Society for Immunotherapy of Cancer

Advances in Cancer Immunotherapy™

Brief Primer for the Patient and Clinician on Use of NGS for Choosing Immunotherapy

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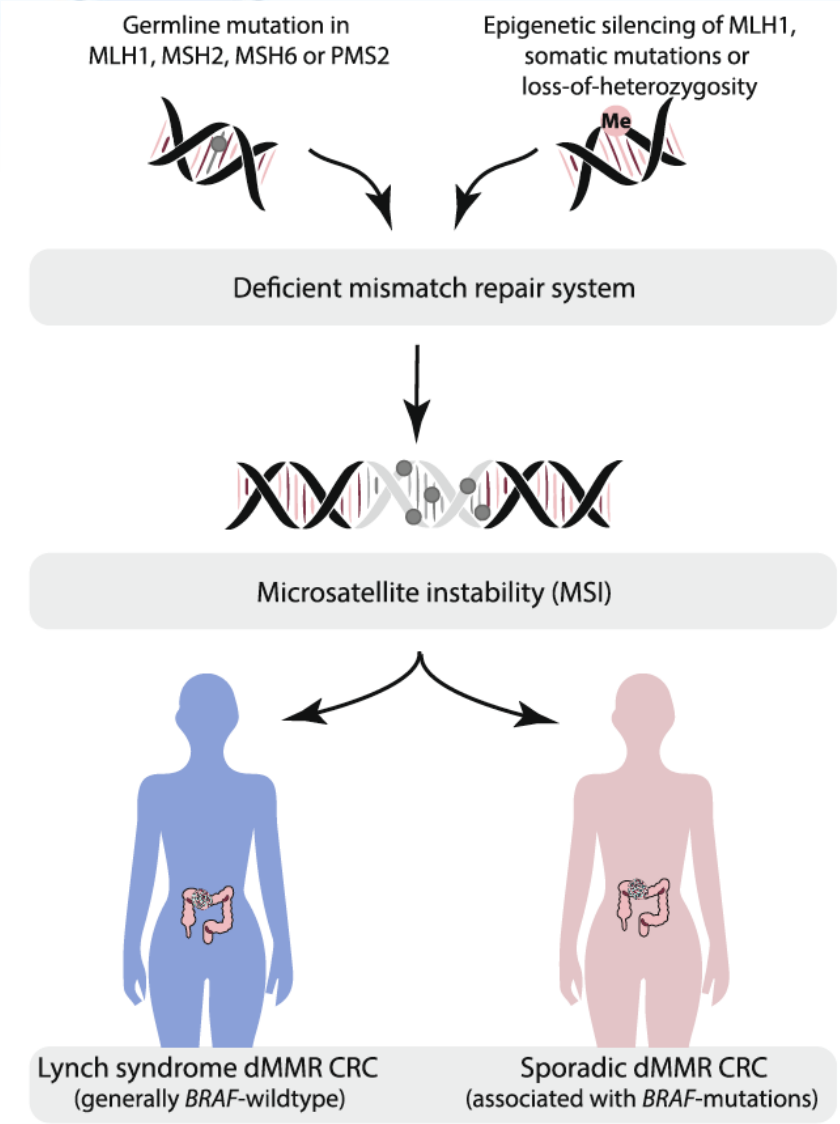
Disclosures

- Immediate family member serves as a consultant in Ophthalmology for:
  - Alcon, Adverum, Gyroscope Therapeutics Limited, Neurogene, and RegenexBio

- I will **not** be discussing non-FDA approved indications during my presentation.

Overview

- Review terminology
- Technologies for determining mismatch repair deficiency and microsatellite instability
- Determining tumor mutational burden
- Germline implications

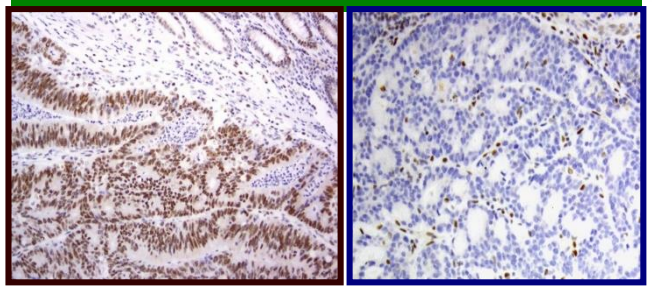


- A deficient MMR system leads to MSI.
- Microsatellites are tandem repeats of nucleotides, which are prone to errors during DNA replication
- Tumor acquired MSI is the hallmark of MMR deficiency.

Wensink et al. *Cancer Treatment Reviews* 2021

Determining MMR-D and MSI: Older Methods

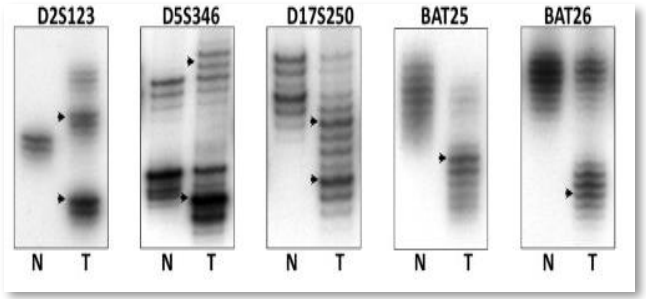
Immunohistochemical (IHC) staining for MMR proteins



- IHC staining detects the presence and absence of MMR proteins (MLH1, MSH2, MSH6, PMS2)
- **MMR-deficiency (MMR-D) is defined at least 1 protein showing loss of expression**

*Pathology Slides, Courtesy of Dr. Jinru Shia

PCR-based Microsatellite instability analysis



- Poymerase chain reactions (PCR) compares the length of nucleotide repeats in tumor cells and normal cells
- Differences in peak patterns between normal vs tumor indicate MSI
- Established set of microsatellites are assessed
- **MSI-H tumors are those showing instability at ≧ 2 markers**

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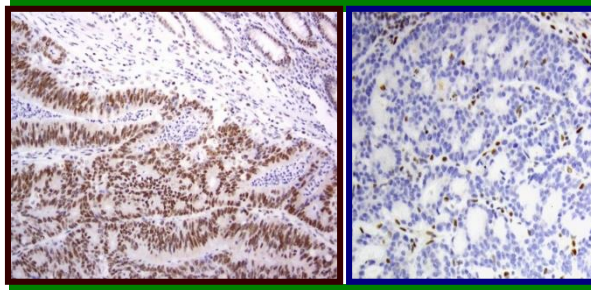
Comparison of IHC and PCR-based MSI Analysis

- 646 tumors analyzed; 88% with CRC

Immunohistochemical staining for MMR proteins



PCR-based Microsatellite instability analysis

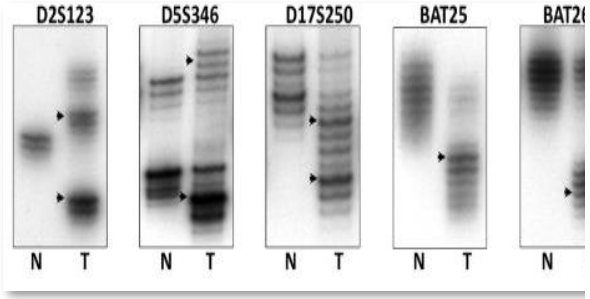


Table 3. Summary of concordances and discordances between immunohistochemical and MSI analysis[a]

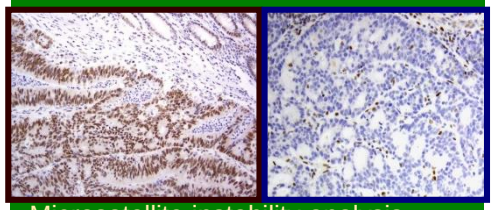
	N (%)	95% CI
MSI-high ($N = 102$)		
IHC shows loss of expression of at least one MMR protein	90 (88.24)	80.35–93.77
IHC shows intact expression of all 4 proteins	12 (11.76)	6.23–19.65
MS-stable ($N = 489$)		
IHC shows loss of expression of at least one MMR protein	1 (0.20)	0.01–1.13
IHC shows intact expression of all 4 proteins	488 (99.80)	98.87–99.99
IHC—loss of at least one MMR protein ($N = 91$)		
MSI-high	90 (98.90)	94.03–99.97
MS-stable	1 (1.10)	0.03–5.97
IHC—intact MMR protein expression ($N = 500$)		
MSI-high	12 (2.40)	1.25–4.15
MS-stable	488 (97.60)	95.85–98.75
Overall agreement		
IHC shows loss of expression of at least one MMR protein and MSI-high or IHC shows intact expression of all 4 proteins and MSI-stable	578 (97.80)	96.27–98.82
IHC shows loss of expression of at least one MMR protein and MSI-stable or IHC shows intact expression of all 4 proteins and MSI-high	13 (2.20)	1.18–3.73

Bartley et al., *Cancer Prev Res:* 5(2) Feb 2012

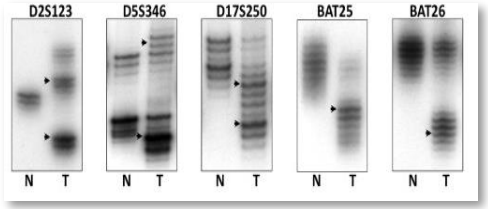
Determination of MSI via Next Generation Sequencing (NGS) Technologies

Older methods:

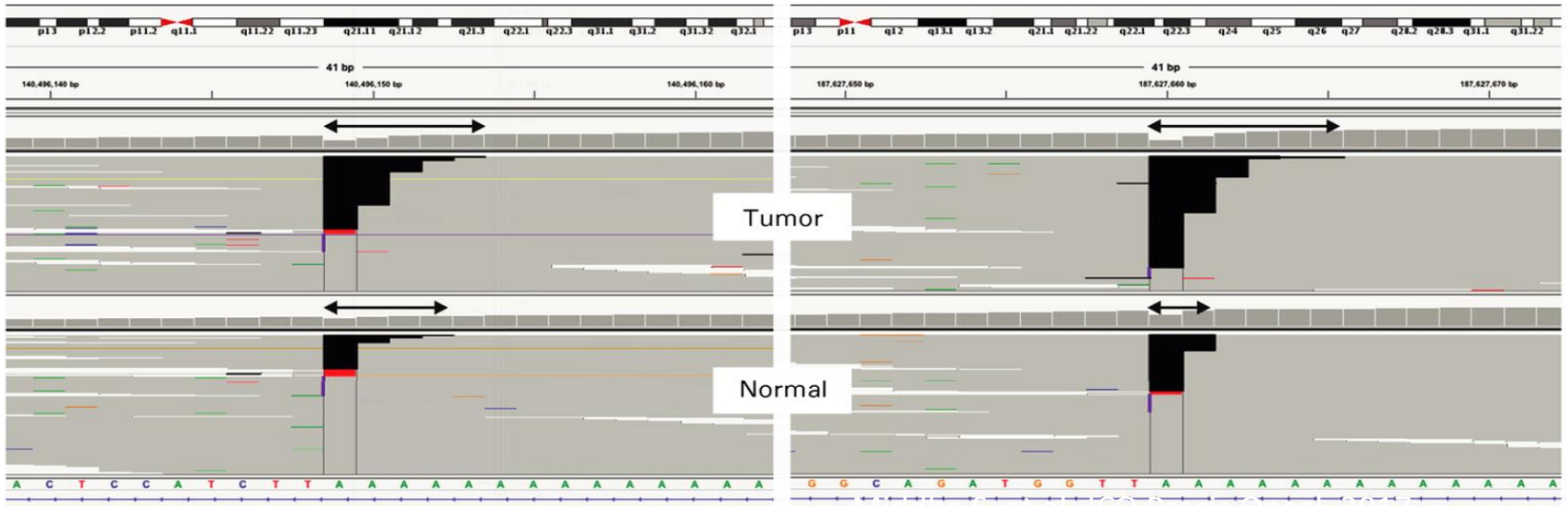
Immunohistochemical staining for MMR proteins



Microsatellite instability analysis (PCR-based assay)



New technique: MSI-NGS



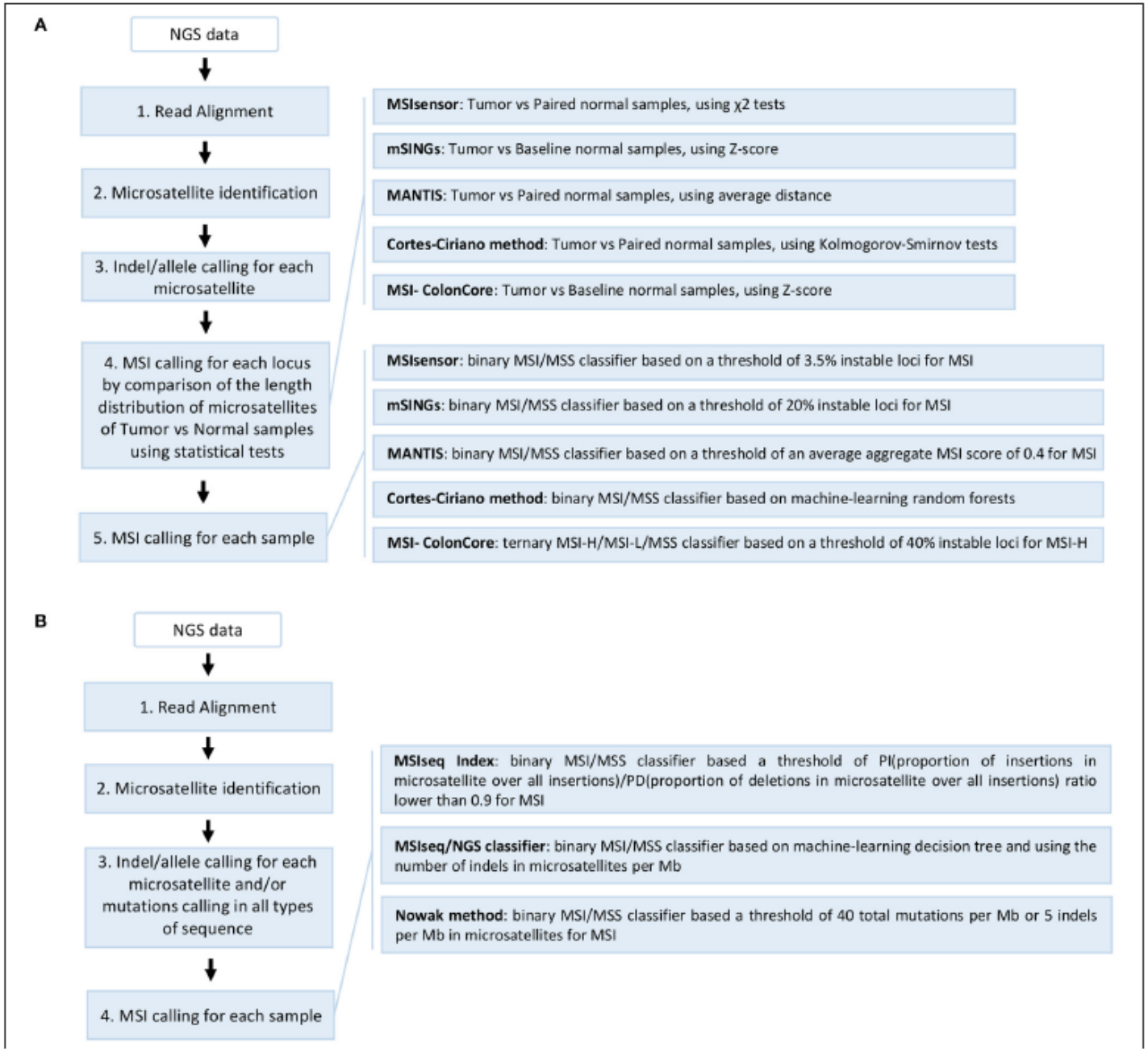
Middha et al., JCO Po 2017

The Appeal of Using Tumor NGS for MSI Analyses

- Incorporation of many microsatellite loci into NGS assays is relatively easy
- Computational MSI analyses may be incorporated into existing NGS pipelines
- Resource efficient in terms of tissue use
- Identification of MSI in tumors not routinely tested for MSI

NGS Technologies for MSI Calling

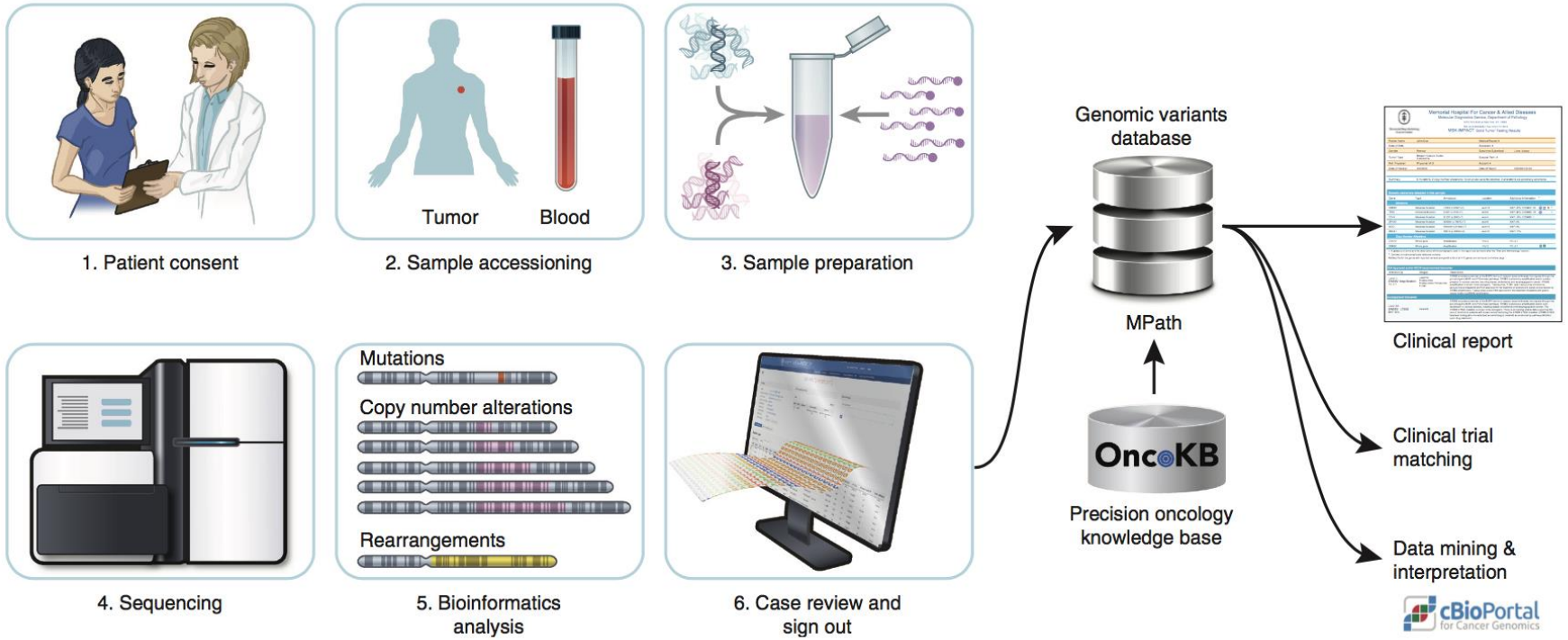
- *Direct* assessment of microsatellite loci in DNA
  - MSISensor, mSINGs, MANTIS etc.
- *Indirect* assessment of MSI via somatic mutations
  - MSIseq, Mutational load, RNA-seq data



Baudrin et al., *Frontiers in Oncology* 2018

MSK-IMPACT Workflow (Integrated Mutation Profiling of Actionable Cancer Targets)

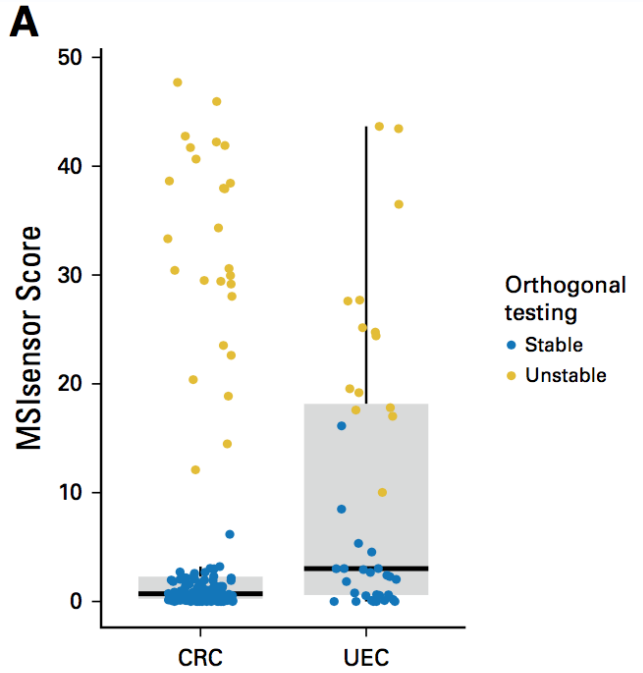
(Zehir et al., *Nat Med* 2017)



MSK-IMPACT™ (Integrated Mutation Profiling of Actionable Cancer Targets) (505 cancer genes)

ABL1	BCL2L11	CENPA	EIF4E	FGFR4	HIST1H3G	KDM5A	MET	NSD1	POLD1	REL	SLX4	TERT
ACVR1	BCL6	CHEK1	ELF3	FH	HIST1H3H	KDM5C	MGA	NTHL1	POLE	REST	SMAD2	TET1
AGO1	BCOR	CHEK2	EP300	FLCN	HIST1H3I	KDM6A	MITF	NTRK1	POT1	RET	SMAD3	TET2
AGO2	BIRC3	CIC	EPAS1	FLT1	HIST1H3J	KDR	MLH1	NTRK2	PPARG	RFWD2	SMAD4	TGFBR1
AKT1	BLM	CMTR2	EPCAM	FLT3	HIST2H3C	KEAP1	MLLT1	NTRK3	PPM1D	RHEB	SMARCA2	TGFBR2
AKT2	BMPR1A	CREBBP	EPHA3	FLT4	HIST2H3D	KIT	MPL	NUF2	PPP2R1A	RHOA	SMARCA4	TMEM127
AKT3	BRAF	CRKL	EPHA5	FOXA1	HIST3H3	KLF4	MRE11A	NUP93	PPP4R2	RICTOR	SMARCB1	TMPRSS2
ALB	BRCA1	CRLF2	EPHA7	FOXF1	HLA-A	KLF5	MSH2	PAK1	PPP6C	RIT1	SMARCD1	TNFAIP3
ALK	BRCA2	CSDE1	EPHB1	FOXL2	HLA-B	KMT2A	MSH3	PAK7	PRDM1	RNF43	SMARCE1	TNFRSF14
ALOX12B	BRD4	CSF1R	ERBB2	FOXO1	HLA-C	KMT2B	MSH6	PALB2	PRDM14	ROS1	SMO	TOP1
ANKRD11	BRIP1	CSF3R	ERBB3	FOXP1	HNF1A	KMT2C	MSI1	PARK2	PREX2	RPS6KA4	SMYD3	TP53
APC	BTK	CTCF	ERBB4	FUBP1	HOXB13	KMT2D	MSI2	PARP1	PRKAR1A	RPS6KB2	SOCS1	TP53BP1
APLNR	CALR	CTLA4	ERCC2	FYN	HRAS	KMT5A	MST1	PAX5	PRKCI	RPTOR	SOS1	TP63
AR	CARD11	CTNNB1	ERCC3	GAB1	ICOSLG	KNSTRN	MST1R	PBRM1	PRKD1	RRAGC	SOX17	TRAF2
ARAF	CARM1	CTR9	ERCC4	GAB2	ID3	KRAS	MTAP	PDCD1	PTCH1	RRAS	SOX2	TRAF7
ARHGAP35	CASP8	CUL3	ERCC5	GATA1	IDH1	LATS1	MTOR	PDCD1LG2	PTEN	RRAS2	SOX9	TRIP13
ARID1A	CBFB	CXCR4	ERF	GATA2	IDH2	LATS2	MUTYH	PDGFRA	PTP4A1	RTEL1	SPEN	TSC1
ARID1B	CBL	CXORF67	ERG	GATA3	IFNGR1	LMO1	MYC	PDGFRB	PTPN11	RUNX1	SPOP	TSC2
ARID2	CCND1	CYLD	ERRFI1	GLI1	IGF1	LYN	MYCL1	PDPK1	PTPRD	RXRA	SPRED1	TSHR
ARID5B	CCND2	CYP19A1	ESR1	GNA11	IGF1R	LZTR1	MYCN	PGBD5	PTPRS	RYBP	SPRTN	U2AF1
ASXL1	CCND3	CYSLTR2	ETAA1	GNAQ	IGF2	MAD2L2	MYD88	PGR	PTPRT	SCG5	SRC	UPF1
ASXL2	CCNE1	DAXX	ETV1	GNAS	IKBKE	MALT1	MYOD1	PHF6	RAB35	SDHA	SRSF2	USP8
ATM	CD274	DCUN1D1	ETV6	GNB1	IKZF1	MAP2K1	NADK	PHOX2B	RAC1	SDHAF2	STAG2	VEGFA
ATR	CD276	DDR2	EZH1	GPS2	IL10	MAP2K2	NBN	PIK3C2G	RAC2	SDHB	STAT3	VHL
ATRX	CD79A	DICER1	EZH2	GREM1	IL7R	MAP2K4	NCOA3	PIK3C3	RAD21	SDHC	STAT5A	VTCN1
ATXN7	CD79B	DIS3	FAM123B	GRIN2A	INHA	MAP3K1	NCOR1	PIK3CA	RAD50	SDHD	STAT5B	WHSC1
AURKA	CDC42	DNAJB1	FAM175A	GSK3B	INHBA	MAP3K13	NEGR1	PIK3CB	RAD51	SERPINB3	STK11	WHSC1L1
AURKB	CDC73	DNMT1	FAM46C	H3F3A	INPP4A	MAP3K14	NF1	PIK3CD	RAD51C	SERPINB4	STK19	WT1
AXIN1	CDH1	DNMT3A	FAM58A	H3F3B	INPP4B	MAPK1	NF2	PIK3CG	RAD51L1	SESN1	STK40	WWTR1
AXIN2	CDK12	DNMT3B	FANCA	H3F3C	INPPL1	MAPK3	NFE2L2	PIK3R1	RAD51L3	SESN2	SUFU	XIAP
AXL	CDK4	DOT1L	FANCC	HGF	INSR	MAPKAP1	NFKBIA	PIK3R2	RAD52	SESN3	SUZ12	XPO1
B2M	CDK6	DROSHA	FAT1	HIST1H1C	IRF4	MAX	NKX2-1	PIK3R3	RAD54L	SETD2	SYK	XRCC2
BABAM1	CDK8	DUSP4	FBXW7	HIST1H2BD	IRS1	MCL1	NKX3-1	PIM1	RAF1	SETDB1	TAP1	YAP1
BAP1	CDKN1A	E2F3	FGF19	HIST1H3A	IRS2	MDC1	NOTCH1	PLCG2	RARA	SF3B1	TAP2	YES1
BARD1	CDKN1B	EED	FGF3	HIST1H3B	JAK1	MDM2	NOTCH2	PLK2	RASA1	SH2B3	TBX3	ZFHX3
BBC3	CDKN2A	EGFL7	FGF4	HIST1H3C	JAK2	MDM4	NOTCH3	PMAIP1	RB1	SH2D1A	TCEB1	ZNRF3
BCL10	CDKN2B	EGFR	FGFR1	HIST1H3D	JAK3	MED12	NOTCH4	PMS1	RBM10	SHOC2	TCF3	ZRSR2
BCL2	CDKN2C	EIF1AX	FGFR2	HIST1H3E	JUN	MEF2B	NPM1	PMS2	RECQL	SHQ1	TCF7L2	
BCL2L1	CEBPA	EIF4A2	FGFR3	HIST1H3F	KBTBD4	MEN1	NRAS	PNRC1	RECQL4	SLFN11	TEK	

MSISensor Validation for MSK-IMPACT (Middha et al., *JCO PO* 2017)



MSIsensor Score	Colorectal Cancer (CRC) ≥ 10 (n = 24)	Colorectal Cancer (CRC) < 10 (n = 114)	Endometrial Cancer (UEC) ≥ 10 (n = 15)	Endometrial Cancer (UEC) < 10 (n = 25)
MMR-D by IHC	11	0	5	0
MMR-P by IHC	0	69	1*	9
MSI-H by PCR	1	0	2	0
MSS by PCR	0	28	0	5
MMR-D by IHC and MSI-H by PCR	12	0	7	0
MMR-P by IHC and MSS by PCR	0	17	0	11
Total Number of Validated Cases	138		40	

- MSISensor score >10 used to define MSI-H designation
- Validation in CRC/UEC showed 99.4% concordance with MSI-PCR and/or MMR-IHC
- MSK-IMPACT: FDA-authorized NGS platform that also incorporates MSI detection using tumor-normal samples

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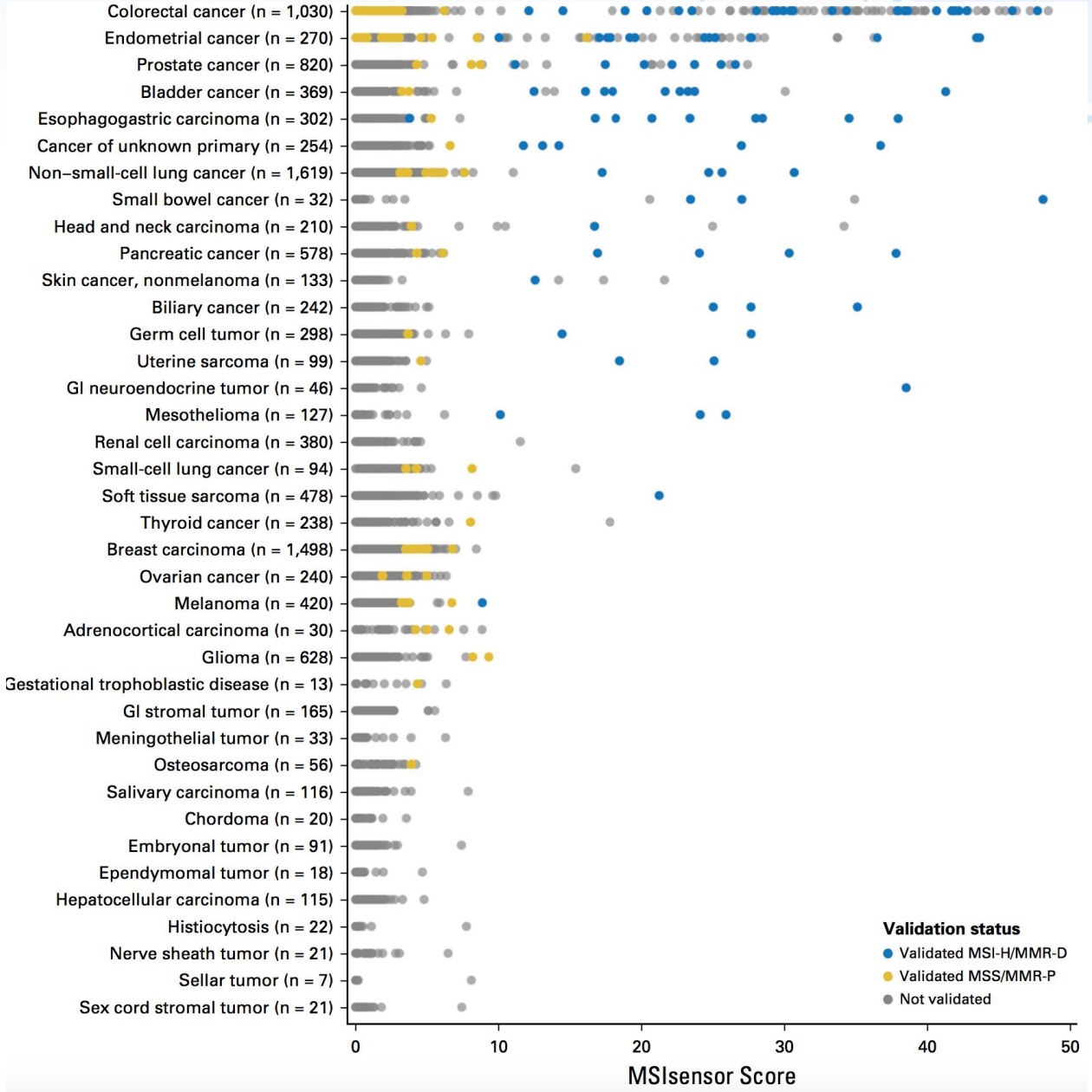
MSISensor Across Different Cancer Types (Middha et al., *JCO PO* 2017)

- 1.8% MSISensor-High
- 35% of MSISensor-High tumors were not CRC or UEC
- 4.8% of non-CRC/UECs with MSISensor score in intermediate range (≥3 and <10)



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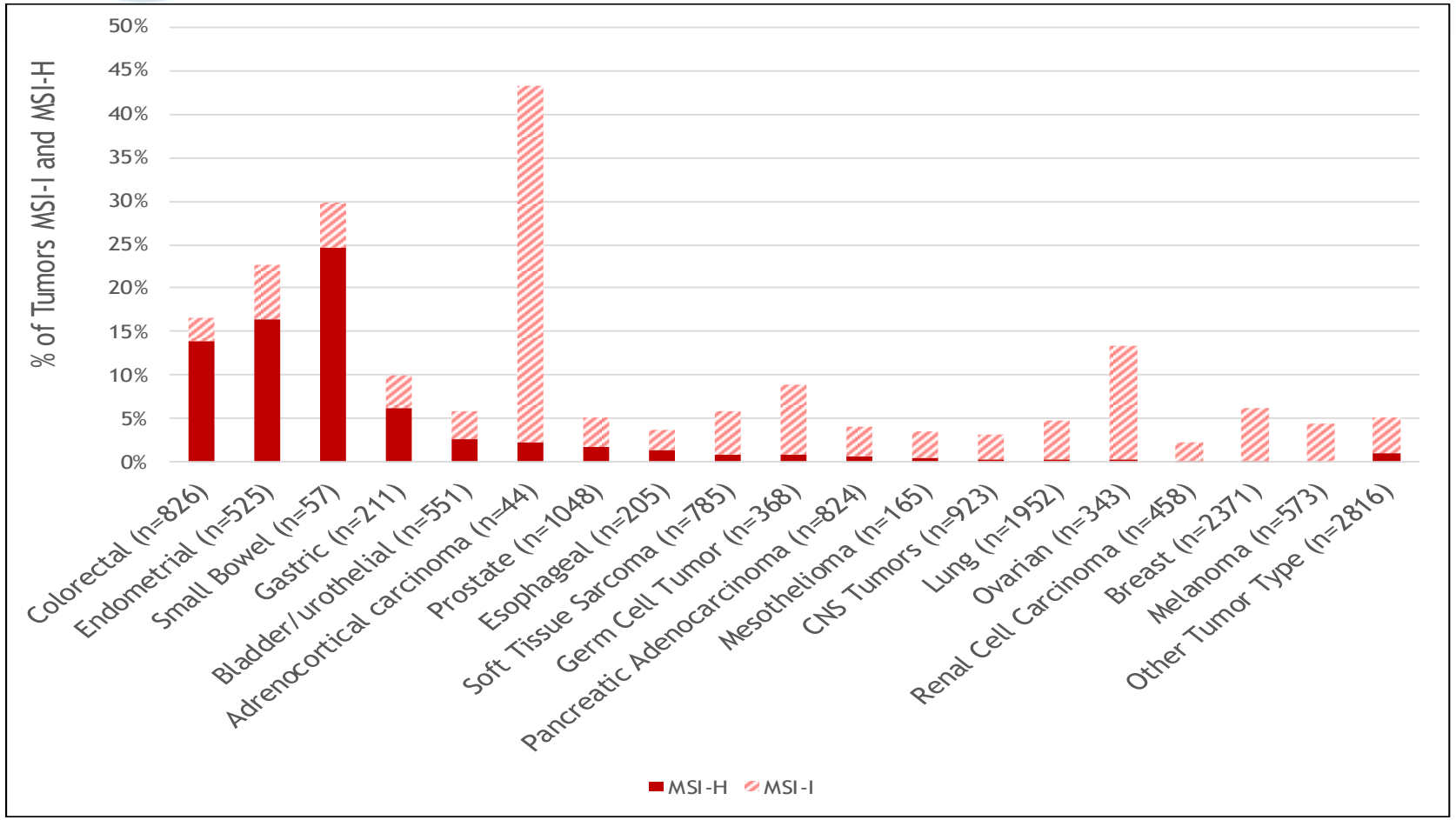
Distribution of MSI Across Cancer Types (Latham et al., JCO 2018)



- MSI-H tumors:
  - small bowel cancer (25%; 14/57)
  - endometrial (16%; 86/525)
  - colorectal (14%; 115/826)
  - gastric (6%; 13/211)

- MSI-H/I tumors
  - Adrenocortical (40%; 18/44)
  - Bladder/urothelial (6%)

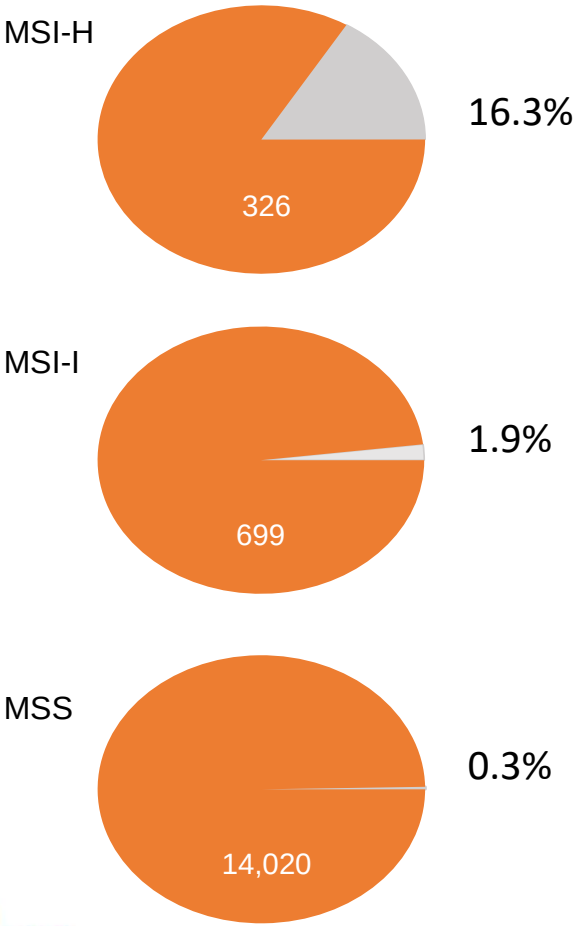
Germline Implications

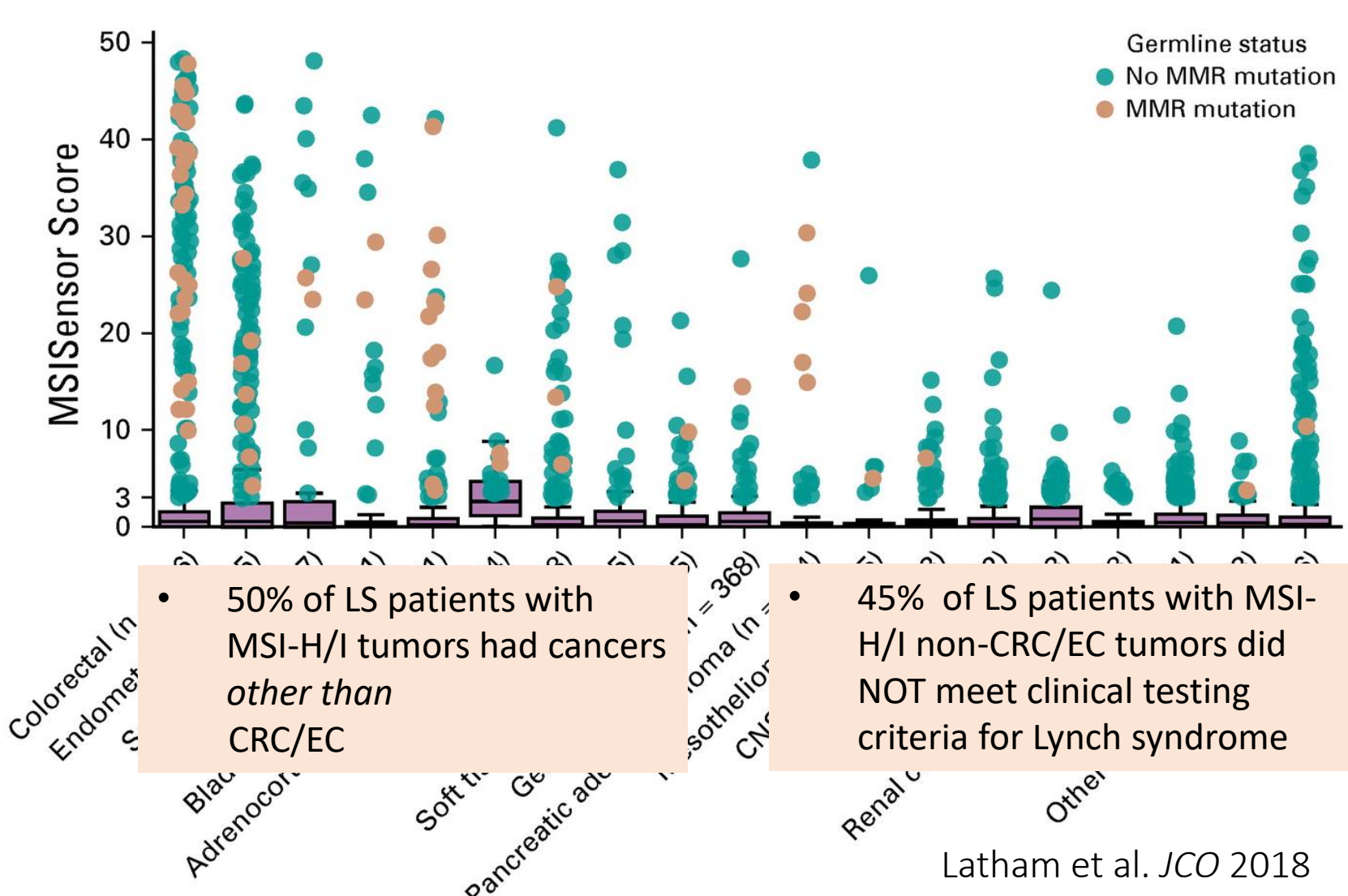
- MSI-PCR and IHC protein expression analysis for MMR-deficiency are not considered genetic germline tests
  - No patient consent required
  - Pathologist-initiated reflexive testing is possible

- *What are the potential germline implications of using NGS technologies for **tumor only** and **tumor/normal paired** sequencing?*

Tumor Profiling and Microsatellite Instability

What is the prevalence of Lynch syndrome according to MSI status?





- 50% of LS patients with MSI-H/I tumors had cancers *other than* CRC/EC
- 45% of LS patients with MSI-H/I non-CRC/EC tumors did NOT meet clinical testing criteria for Lynch syndrome

Latham et al. *JCO* 2018

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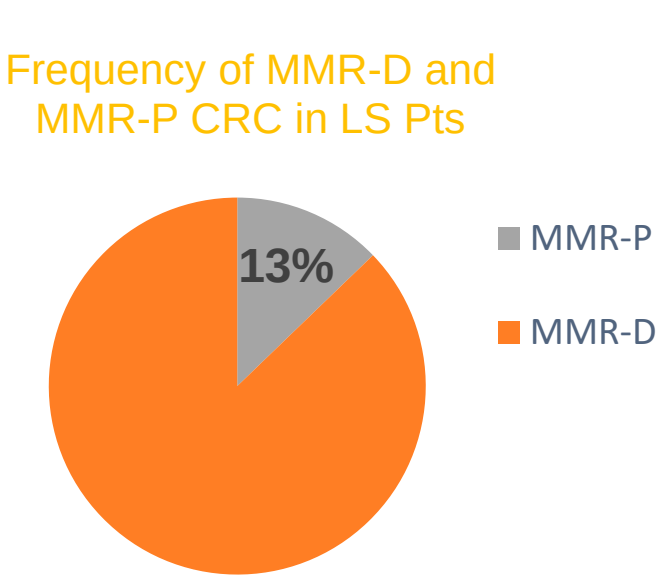
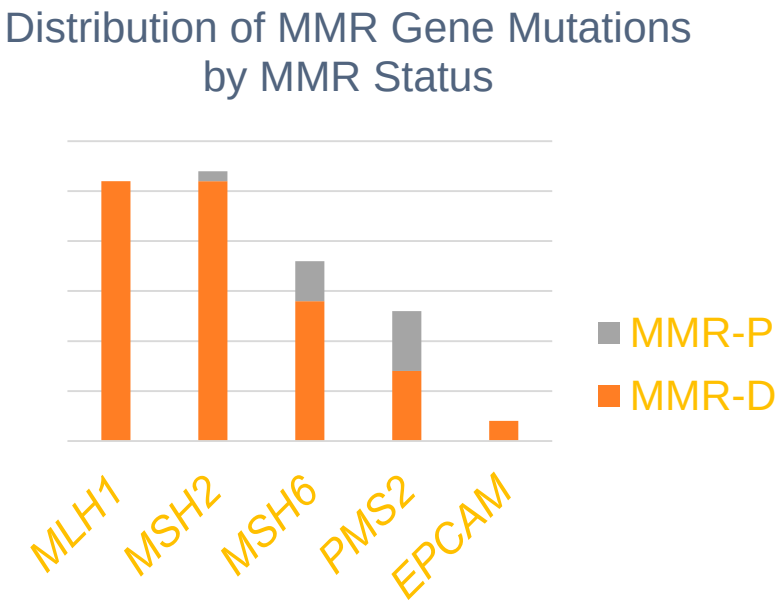
Colorectal Cancer in Patients with Lynch Syndrome

- **13% of CRCs in Lynch syndrome patients were MMR-P**
  - 91% with *PMS2/MSH6*
  - Later age at presentation
  - **Lynch patients should have confirmation of MMR/MSI status given implications for treatment and risk for CRC in family members**

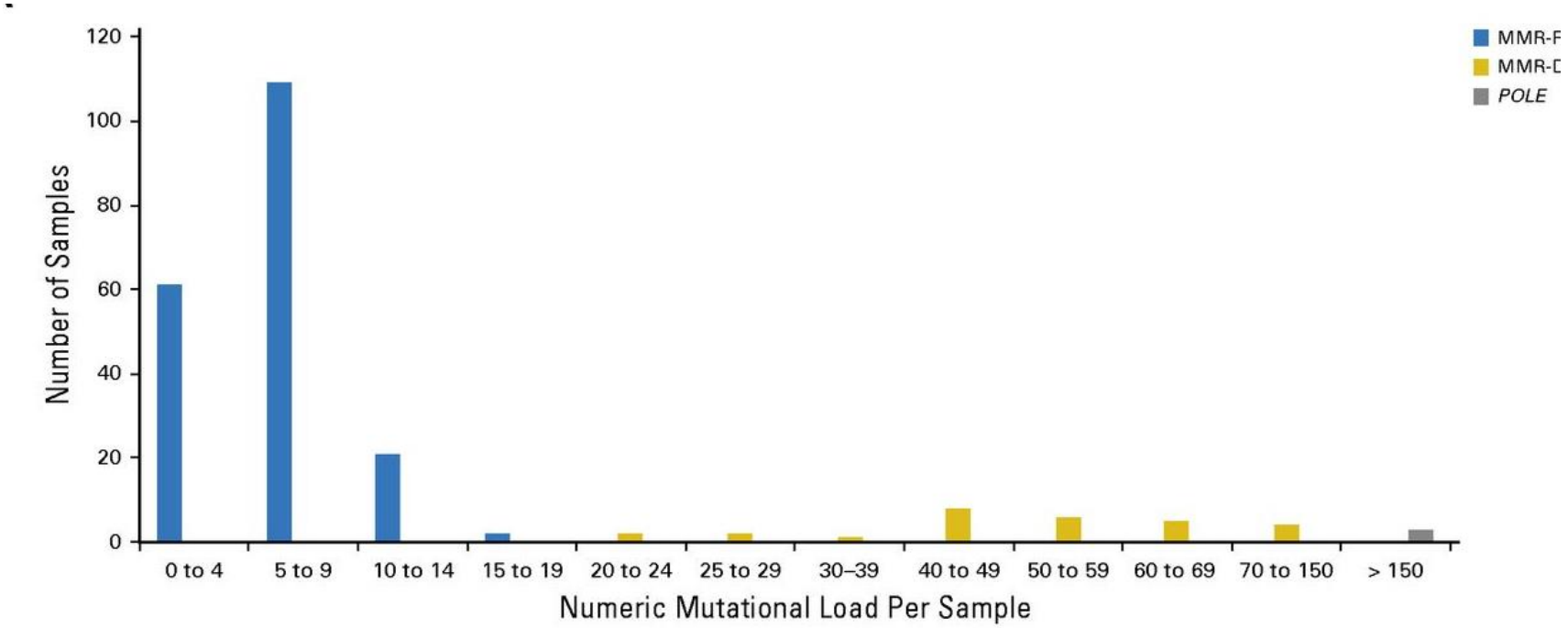




Ranganathan M, Latham A. CGA 2020

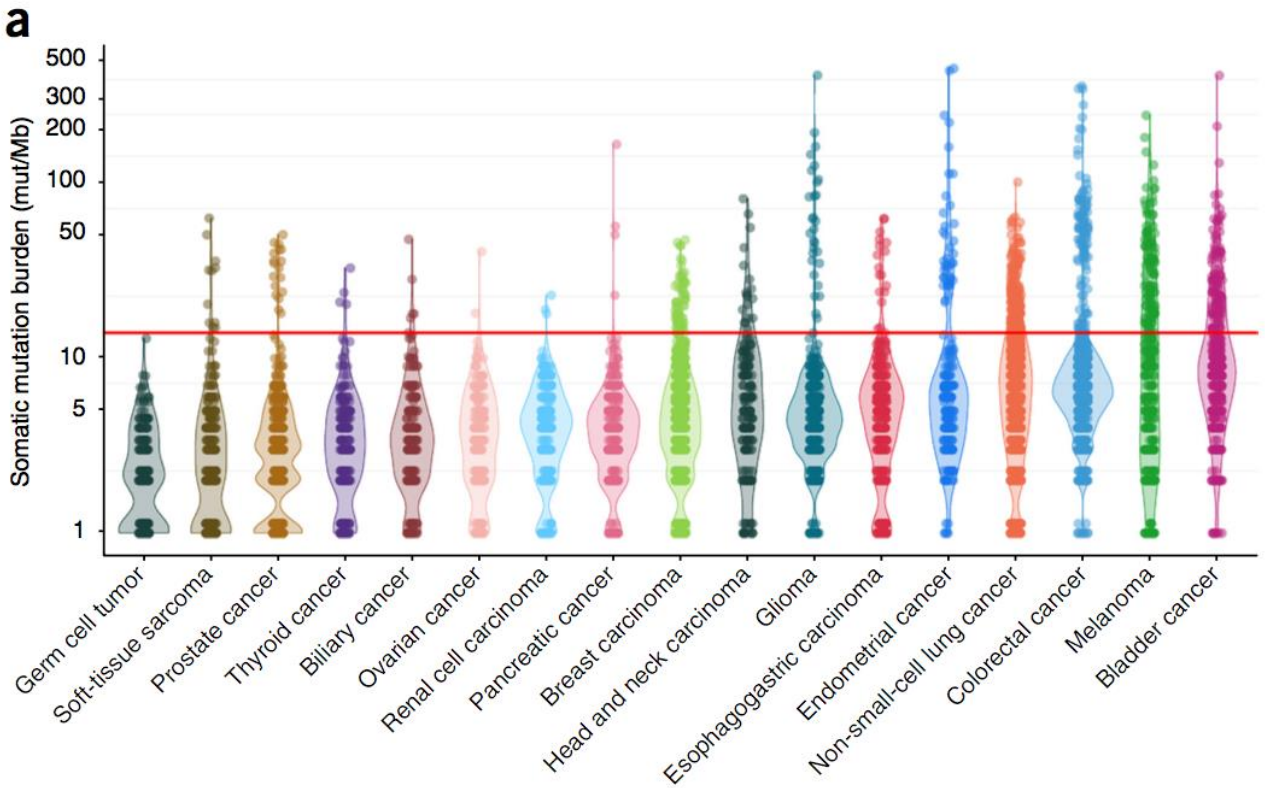
Tumor Mutational Burden In Colorectal Tumors

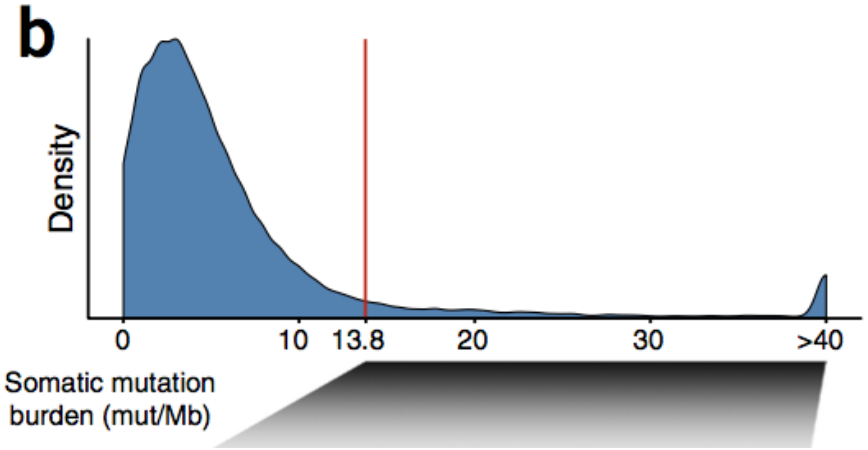
- Tumor mutational burden predicts DNA MMR-deficiency in CRC



Stadler et al. *JCO* 2016;34:2141-2147

MUTATIONAL LANDSCAPE IN 10,000 METASTATIC CANCER PATIENTS (ZEHIR ET AL., *NAT MED* 2017)





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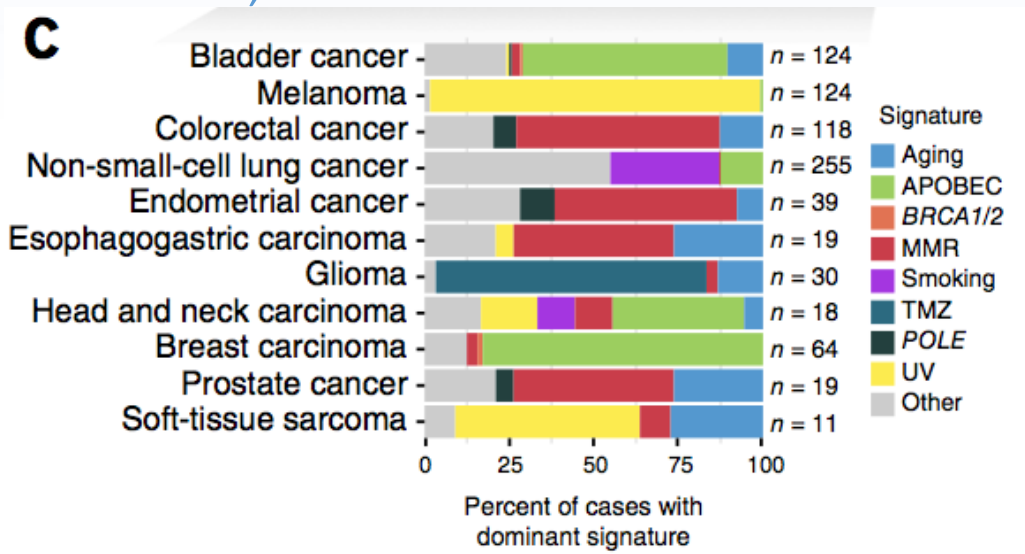

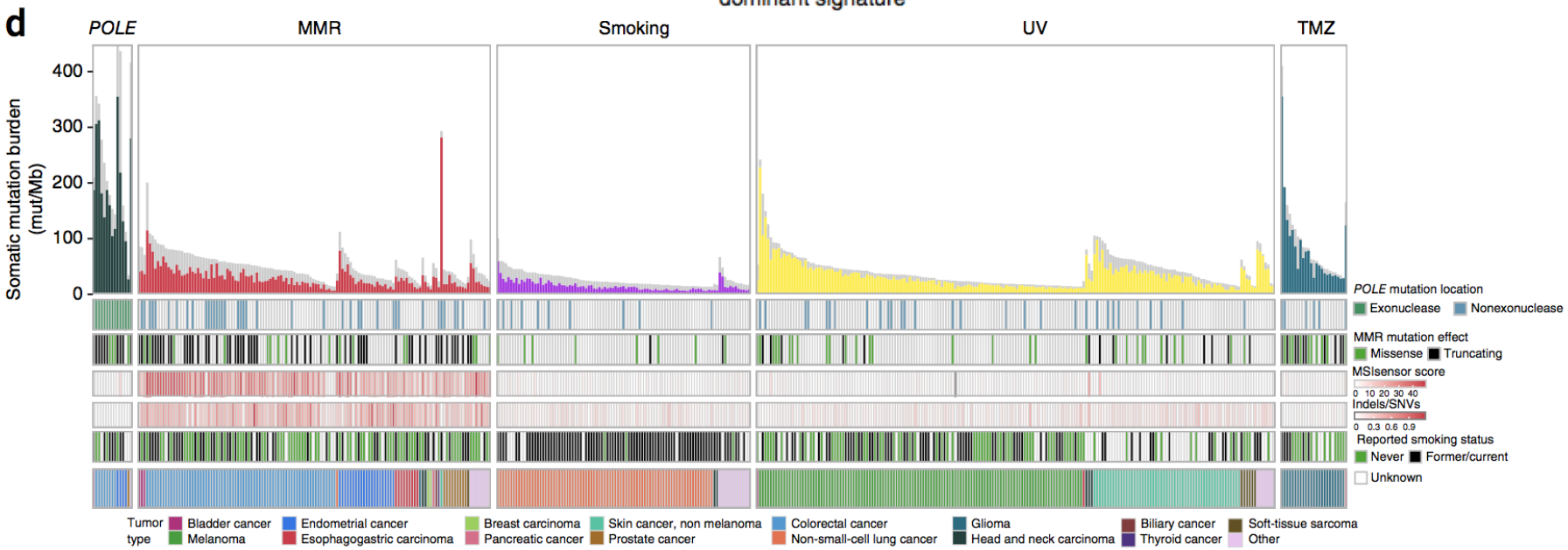

Summary: NGS based MSI Detection

Pros

- More economical use of available tissue material
- Simultaneous somatic mutation profile
- Incorporation of many microsatellites
- Potential for improved MSI detection pan-cancer
- Facilitates evaluation of Lynch syndrome
- Cost?

Cons

- Turn-around time
- Amount of tumor needed
- Adequate tumor purity
- Depth of coverage
- Extent of patient consent
- Incidental findings
- Validation across all cancer types
- Widespread availability
- Cost?

Conclusions

- Technologies for detecting MMR-D/MSI include:
  - IHC for the MMR proteins
  - MSI-PCR
  - MSI-NGS
- No technology is perfect! If high-suspicion of MMR-D/MSI, orthogonal testing is reasonable to pursue given the important implications of MMR-D/MSI for cancer treatment
- While the majority of MMR-D/MSI is driven by an epigenetic event, work up for Lynch syndrome in patient with unexplained MMR-D/MSI should be pursued.
- High tumor mutational burden is not a surrogate for MMR-D/MSI.
- With continued improvement in existing sequencing and computational technologies, increasing accuracy of MMR-D/MSI is anticipated.