitoring

Modified from SRMAtlas

Metabolomics cohort design and specimen collection



Overview of the profiles of polar metabolites in two cancer types

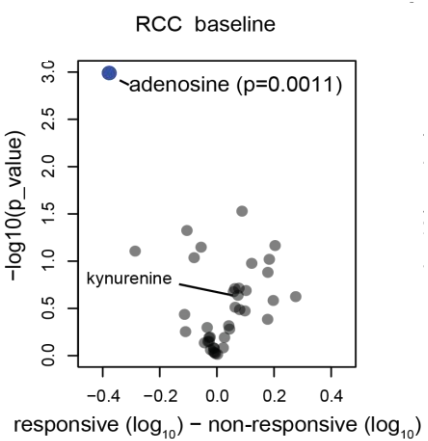


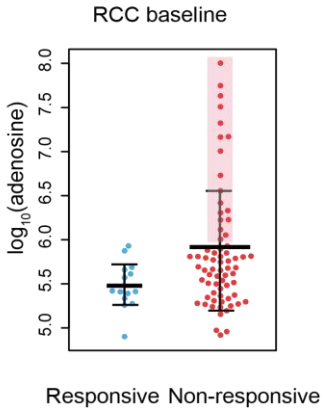
- Serum metabolic landscapes cluster by cancer type / lineage

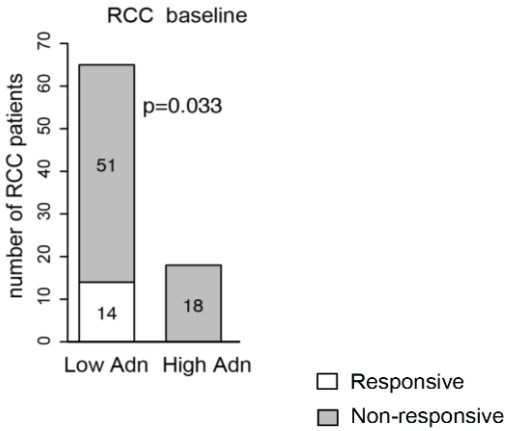
What are the serum metabolic features correlating with response in RCC?

Compare metabolite levels between responders (CR, PR) and non-responders (SD, PD) to nivolumab

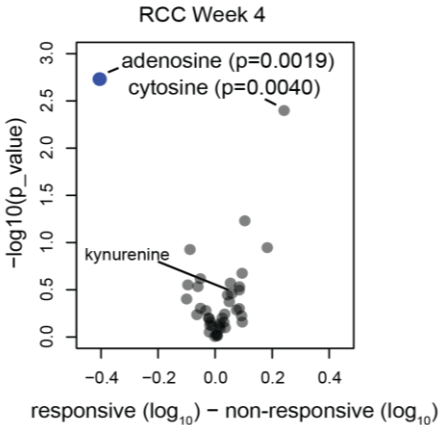
At baseline:

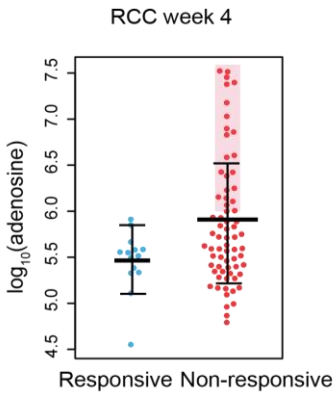


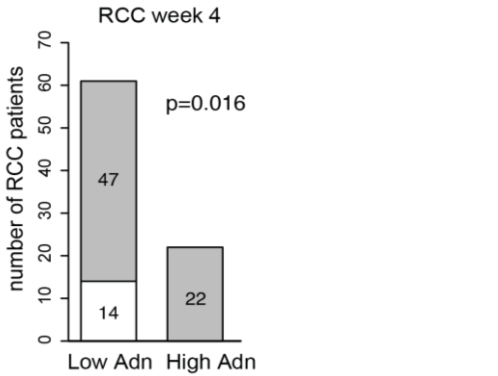




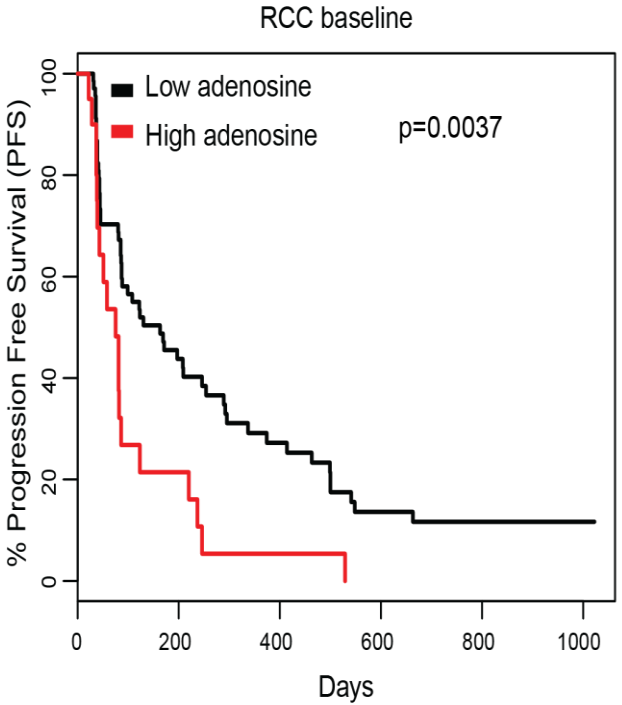
At week 4:







Higher baseline serum adenosine is significantly associated with worse PFS in nivolumab-treated RCC patients



Binary adenosine feature	No. of Patients	Median PFS months (95% CI)	
High adenosine	20	2.5 (1.4-7.3)	p=0.0037
Low adenosine	71	5.4 (2.9-9.6)	

Continuous adenosine feature	Hazard ratio, 1.83 (95% CI, 1.28-2.62), p=0.0022

High serum adenosine levels are associated with an intrinsically immune-suppressive transcriptional state in renal tumors

RCC tumor biopsies (n = 34)

↓

RNAseq

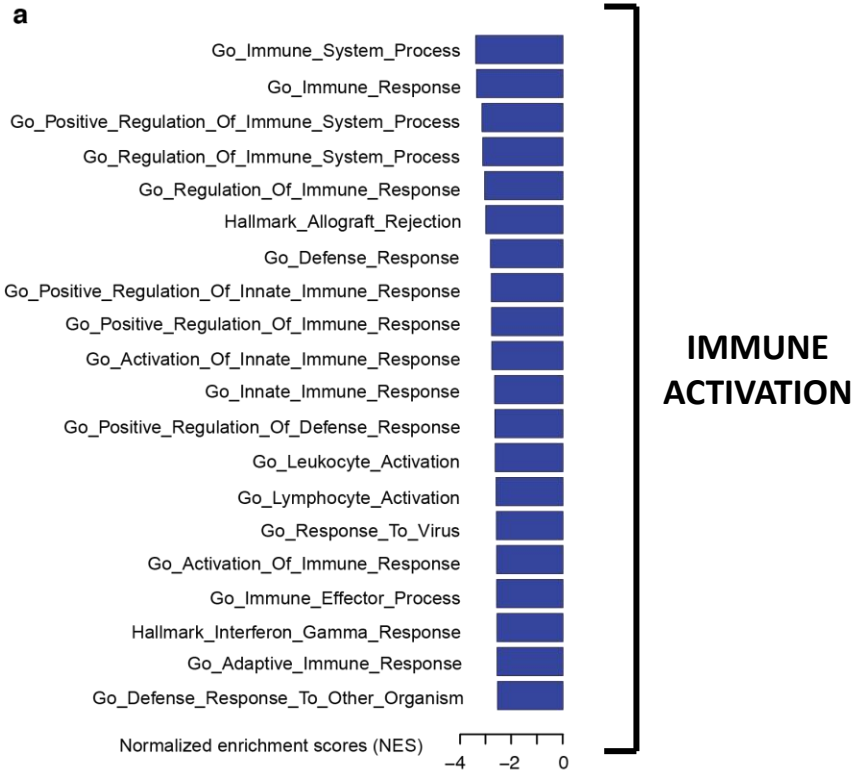
↓

Genes that correlate with serum adenosine levels

↓

Pathway analysis (GSEA on 5,000 Gene Ontology and Hallmark gene sets)

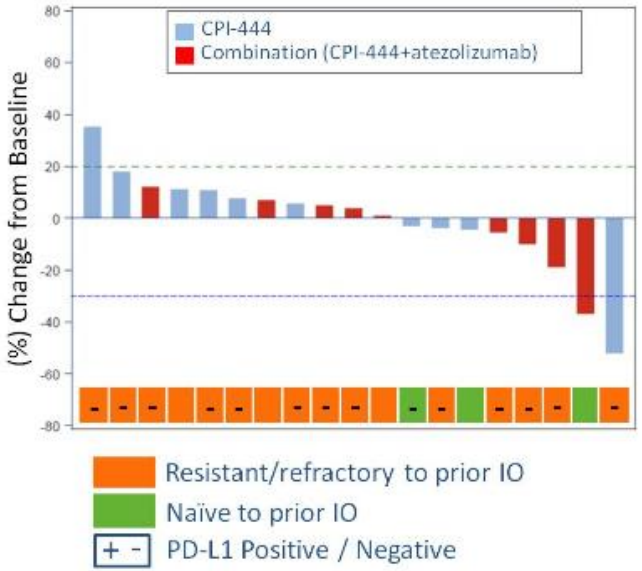
Top 20 pathways *negatively* correlated with serum adenosine levels

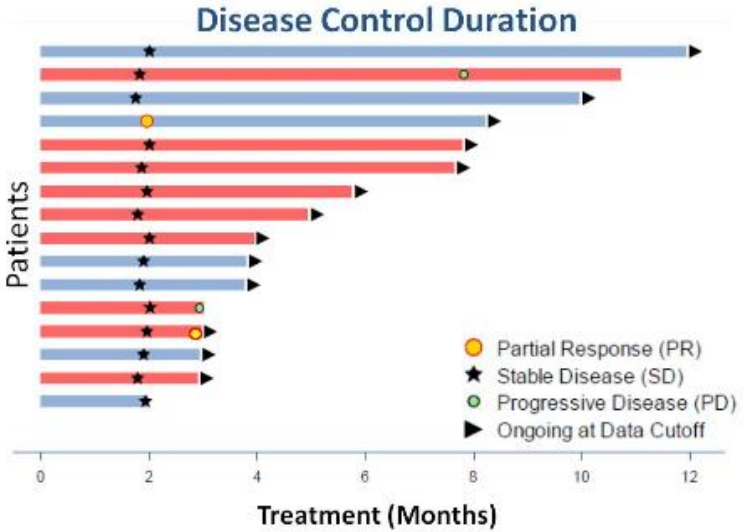


Combination of adenosinergic pathway inhibitors with immune-checkpoint blockade (A2AR inhibitor CPI-444 and PDL1 inhibitor atezolizumab)

Phase 1/1b Trial with CPI-444: Disease Control in Renal Cell Cancer

Partial responses can be seen in an anti-PD-1 progressing and naïve patients





Ongoing efforts with biomarkers in mRCC

- Exciting “potential” biomarkers at the:
  - IHC (protein level), mostly around PD-L1
  - WES/Genomics
  - RNA seq
  - Blood (e.g. metabolomics, T-cell repertoire)
  - ERVs
  - Microbiota*
- RCC currently does not have a “one size fits all biomarker”
  - Need larger samples, ideally from phase III trial with a non-IO control
- Combinations IO/IO, IO/VEGF can potentially complicate biomarkers discovery in mRCC

Microbiome

- "Gut microbiome and its role in host response to Immune Checkpoint Blockade for metastatic RCC"
  - PI: A. Lalani (JCC) with M. Surette (Farncombe)
  - In collaboration with B. Routy, others… and you?
  - Prospective, pilot study of fecal sample collection at start and on-treatment with immunotherapy
    - 16S RNA, metagenomic analysis
    - Merged with granular clinical data

Ongoing efforts with biomarkers in mRCC

- Exciting “potential” biomarkers at the:
  - IHC (protein level), mostly around PD-L1
  - WES/Genomics
  - RNA seq
  - Blood (e.g. metabolomics, T-cell repertoire)
  - Microbiota*
  - ERVs

- RCC currently does not have a “one size fits all biomarker”
  - Need larger samples, ideally from phase III trial with a non-IO control

- Combinations IO/IO, IO/VEGF can potentially complicate biomarkers discovery in mRCC
  - Other modalities: What about role of (immune potentiating) SBRT?

Cytoreductive **S**tereotactic **H**ypofractionated **R**adiotherapy With Combination **I**pilumimab/**N**ivolumab for Metastatic **K**idney Cancer (CYTOSHRINK)

Study Co-Chairs: A. Swaminath, A. Lalani, S. Hotte

McMaster University HEALTH SCIENCES

ONTARIO CLINICAL ONCOLOGY GROUP

Juravinski Cancer Centre HAMILTON HEALTH SCIENCES

CYTOSHRINK – Schema and endpoints

N=78

Inclusion criteria:
1. Biopsy proven metastatic renal cell carcinoma
2. Intermediate/poor risk disease based on IMDC criteria
3. Primary kidney lesion amenable to SBRT

Exclusion criteria:
1. Patient eligible for cytoreductive nephrectomy
2. Patient not eligible for first line immunotherapy
3. Previous abdominal radiation precluding SBRT

RANDOMIZATION

2:1

Experimental arm:
Cycle 1 Ipilimumab/Nivolumab
followed by

SBRT to primary kidney lesion
(30-40 Gy in 5 fractions)
followed by

Cycle 2-4 Ipilimumab/Nivolumab

Maintenance nivolumab per standard of care

Control arm:
Cycle 1-4 Ipilimumab/Nivolumab

Maintenance nivolumab per standard of care

1º endpoint:
1-year PFS rate
(75% experimental vs 50% control, 80% power, 2-sided α=0.1)

Key 2º endpoints:
Safety
ORR
QOL
Correlatives

Planned correlatives

- Circulating blood markers:
  - Baseline IL-6 and other cytokines/angiokines
  - Germline DNA and ctDNA
  - PBMCs
- Stool Microbiome:
  - 16S RNA
  - metagenomics






Summary

- Many exciting genomic correlates of ICB response → need validation: functional preclinical models, prospective clinical cohorts
  - Clinically annotated data w/ diversity of race, ethnicity, age, tumour histology…
- Future efforts focus on coupling with epigenomics, proteomics, metabolomics, microbiome
- Bioinformatic approaches needed to coordinate array of information
  - develop risk scores to capture driving alterations influencing ICB response
- Ultimately, we seek to maintain the remarkable recent progress in bringing rational options to our RCC patients

THANK YOU

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