

Clinical research on genomic profiling using the Oncomine™ Comprehensive Assay Plus

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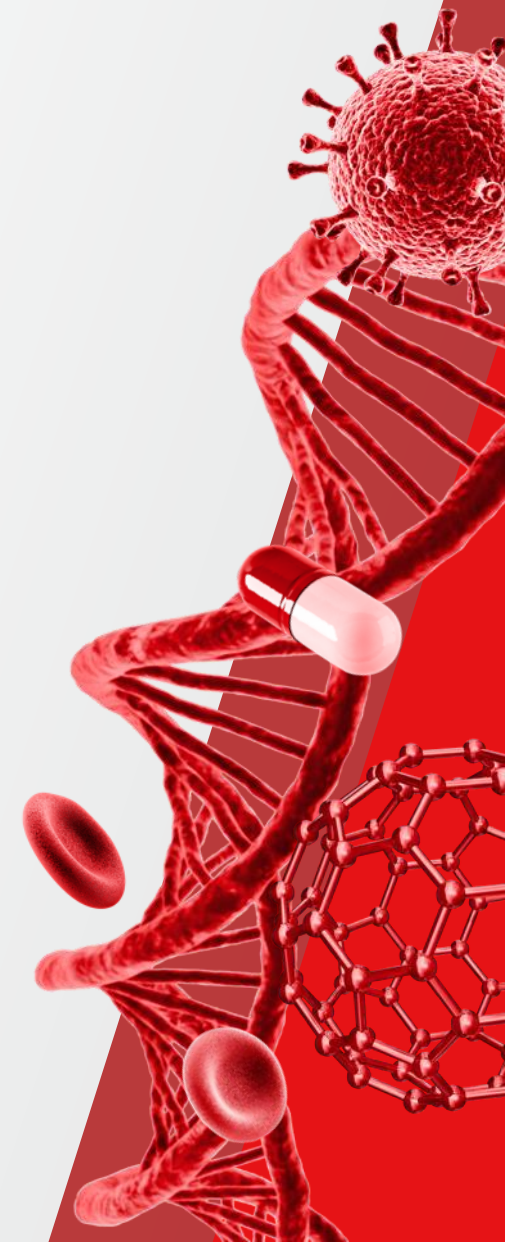
Institute for pathology medica AG

Zurich, Switzerland

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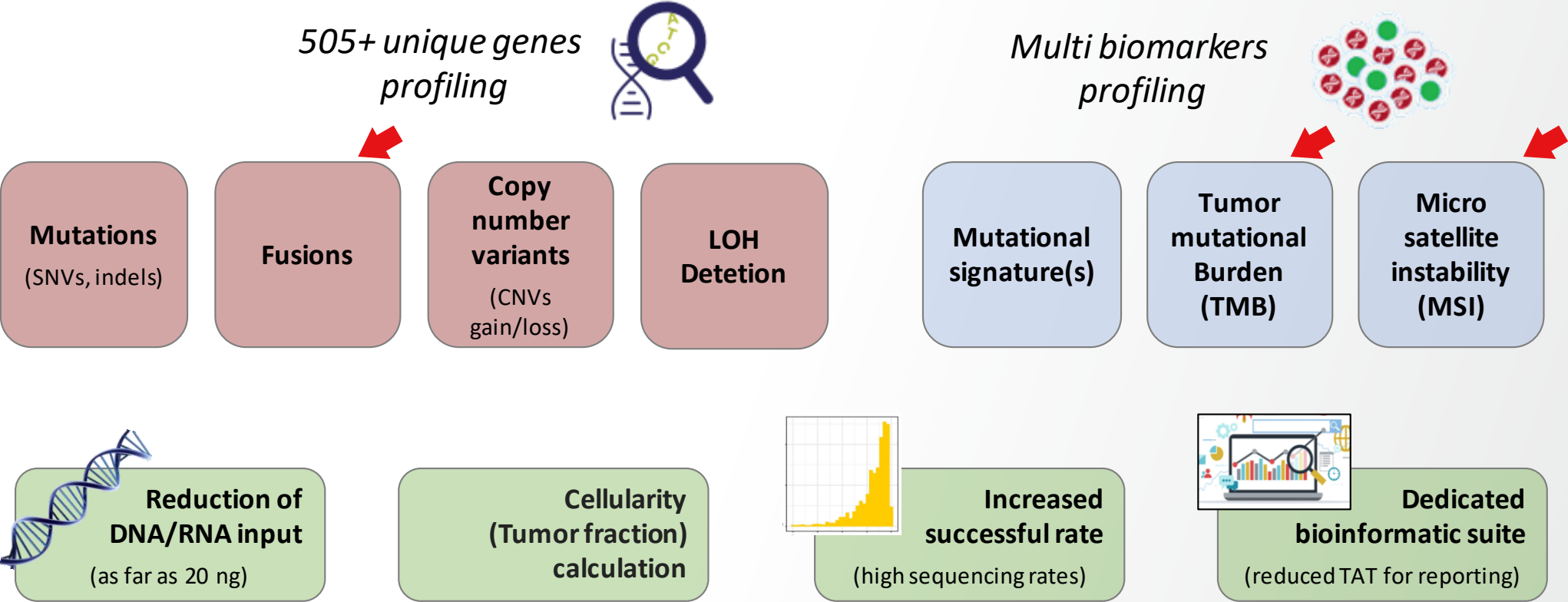
Speaker is provided honorarium for its presentation.

Introduction to the Oncomine™ Comprehensive Assay Plus



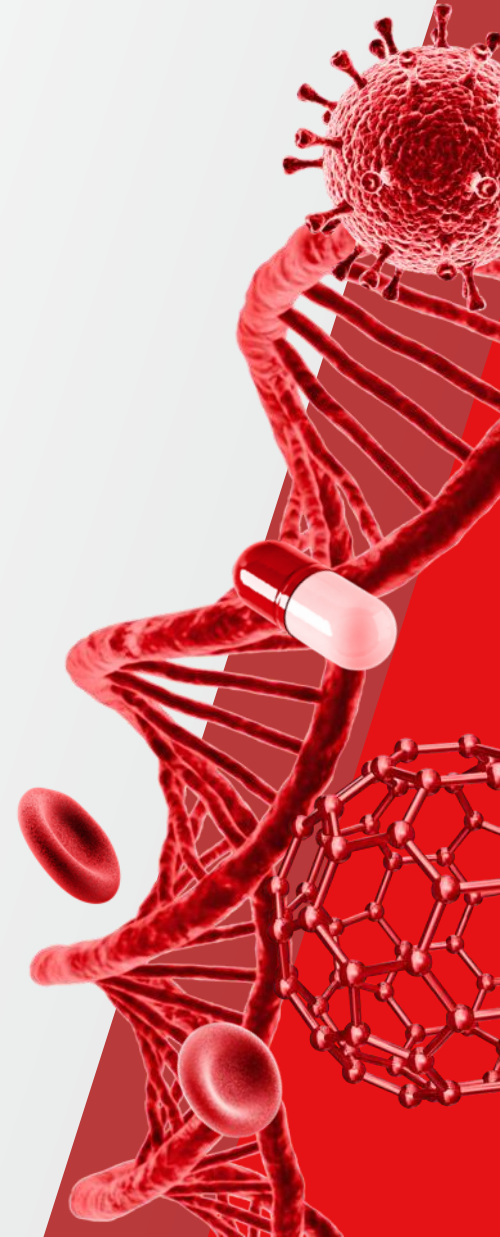
Overview Oncomine™ Comprehensive Assay Plus (OCA Plus)

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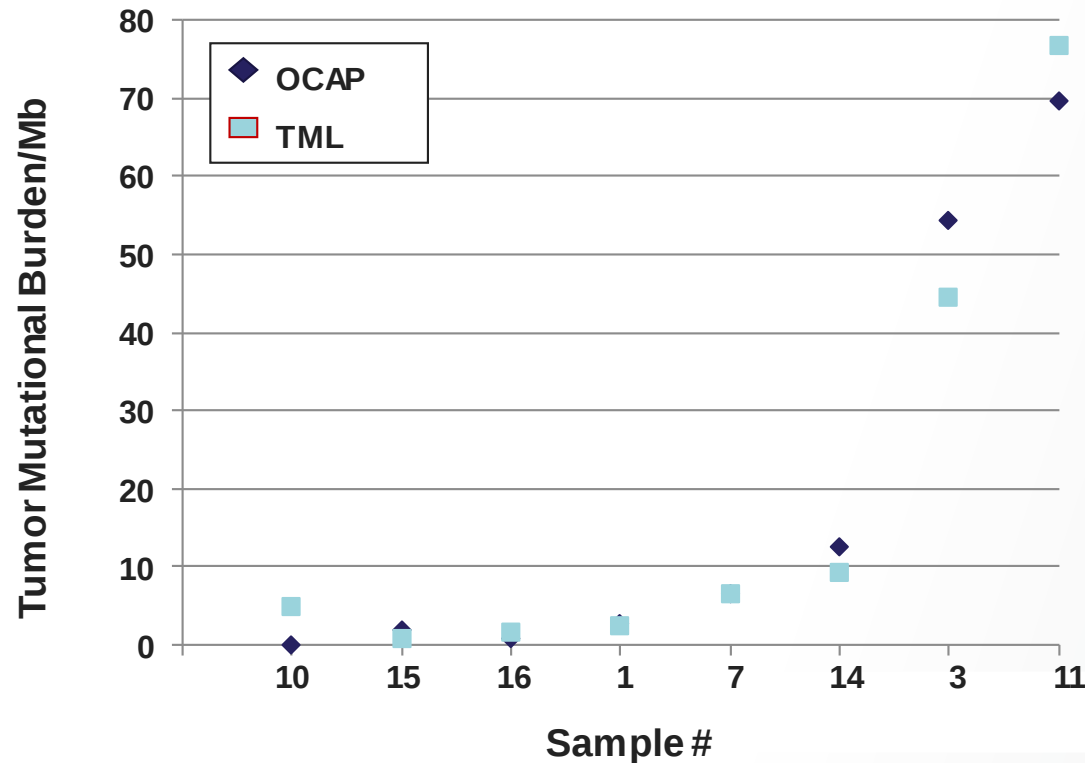
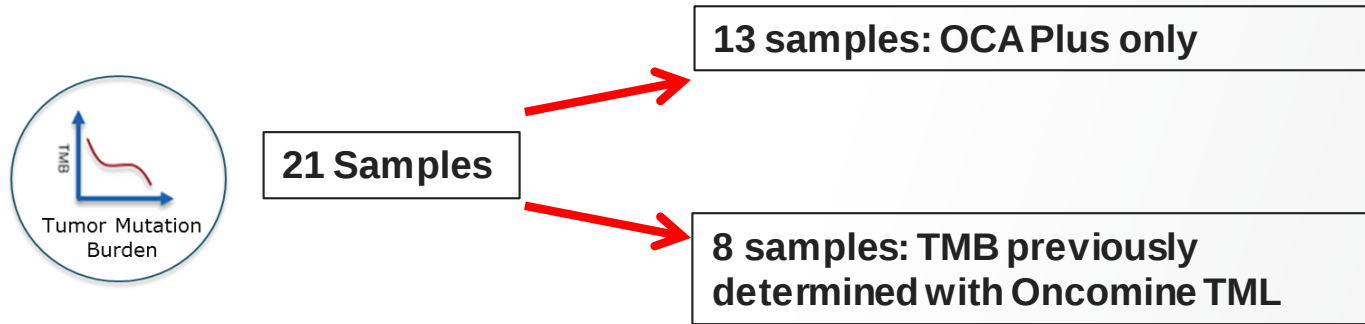


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Assessment of Tumor Mutational Burden (TMB)

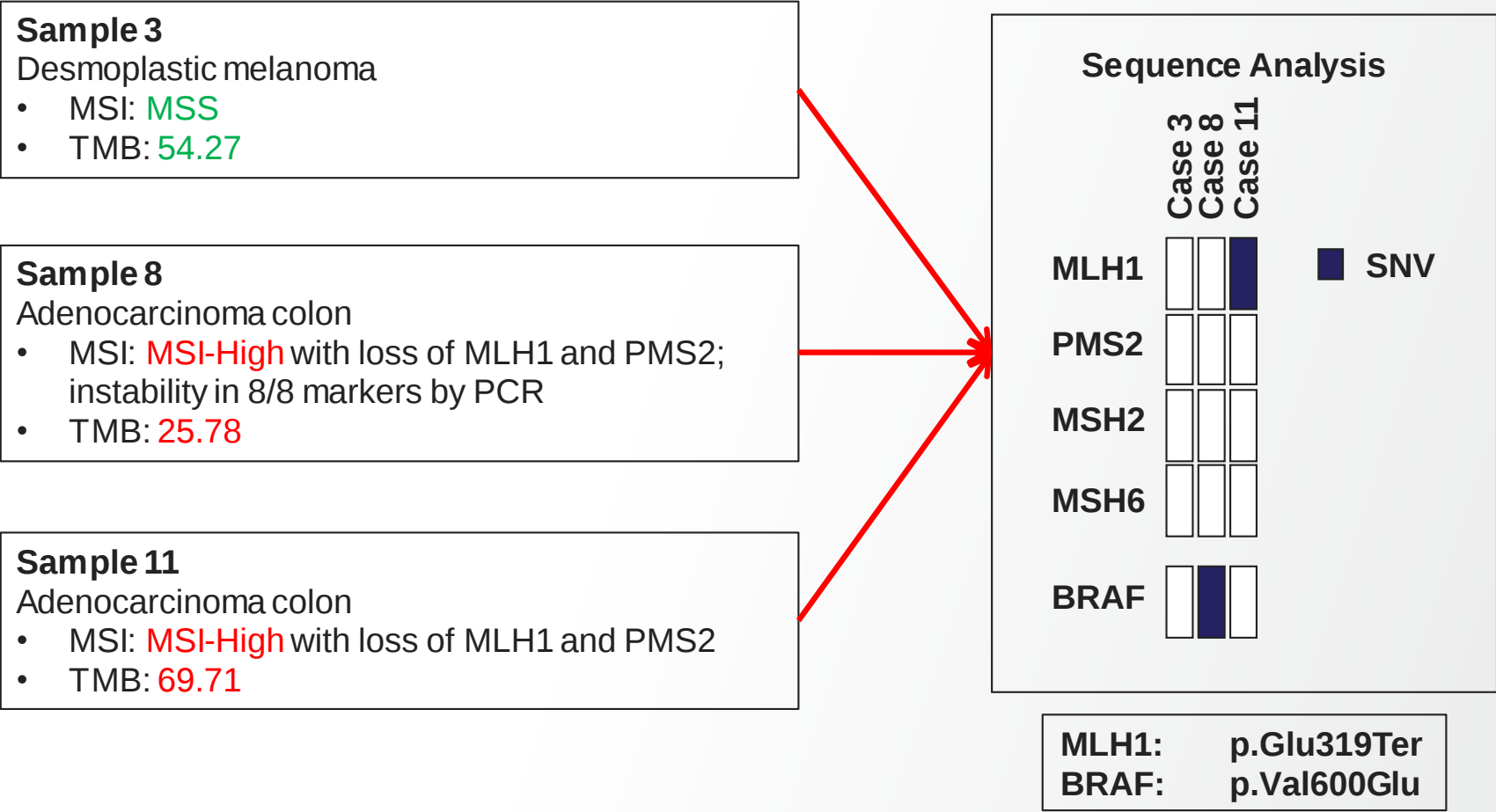


Comparison of Tumor Mutational Burden (TMB) in samples tested with Oncomine Tumor Mutational Load (TML) and OCA Plus Assay

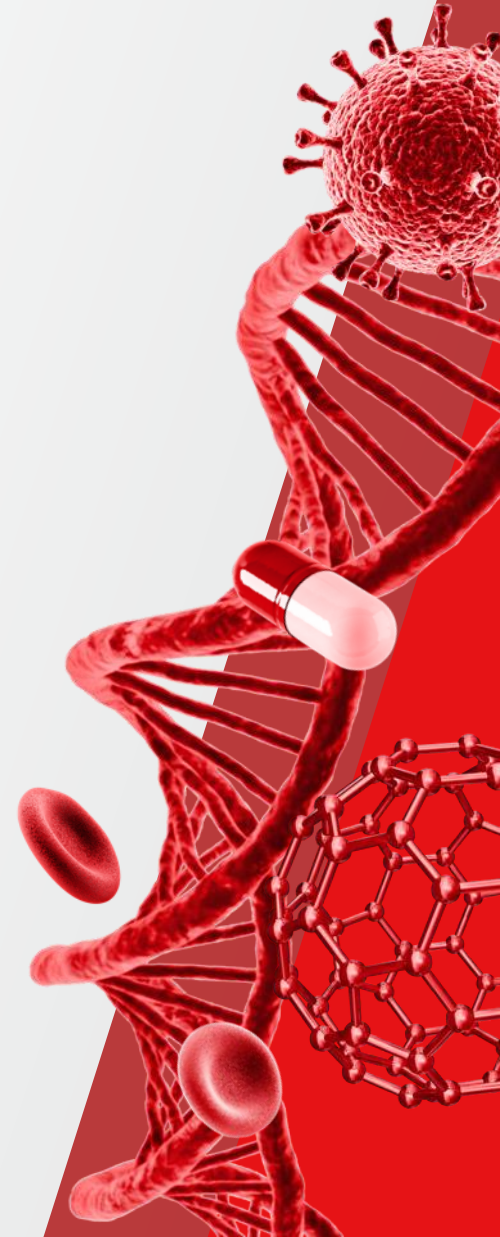


- Sample 10 Adenocarcinoma appendix
- Sample 15 Uveal melanoma
- Sample 16 Carcinoma of the adrenal gland
- Sample 1 Adenocarcinoma pancreas
- Sample 7 Adenocarcinoma lung
- Sample 14 Neuroendocrine carcinoma NOS
- Sample 3 Desmoplastic melanoma
- Sample 11 Adenocarcinoma colon

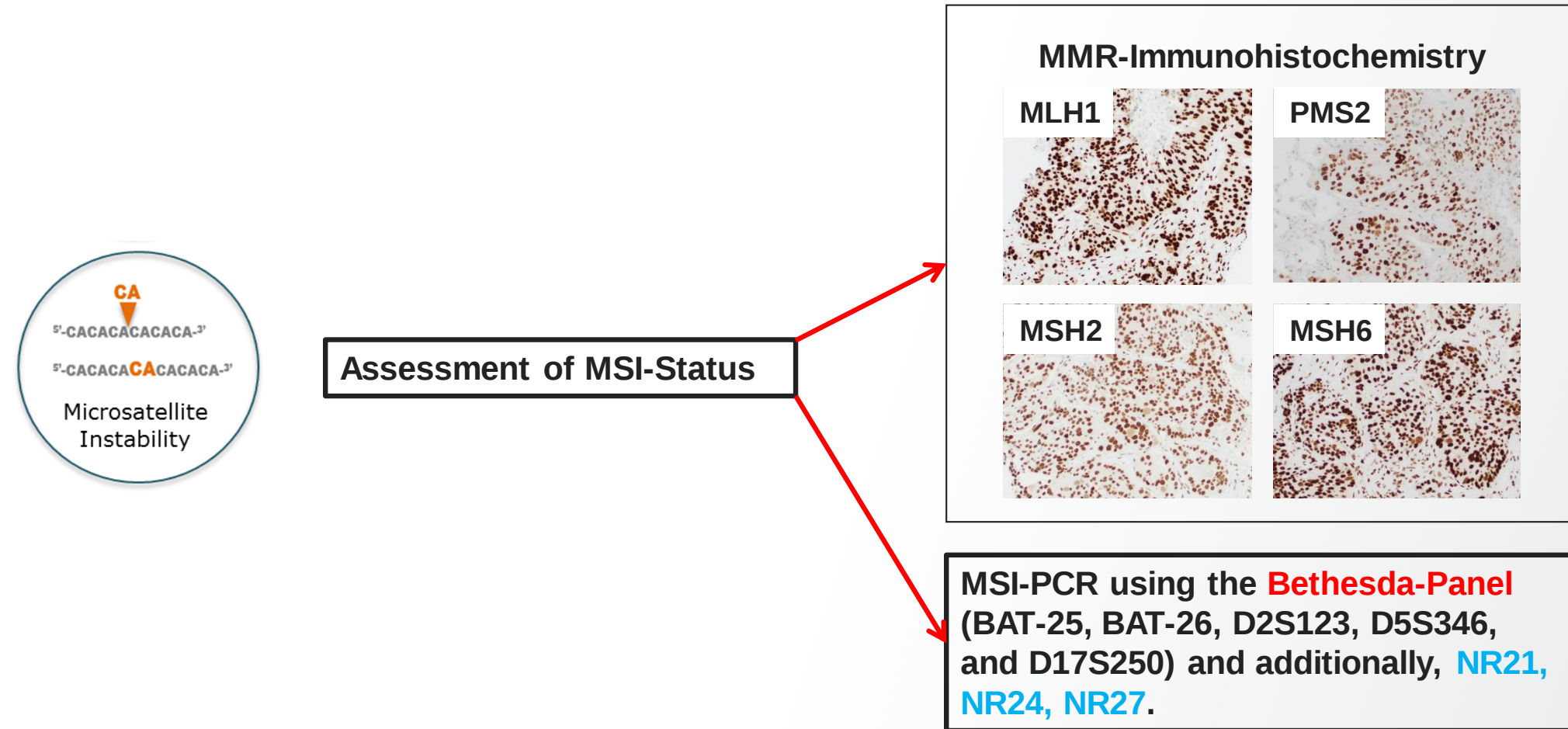
Correlation of TMB High Samples with MSI



Assessment of MSI-Status



MSI-Status Determined by OCA Plus in Comparison with MMR-Immunohistochemistry (MMR-IHC) and/or MSI-PCR

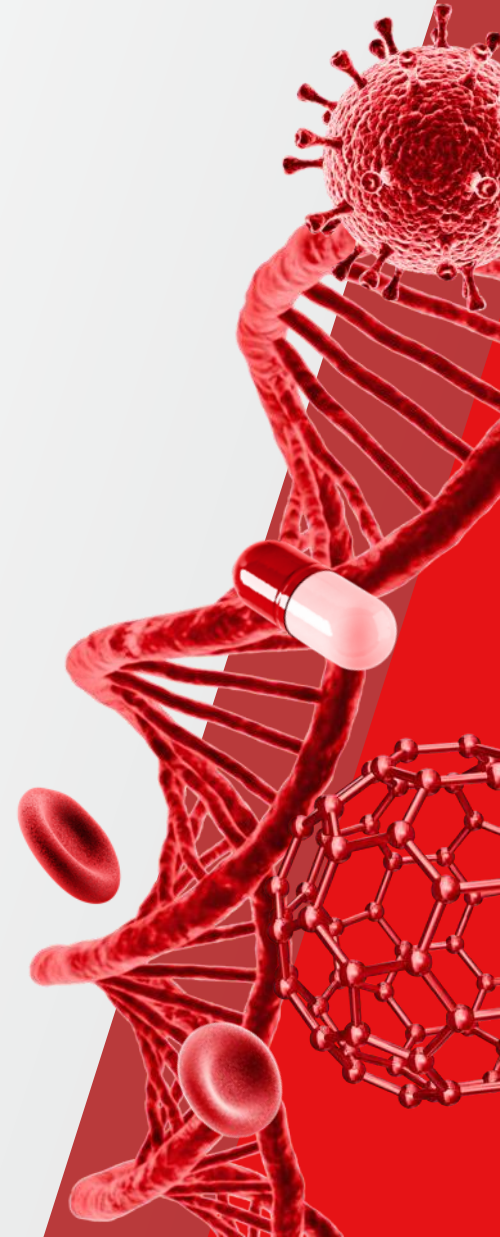


Sample	MMR-IHC	MSI-PCR	MSI-Score (OCA Plus)	MSI-Status
1	No loss	n.d.	8.3	MSS
3	No loss	n.d.	3.83	MSS
4	No loss	n.d.	0	MSS
5	No loss	MSS	0	MSS
9	indeterminate	MSS	6.11	MSS
10	Not done	MSS	1.73	MSS
12	No loss	n.d.	0.78	MSS
13	No loss	n.d.	0	MSS
15	No loss	n.d.	5.94	MSS
16	No loss	n.d.	0	MSS
17	No loss	n.d.	1.58	MSS
18	No loss	n.d.	0	MSS
19	No loss	n.d.	0	MSS
20	No loss	n.d.	7.6	MSS
21	No loss	n.d.	9.44	MSS
8	MLH1/PMS2 Loss	MSI-High	91.59	MSI-High
11	MLH1/PMS2 Loss	n.d.	47.81	MSI-High

CA
 5'-CACACACACACA-3'
 5'-CACACA**CA**CACACA-3'

Microsatellite
 Instability

Fusions – introduction to the topic



High frequency of recurrent gene fusions in malignant tumors

- **Diagnostic and prognostic**

- ❖ Sarcomas
- ❖ Hematologic malignancies
- ❖ Salivary gland tumors

- **Therapeutic**

- ❖ Frequent involvement of kinases
- ❖ Ideal for targeted therapy

- **Fusion detection**

- ❖ Cytogenetics
- ❖ FISH
- ❖ Immunohistochemistry
- ❖ RT-PCR

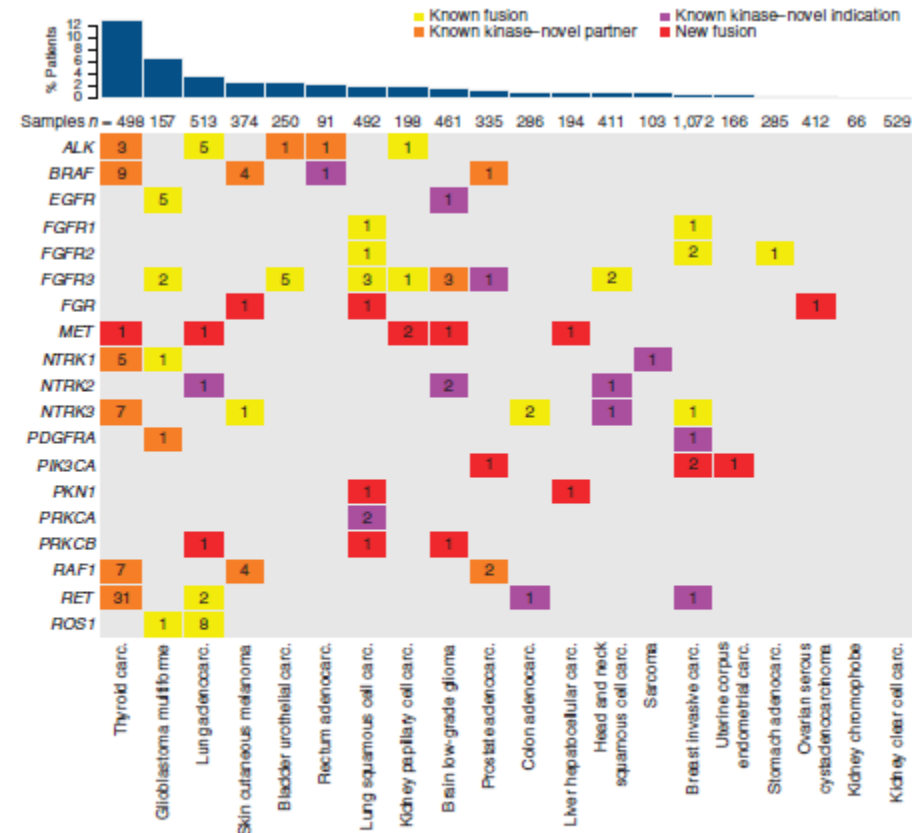
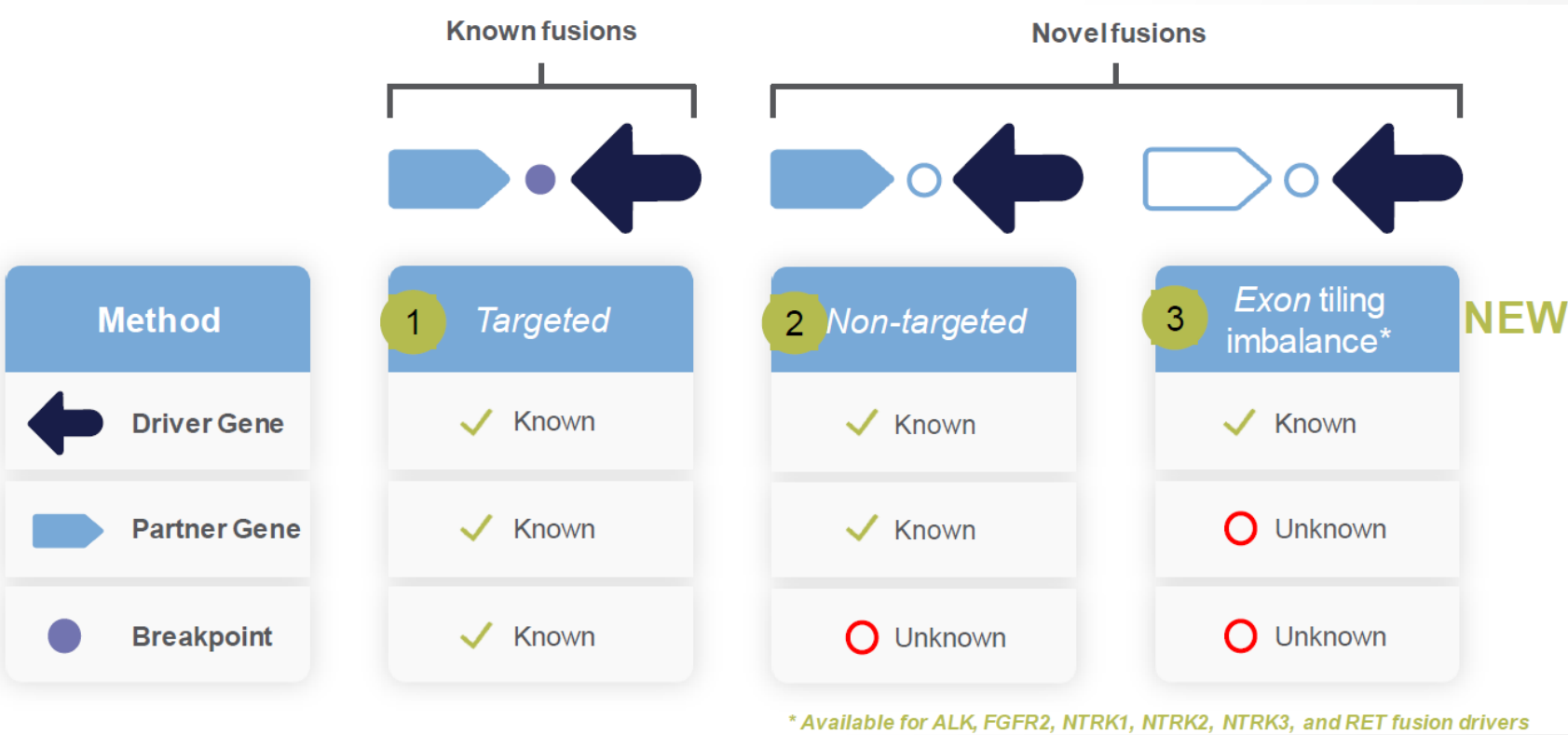


Figure 1 | Landscape of recurrent kinase fusions in solid tumours. Tumour types are indicated at the bottom and ordered by frequency of samples harbouring recurrent fusions (%; bar chart at the top). For each gene, the number of fusions found in TCGA samples is displayed in the matrix and coloured by the type of novelty: yellow denotes kinase fusions that have been described previously in this particular indication; orange denotes kinase fusions for which one or more partner genes are novel but the indication is not; purple denotes a novel indication for a particular kinase fusion regardless of the identity of the partner gene; red denotes novel, recurrent kinase fusions.

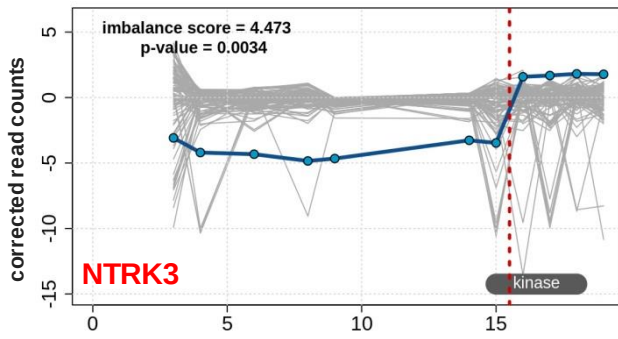
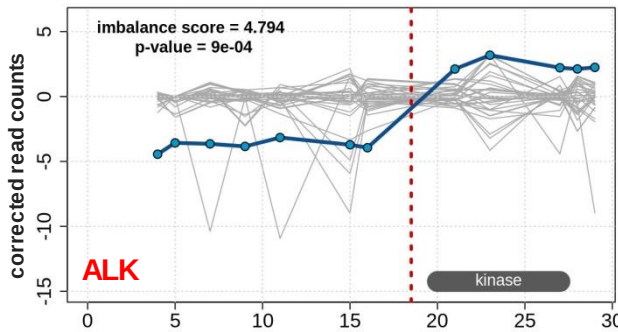
Stransky N et al. Nature Communications. 2014. 5 (4846)

Detection of known and novel fusions

- Known Fusions: 49 fusion driver genes with 1362 isoforms



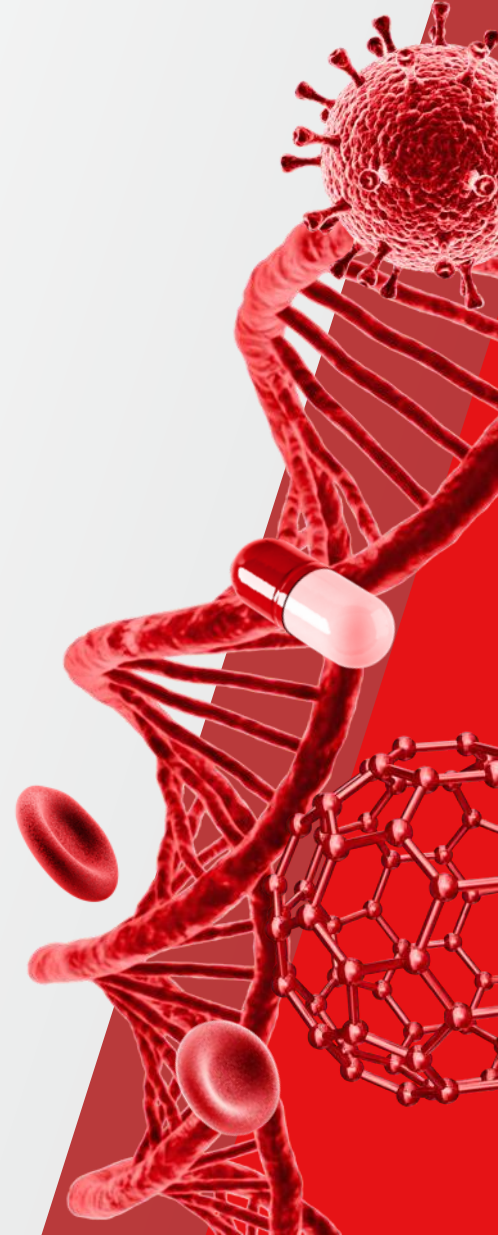
Exon tiling imbalance →
ALK, FGFR2, NTRK1, NTRK2, NTRK3, RET



modified from Thermo Fisher OCA Plus Infographic

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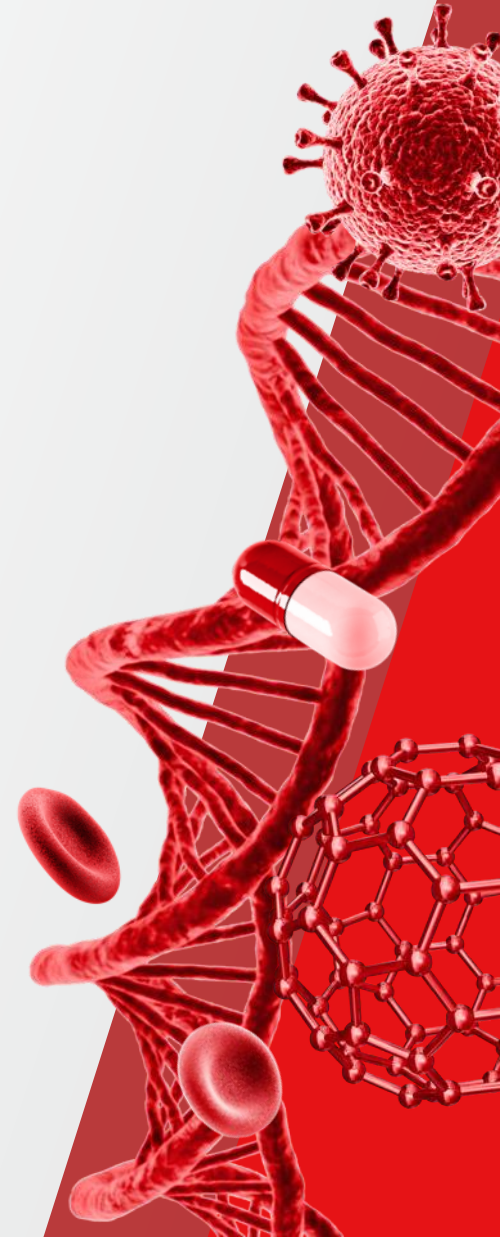
Set-Up for Fusion Assay Performance Assessment



- **Seraseq® Fusion RNA Mix v4 containing 18 clinically relevant fusions**
- **13 clinical samples with known fusions (previously detected using Oncomine™ Focus Assay or Oncomine™ Comprehensive Assay v3)**

Sample ID	Diagnosis	FNA/Biopsy	Material	Fusion Driver
F1	Adenocarcinoma lung	EBUS-TBNA; lymph node	FFPE (cell block)	ROS1
F2	Adenocarcinoma lung	EBUS-TBNA; lymph node	FFPE (cell block)	ALK
F3	Adenocarcinoma lung	Biopsy lung	FFPE	MET Exon 14 skipping
F4	Adenocarcinoma lung	FNA LK cervical	FFPE (cell block)	ROS1
F5	Adenocarcinoma lung	Biopsy lung	FFPE	ROS1
F6	Adenocarcinoma prostate	Biopsy bone	FFPE	ERG
F7	Squamous cell carcinoma lung	Biopsy lung	FFPE	MET Exon 14 skipping
F8	Adenocarcinoma (CUP)	Biopsy lymph node	FFPE	MET Exon 14 skipping
F9	Adenocarcinoma lung	EBUS-TBNA lymph node	Cells suspended in CytoLyt™ solution (Hologic)	ALK
F10	Adenocarcinoma prostate	Biopsy bone	FFPE	ERG
F11	Mammary analogue secretory carcinoma salivary gland	FNA parotid gland	FFPE (cell block)	NTRK 3
F12	Adenocarcinoma prostate	Biopsy prostate	FFPE	ERG
F13	Adenocarcinoma prostate	Resection prostate	FFPE	ERG

Assessment of Fusions QC parameters

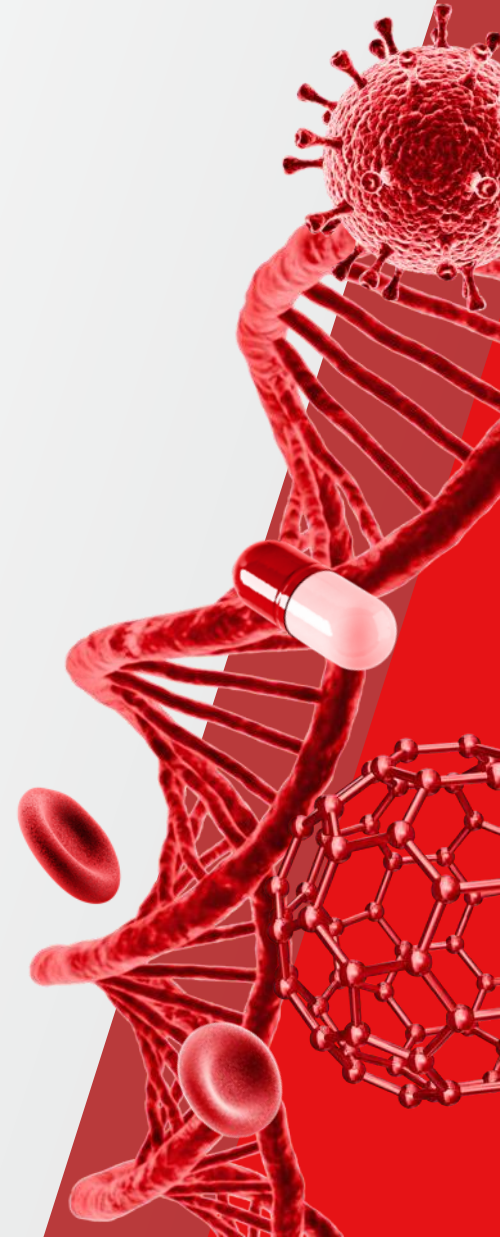


- For each of the 14 samples tested
 - Input RNA → 20ng
 - Number of reads assigned per sample → 3'000'000

	median	min	max
Read length (>50 bp)	68.5	53	87
Total mapped reads (>500'000)	4'261'887	1'782'326	9'246'948
Mapped reads pool 1 (>100'000)	2'466'513	809'604	4'398'648
Mapped reads pool 2 (>100'000)	1'901'365	461'641	6'433'037

Evaluation expression controls in samples tested		
Number of expression controls present (>15 reads)	13 samples 5/5	1 sample 4/5
Median reads all expression controls	1'023'838	min 79'278 max 2'230'981

Targeted fusion detection in Seraseq® Fusion RNA Mix v4 and 13 clinical samples



Targeted fusion detection Seraseq® Fusion RNA Mix v4

Genes	COSMIC ID	Read counts	% total mapped reads
LMNA(2) - NTRK1(11)		97'325	1.44
TFG(5) - NTRK1(10)	COSF1323	92'975	1.38
TPM3(7) - NTRK1(10)	COSF1329	143'516	2.12
ETV6(5) - NTRK3(15)	COSF571.2	130'690	1.93
EML4(13) - ALK(20)	COSF408.2	61'494	0.91
CCDC6(1) - RET(12)	COSF1271.1	38'663	0.57
KIF5B(24) - RET(11)	COSF1262.1	285'853	4.23
NCOA4(7) - RET(12)	COSF1491.1	72'492	1.07
CD74(6) - ROS1(34)	COSF1200.1	193'670	2.86
SLC34A2(4) - ROS1(34)	COSF1198	119'244	1.76
FGFR3(17) - BAIAP2L1(2)	COSF1346	104'062	1.54
FGFR3(17) - TACC3(11)	COSF1348.1	263'920	3.90
EGFR(24) - SEPT14(10)		55'505	0.82
SLC45A3(1) - BRAF(8)	COSF871	105'036	1.55
PAX8(9) - PPARG(2)	COSF1217	135'519	2.00
TMPRSS2(1) - ERG(2)	COSF23.1	117'802	1.74
EGFR(1) - EGFR(8)		236'370	3.50
MET(13) - MET(15)		147'436	2.18

- **Presence of**
 - **16/16 targeted fusions**
 - **2/2 intragenic rearrangements**
- **In addition presence of**
 - **9 non-targeted fusions in ALK, RET, ROS, FGFR3**
 - **Read counts of non-targeted fusions 536-1537 (<0.03% of TMR)**

Targeted fusion detection in clinical samples

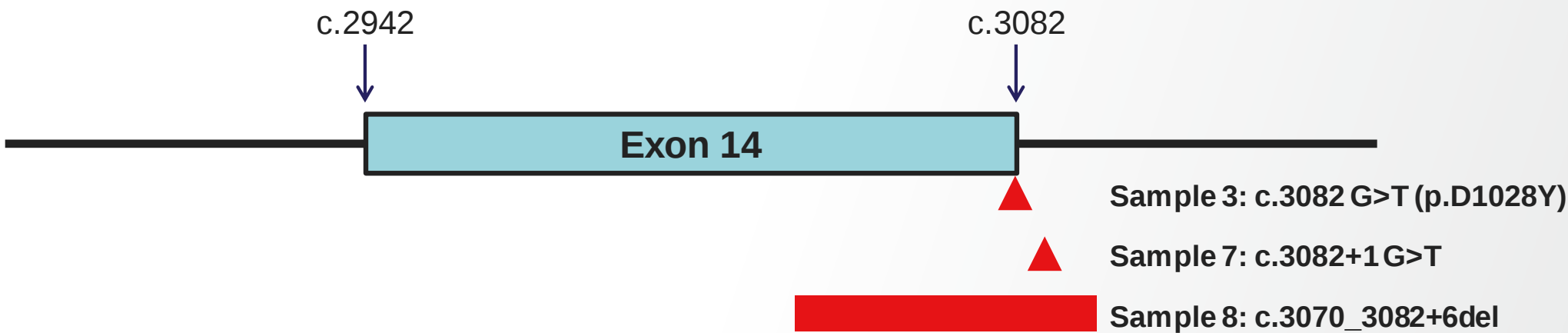
➤ **ROS1-Fusions: confirmed in 3/3 samples**

	Oncomine Comprehensive Assay Plus		Oncomine Focus Assay	
Fusions detected Sample F1	Read Count	% TMR	Read Count	% TMR
GOPC(8) - ROS1(35) COSF1139.1	27058	0.66	2780	0.85
Fusions detected Sample F4	Read Count	% TMR	Read Count	% TMR
SLC34A2(4) - ROS1(32) COSF1196	404727	17.89	114021	69.40
SLC34A2(4) - ROS1(34) COSF1198	31336	1.38	135	0.08
Fusions detected Sample F5	Read Count	% TMR	Read Count	% TMR
SDC4(4) - ROS1(32) COSF1278	1767921	36.12	251703	32.76
SDC4(4) - ROS1(34) COSF1280	42817	0.87	6183	0.80

➤ **TMPRSS2-ERG Fusions: confirmed in 4/4 samples**

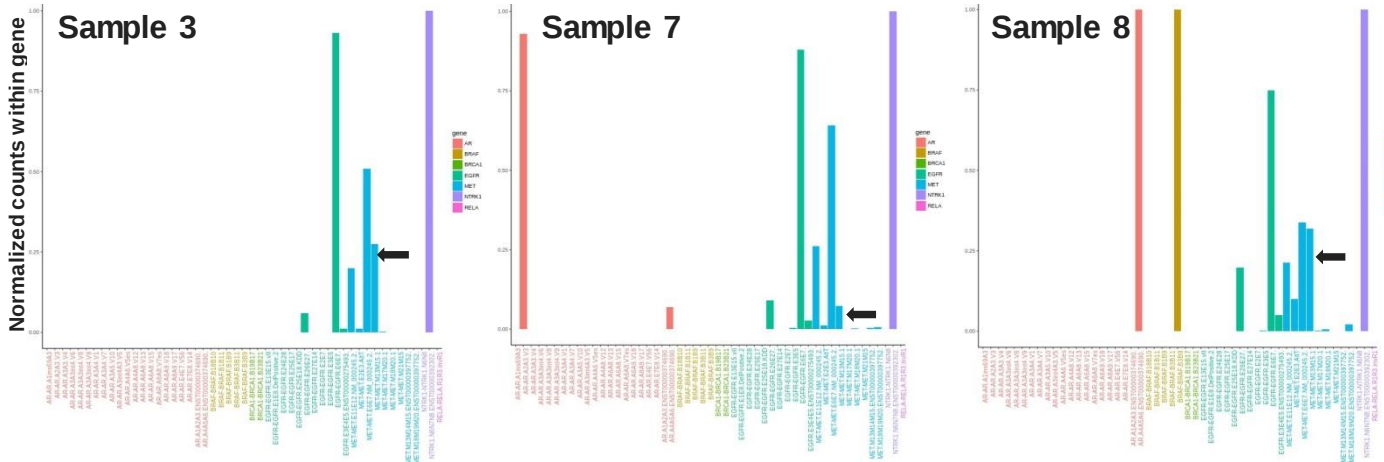


MET Exon 14 skipping in samples F3, F7 and F8



Analysis MET(13) – MET (15) expression		
	Read Count	% TMR
Sample 3	404727	17.89
Sample 7	31336	1.38
Sample 8	42817	0.87

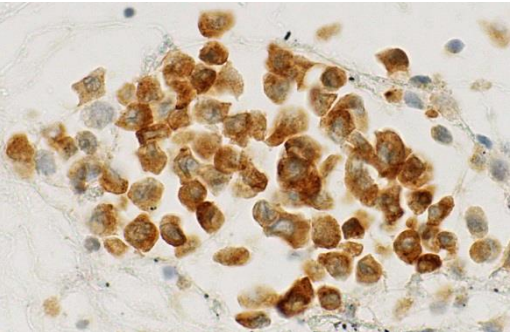
RNA Exon Variant



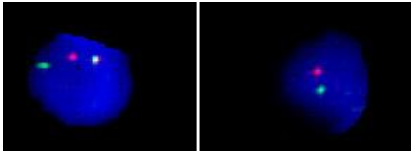
→Met-Met.M13.M15

Detection of ALK-fusions in samples 2 and 9

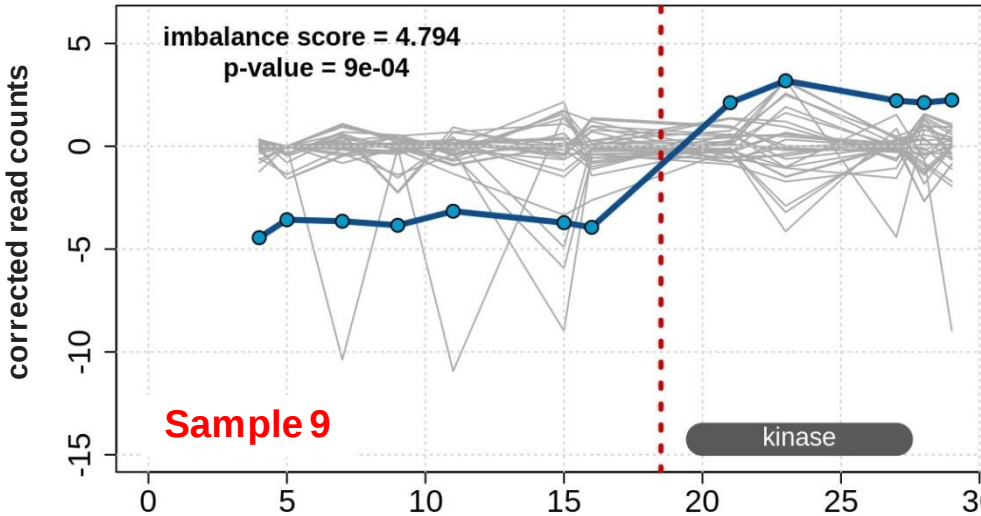
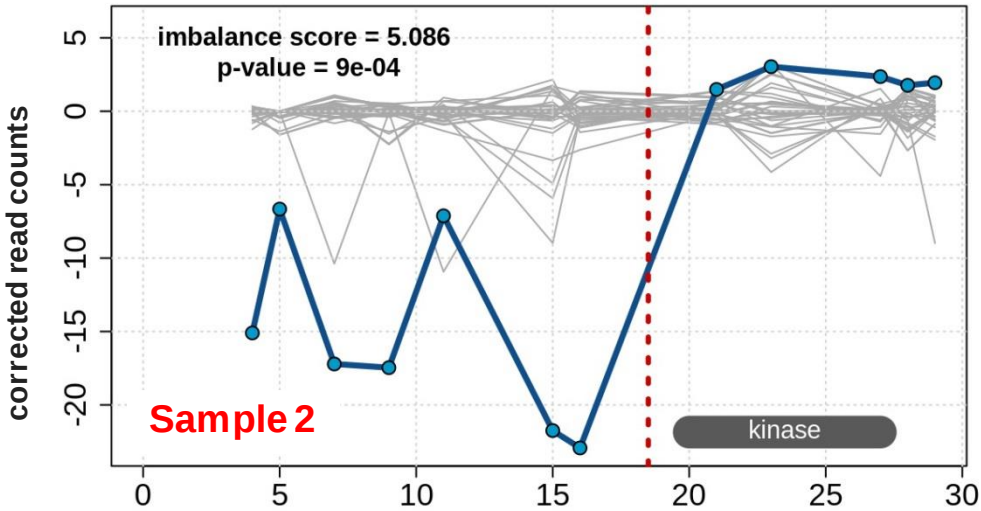
	Oncomine Comprehensive Assay Plus		Oncomine Focus Assay	
Fusions detected Sample 2	Read Count	% TMR	Read Count	% TMR
EML4(6) - ALK(20) AB374361.1	34123	0.37	34677	11.50
EML4(7) - ALK(20) AB374362.1	17823	0.20	26038	8.63
EML4(2) - ALK(20) COSF478.1			132	0.04
Fusions detected Sample 9	Read Count	% TMR	Read Count	% TMR
EML4(13) - ALK(20) COSF408.1	78823	2.14	130051	16.74
EML4(13) - ALK(20) Non-targeted	2553	0.07		



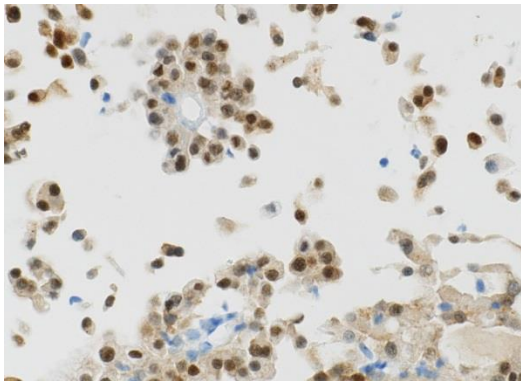
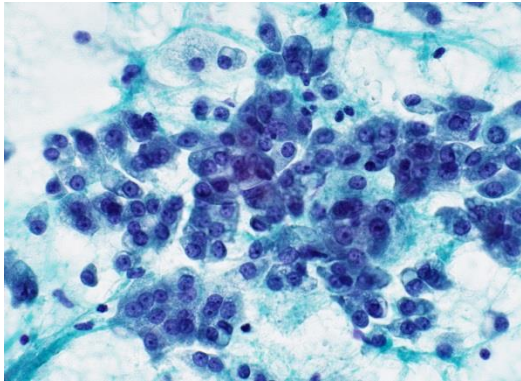
ALK immunohistochemistry and ALK FISH break-apart



Sample coverage for imbalance analysis



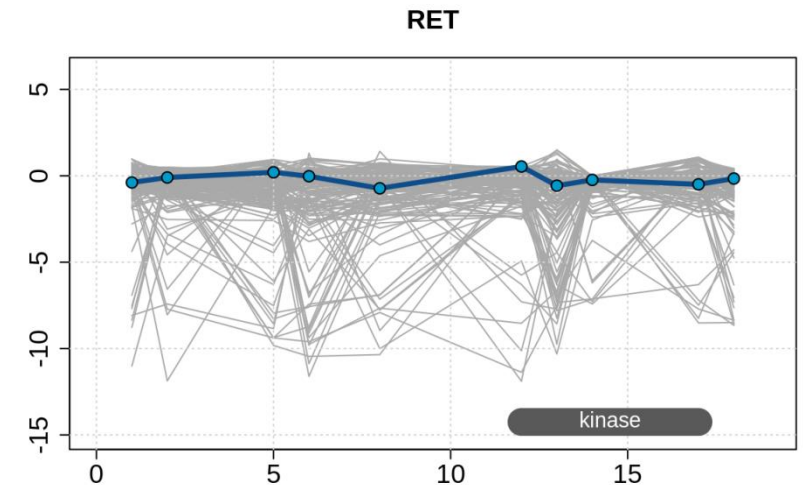
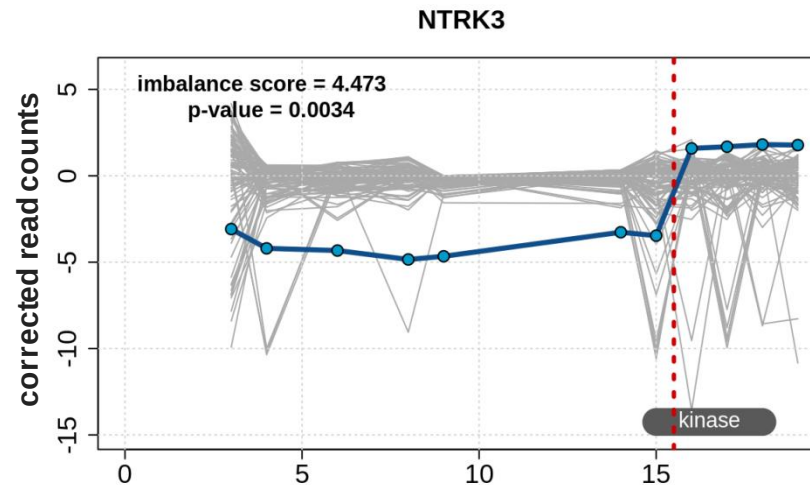
Detection of ETV6(5) - NTRK3(15) fusion in sample F11



FNA parotid gland

- Mammary analogue secretory carcinoma salivary gland
- Immunohistochemistry: Pan-TRK expression

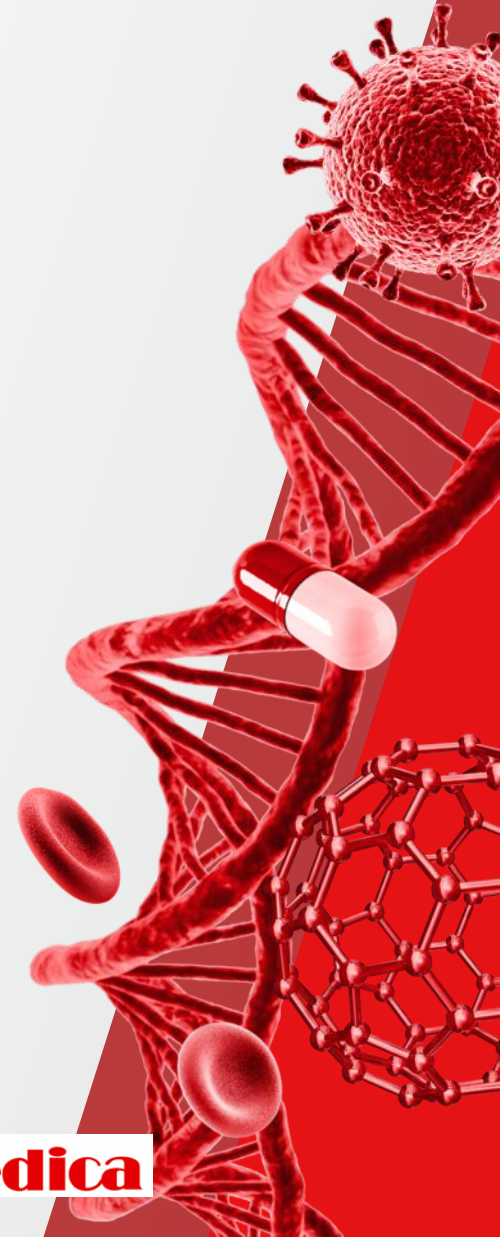
	Oncomine Comprehensive Assay Plus		Oncomine Focus Assay	
Fusion detected	Read Count	% TMR	Read Count	% TMR
ETV6(5) - NTRK3(15) COSF571.2	488'744	10.74	347'975	26.92
ETV6(4) - NTRK3(15) COSF823.2	460	0.01	1'077	0.08
ETV6(5) - RET(12) not-targeted	1'456	0.03		



Summary

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- TMB results determined by Oncomine Comprehensive Assay Plus matched the results previously obtained with TML Assay. High TMB values correlated with biological/genetic characteristics of the tumors (e.g. MSI-high tumors, desmoplastic melanoma)
- Highly reproducible determination of MSI by Oncomine Comprehensive Assay Plus compared to IHC for MMR- proteins and/or MSI-PCR
- Detection of 16/16 fusions and 2/2 intragenic rearrangements in the RNA Fusion Control Mix (SeraSeq).
- Detection of 13/13 fusions and intragenic rearrangements in clinical samples
- Correct prediction of ALK and NTRK3 break-points by exon-tiling

■ Acknowledgements

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