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29 JUNE–2 JULY 2022

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LBA SO-34

Impact of $BRAF^{V600E}$ mutation on immunologic characteristics of the tumor microenvironment (TME) and associated genomic alterations in patients with microsatellite instability-high (MSI-H) or mismatch-repair–deficient (dMMR) colorectal cancer (CRC)

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Background

- Immune checkpoint inhibitors radically changed clinical management of patients with MSI/dMMR CRC. [1,2]
- The *BRAF*^{V600E} mutation is associated with the hypermethylator phenotype CIMP (CpG island methylator phenotype), which can also lead to the microsatellite instability-high (MSI-H) phenotype through hypermethylation of the MLH1 gene promoter.
- Coexisting *BRAF*^{V600E} mutation and MSI-H/dMMR status intertwine and constitute the CMS1 molecular subtype of colorectal cancer.
- The incidence of MSI-H *BRAF*^{V600E}-mutated mCRC in the total population is estimated to be approximately 1% to 2%.[3]
- However, the Impact of *BRAF*^{V600E} mutation on immunologic characteristics of the tumor microenvironment (TME) and associated genomic alterations in patients with MSI-H/dMMR colorectal cancer (CRC), has not been well described.

[1]Andre T. et al, NEJM 2020.
[2]Overman MJ, et al. Lancet Oncol. 2017
[3]Venderbosch S et al. Clin Cancer Res 2014;20:5322-30

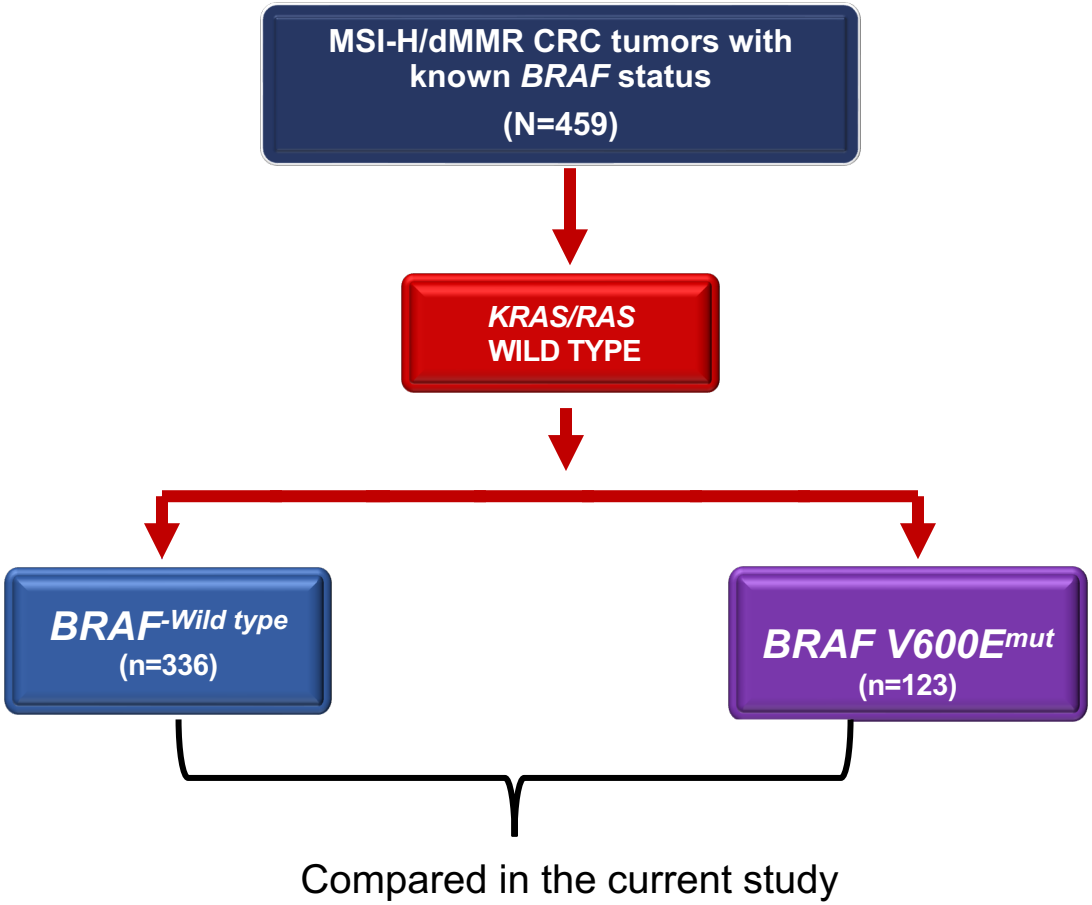
Objectives

- Primary Aim:
  - To examine the impact of *BRAF*^{V600E} mutation on immunologic characteristics of the tumor microenvironment (TME) in patients with MSI-H/dMMR CRC.
- Secondary Aims :
  - To describe associated genomic alterations with *BRAF*^{V600E}, and
  - Examine the relationship between *BRAF*^{V600E} and immuno-oncology (IO) biomarkers
  - To examine the impact of *KRAS* mutation on TME in patients with MSI-H/dMMR CRC.

Methods

- A retrospective review of patients with MSI-H/dMMR CRC that underwent Tempus xT next-generation sequencing was performed. The Tempus platform was employed for the following:
  - MSI-H was determined by assessment of 239 loci and dMMR by IHC
  - Tumor mutational burden (TMB), neoantigen tumor burden (NTB, NeoScan), PD-L1 expression, immune infiltration, and canonical immuno-metabolomic pathways were assessed
- Chi-squared/Fisher's Exact tests or Wilcoxon rank-sum tests compared:
  - IO-biomarkers between *BRAF*^{V600E} and *BRAF* wild-type groups
  - And prevalence of other oncogenes between *BRAF*^{V600E} and *BRAF* wild-type groups (false-discovery corrected)
- Gene Set Variation Analysis (GSVA) was implemented to calculate enrichment scores of a priori defined immuno-metabolomic pathways. Differential expression analysis compared pathway enrichment scores between *BRAF*^{V600E} and *BRAF* wild-type groups.

Results

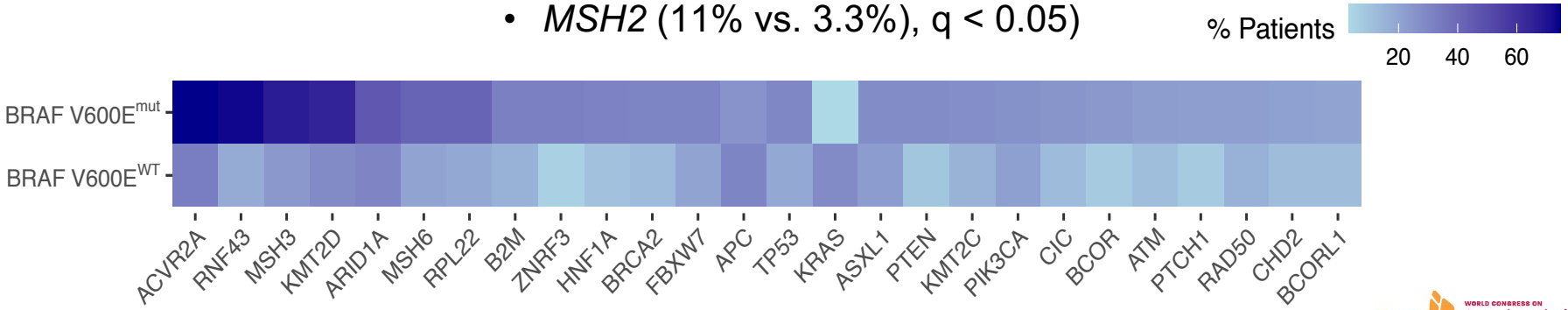

MSI-H/dMMR CRC tumors with known BRAF status
(N=459)
KRAS/RAS WILD TYPE
BRAF-Wild type
(n=336)
BRAF V600Emut
(n=123)
Compared in the current study

Clinical Characteristics

Characteristic	Overall, N=459	*BRAF*^{WT}, n=336	*BRAF V600E*^{mut}, n=123
***Gender, n (%)**			
Female	269 (59%)	185 (55%)	84 (69%)
Unknown	1	0	1
***Age, Median (IQR)**	69 (57, 78)	62 (51, 73)	76 (70, 85)
Unknown	126	108	18
Race, n (%)			
White	227 (83%)	170 (82%)	57 (86%)
Black/African American	20 (7.3%)	16 (7.7%)	4 (6.1%)
Asian	3 (1.1%)	3 (1.4%)	0 (0%)
Other	24 (8.8%)	19 (9.1%)	5 (7.6%)
Unknown	185	128	57
Stage, n (%)			
Stage I	19 (5.0%)	17 (6.0%)	2 (2.0%)
Stage II	81 (21%)	60 (21%)	21 (21%)
Stage III	90 (24%)	63 (22%)	27 (27%)
Stage IV	190 (50%)	141 (50%)	49 (49%)
Unknown	79	55	24

Oncogenic co-mutations in *BRAF V600E*^{mut} vs. *BRAF*^{wt} *CRC*

- Significant differences in co-occurring genomic mutations with *BRAF V600E*^{mut} compared to *BRAF*^{wt} in MSI-H/dMMR CRC were observed in the following genes:
  - *MSH6* (42% vs. 20%, q < 0.05),
  - *B2M* (33% vs. 16%, q < 0.05),
  - *ATM* (23% vs. 12%, q < 0.05),
  - *TP53* (30% vs. 19%, q < 0.05),
  - *MSH2* (11% vs. 3.3%), q < 0.05)



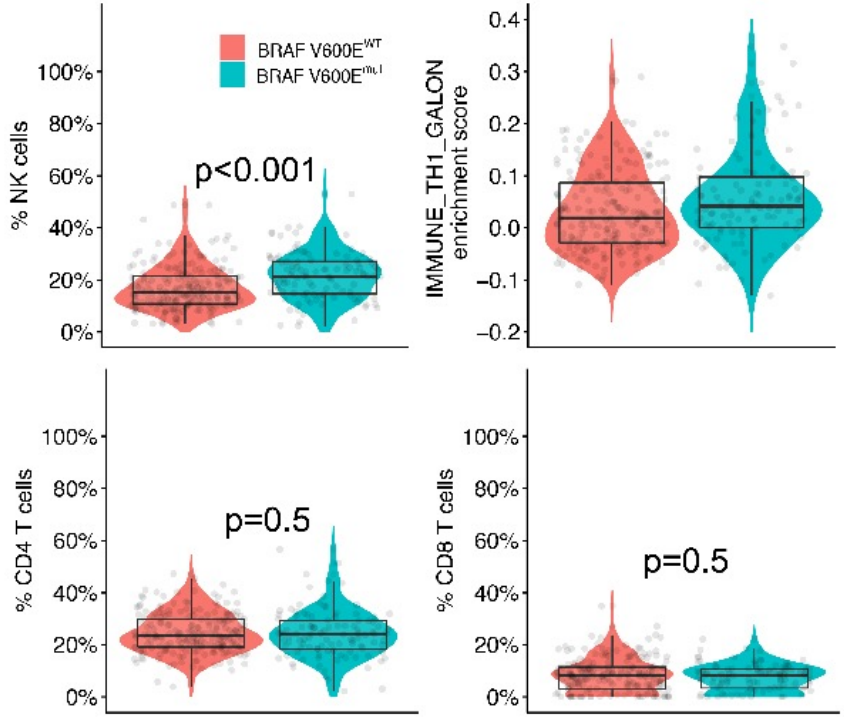
BRAF^{wt} and ^{mut} MSI-H CRC tumors have the same Neoantigen Mutation Burden

Characteristic	Overall, N=459	*BRAF*^{WT}, n=336	*BRAF V600E*^{mut}, n=123	p-value
MSI-H, n (%)	444 (98%)	321 (97%)	123 (100%)	0.041*
Unknown	4	4	0	
TMB-H, n (%)	428 (96%)	305 (95%)	123 (100%)	0.008*
Unknown	15	15	0	
NTB, Median (IQR)	15 (10, 20)	15 (10, 20)	15 (12, 20)	0.4
Unknown	38	35	3	
†**PDL-1+, n (%)**	37 (26%)	27 (23%)	10 (36%)	0.2
Unknown	316	221	95	

TMB: *tumor mutational Burden (Mb^{-1})****; NTB:*** *Neoantigen mutation burden (Mb^{-1})*

Tumor Immune Microenvironment

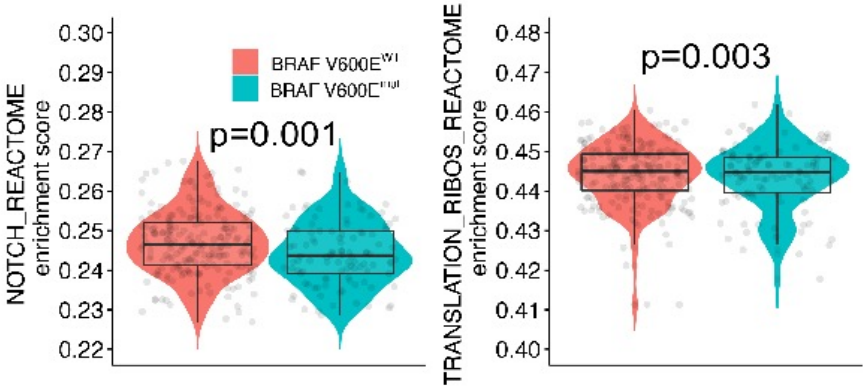
BRAF V600E^{mut} Impacts Colorectal Tumor Immune Microenvironment*

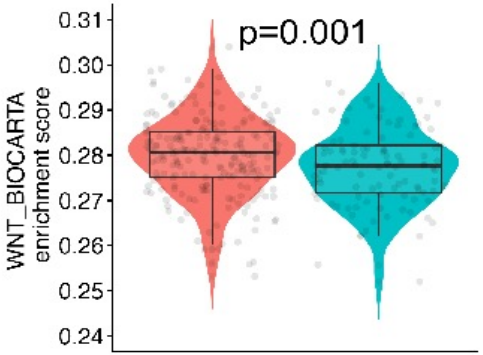


- The proportion of natural killer (NK) cells was significantly higher in *BRAF V600E*^{mut} compared with *BRAF*^{wt} (median 21% vs. 15%, p <0.001); however, no significant differences were found amongst CD4+ and CD8+ T cells.
- IMMUNE_TH1_GALON showed significant up-regulation in *BRAF V600E*^{mut} tumors

*Immune cells were estimated through RNA-seq

Cancer Stem Cell Pathways Are Downregulated in *BRAF V600E*^{mut} Tumor

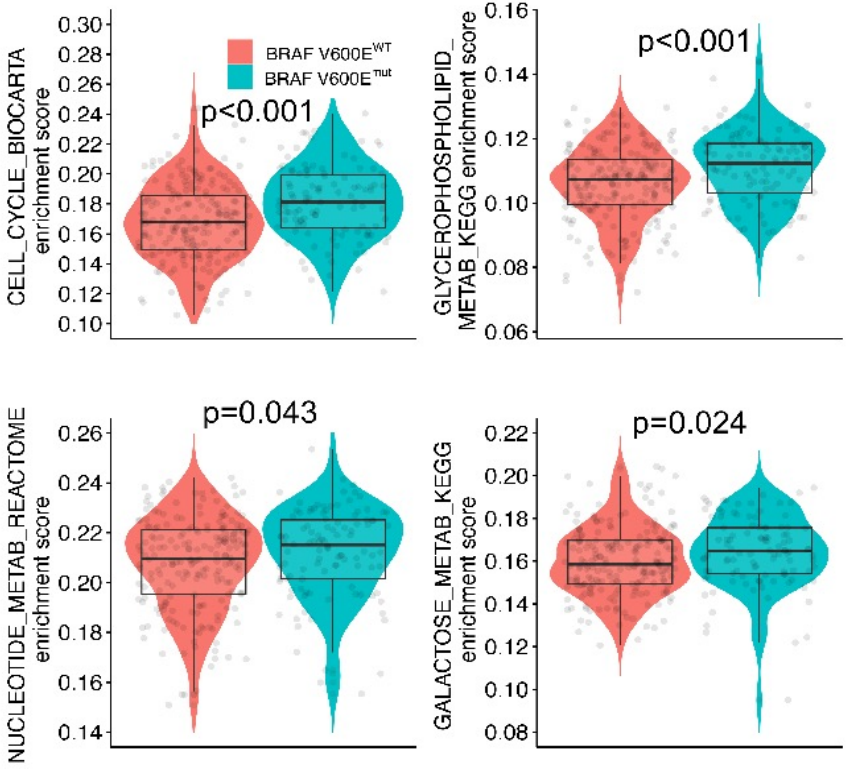




Cancer Stem Cell pathways showed significant downregulation including Notch and WNT/Catenin signaling pathways.

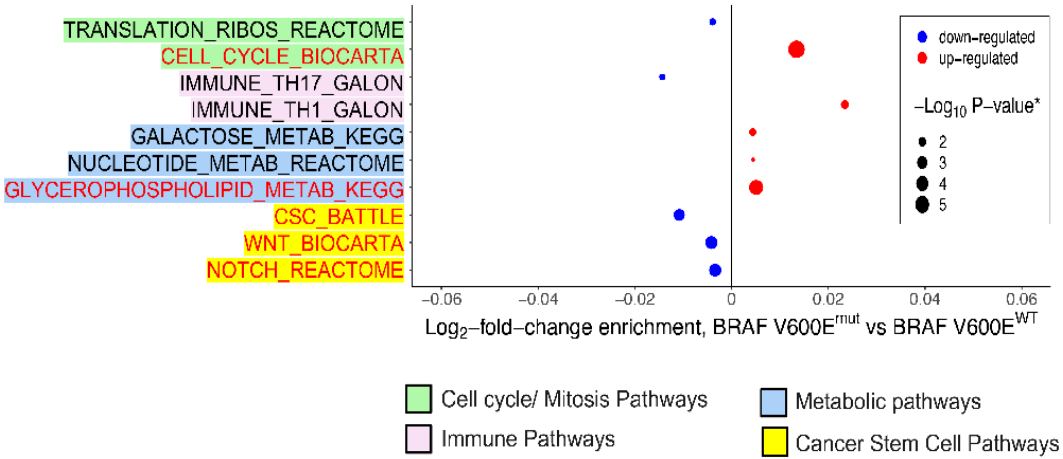
**P values* are unadjusted for false discovery

BRAF V600E^{mut} CRCs Show Accelerated Growth and Metabolic Reprograming



Significant upregulation of 5 pathways among *BRAF V600E*^{mut} tumors, 4 of which were cyclin-dependent cell signaling, glycerophospholipid metabolism, galactose metabolism and nucleotide metabolism.

Immune-related Pathways Differentially Expressed by *BRAF V600E*^{mut} Status



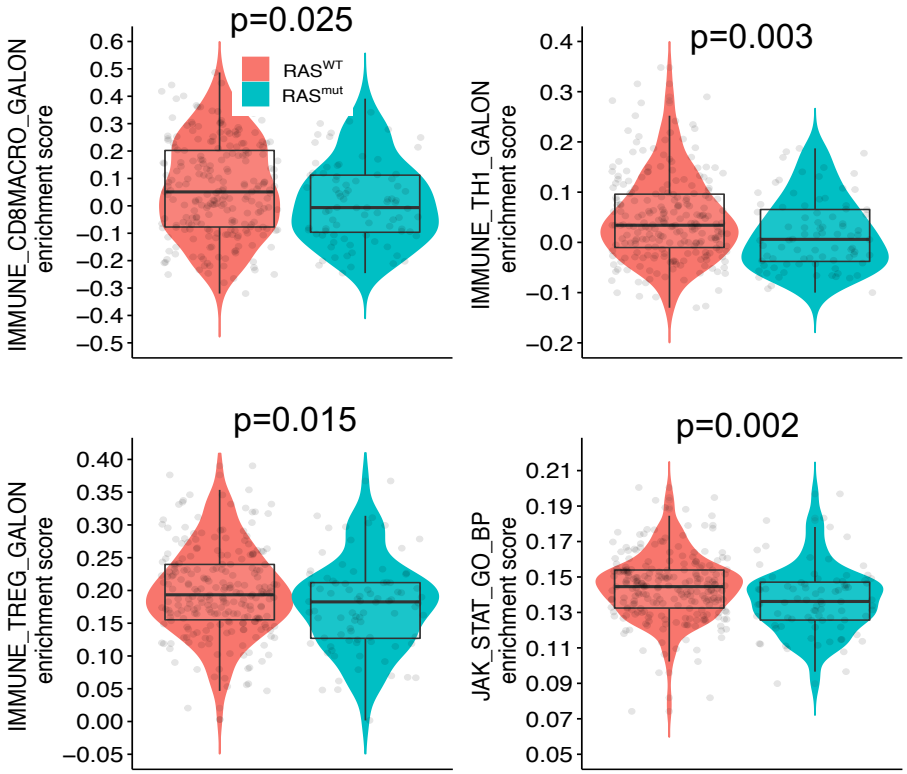
Pathway enrichment scores computed through GSVA were compared between *BRAF V600E*^{mut} and *BRAF*^{wt} via differential expression analysis. Differentially expressed pathways (at 5% alpha level) are shown and pathways differentially expressed after false discovery adjustment are represented in red text. Gene set analysis of canonical immune pathways showed limited differences in tumor inflammation between *BRAF V600E*^{mut} and WT tumor.

RAS^{mut} CRC tumors have lower Neoantigen Mutation Burden than *RAS*^{wt}

Characteristic	Overall, N=463	*RAS*^{WT}, n=353	*RAS*^{mut}, n=110	p-Value
MSI-H, n (%)	446 (97%)	340 (97%)	106 (97%)	0.8
Unknown	5	4	1	
TMB-H, n (%)	432 (96%)	328 (97%)	104 (95%)	0.2
Unknown	15	15	0	
***NTB, Median (IQR)**	15 (10, 20)	16 (12, 20)	12 (9, 18)	<0.001
Unknown	39	36	3	
†**PDL-1+, n (%)**	39 (27%)	33 (31%)	6 (15%)	0.058
Unknown	318	247	71	

TMB: *tumor mutational Burden (Mb^{-1})****; NTB:*** *Neoantigen mutation burden (Mb^{-1})*

RAS^{mut} MSI-H CRC tumors are less inflamed than *RAS*^{wt}

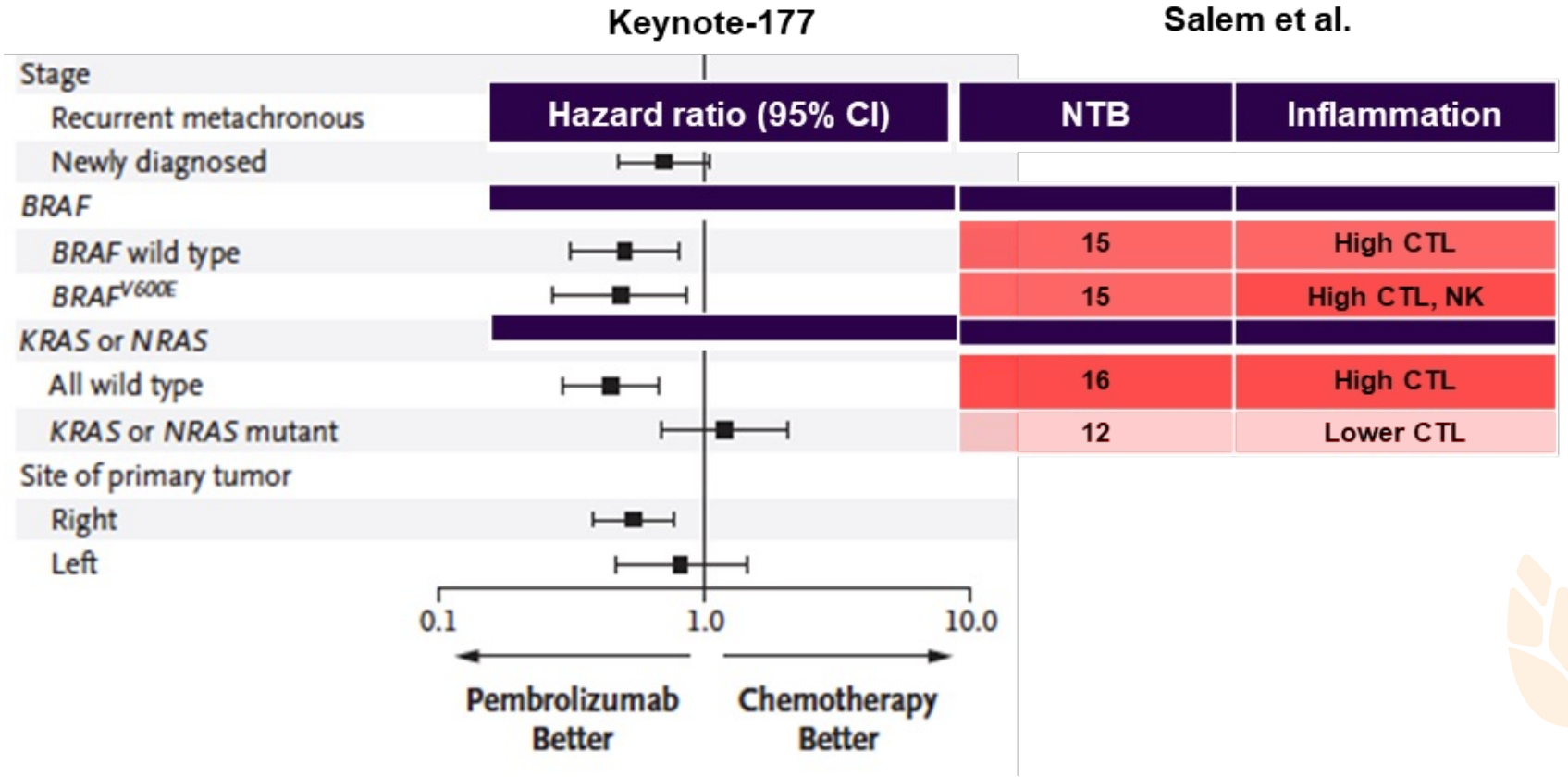


Tumor inflammation is mainly downregulated in *RAS*^{mut} tumors, including key pathways: cytokine signaling (JAK-STAT, TH1), and adaptive immune events (CD8+ T cell, Tregs).

Limitations

- The retrospective nature of the analysis
- Treatment and outcome data were not available for the entirety of this dataset
- We used a TMB cut-off of 10 mut/Mb to define TMB-high vs. -low across all tumor types but the optimal threshold, if any, to identify a cancer as TMB-high for ICI treatment selection remains the subject of much debate
- Missing data on the PD-L1 expression

Summary of Findings



Conclusions

- *BRAF V600E*^{mut} and *BRAF*^{wt} MSI-H/dMMR CRCs exhibited similar NTB and T cell infiltration. They exhibited hyperproliferative characteristics associated with broad metabolic reprogramming.

- *BRAF V600E*^{mut} and *BRAF*^{wt} MSI-H/dMMR CRCs appeared to have similar immunologic characteristics within their microenvironment. They are equally likely to respond well to immune checkpoint inhibitors.

- *RAS*^{mut} MSI-H/dMMR CRCs had lower neoantigen production and lower tumor inflammation than *RAS*^{WT} tumors.

- Overall, these data suggest that *RAS*^{mut} CRCs are less immunogenic than *BRAF V600E*^{mut} MSI-H/dMMR CRCs and have a microenvironment that may be less sensitive to immune checkpoint blockade.

- Further studies are required to validate this finding.

THANK YOU