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CMS subtype characterized by high TMB shows immunosuppressive microenvironment that implies resistance to immunotherapy

CONTRIBUTING RESEARCHERS

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BACKGROUND

Tumor mutational burden (TMB) is emerging as an **important biomarker for immune checkpoint therapy (ICT)** response. Yet even in the context of high TMB, **ICT are likely ineffective in an immuno-suppressed microenvironment**. Here we demonstrate that a well-characterized subtype of CRC, **CMS2,** associated with Wnt pathway activation, **is immunosuppressive despite high TMB**.

METHODS

- Tumor/normal-paired DNAseq (WGS or WES) and deep RNAseq (~200x10^{6}reads per tumor) was performed on 464 GI tumors from a commercial database

- TMB was calculated by observing all somatic-specific non-synonymous exonic mutations as previously established (Rizvi et al, 2015)

- Each sample was assigned to one of the colorectal Consensus Molecular subtypes (CMS) based on RNA classification

- A curated panel of 109 genes that discriminate between 22 immune subsets was identified. A database containing 1467 unselected tumors was used to define a distribution of expression for each signature

- Somatic-specific pathogenic/likely pathogenic mutations were identified using ClinVar annotations

- Significant enrichment was analyzed between immune subsets, CMS types, TMB status, and somatic mutational status

RESULTS

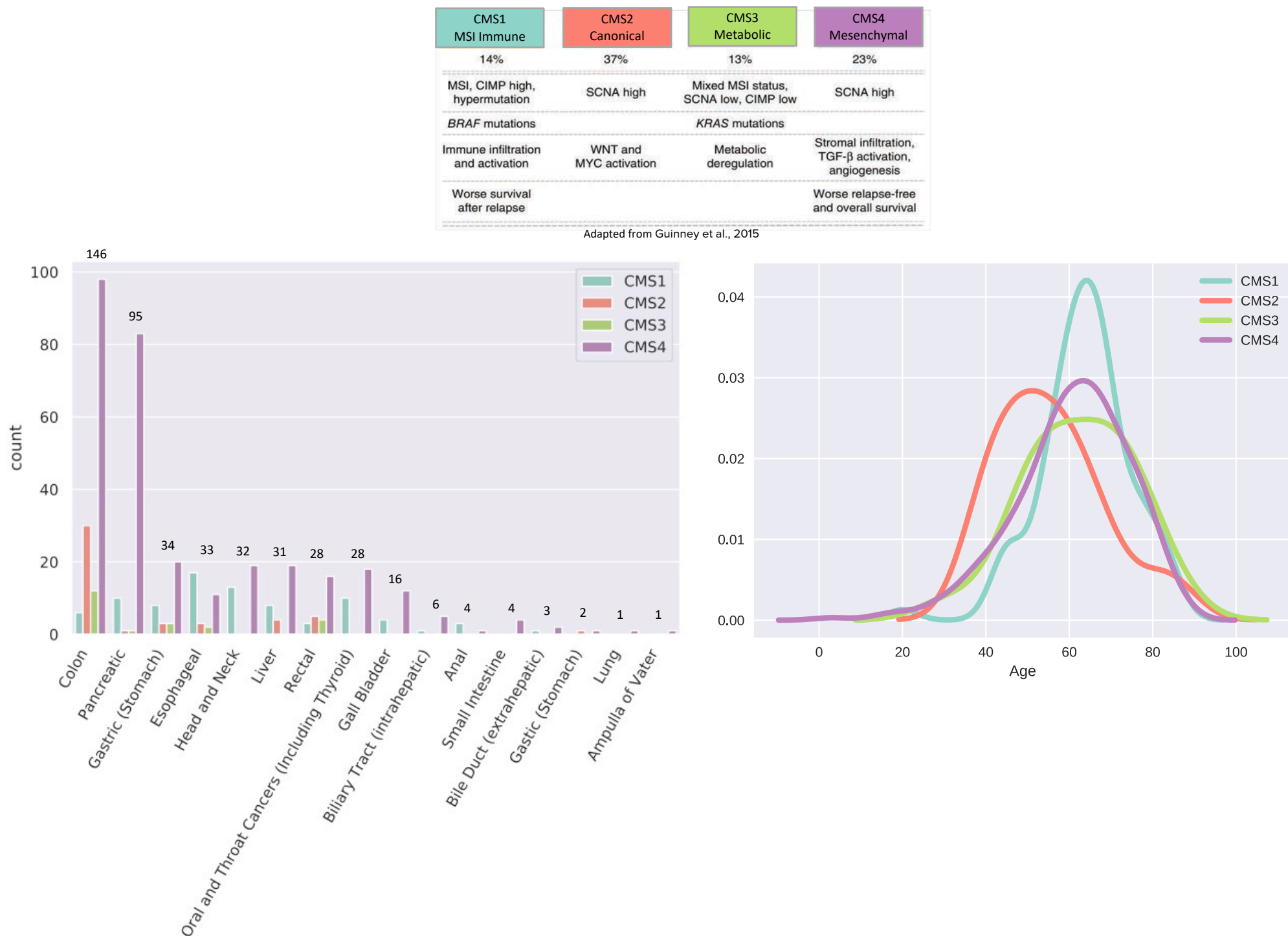Figure 1. **NantHealth clinical GI cases database**

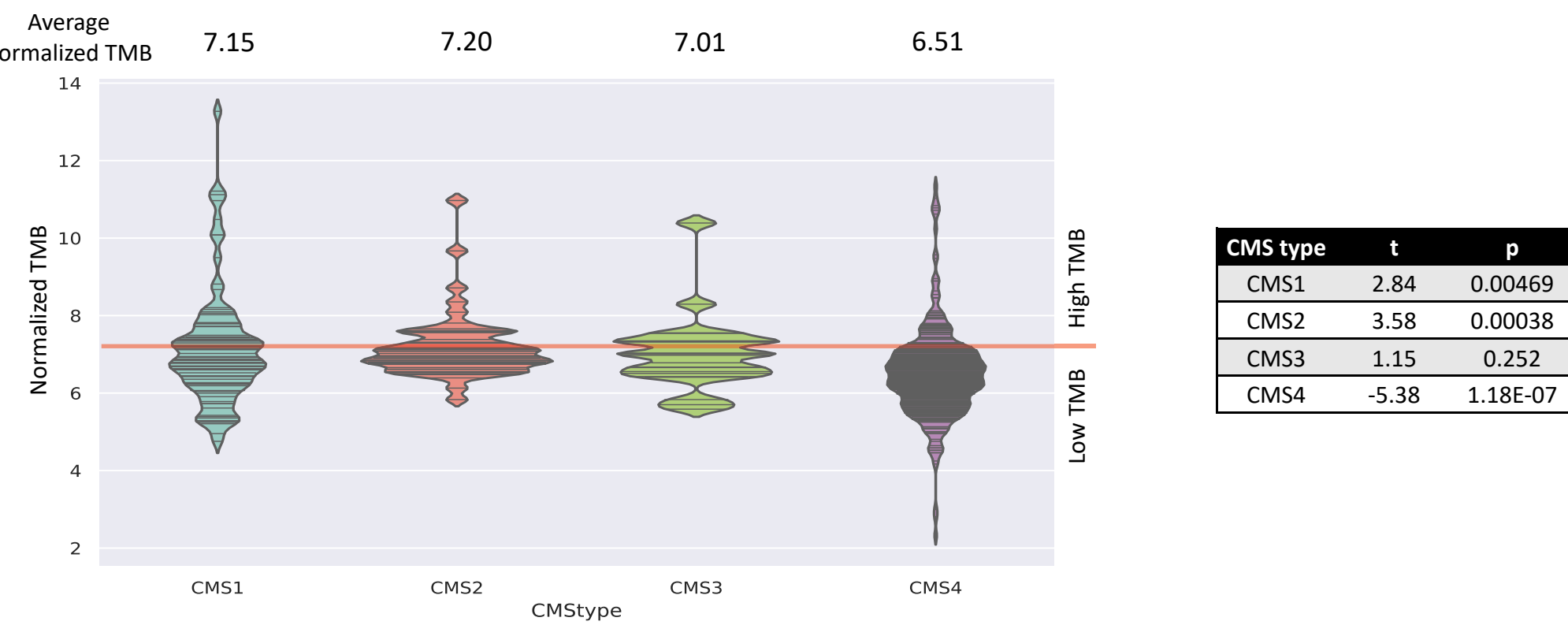Figure 2. **CMS1 and CMS2 have significantly elevated TMB**

CMS type	t	p
CMS1	2.84	0.00469
CMS2	3.58	0.00038
CMS3	1.15	0.252
CMS4	-5.38	1.18E-07

Figure 3. **CMS1 has high checkpoint expression, CMS2 has low checkpoint expression**

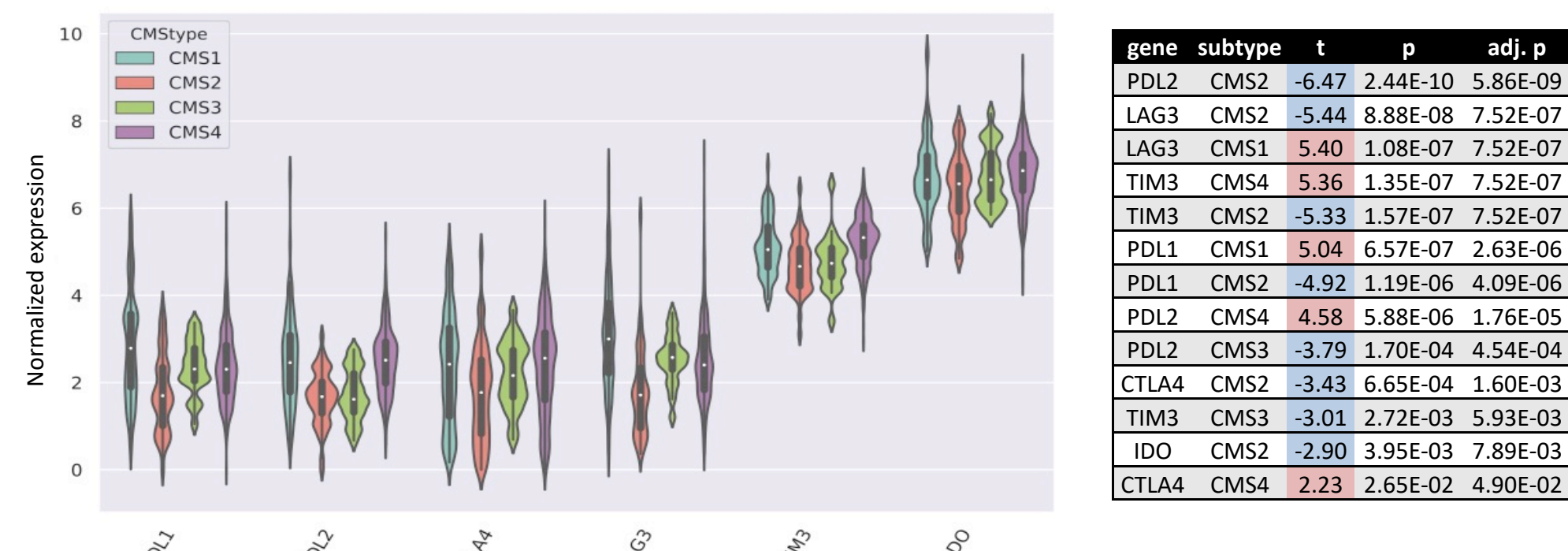| gene	subtype	t	p	adj. p
PDL2	CMS2	-6.47	2.44E-10	5.86E-09
LAG3	CMS2	-5.44	8.88E-08	7.52E-07
LAG3	CMS2	5.40	1.08E-07	7.52E-07
TIM3	CMS2	5.36	1.35E-07	7.52E-07
TIM3	CMS2	-5.33	1.57E-07	7.52E-07
PDL1	CMS2	5.04	6.57E-07	2.63E-06
PDL1	CMS2	-4.92	1.19E-06	4.09E-06
PDL2	CMS4	4.58	5.88E-06	1.76E-05
CTLA4	CMS2	-3.79	1.70E-04	4.54E-04
CTLA4	CMS2	-3.43	6.65E-04	1.60E-03
TIM3	CMS3	-3.01	2.72E-03	5.93E-03
IDO	CMS2	-2.90	3.95E-03	7.89E-03
CTLA4	CMS4	2.23	2.65E-02	4.90E-02

Figure 4. **CMS2 is significantly associated with lower inferred immune infiltrate activity, especially in effector memory cells**

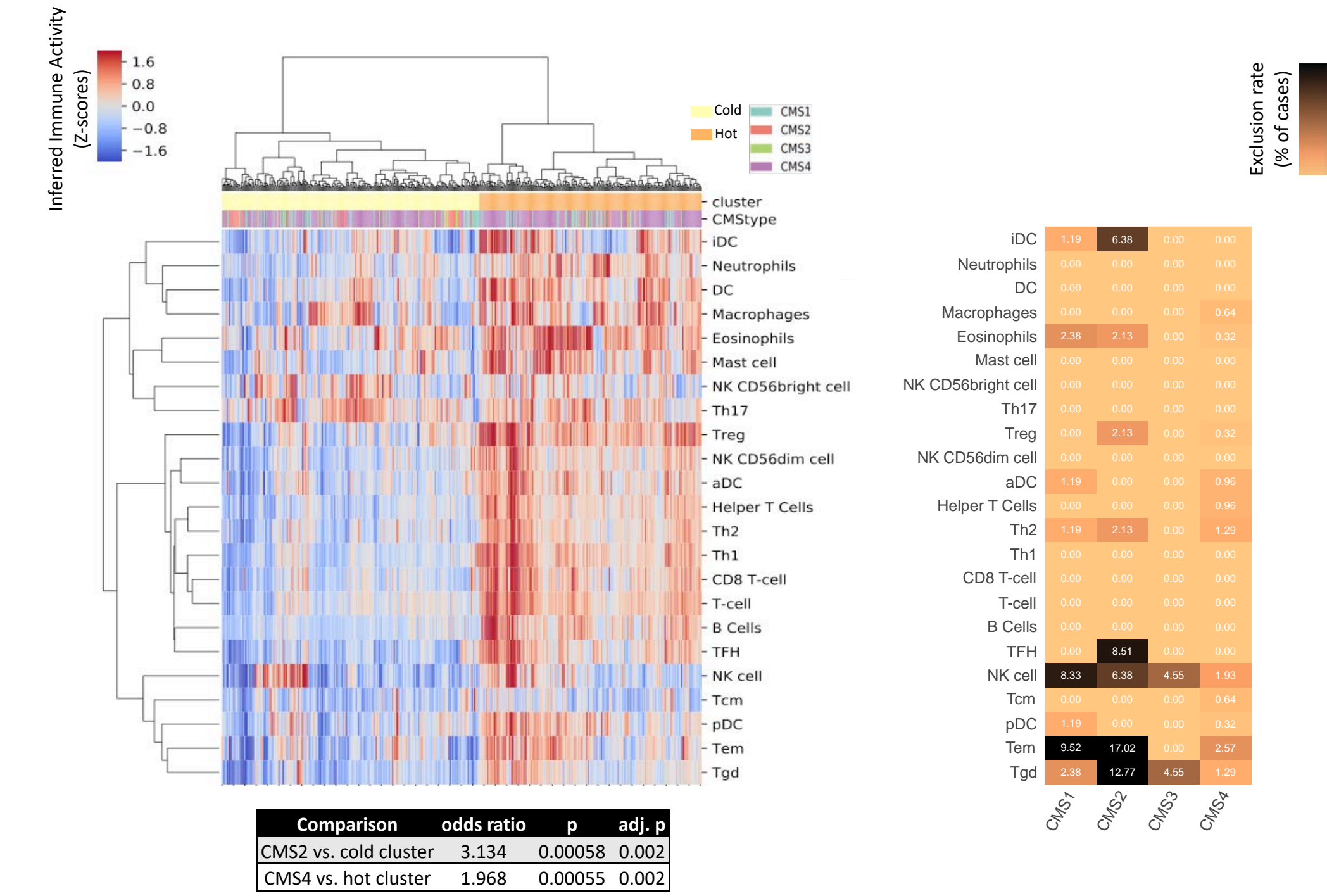| Comparison	odds ratio	p	adj. p
CMS2 vs. cold cluster	3.134	0.00058	0.002
CMS4 vs. hot cluster	1.968	0.00055	0.002

Figure 5. **CMS2 is significantly associated with likely pathogenic Wnt-family APC mutations**

comparison	odd ratio	p	adj. p
CMS2 vs. APC	8.09	1.12E-10	1.34E-08
CMS2 vs. TP53	6.86	1.71E-08	1.02E-06
CMS1 vs. KMT2D	5.70	2.06E-05	8.22E-04
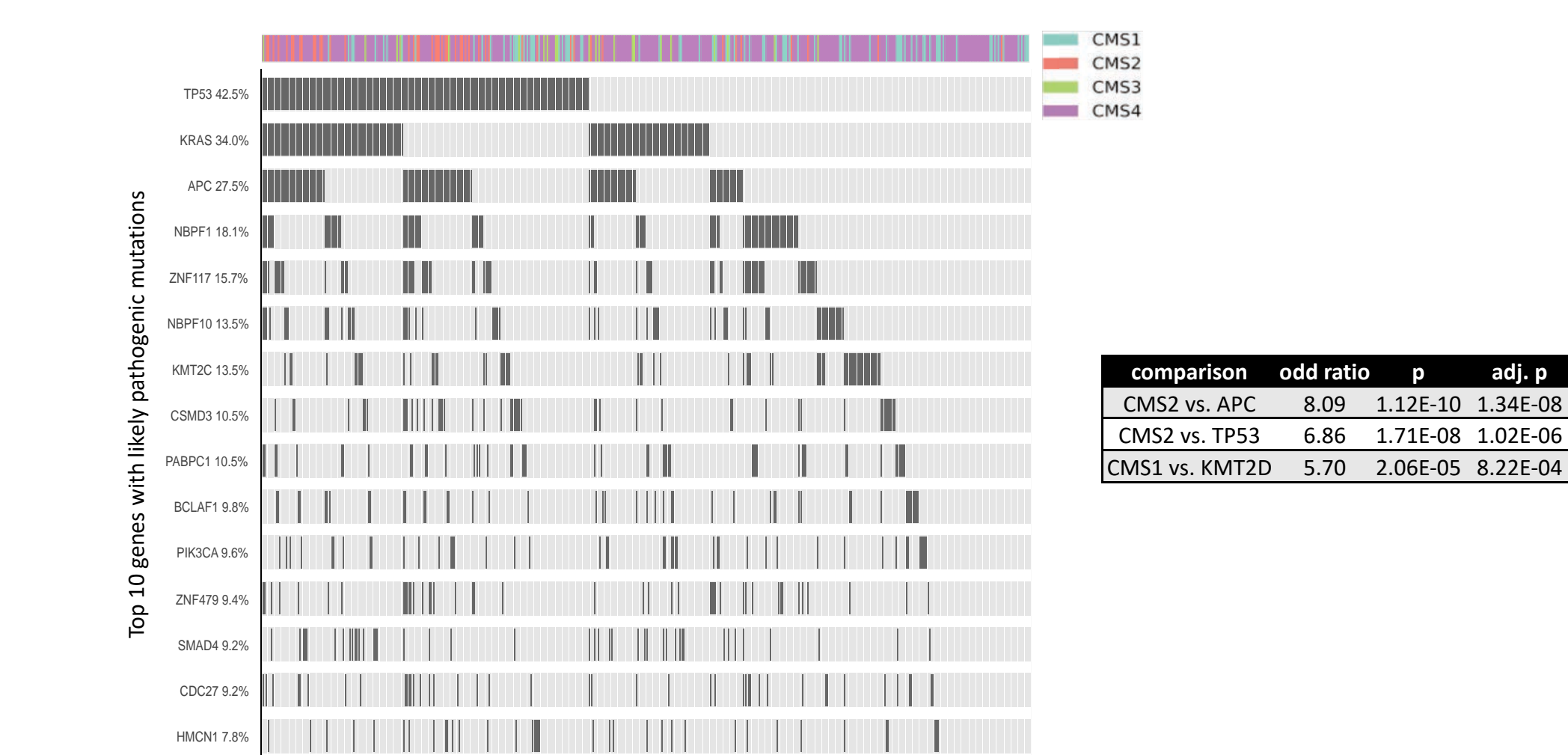
Figure 6. **WNT activity can modulate immune response through Tcm→Tem blockade**

Adapted from Gattinoni et al, 2010
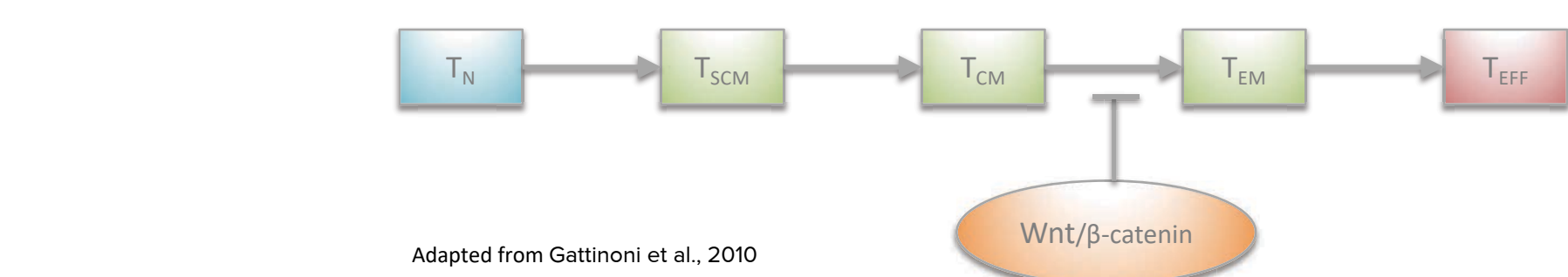
KEY FINDINGS

- CMS1 & CMS2 have significantly higher TMB

- CMS1 (MSI-enriched) expresses selected TME markers more than other subtypes

- Perplexingly, CMS2 had significantly lower expression of 6 targetable checkpoint markers

- As expected, CMS2 tumors were significantly enriched for likely pathogenic variants in the Wnt-associated gene APC

- Immune-deconvolution indicated substantial exclusion of Tem cells from CMS2 tumors, in line with Wnt/β-catenin blockade of Tcm→Tem maturation for immunoreactivity.

CONCLUSIONS:

The most common subtype of CRC, CMS2 (~37% in unselected populations), is highly immunosuppressive despite high TMB. ICT is only effective in an immunologically active microenvironment. TMB alone as a biomarker likely is insufficient to indicate the effectiveness of immunotherapy.

References

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