Comprehensive genomic profiling with the Oncomine™ Comprehensive Plus Assay

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Conflict of interest statement

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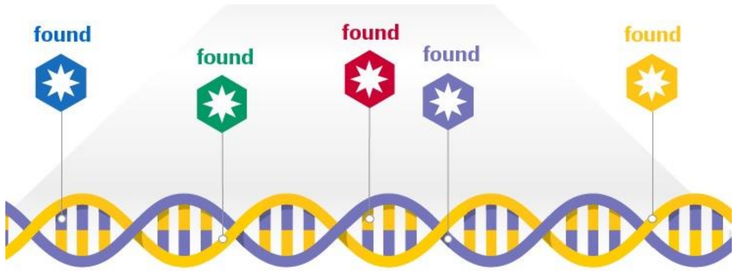
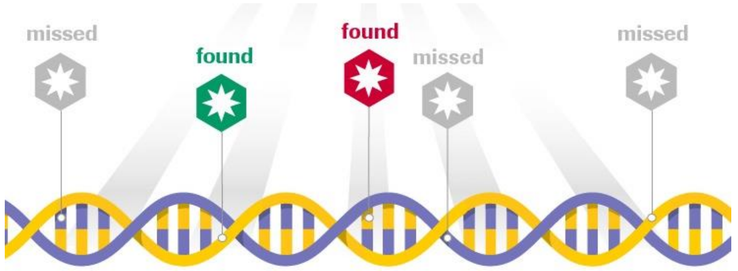
Genetic Biomarker Testing Strategies

Comprehensive Genome Profiling (>300 Genes)	Hotspot Profiling
	
✓**One panel for all** tumor types ✓Broader coverage including potential **relevant biomarkers** ✓Includes **additional information** such as **TMB, MSI**, and **HRD**	✓**Rapid turnaround time** (automated in 1 – 2 days) ✓Focus on key **relevant biomarkers** ✓**Cost-efficient**

ESMO recommendations for using NGS on metastatic cancers samples

1. **Tumor-specific** testing recommended for **daily practice.** Recommendation to assess **TMB in some indications.**

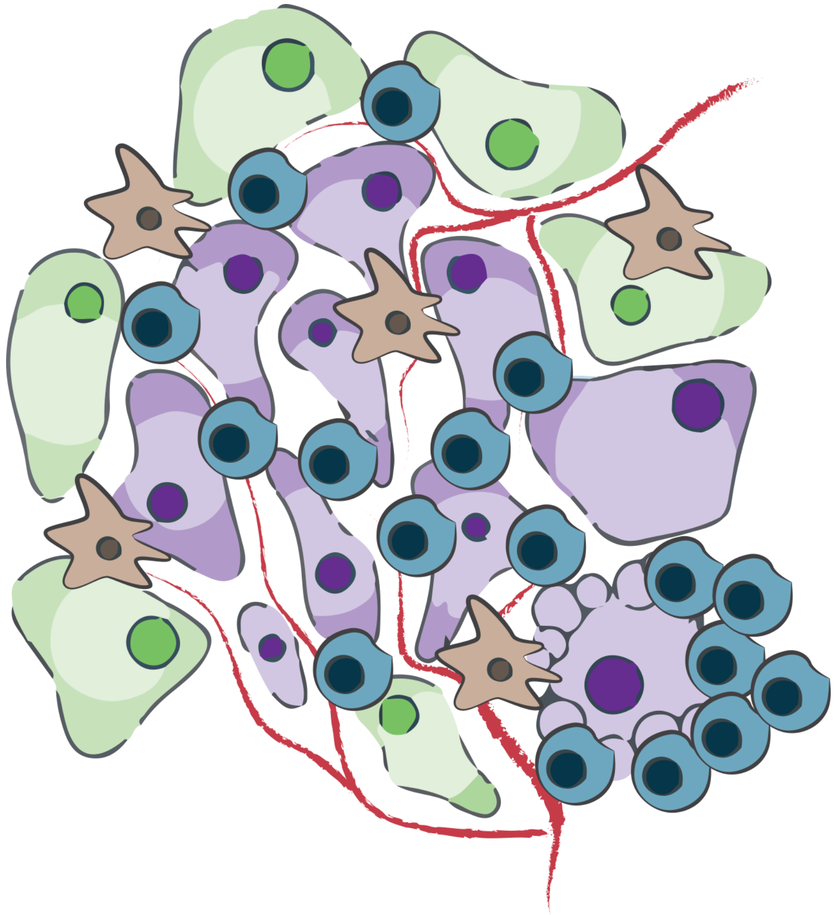
2. **Large panel** testing recommended for **clinical research centres** with the aim to increase **access to drugs and accelerate drug development.**

Slide courtesy of Dr. P. Jermann

Illustrations from https://www.roche.com/dam/jcr:06f059f3-e3db-4df1-b8fa-72196a6e66a5/en/hot-spot-test.jpg and https://www.roche.com/dam/jcr:336a1ed3-5ddb-4ff6-b38e-e03a9dffb615/en/comprehensive-profiling.jpg, accessed 27/08/2020

Mosele et al., 2020, https://doi.org/10.1016/j.annonc.2020.07.014

NGS-based biomarkers

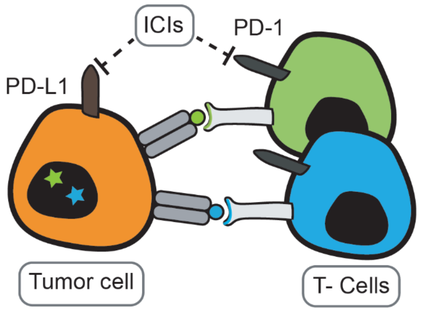


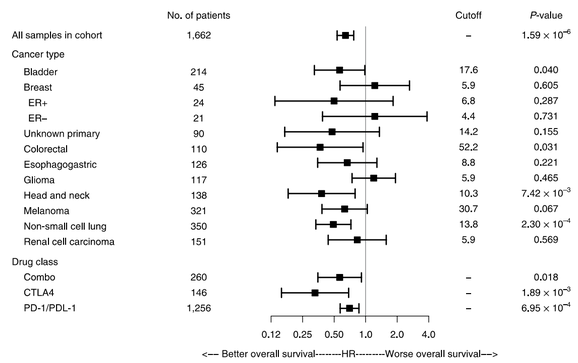
- **Mutations (SNVs, indels – DNA level)**
- **Copy Number Variations (CNVs, DNA level)**
- **Fusions (RNA level)**
- Tumor Mutational Burder (TMB)
- Microsatellite Instability (MSI)
- Genomic instability: loss of heterozigosity (LOH) and others (TAI, LST) for homologous-repair deficiency (HRD)

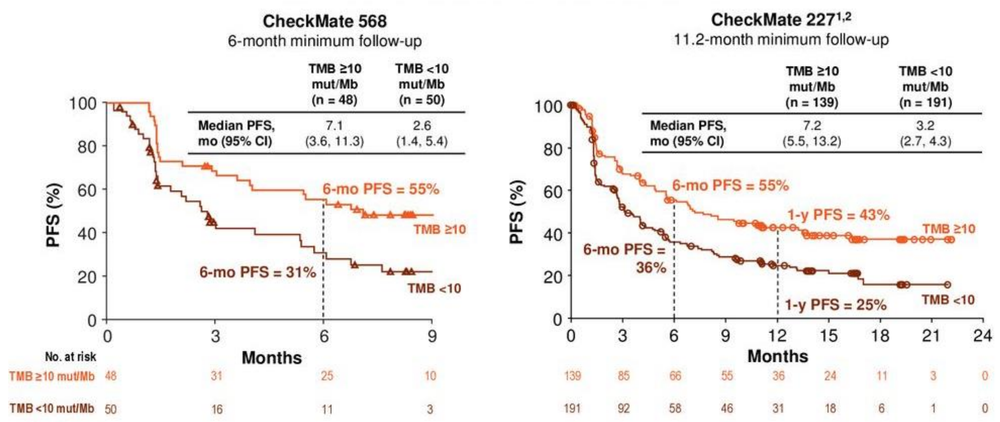
Comprehensive Genomic Profiling

Biomarkers are evolving to recapitulate the complexity of tumors

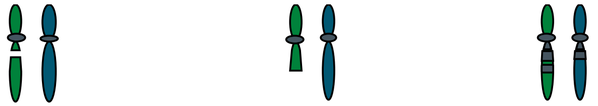
Tumor Mutational Burden (TMB)



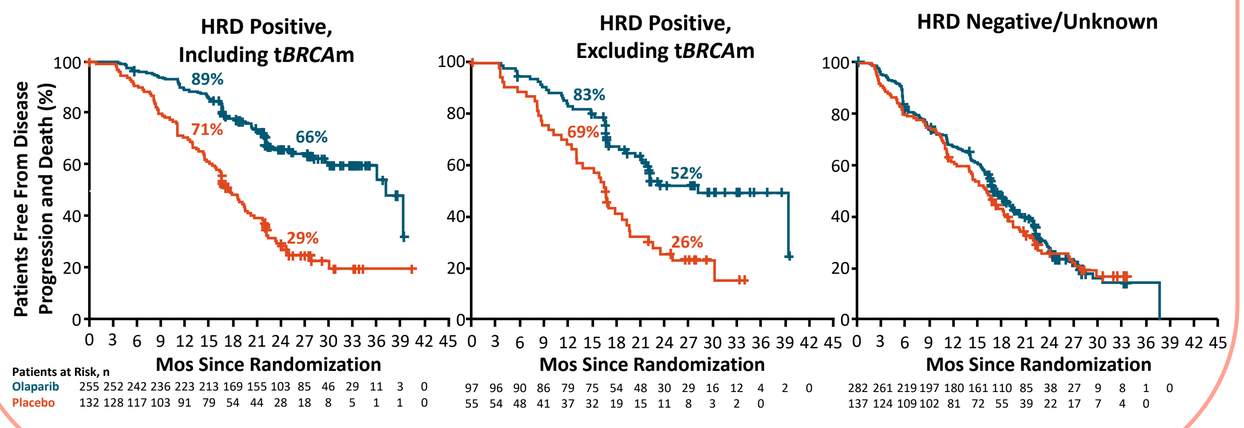




Genomic Instability/HRD score



GIS>42, Myriad MyChoice, PAOLA-1 study



Ramalingam, Cancer Res, 2018; Hellman, NEJM, 2018; Samstein R. et al. Nat. Gen., 2019

Slide credit: clinicaloptions.com

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MSI-H/MSS Markers for Microsatellite Instability (MSI)

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MET exon skipping detection at DNA and RNA level

Cellularity (Tumor fraction) calculation

46 genes in **Homologous Recombination Repair** pathway

Loss of heterozygosity (LOH) detection

Mutational signature analysis

CNV gain genes						Full CDS coverage						
	Hotspot genes											
CNV gain only	CNV gain and hotspot			Hotspot only		CNV loss						CDS only
ABCB1	ABL1	FGFR4	PIK3C2B	ACVR1	MYOD1	ABRAXAS1	CDKN1A	FANCA	MAP3K1	POLE	SOX9	CALR
CTNND2	ABL2	FLT3	PIK3CA	ATP1A1	NSD2	ACVR1B	CDKN1B	FANCC	MAP3K4	POT1	SPEN	CIITA
DDR1	AKT1	FLT4	PIK3CB	AURKB	NT5C2	ACVR2A	CDKN2A	FANCD2	MAPK8	PPM1D	STAG2	CYP2D26
EMSY	AKT2	FOXA1	PIK3R2	BCR	NTRK2	ADAMTS12	CDKN2B	FANCE	MEN1	PPP2R2A	STK11	ERCC5
FGF3	AKT3	GATA2	PIM1	BMP5	NUP93	ADAMTS2	CDKN2C	FANCF	MGA	PRDM1	SUFU	FAS
FGF4	ALK	GNAS	PLCG1	BTK	PAX5	AMER1	CHEK1	FANCG	MLH1	PRDM9	TAP1	ID3
FGF9	AR	H3F3A	PPP2R1A	CACNA1D	PIK3CD	APC	CHEK2	FANCI	MLH3	PRKAR1A	TAP2	KLHL13
FGF19	ARAF	H3F3B	PPP6C	CD79B	PIK3CG	ARHGAP35	CIC	FANCL	MRE11	PTCH1	TBX3	MTUS2
FGF23	AURKA	IDH2	PRKACA	CSF1R	PTPRD	ARID1A	CREBBP	FANCM	MSH2	PTEN	TCF7L2	PSMB8
FYN	AURKC	IKBKB	PTPN11	CTNNB1	RGS7	ARID1B	CSMD3	FAT1	MSH3	PTPRT	TET2	PSMB9
GLI3	AXL	IL7R	PXDNL	CUL1	RHOA	ARID2	CTCF	FBXW7	MSH6	RAD50	TGFBR2	PSMB10
IGF1R	BCL2	KDR	RAC1	CYSLTR2	RPL10	ARID5B	CTLA4	FUBP1	MTAP	RAD51	TNFAIP3	RNASEH2C
MCL1	BCL2L12	KIT	RAF1	DGCR8	SIX1	ASXL1	CUL3	GATA3	MUTYH	RAD51B	TNFRSF14	RPL5
MDM2	BCL6	KLF5	RARA	DROSHA	SIX2	ASXL2	CUL4A	GNA13	NBN	RAD51C	TP53	RPL22
MYCL	BRAF	KRAS	RET	E2F1	SNCAIP	ATM	CUL4B	GPS2	NCOR1	RAD51D	TP63	RUNX1T1
RPS6KB1	CARD11	MAGOH	RHEB	EPAS1	SOS1	ATR	CYLD	HDAC2	NF1	RAD52	TPP2	SDHC
RPTOR	CBL	MAPK1	RICTOR	FGF7	SOX2	ATRX	CYP2C9	HDAC9	NF2	RAD54L	TSC1	SOCS1
YAP1	CCND1	MAP2K1	RIT1	FOXL2	SRSF2	AXIN1	DAXX	HLA-A	NOTCH1	RASA1	TSC2	STAT1
YES1	CCND2	MAX	ROS1	FOXO1	STAT5B	AXIN2	DDX3X	HLA-B	NOTCH2	RASA2	USP9X	TMEM132D
	CCND3	MDM4	SETBP1	GLI1	TAF1	B2M	DICER1	HNF1A	NOTCH3	RB1	VHL	UGT1A1
	CCNE1	MECOM	SF3B1	GNA11	TGFBR1	BAP1	DNMT3A	INPP4B	NOTCH4	RBM10	WT1	ZBTB20
	CDK4	MEF2B	SLCO1B3	GNAQ	TRRAP	BARD1	DOCK3	JAK1	PALB2	RECQL4	XRCC2	
	CDK6	MET	SMC1A	HIF1A	TSHR	BCOR	DPYD	JAK2	PARP1	RNASEH2A	XRCC3	
	CHD4	MITF	SMO	HIST1H1E	WAS	BLM	DSC1	JAK3	PARP2	RNASEH2B	ZFHX3	
	DDR2	MPL	SPOP	HIST1H2BD		BMPR2	DSC3	KDM5C	PARP3	RNF43	ZMYM3	
	EGFR	MTOR	SRC	HIST1H3B		BRCA1	ELF3	KDM6A	PARP4	RPA1	ZRSR2	
	EIF1AX	MYC	STAT3	HRAS		BRCA2	ENO1	KEAP1	PBRM1	RUNX1		
	ERBB2	MYCN	STAT6	IDH1		BRIP1	EP300	KMT2A	PDCD1	SDHA		
	ERBB3	MYD88	TERT	IL6ST		CASP8	EPCAM	KMT2B	PDCD1LG2	SDHB		
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	ESR1	NRAS	TPMT	IRS4		CD274	ERAP1	KMT2D	PGD	SETD2		
	EZH2	NTRK1	U2AF1	KCNJ5		CD276	ERAP2	LARP4B	PHF6	SLX4		
	FAM135B	NTRK3	USP8	KLF4		CDC73	ERCC2	LATS1	PIK3R1	SMAD2		
	FGFR1	PCBP1	XPO1	KNSTRN		CDH1	ERCC4	LATS2	PMS1	SMAD4		
	FGFR2	PDGFRA	ZNF217	MAP2K2		CDH10	ERRFI1	MAP2K4	PMS2	SMARCA4		
	FGFR3	PDGFRB	ZNF429	MED12		CDK12	ETV6	MAP2K7	POLD1	SMARCB1		

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FGF9	AR	H3F3A	PPP2R1A	CACNA1D	PIK3CD	APC	CHEK2	FANCI	MLH3	PRKAR1A	TAP2	KLHL13
FGF19	ARAF	H3F3B	PPP6C	CD79B	PIK3CG	ARHGAP35	CIC	FANCL	MRE11	PTCH1	TBX3	MTUS2
FGF23	AURKA	IDH2	PRKACA	CSF1R	PTPRD	ARID1A	CREBBP	FANCM	MSH2	PTEN	TCF7L2	PSMB8
FYN	AURKC	IKBKB	PTPN11	CTNNB1	RGS7	ARID1B	CSMD3	FAT1	MSH3	PTPRT	TET2	PSMB9
GLI3	AXL	IL7R	PXDNL	CUL1	RHOA	ARID2	CTCF	FBXW7	MSH6	RAD50	TGFBR2	PSMB10
IGF1R	BCL2	KDR	RAC1	CYSLTR2	RPL10	ARID5B	CTLA4	FUBP1	MTAP	RAD51	TNFAIP3	RNASEH2C
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YAP1	CCND1	MAP2K1	RIT1	FOXL2	SRSF2	AXIN1	DAXX	HLA-A	NOTCH1	RASA1	TSC2	STAT1
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	CCND3	MDM4	SETBP1	GLI1	TAF1	B2M	DICER1	HNF1A	NOTCH3	RB1	VHL	UGT1A1
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	CDK4	MEF2B	SLCO1B3	GNAQ	TRRAP	BARD1	DOCK3	JAK1	PALB2	RECQL4	XRCC2	
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	EIF1AX	MYC	STAT3	HRAS		BRCA2	ENO1	KEAP1	PBRM1	RUNX1		
	ERBB2	MYCN	STAT6	IDH1		BRIP1	EP300	KMT2A	PDCD1	SDHA		
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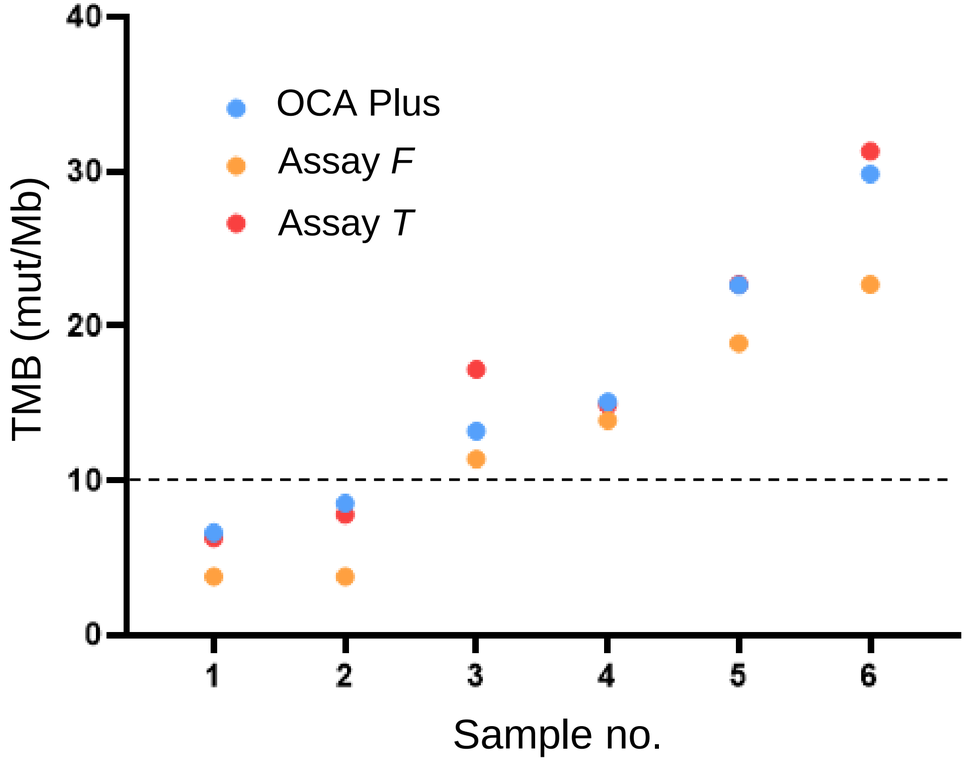
Tumor Mutational Burden with OCAPlus

Robust TMB estimation

Comparison of TMB between OCA Plus, in-house assay *T* and commercial service provider *F*:

- 6 samples with TMB ranging from 3 to 33 mut/Mb
- Ion Reporter™ OCAPlus w1.0 workflow
- **Good concordance** between all three assays
- In-house test T & OCA Plus show best concordance
- **Same clinical classification (TMB-high** cut-off = 10 mut/Mb)

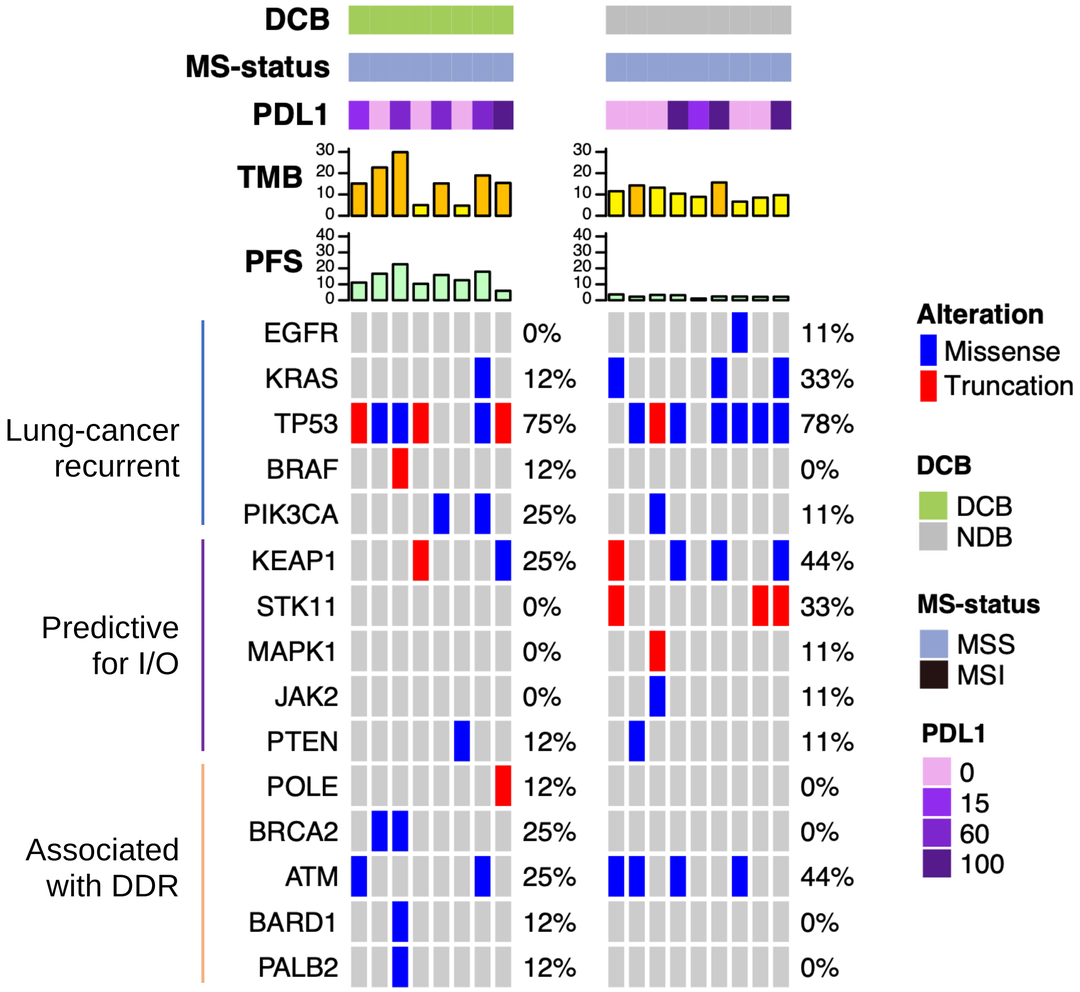


TMB assessment with the OCA Plus is concordant with other assays of similar size

OCAPlus: NSCLC - TMB and relevant alterations

Oncomine™ Comprehensive Assay Plus tested on **17 NSCLC** samples**,** ICI treated retrospective cancer case studies: response and PD-L1 status available. (8 durable clinical benefit (DCB), 9 no durable benefit (NDB).

- Analysis of TMB, mutations, MS-status.
- TMB would misclassify 2/8 DCB and 2/9 NDB.
- KRAS+KEAP1+STK11 detected in NDB samples.
- STK11 truncating mutations in NDB only

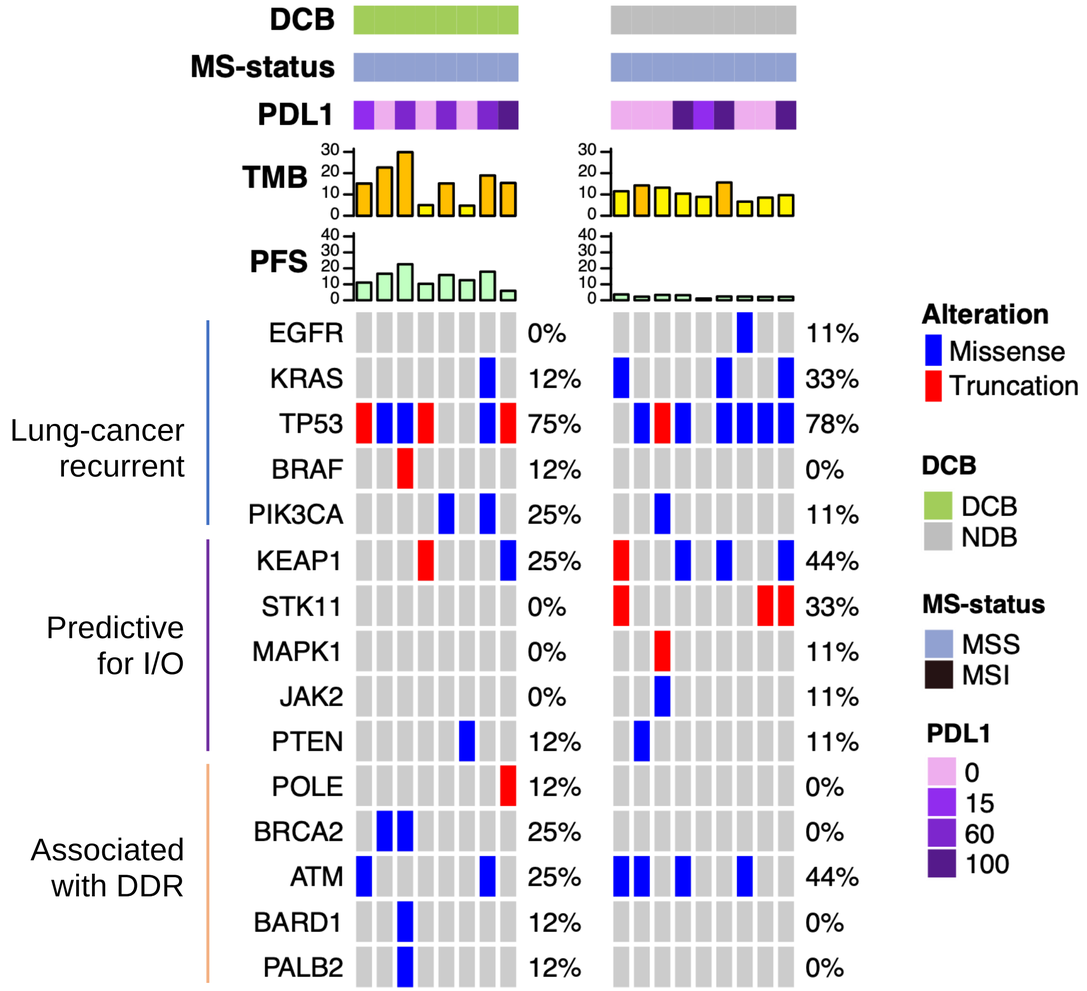


**DCB = PFS > 6 months*

OCAPlus: NSCLC - TMB and relevant alterations

Resistance/response to ICIs

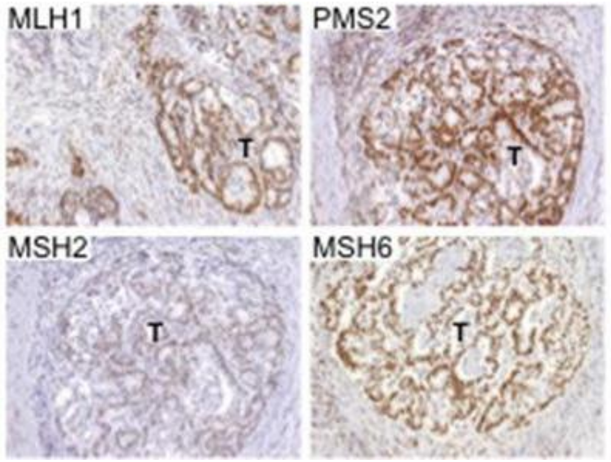
B2M	Antigen Presentation
TAP1	Antigen Presentation
TAP2	Antigen Presentation
HLA-A	Antigen Presentation
TAPBP	Antigen Processing
JAK1	Resistance/Response to ICIs
JAK2	Resistance/Response to ICIs
JAK3	Resistance/Response to ICIs
STAT1	Resistance/Response to ICIs
SOCS1	Resistance/Response to ICIs
CD274	PDL1
PDCD1	PD1
PTEN	Resistance/Response to ICIs
STK11	Resistance/Response to ICIs
KEAP1	Resistance/Response to ICIs

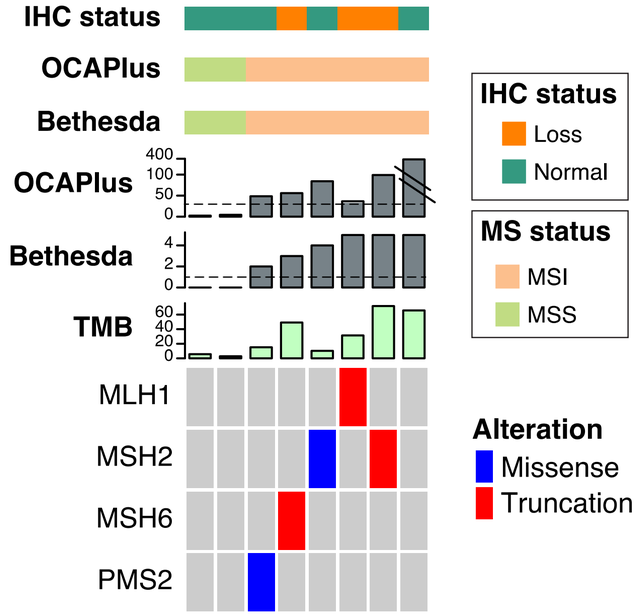


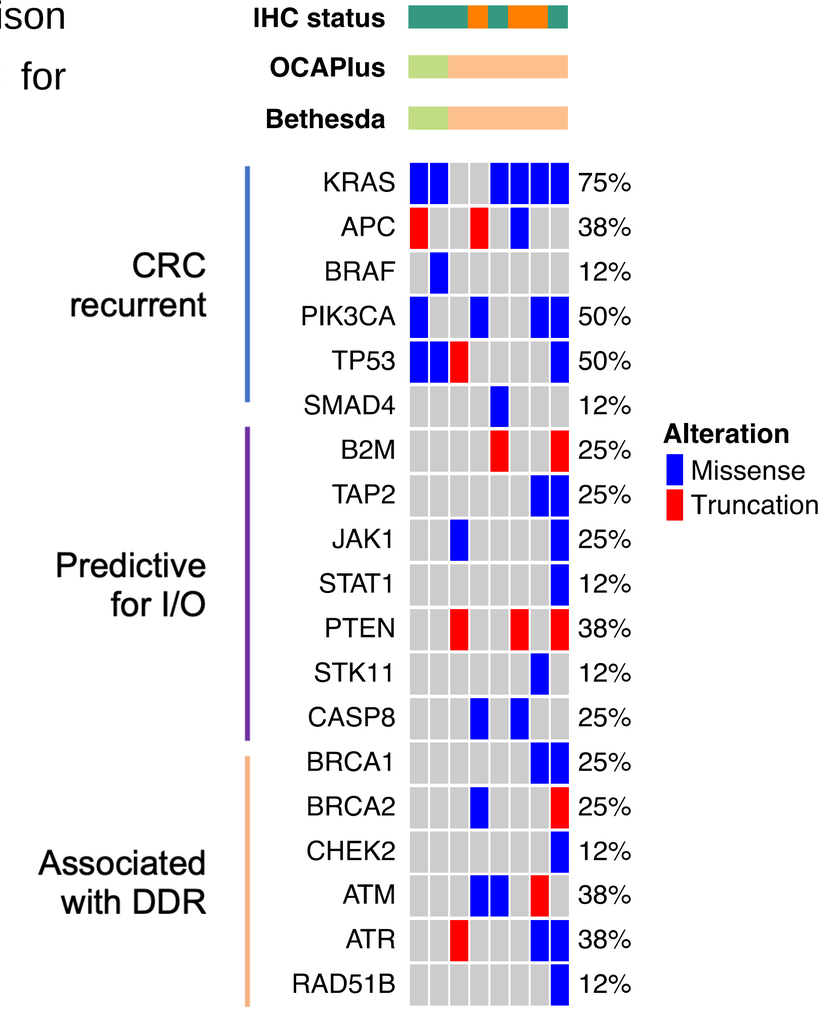
**DCB corresponds to PFS > 6 months*

OCAPlus: CRC - MSI and relevant alterations

Oncomine™ Comprehensive Assay Plus tested on **8 colorectal cancer** samples: comparison with Bethesda panel (NCI; *BAT-25*, *BAT-26, D2S123*, *D5S346*, and *D17S250*) and IHC for MMR proteins.







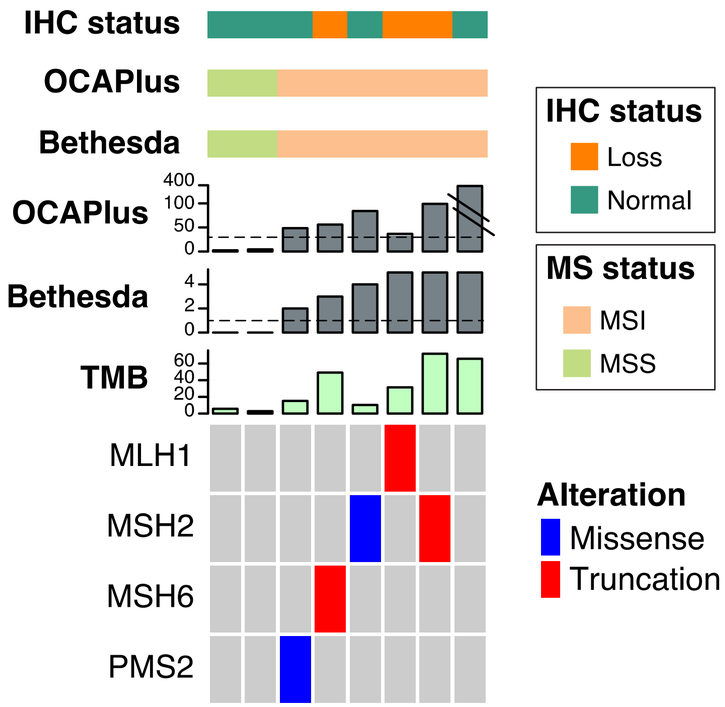
- MMR protein loss (IHC) = truncating mutations detected by OCAPlus
- Good correlation with MS-score and number of unstable Bethesda panel markers

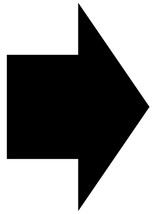
*Credit for MMR IHC image: Aldo Scarpa.

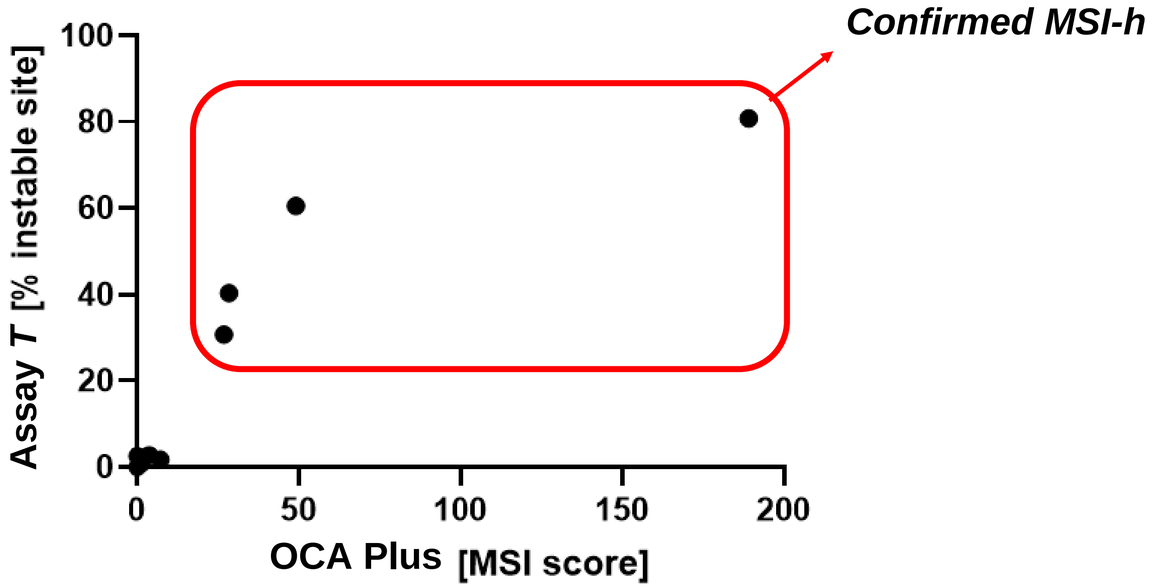
Unpublished results

OCAPlus: CRC - MSI

Oncomine™ Comprehensive Assay Plus tested on **8 colorectal cancer** samples: comparison with NGS assay T.



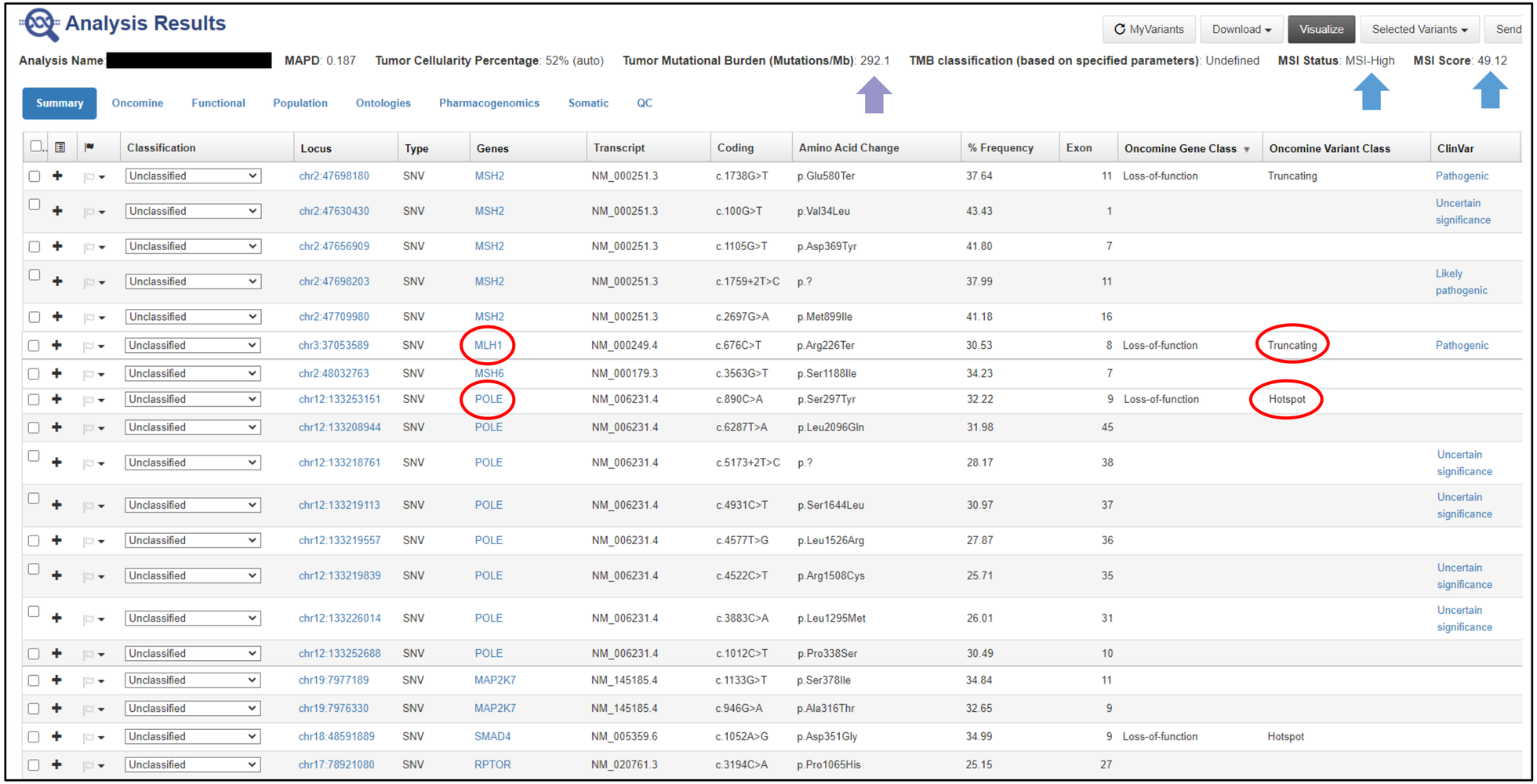




* Analysis performed on Ion Reporter with early access 2.1 workflow

Good concordance between OCA Plus, Bethesda PCR, and in-house NGS assay T

OCAPlus: mutational signature



Analysis Results

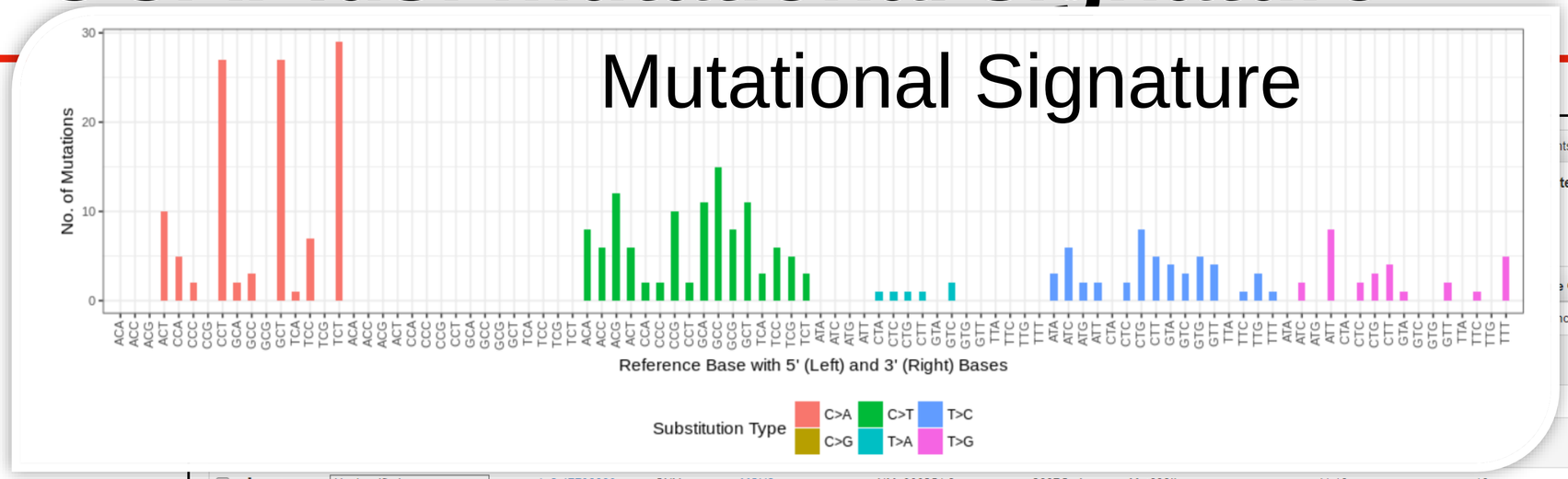
MyVariants | Download | Visualize | Selected Variants | Send

Analysis Name [redacted] **MAPD**: 0.187 **Tumor Cellularity Percentage**: 52% (auto) **Tumor Mutational Burden (Mutations/Mb)**: 292.1 **TMB classification (based on specified parameters)**: Undefined **MSI Status**: MSI-High **MSI Score**: 49.12

Summary | Oncomine | Functional | Population | Ontologies | Pharmacogenomics | Somatic | QC

Classification	Locus	Type	Genes	Transcript	Coding	Amino Acid Change	% Frequency	Exon	Oncomine Gene Class	Oncomine Variant Class	ClinVar
Unclassified	chr2:47698180	SNV	MSH2	NM_000251.3	c.1738G>T	p.Glu580Ter	37.64	11	Loss-of-function	Truncating	Pathogenic
Unclassified	chr2:47630430	SNV	MSH2	NM_000251.3	c.100G>T	p.Val34Leu	43.43	1			Uncertain significance
Unclassified	chr2:47656909	SNV	MSH2	NM_000251.3	c.1105G>T	p.Asp369Tyr	41.80	7			
Unclassified	chr2:47698203	SNV	MSH2	NM_000251.3	c.1759+2T>C	p.?	37.99	11			Likely pathogenic
Unclassified	chr2:47709980	SNV	MSH2	NM_000251.3	c.2697G>A	p.Met899Ile	41.18	16			
Unclassified	chr3:37053589	SNV	MLH1	NM_000249.4	c.676C>T	p.Arg226Ter	30.53	8	Loss-of-function	Truncating	Pathogenic
Unclassified	chr2:48032763	SNV	MSH6	NM_000179.3	c.3563G>T	p.Ser1188Ile	34.23	7			
Unclassified	chr12:133253151	SNV	POLE	NM_006231.4	c.890C>A	p.Ser297Tyr	32.22	9	Loss-of-function	Hotspot	
Unclassified	chr12:133208944	SNV	POLE	NM_006231.4	c.6287T>A	p.Leu2096Gln	31.98	45			
Unclassified	chr12:133218761	SNV	POLE	NM_006231.4	c.5173+2T>C	p.?	28.17	38			Uncertain significance
Unclassified	chr12:133219113	SNV	POLE	NM_006231.4	c.4931C>T	p.Ser1644Leu	30.97	37			Uncertain significance
Unclassified	chr12:133219557	SNV	POLE	NM_006231.4	c.4577T>G	p.Leu1526Arg	27.87	36			
Unclassified	chr12:133219839	SNV	POLE	NM_006231.4	c.4522C>T	p.Arg1508Cys	25.71	35			Uncertain significance
Unclassified	chr12:133226014	SNV	POLE	NM_006231.4	c.3883C>A	p.Leu1295Met	26.01	31			Uncertain significance
Unclassified	chr12:133252688	SNV	POLE	NM_006231.4	c.1012C>T	p.Pro338Ser	30.49	10			
Unclassified	chr19:7977189	SNV	MAP2K7	NM_145185.4	c.1133G>T	p.Ser378Ile	34.84	11			
Unclassified	chr19:7976330	SNV	MAP2K7	NM_145185.4	c.946G>A	p.Ala316Thr	32.65	9			
Unclassified	chr18:48591889	SNV	SMAD4	NM_005359.6	c.1052A>G	p.Asp351Gly	34.99	9	Loss-of-function	Hotspot	
Unclassified	chr17:78921080	SNV	RPTOR	NM_020761.3	c.3194C>A	p.Pro1065His	25.15	27			

OCAPlus: mutational signature

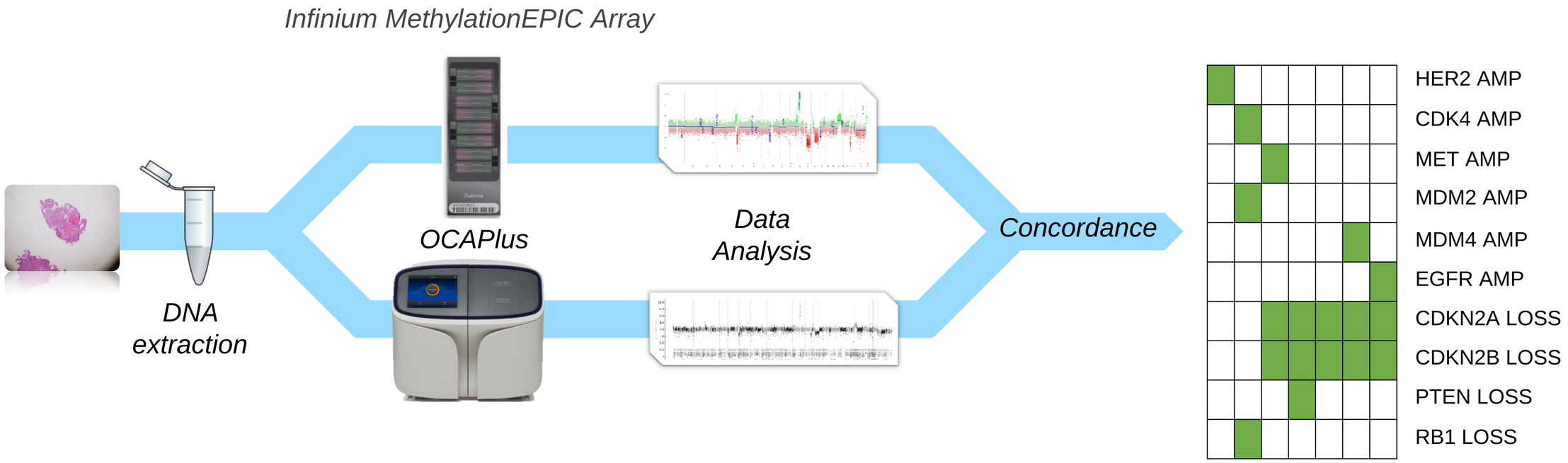


OCAPlus: copy number variants

NGS is routinely employed in Clinical Research for the detection of clinically relevant variants in cancer specimens.

Copy-number variation (CNV) analysis through NGS has not yet been established to meet clinical standards.

Methods currently used in the clinics: arrays (array-CGH, single-nucleotide polymorphism (SNP) arrays, OncoScan™ CNV Plus kit).

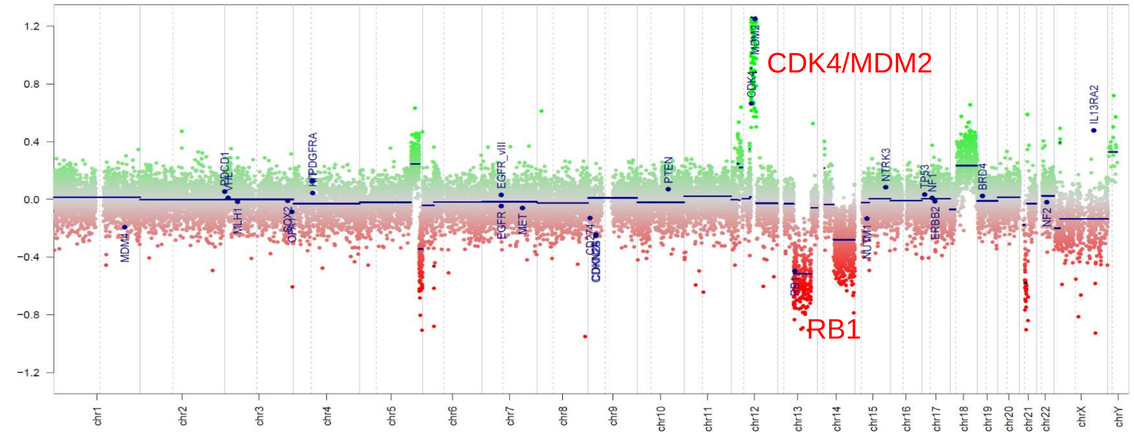


Source: Thermo Fisher Scientific

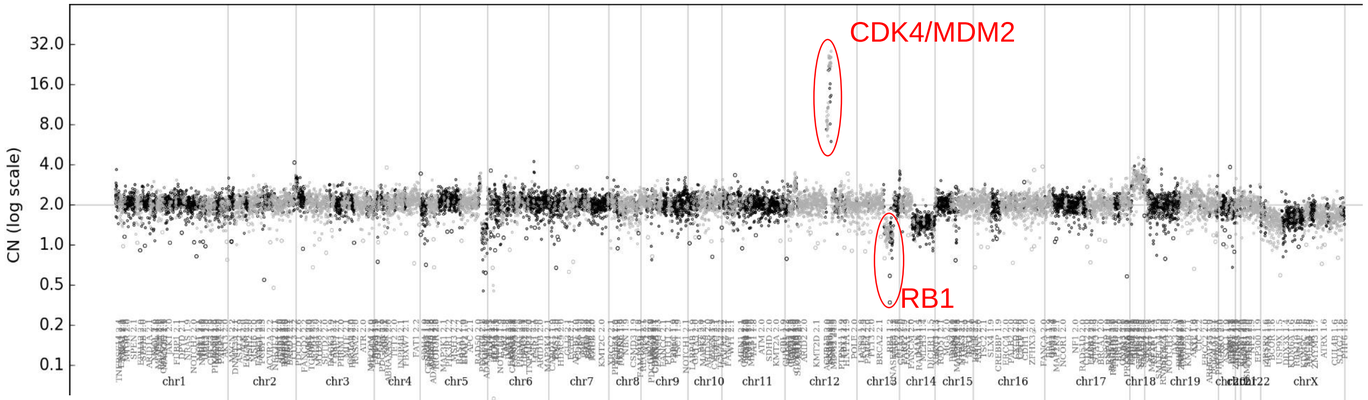
OCAPlus: copy number variants

Example: Glioblastoma sample

InfiniumArray

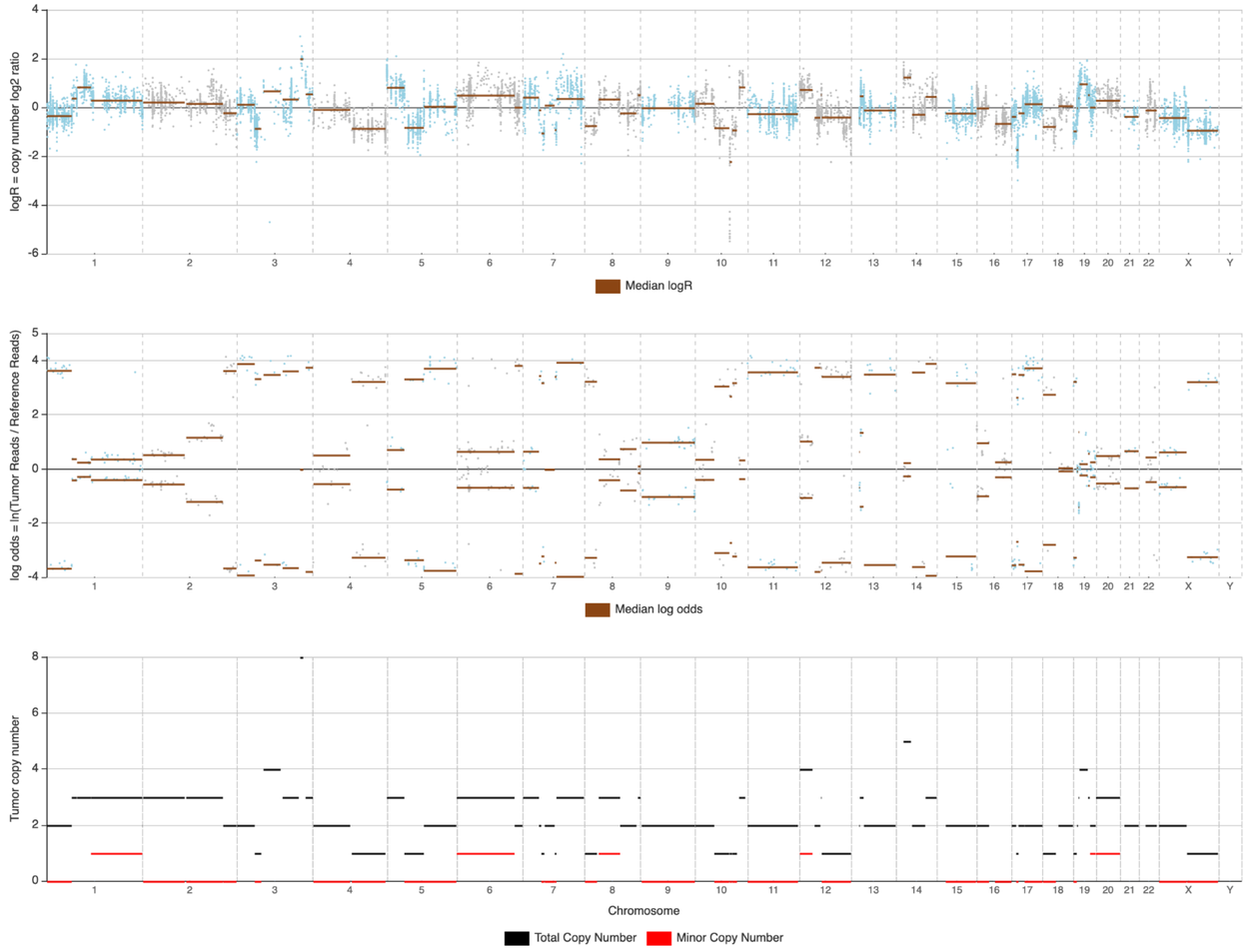


OCAPlus



Good concordance between OCA Plus and array for CNV analysis.

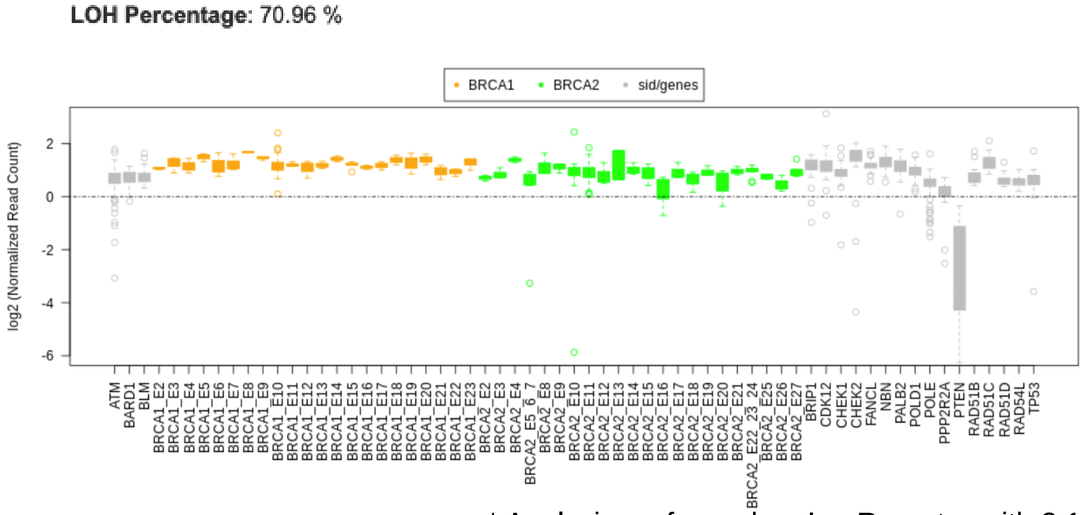
OCAPlus: percentage LOH and HRD



Clinical research sample, high percentage LOH and good quality segmentation profile.

No BRCA1/2 mutation, however potential PTEN loss (PTEN associated with high HRD score, Takaya et al. 2020, Mansour et al. 2018, McEllin et al. 2010).

Need for validation of percentage LOH.



* Analysis performed on Ion Reporter with 2.1 workflow

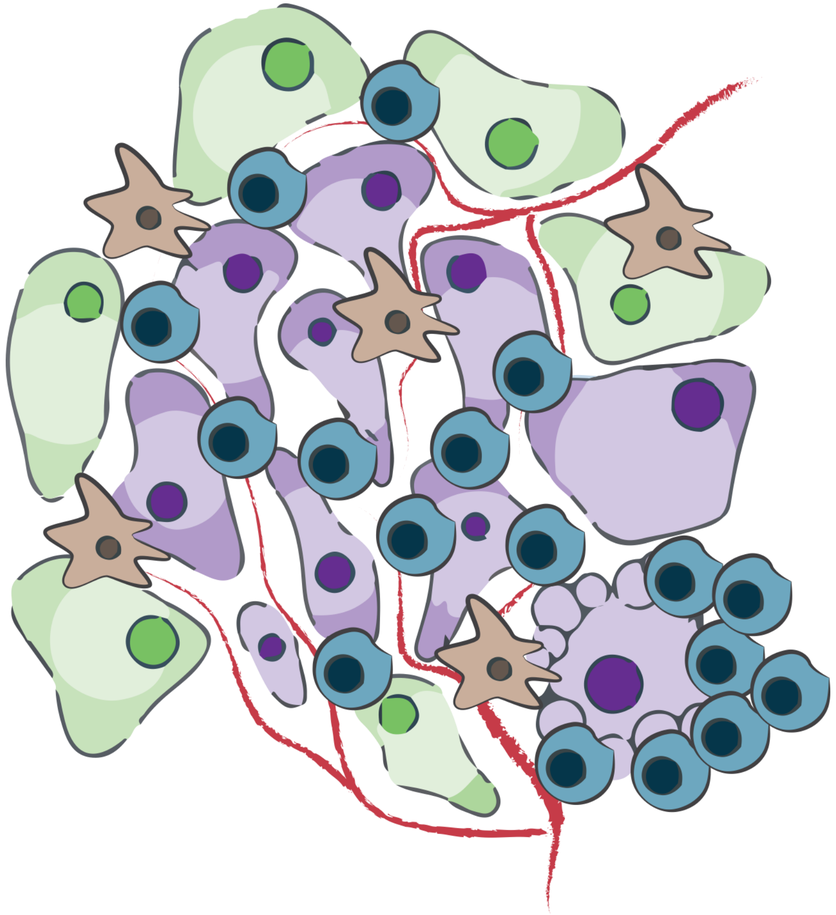
Comprehensive Genomic Profiling

Conclusion

Higher probability of identifying relevant biomarkers

! Need for clinical validation and clinical utility assessment

Standardization and harmonization between different assays, in addition to assessment of clinical utility in large prospective clinical studies, will be key for establishing novel biomarkers and CGP in routine cancer care.



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