Universitätsspital Basel

Biomarkers for immuno-oncology: tumour mutational load and beyond.

The impact of a multidimensional approach

Philip Jermann, PhD
Head of Molecular Assay Development Unit
Institute of Medical Genetics and Pathology
University Hospital Basel, Switzerland

Universitätsspital Basel

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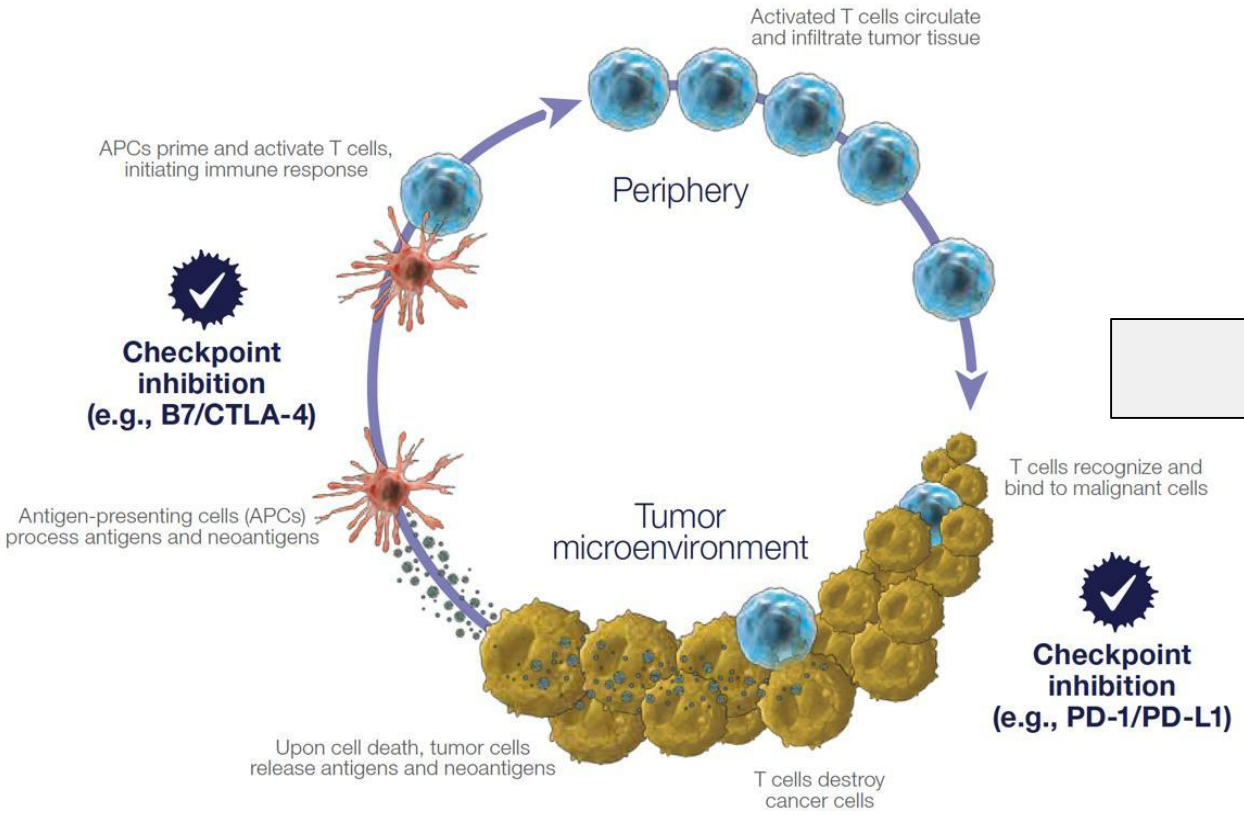
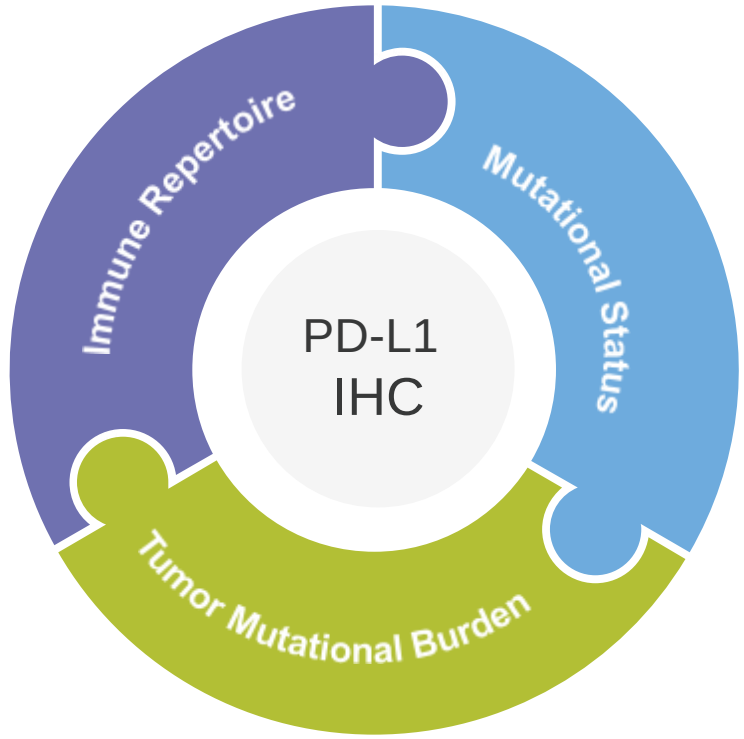
Understanding Tumor-Immune Interactions Through NGS

Using genomic assays to improve the success of immunotherapy





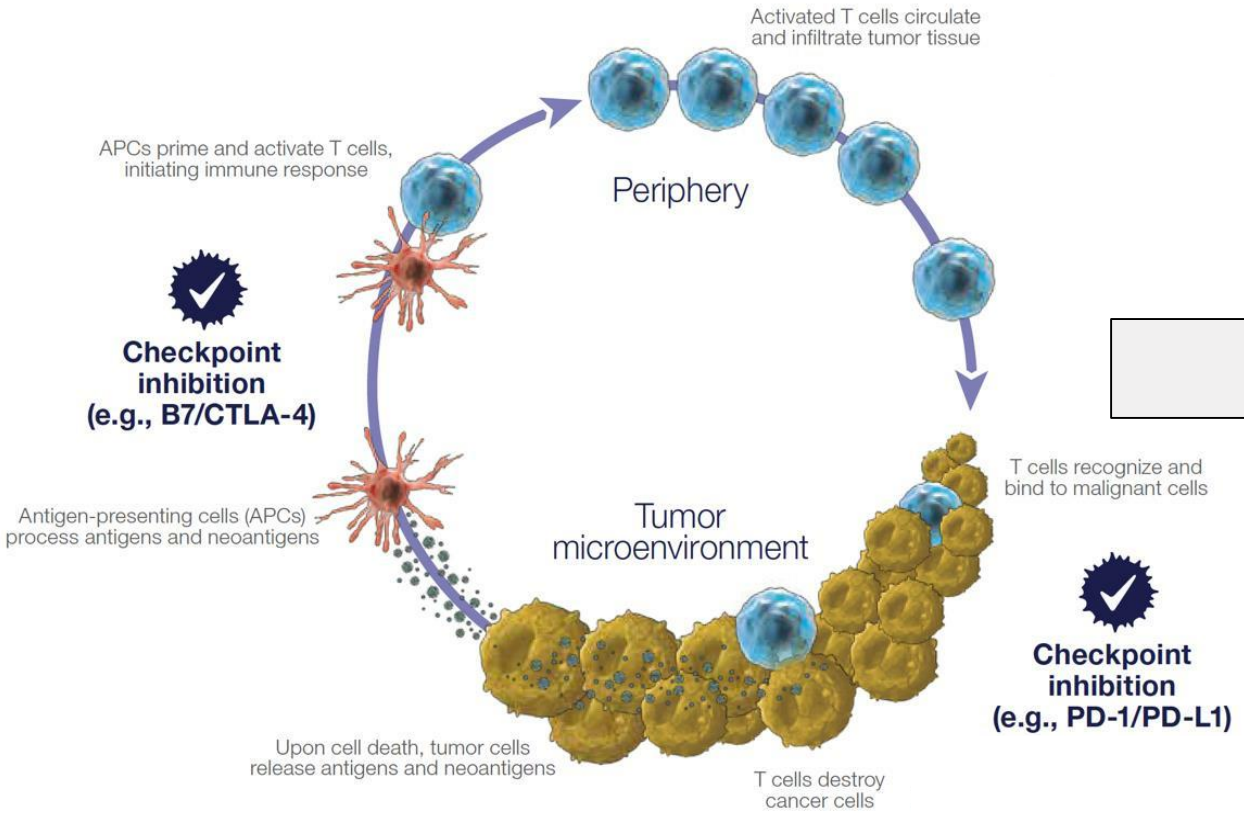
Tumor-Immune interactions are complex and warrants a multi-marker approach

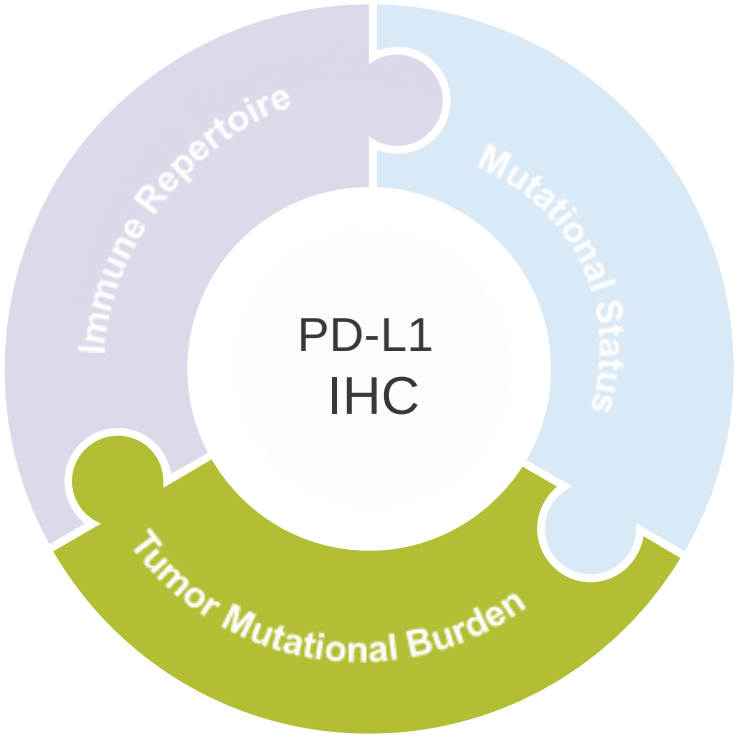
NGS assays broaden our understanding of the biology beyond traditional IHC biomarkers

Source: Thermo Fisher Scientific

Understanding Tumor-Immune Interactions Through NGS

Using genomic assays to help improve the understanding of the success of immunotherapy





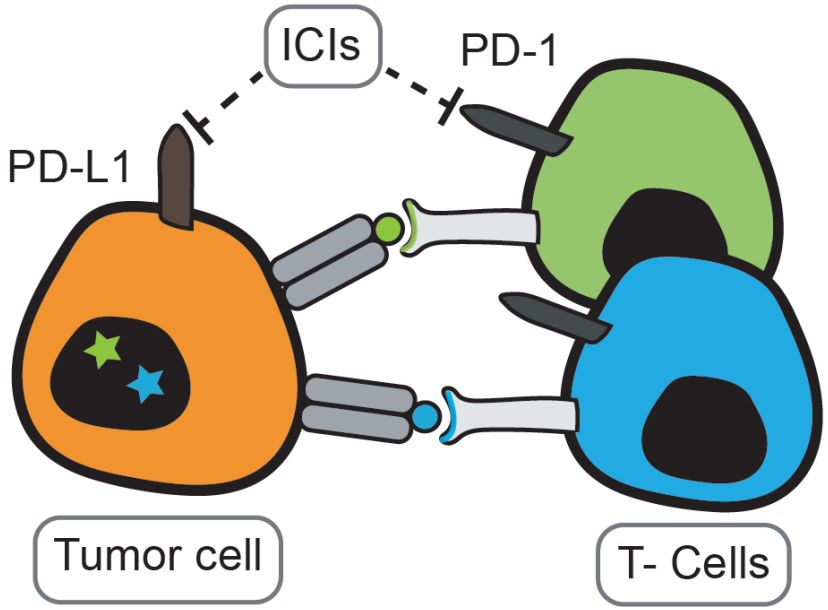
Tumor-Immune interactions are complex and warrants a multi-marker approach

NGS assays broaden our understanding of the biology beyond traditional IHC biomarkers

Source: Thermo Fisher Scientific

Promises: what and why TMB

An assessment of the number of somatic mutations within a tumor genome



The number of mutations represents an INDIRECT measure of neoantigens load.

What: Tumor Mutational Burden (TMB) measures the number of somatic mutations per Megabase (Mb) of tumor genome. This number should correlate to the neoantigen burden.

Why: The presence of tumor-specific neoantigens is associated with increased immunogenicity *[Heemskerk B. 2013]*, leading to the hypothesis that tumors presenting a higher number of neoantigens may respond better to immunotherapy *[Gubin MM. 2015, Schumacher TN. 2015, Grizzi G. 2017].*

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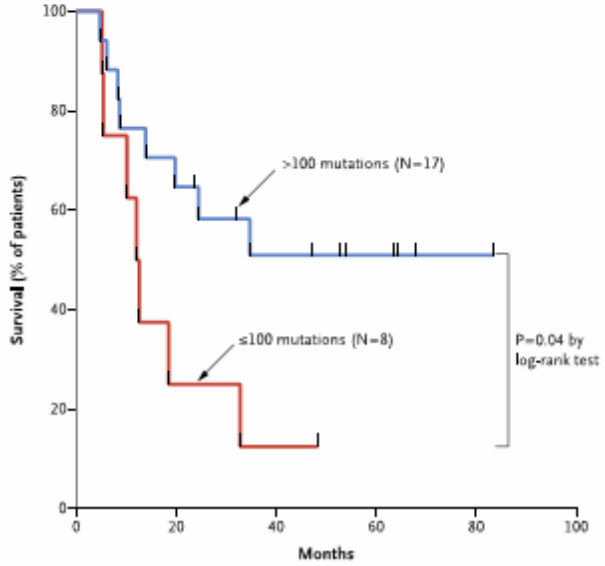
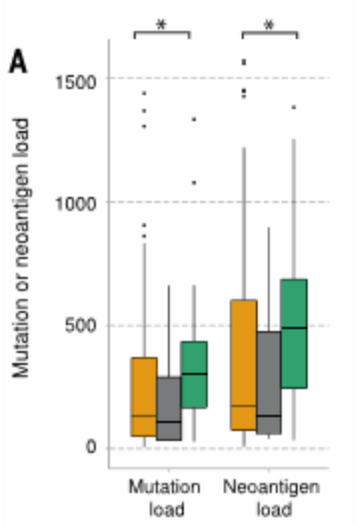
Promises: early evidence and clinical research studies

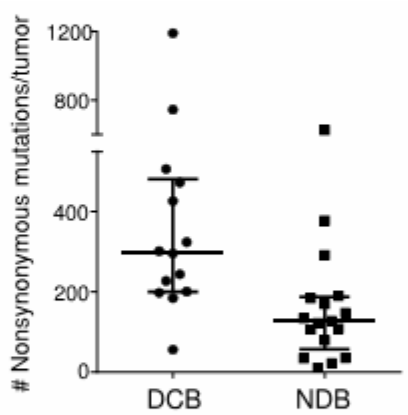
High TMB correlates with response to ICI treatment – Whole E xome Sequencing

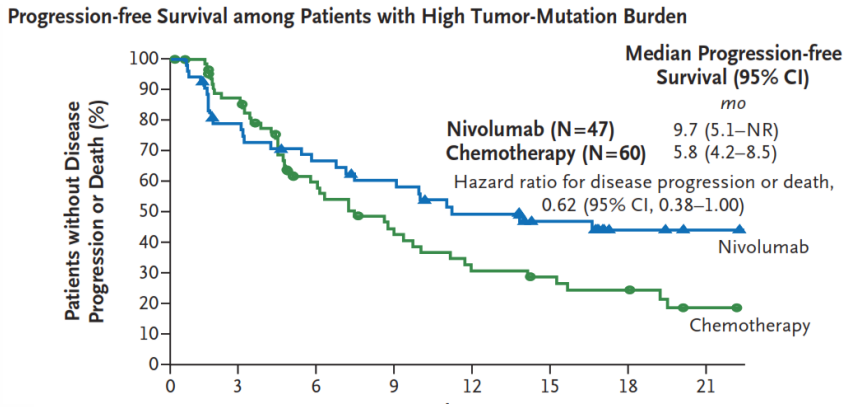
Melanoma:
Genomic correlation of response to CTLA-4 blockade in metastatic melanoma[1,2]

NSCLC:
Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer [3,4]









CheckMate 026

Snyder 2014
TMB > 100 mut/exome

Van Allen 2015
TMB > 197 mut/exome

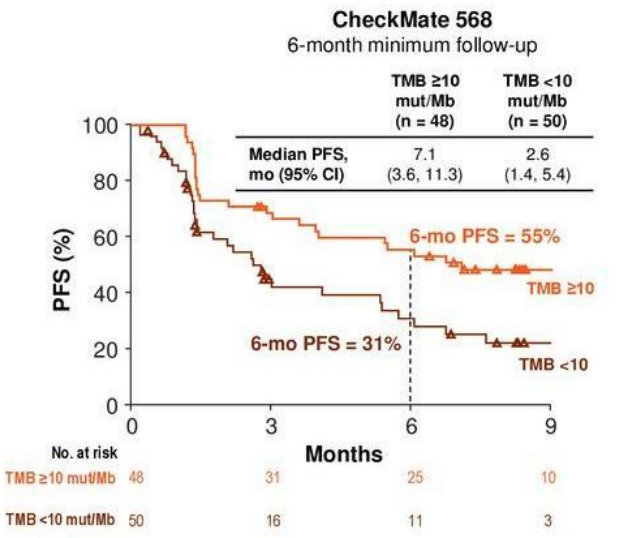
Rizvi 2015
TMB > 205 mut/exome

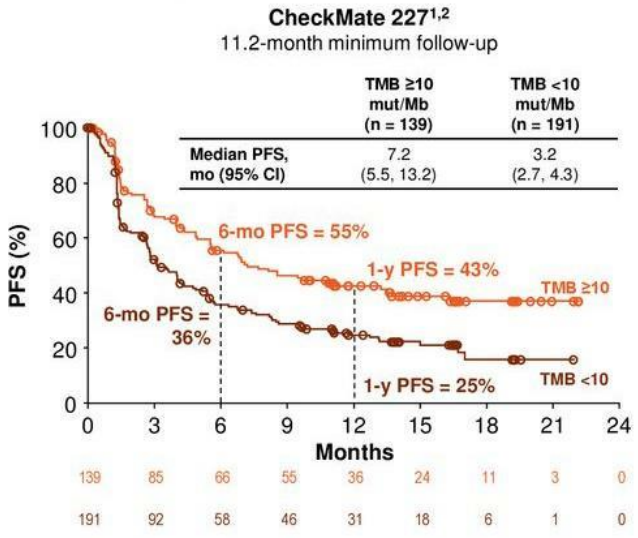
Carbone 2017
TMB > 242 mut/exome

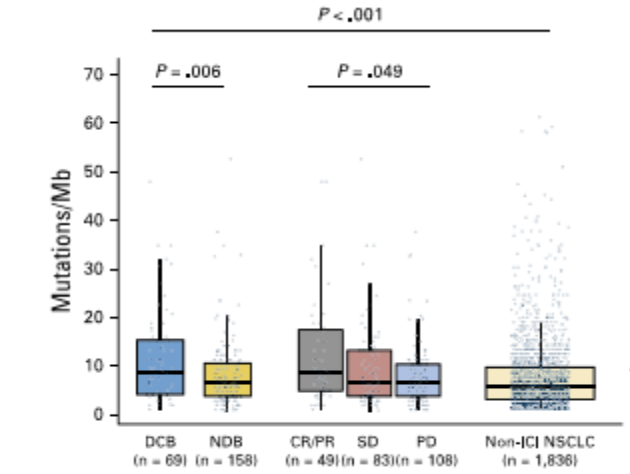
[1]Snyder et al. N Eng J Med (2014); [2]Van Allen at al. Science (2015); [3]Rizvi et al. Science (2015); [4]Carbone et al. NEJM (2017)

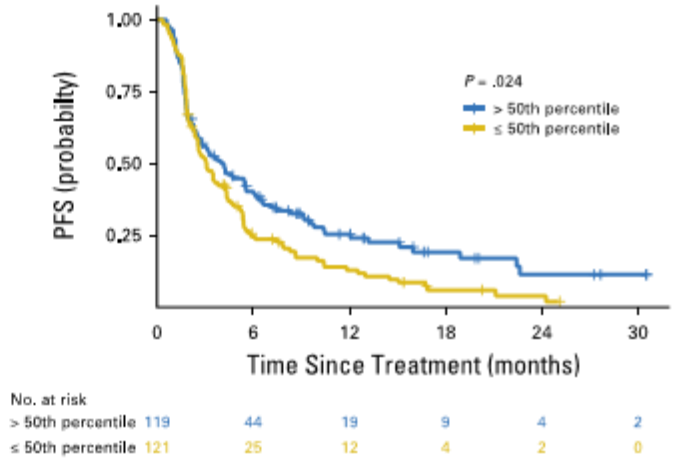
Promises: early evidence and clinical research studies

High TMB correlates with response to ICI treatment – Targeted Sequencing









- **CheckMate 568** (Ramalingam, Cancer Res, 2018):

1st line PD-1 + CTLA-4 blockade (nivo + ipi), phase II.

TMB evaluated with Foundation Medicine (TMB >10 mut/Mb)

Median PFS (TMB high vs low): 7.1 vs 2.6 months.

- **CheckMate 227** (Hellman, NEJM, 2018):

1st line PD-1 + CTLA-4 blockade (nivo + ipi), phase III

TMB evaluated with Foundation Medicine (TMB >10 mut/Mb)

Median PFS (TMB high vs low): 7.2 vs 5.5 months (final).

- **MSK** (Rizvi, JCO, 2018):

Mixed lines, PD-1 mono / + CTLA-4 blockade, retrospective

TMB evaluated with MSK assay (TMB >7.3 mut/Mb)

Feasibility: issues for the application into clinical research

What is TMB high? The assay makes the difference

Table 2 Examples of NGS gene panels in development or currently available to assess TMB

Status	Test name	Number of genes	Coverage (Mb)*	Gene variants	Sample type
FDA-approved or authorised diagnostic assays†	MSK-IMPACT[15 56 68]	468	1.5	SNVs, indels, rearrangements/ fusions, CNAs, parallel analysis of genomic signatures (eg, TMB and dMMR/MSI)	FFPE
	Foundation Medicine FoundationOne CDx[14 49]	324	0.8	SNVs, indels, CNAs, select rearrangements, parallel analysis of genomic signatures (eg, TMB and dMMR/MSI)	FFPE
Commercial assays for research use only	Caris Molecular Intelligence[132]	592	1.4	Somatic missense mutations	FFPE
	Illumina TruSight 500 gene panel[133]	500	2.0	SNVs and indels	FFPE
	Thermo Fisher Scientific Oncomine Tumor Mutation Load Assay[77]	409	1.7	SNVs	FFPE
	NEO New Oncology NEOplus v2 RUO[134]	>340	1.1	SNVs, indels, fusions, CNAs, parallel analysis of TMB, MSI, and driver mutations	FFPE
	Foundation Medicine FoundationOne[50]	315	1.1	SNVs, indels, CNAs, select gene rearrangements, genomic signatures for MSI and TMB	FFPE
	Foundation Medicine bTMB assay[88 122]	394	1.1	SNVs	Blood
	TruSight Tumor 170[135]	170	0.5	Fusions, splice variants, SNVs, indels, amplifications	FFPE
	QIAGEN GeneRead DNAseq Comprehensive Cancer Panel[97]	160	0.7	SNVs, CNAs, indels, and fusions	FFPE
	NEO New Oncology NEOplus[105 136]	94		SNVs, indels, CNAs, rearrangements, and fusions	FFPE

Büttner R, et al. ESMO Open (2019)

Assays for TMB evaluation differ in:

- Genes (number and content)
- Coverage (genomic/exonic area)
- Variants included: SNVs + Indel or only SNVs
- Non-synonymous only or + synonymous
- Limit of detection for mutation count (5 or 10% variant allelic frequency)
- Input required (from 20 ng to >100 ng DNA)
- Sample type

Direct influence on TMB value and cut-off for TMB high

Retrospective analysis of NSCLC samples treated with ICIs

Retrospective study to evaluate TMB as a potential biomarker for ICI treatment

76 clinical research samples, advanced NSCLC, treated with immune checkpoint inhibitors.

Retrospective research sample collection.
Tissue blocks prior to treatment.

Sample characteristics	All patients (n = 76) No. (%)	TMB Low & Int (n = 51) No. (%)	TMB High (n = 25) No. (%)	p - value
Age (yr)				0.907
Median (Range)	66 (31-90)	65 (49-79)	67 (31-90)	
Sex (N)				0.615
Male	47 (62)	30 (59)	16 (68)	
Female	29 (38)	21 (41)	8 (32)	
Tumor histology at diagnosis (N)				
Adenocarcinoma				>0.999
Squamous cell carcinoma	70 (92)	47 (92)	23 (92)	
Tumor type (N)	6 (8)	4 (8)	2 (8)	
Primary tumor				0.043
Metastasis/ Lymph node	47 (62)	36 (71)	11 (44)	
Tumor cell content (%)	29 (38)	15 (29)	14 (56)	
Median (Range)				
Immunotherapy (N)				0.213
Nivolumab	60 (20 - 95)	60 (20-95)	60 (20-90)	
Pembrolizumab				>0.999
Atezolizumab	60 (79)	40 (78)	20 (80)	
Other	10 (13)	9 (18)	1 (4)	
Number of lines before I-O (N)	3 (4)	2 (4)	1 (4)	
First (0)	3 (4)	0 (0)	3 (12)	
Second (1)				
Third (2)				0.724
Fourth (3)	11 (14)	7 (14)	4 (16)	
not available	39 (51)	30 (59)	9 (36)	
Smoking status (N)	10 (13)	6 (12)	4 (16)	
Never	2 (3)	0 (0)	2 (8)	
Current/former	13 (17)	8 (16)	5 (20)	
not available				
PD-L1 (N)				0.155
< 1%	10 (13)	9 (18)	1 (4)	
≥ 1%	60 (79)	39 (76)	21 (84)	
not available	6 (8)	3 (6)	3 (12)	
Durable clinical benefit (N)				>0.999
DCB	28 (37)	19 (37)	9 (36)	
No DCB	39 (51)	27 (53)	12 (48)	
	9 (12)	5 (10)	4 (16)	
	32 (42)	16 (31)	16 (64)	0.013
	44 (58)	35 (69)	9 (36)	

Alborelli et al., The Journal of Pathology, 2019

Retrospective analysis of NSCLC samples treated with ICIs

Retrospective study to evaluate TMB as a potential biomarker for ICI treatment

76 clinical samples, advanced NSCLC, treated with immune checkpoint inhibitors.

Retrospective sample collection. Tissue blocks prior to treatment.

Target enrichment using Oncomine™ TML Assay*

Sequencing 1000X depth of coverage.

TMB calculation based on non-synonymous SNVs and InDels using Ion Reporter™ Software*.

- *1.22 Mb coding regions*
- *409 cancer-related genes*
- *Low DNA input requirement (20 ng)*
- *Automated analysis workflow in Ion Reporter Software**
- *Detection of clinically relevant mutation*

**For Research Use Only Not For Use in Diagnostic Procedures*

The Oncomine™ Tumor Mutation Load Assay

How does the TML assay compare to WES?

Original Article

A scalable solution for tumor mutational burden from formalin-fixed, paraffin-embedded samples using the Oncomine Tumor Mutation Load Assay

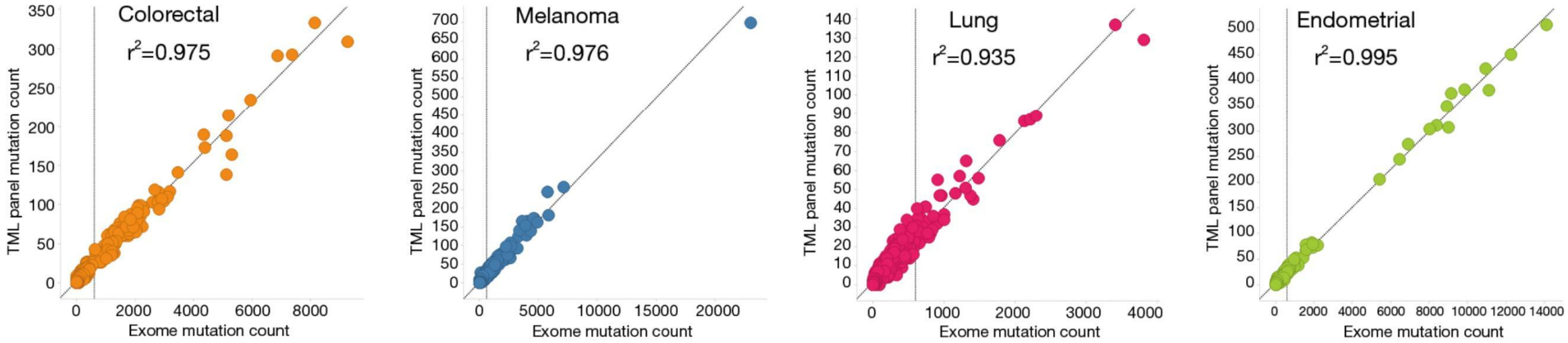
Ruchi Chaudhary[1], Luca Quagliata[2], Jermann Philip Martin[2], Ilaria Alborelli[2], Dinesh Cyanam[1], Vinay Mittal[1], Warren Tom[1], Janice Au-Young[1], Seth Sadis[1], Fiona Hyland[1]

[1]Thermo Fisher Scientific, Waltham, Massachusetts, USA; [2]Institute of Pathology, University Hospital Basel, 4031 Basel, Switzerland

Contributions: (I) Conception and design: R Chaudhary; (II) Administrative support: R Chaudhary; (III) Provision of study materials or patients: L Quagliata, JP Martin, I Alborelli, W Tom, J Au-Young; (IV) Collection and assembly of data: R Chaudhary; (V) Data analysis and interpretation: R Chaudhary, D Cyanam; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Ruchi Chaudhary. Thermo Fisher Scientific, Waltham, Massachusetts, USA. Email: ruchi.chaudhary@thermofisher.com.

TLCR TRANSLATIONAL LUNG CANCER RESEARCH
AN OPEN ACCESS JOURNAL FOCUSING ON CLOSING THE GAP BETWEEN "BENCH AND BEDSIDE"

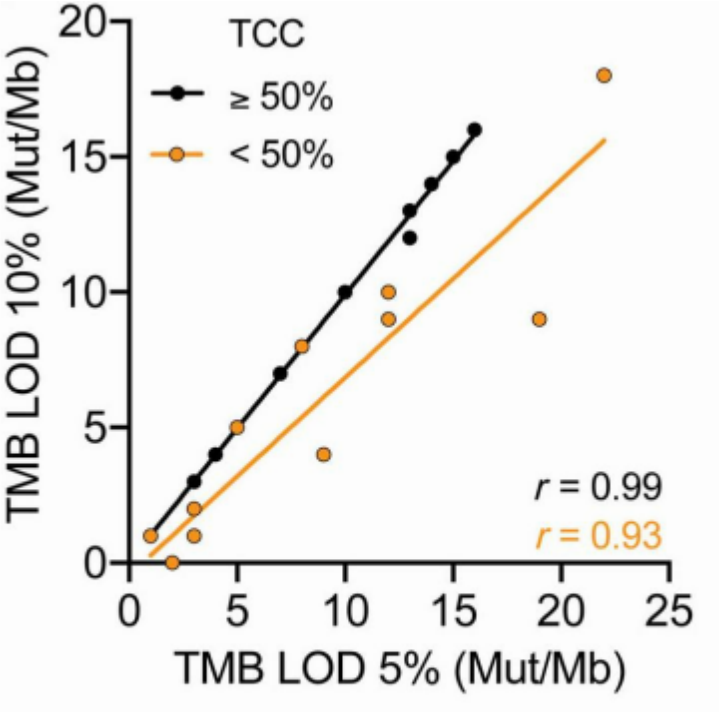


Consistent and robust correlation values with WES across different tumor types

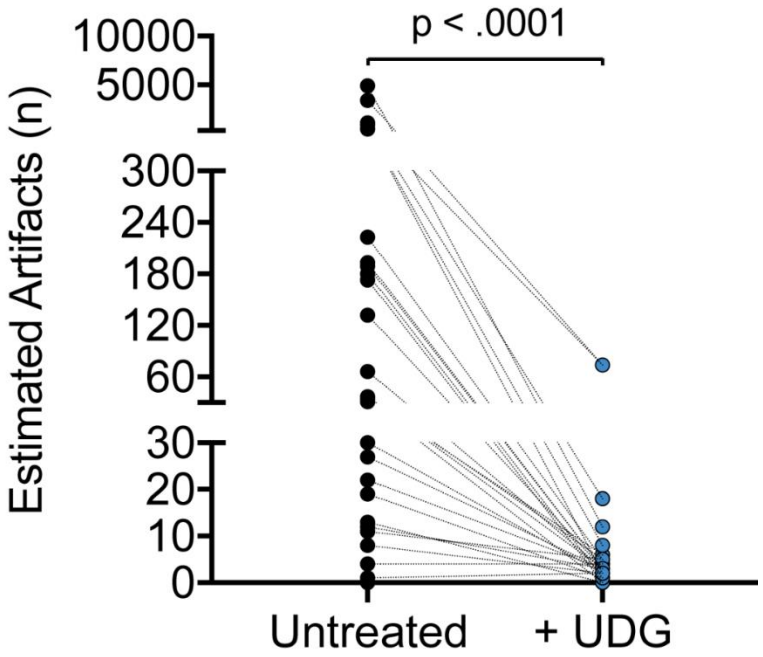
Retrospective analysis of NSCLC treated with ICIs

Pre-analytical factors influence TMB estimation

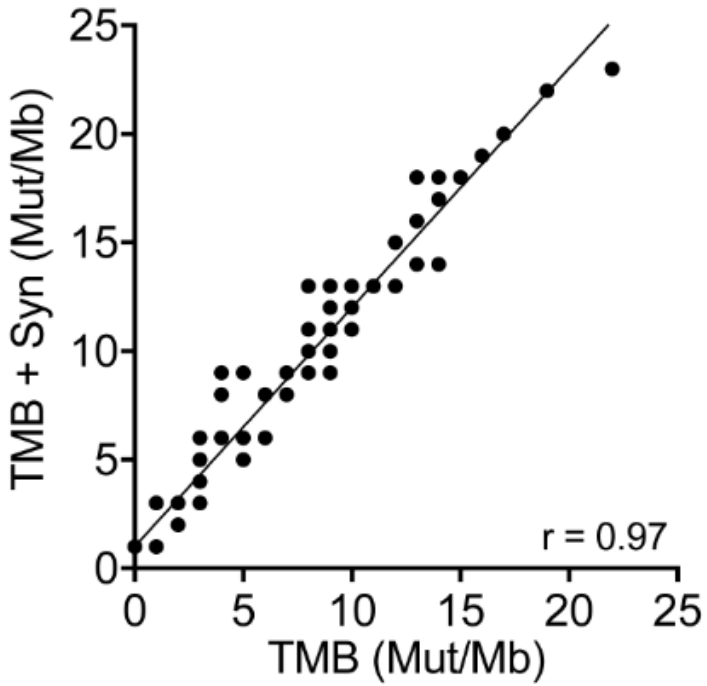
Tumor Cell Content (TCC) influences the choice of Limit Of Detection (LOD)



UDG treatment reduces rejection rate due to deamination artefacts*



High correlation between analyses with and without inclusion of synonymous mutations.



*Potential deamination artefacts defined as G:C>A:T mutations with < 15% allelic frequency

Alborelli I., Leonards K., Rothschild S.I., Savic Prince S., et al. The Journal of Pathology, 2019

Retrospective analysis of NSCLC treated with ICIs

TMB correlates with response to ICI therapy

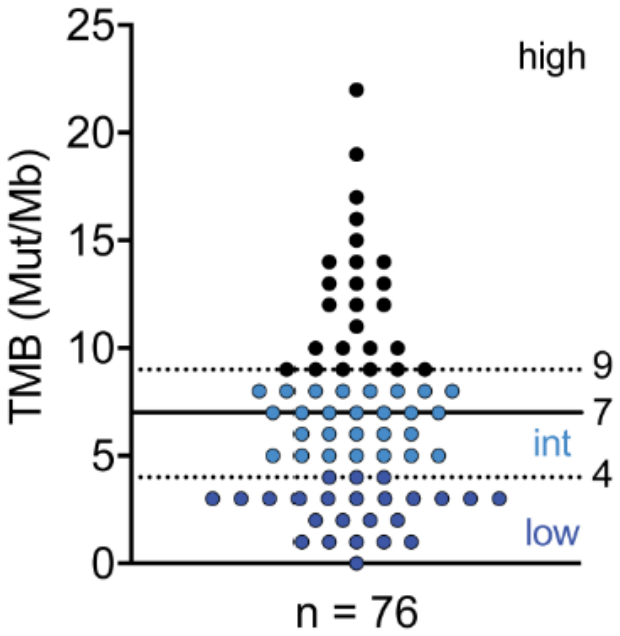
76 NSCLC treated with ICIs, 32 = DCB*, 44 no DCB, 20 ng input DNA

Oncomine™ TML Assay, 409 genes, 1.22 Mb coding region

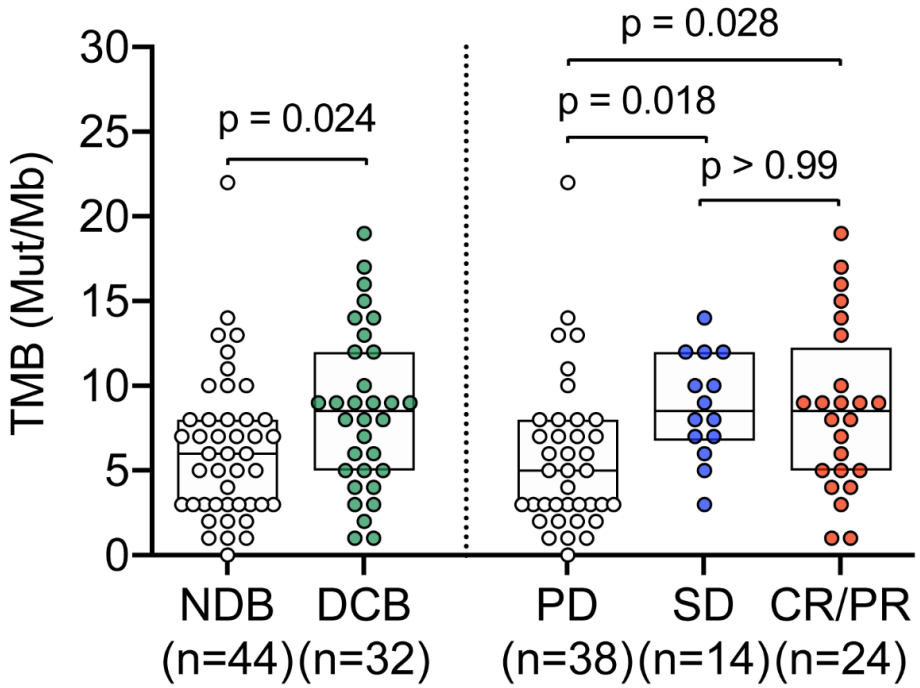
TMB: Non-synonymous SNVs and indels / Mb, using Ion Reporter™.

Correlation with ICI response data, predictive power

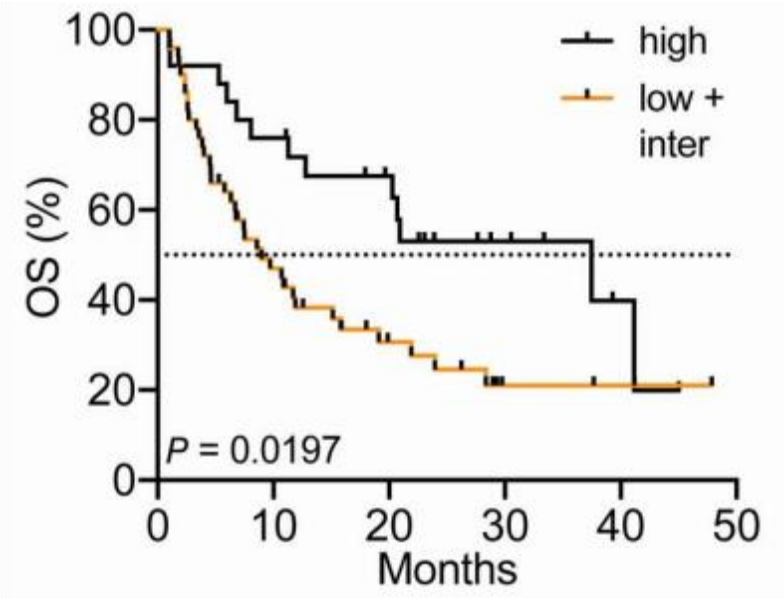
TMB distribution In our cohort



TMB status correlates with response



Increased PFS and OS in TMB-high samples

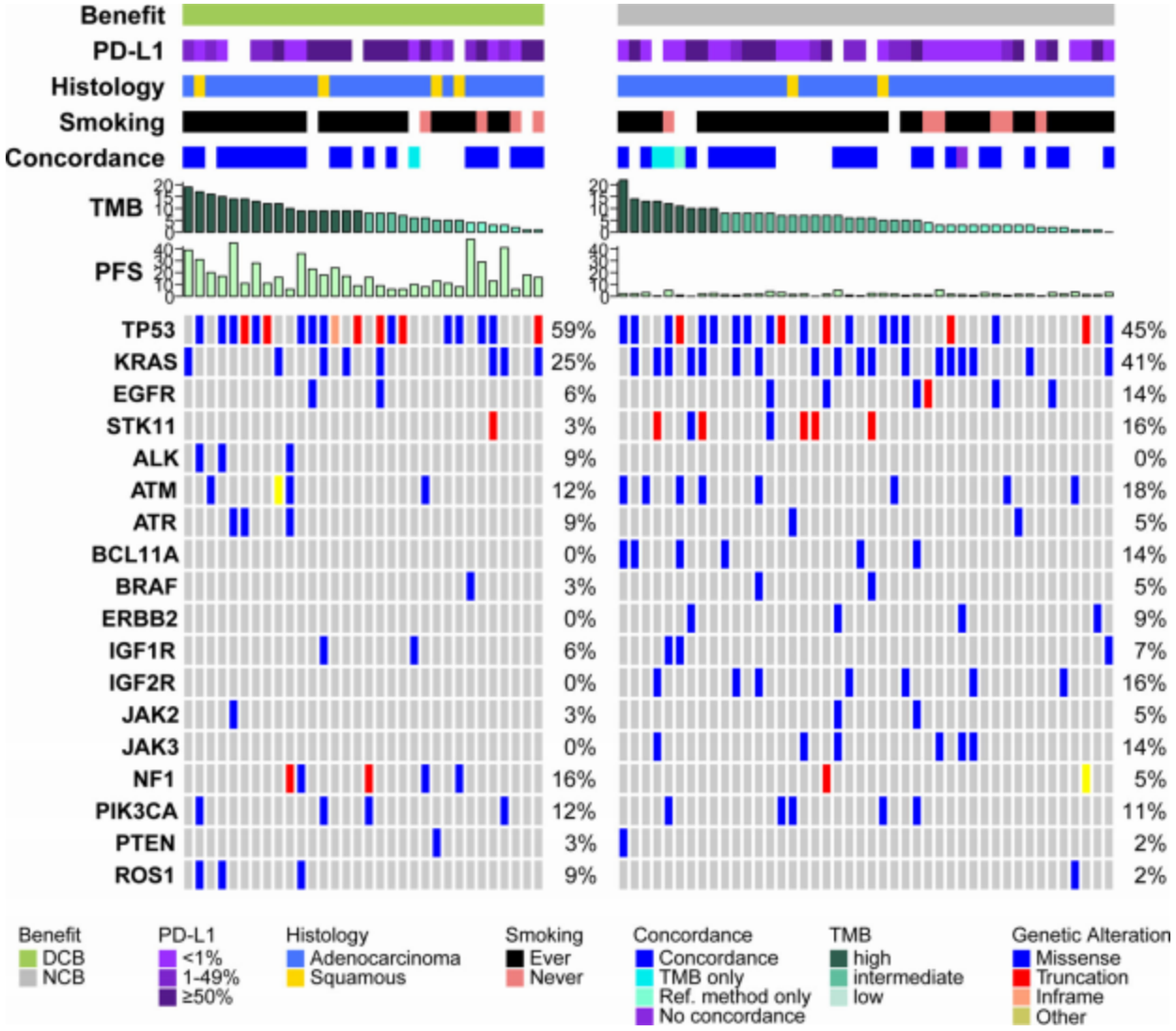


*DCB: PFS > 6 months

Alborelli I., Leonards K., Rothschild S.I., Savic Prince S., et al. The Journal of Pathology, 2019

Retrospective analysis of NSCLC treated with ICIs

Molecular and clinical features associated with response



Oncogenic driver mutations (EGFR, BRAF, KRAS, ERBB2) are more likely to associate with no response.

STK11 alterations are often found in non-responders, in combination with KRAS/KEAP1 mutations.

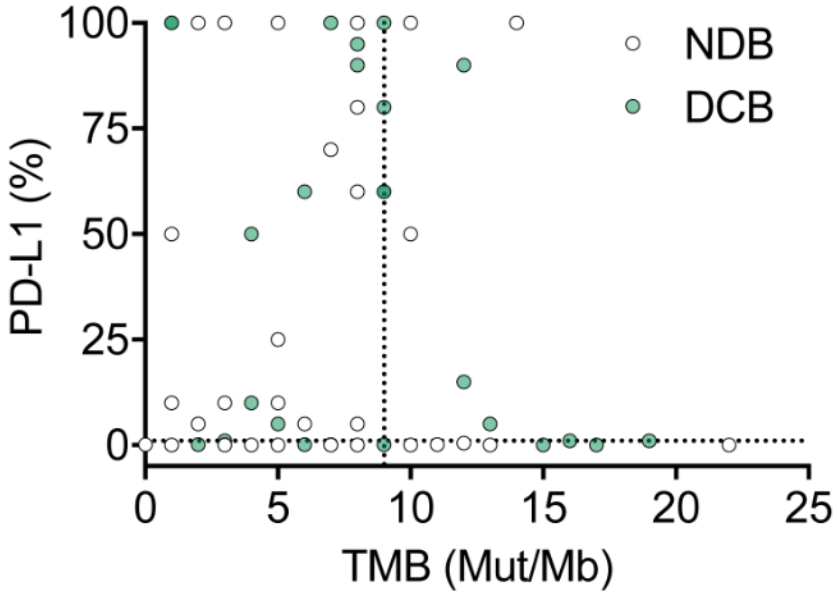
B2M	Antigen Presentation
TAP1	Antigen Presentation
TAP2	Antigen Presentation
HLA-A	Antigen Presentation
TAPBP	Antigen Processing
JAK1	Resistance/Response to ICIs
JAK2	Resistance/Response to ICIs
JAK3	Resistance/Response to ICIs
STAT1	Resistance/Response to ICIs
SOCS1	Resistance/Response to ICIs
CD274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

Alborelli I., Leonards K., Rothschild S.I., Savic Prince S., et al. The Journal of Pathology, 2019

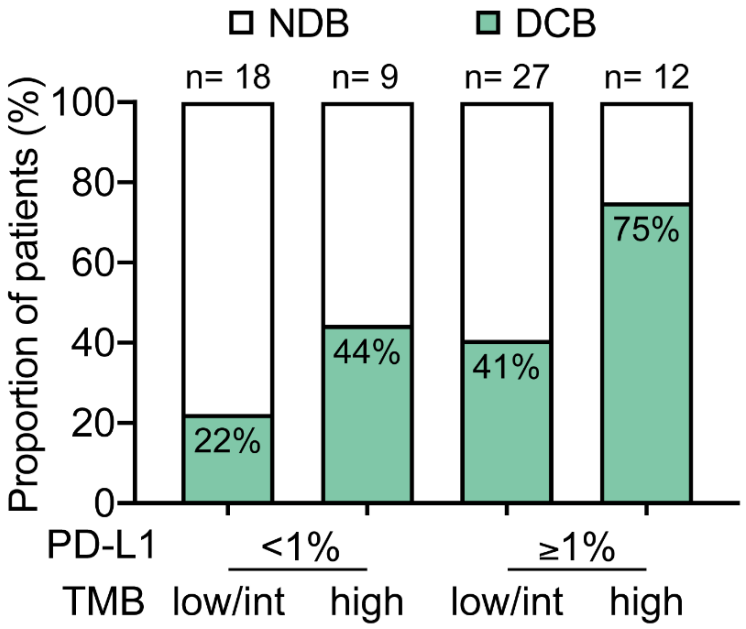
Retrospective analysis of NSCLC treated with ICIs

Combination of TMB and PD-L1

No correlation between PD-L1 and TMB

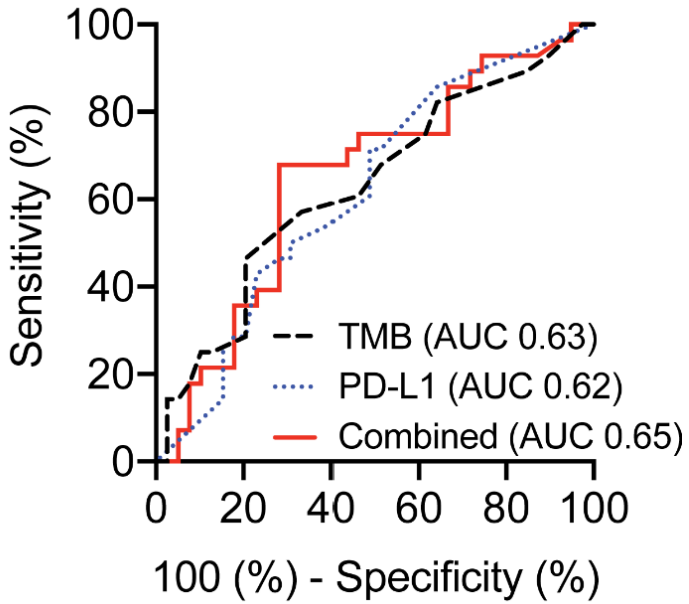


Improved stratification upon combination of PD-L1 and TMB



*TMB alone= 64%DCB

ROC analysis confirms feasibility of combinatorial approach



Alborelli I., Leonards K., Rothschild S.I., Savic Prince S., et al. The Journal of Pathology, 2019

Retrospective analysis of NSCLC treated with ICIs

Combination of TMB and PD-L1

No correlation between PD-L1 and TMB

Improved stratification upon

ROC analysis confirms feasibility
roach

Original Paper | Full Access

Tumor mutational burden assessed by targeted NGS predicts clinical benefit from immune checkpoint inhibitors in non-small cell lung cancer

Ilaria Alborelli, Katharina Leonards, Sacha I. Rothschild, Laura P. Leuenberger, Spasenija Savic Prince, Kirsten D. Mertz, Severin Poechtrager, Martin Buess, Alfred Zippelius, Heinz Läubli, Jasmin Haegele, Markus Tolnay, Lukas Bubendorf, Luca Quagliata, Philip Jermann ... See fewer authors

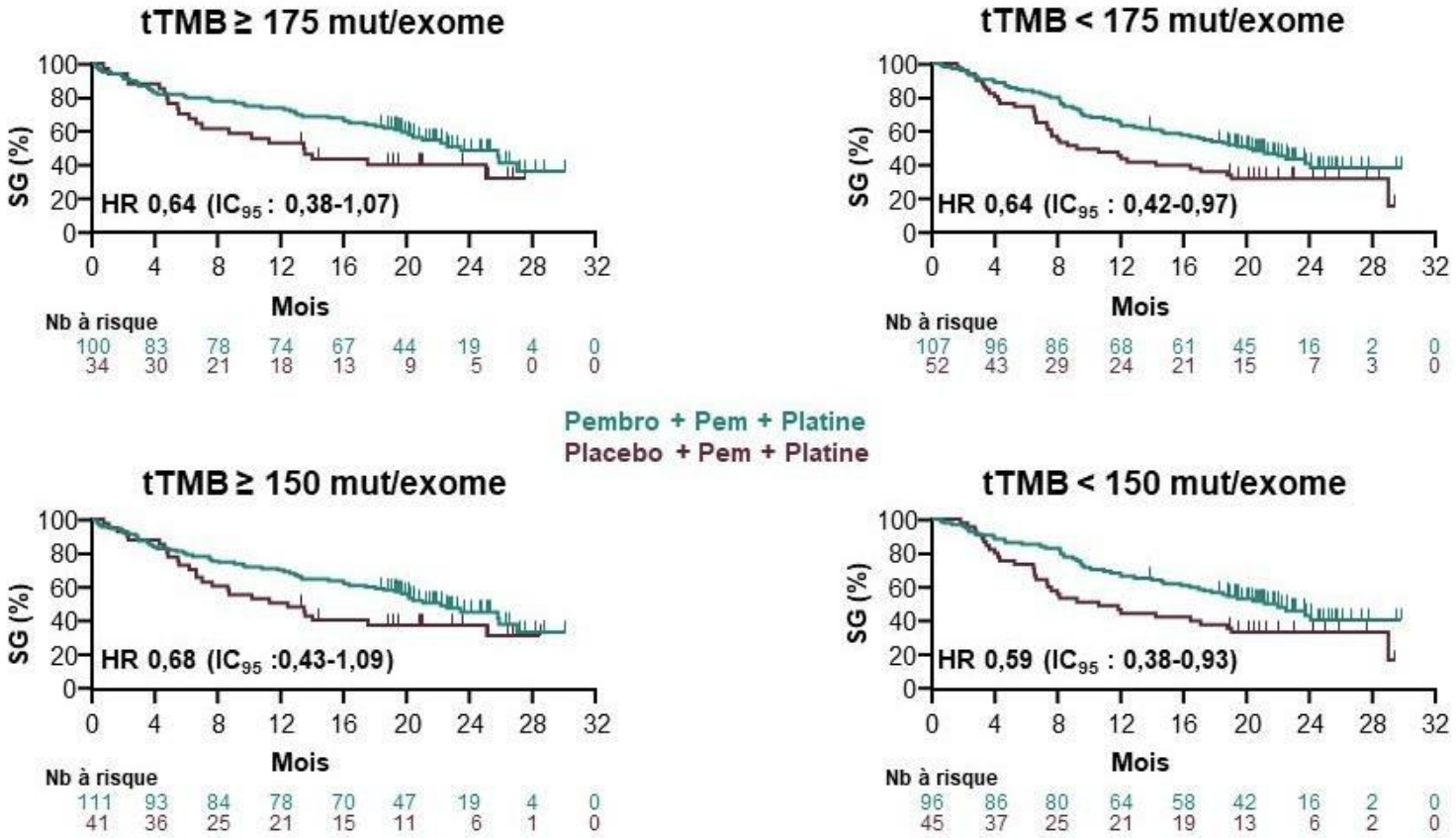
First published: 30 August 2019 | https://doi.org/10.1002/path.5344

*TMB alone= 64%DCB

Alborelli I., Leonards K., Rothschild S.I., Savic Prince S., et al. The Journal of Pathology, 2019

The future: will TMB still be relevant?

Broken Promises: OS and Keynote 189



CheckMate 227:

No significant difference in OS for patients with TMB ≥10 mut/Mb versus TMB <10 mut/Mb.

Keynote 189:

Pembrolizumab plus chemotherapy showed **survival benefit in TMB-high and -low** subgroups for both squamous and non-squamous NSCLC.

Where to go from here?

Hellman MD, Ciuleanu T, Pluzanski A, et al. NEJM 2018; Paz-Ares L, Langer CJ, Novello S, ESMO 2019

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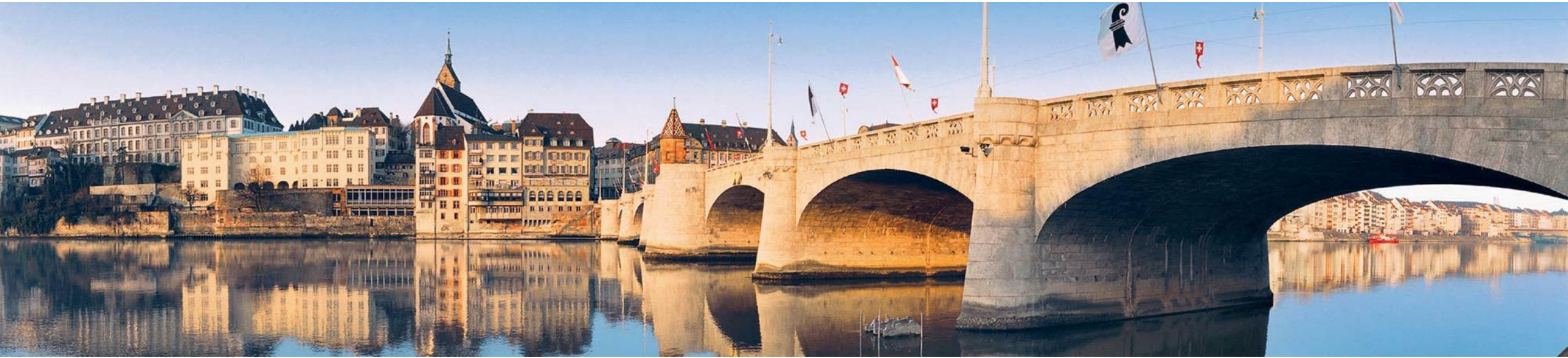
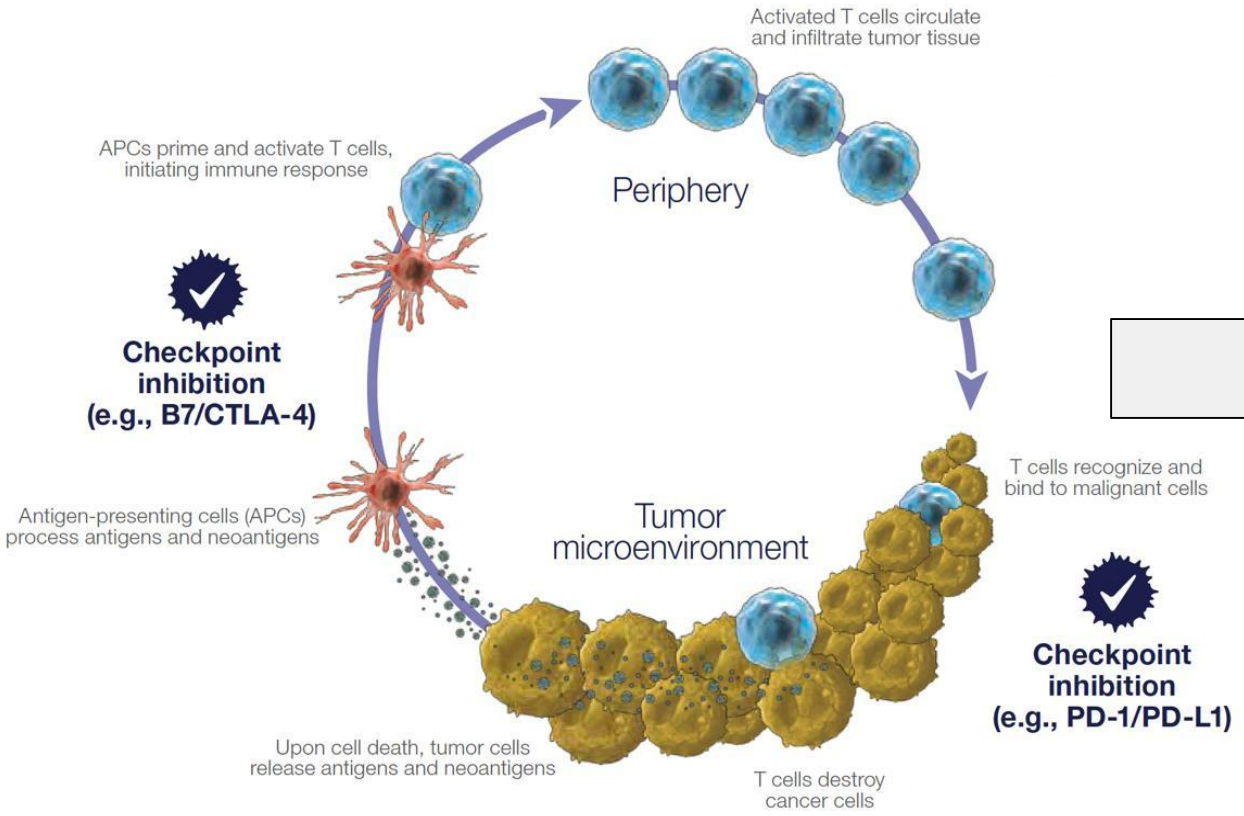
Looking at the T-Cell repertoire

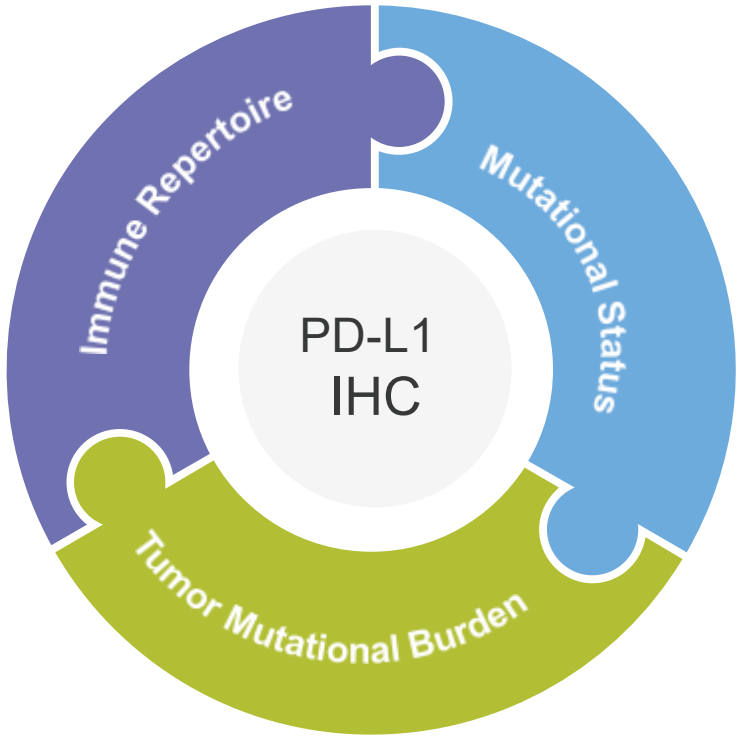
A potential I/O biomarker?



Understanding Tumor-Immune Interactions Through NGS

Focusing on T-Cells





Tumor-Immune interactions are complex and warrants a multi-marker approach

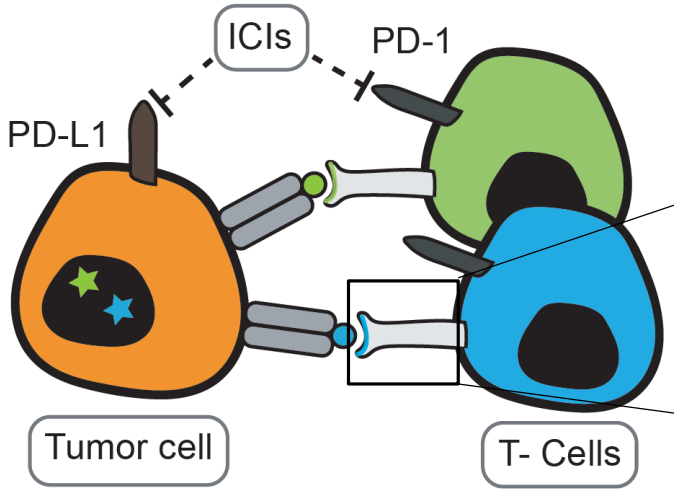
NGS assays broaden our understanding of the biology beyond traditional IHC biomarkers

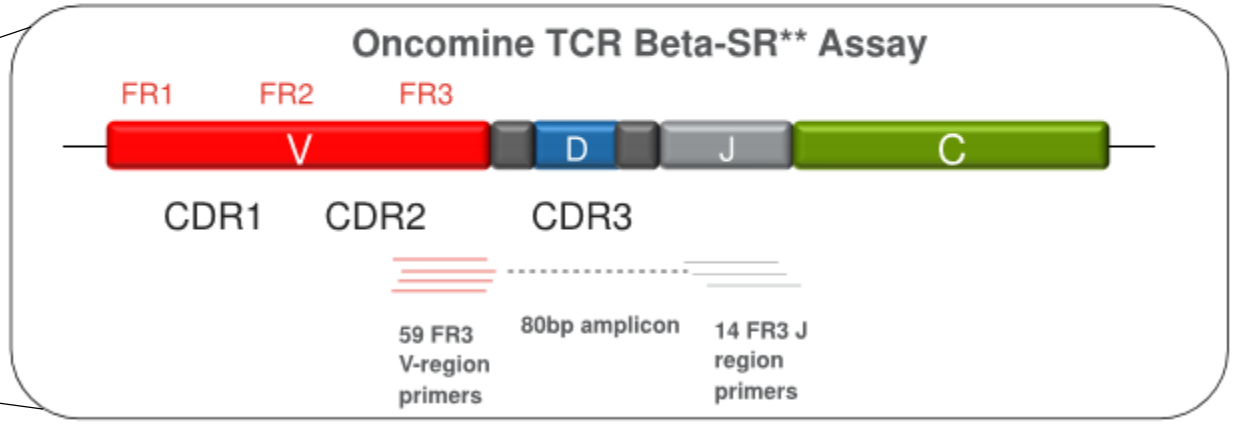
Source: Thermo Fisher Scientific

TCR-beta repertoire convergence and evenness are associated with response to immune checkpoint inhibitors

K. Leonards[1], T. Looney[2], I. Alborelli[1], S. Rothschild[3], S. Savic Prince[4], K. Mertz[4], A. Zippelius[5], L. Bubendorf[4], P. Jermann[1]

(1) Pathology, University Hospital Basel (2) Thermo Fisher Scientific, (3) Oncology, University Hospital Basel, (4) Pathology, Cantonal Hospital Basel-Landschaft, (5) DBM Cancer Immunology, University Hospital Basel





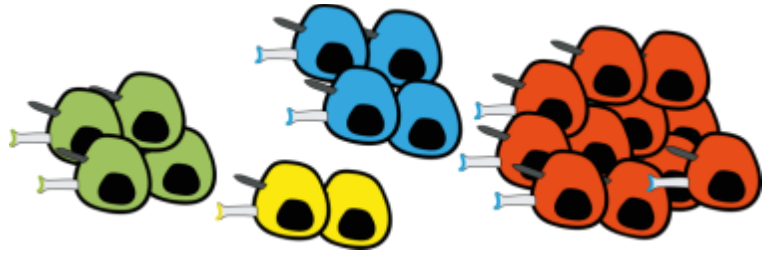
TCR Convergence

Measures TCRs with identical AA sequence but different NT sequence

V-Gene	CDR3 AA	CDR3 NT	Frequency
TRBV7-8	ASSLGQAYEQY	GCCAGCAGCTTAGGTCAGGCATACGAGCAGTAC	1.8E-03
TRBV7-8	ASSLGQAYEQY	GCCAGCAGCTTGGGACAGGCCTACGAGCAGTAC	4.8E-04
TRBV7-8	ASSLGQAYEQY	GCCAGCAGCTTAGGGCAGGCCTACGAGCAGTAC	9.9E-05

TCR Evenness

Measures clonal uniformity



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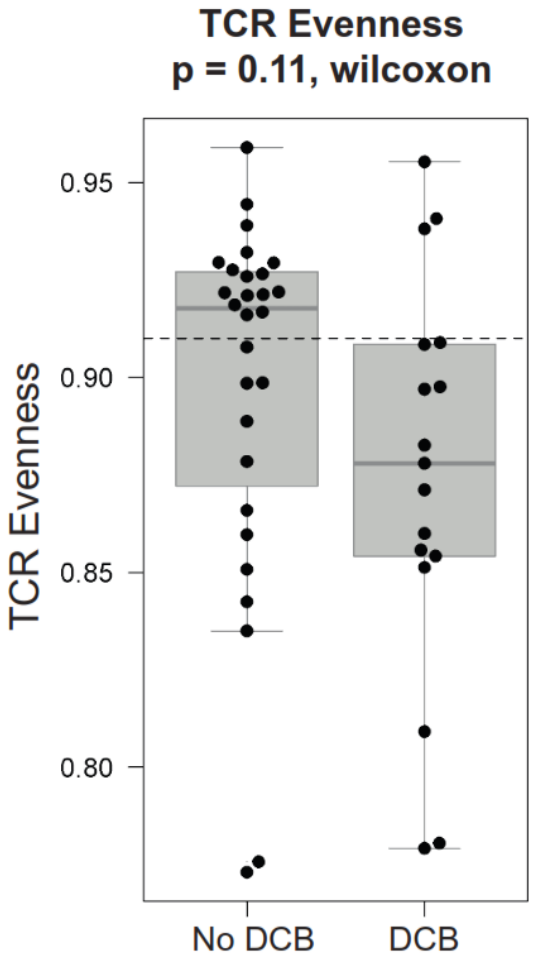
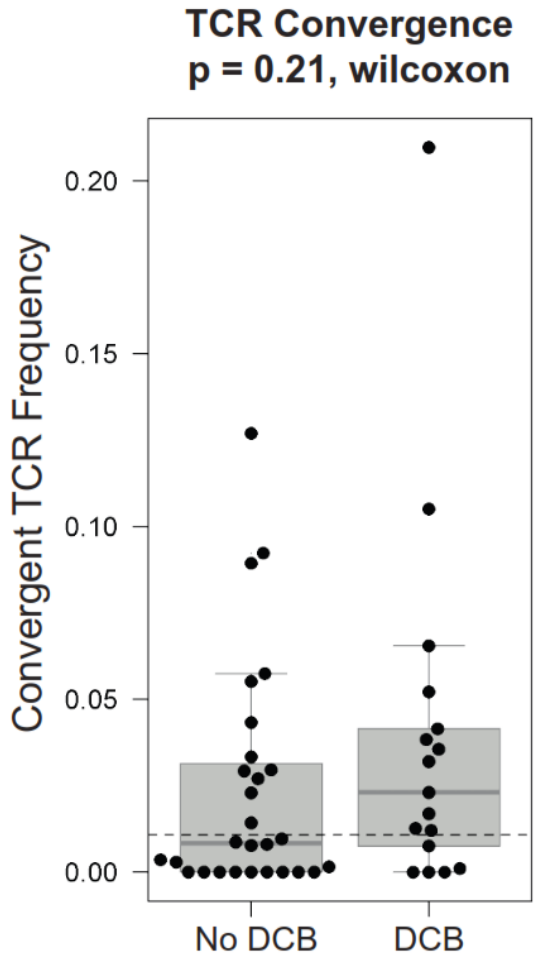
45 NSCLC patients treated with ICI therapy. 17 DCB*, 28 no DCB 150ng RNA input

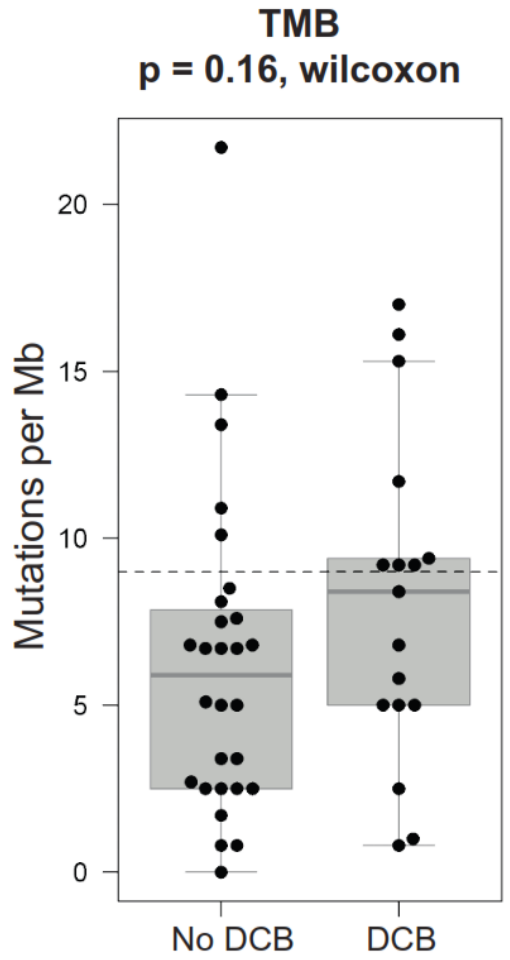
Sequencing on Ion Genestudio S5 targeting at 2M reads per sample

Data Analysis on Ion Reporter using TCRB SR workflow to identify TCR Repertoire features

Correlation with Tumor Mutation Burden (Oncomine TMB Assay) and PD-L1 IHC







*DCB: Durable Clinical Benefit (PFS > 6 months)

TCR Convergence and Evenness are associated with clinical outcome of immune checkpoint inhibitor therapy.

TCR repertoire features and response to immune checkpoint inhibitors

Logistic Regression Model combining Evenness and Convergence performs best

45 NSCLC patients treated with ICI therapy. 17 DCB*, 28 no DCB 150ng RNA input

➤

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➤

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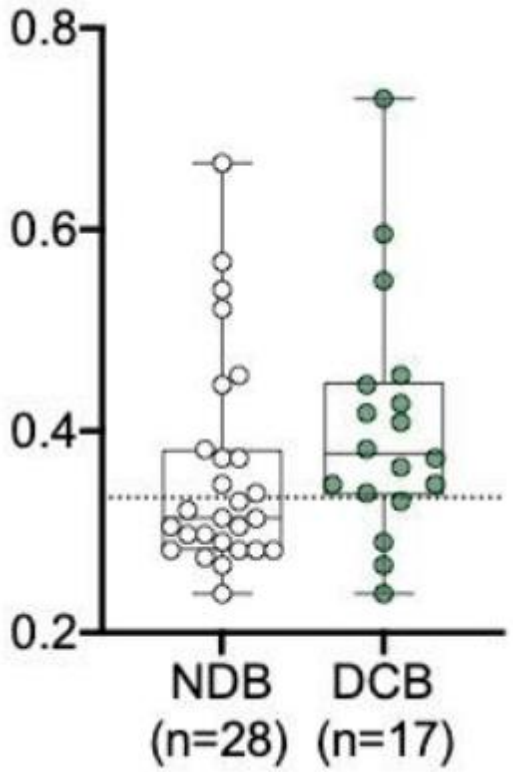
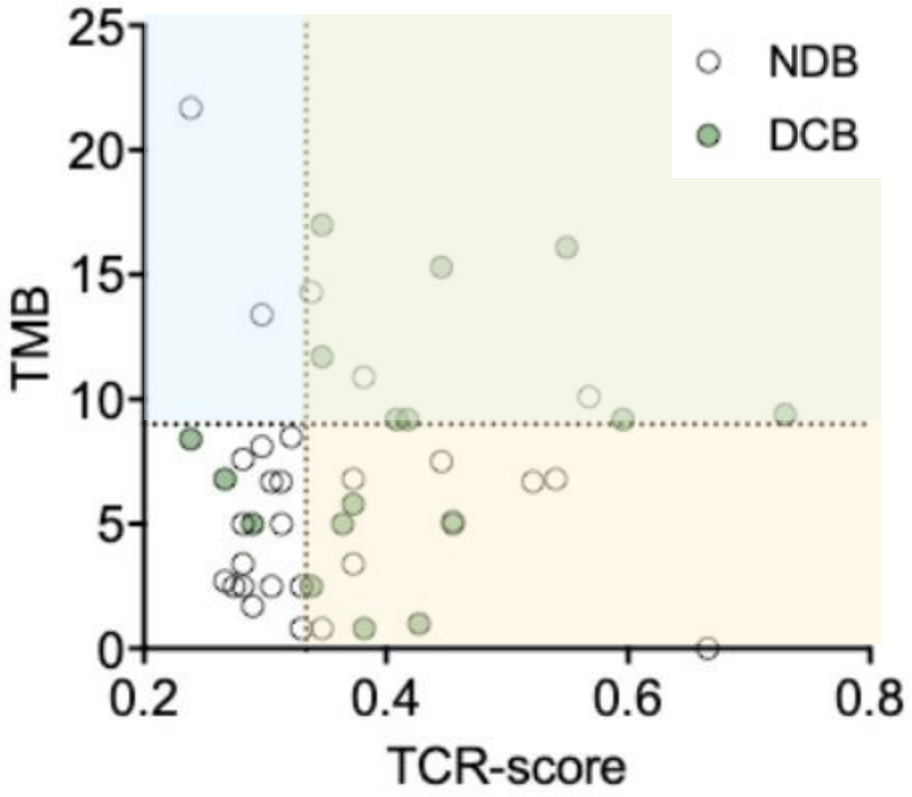
➤

Correlation with Tumor Mutation Burden (Oncomine TMB Assay) and PD-L1 IHC

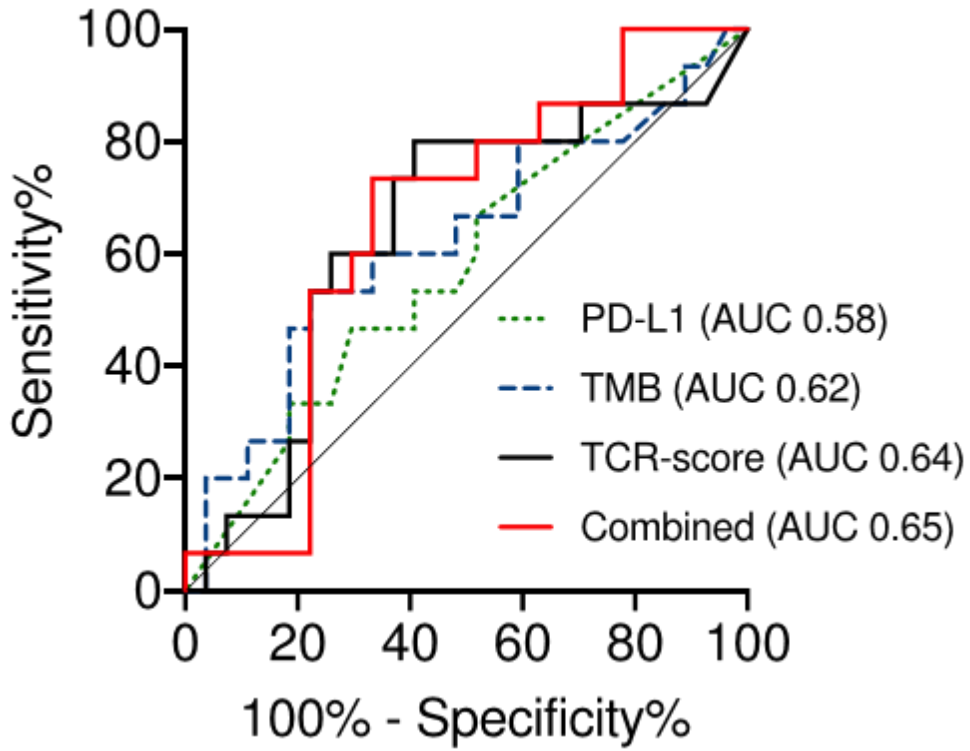
TCR Score
P = 0.06, wilcoxon



TCR Score vs. TMB



ROC Analysis
(single biomarker vs multivariate)



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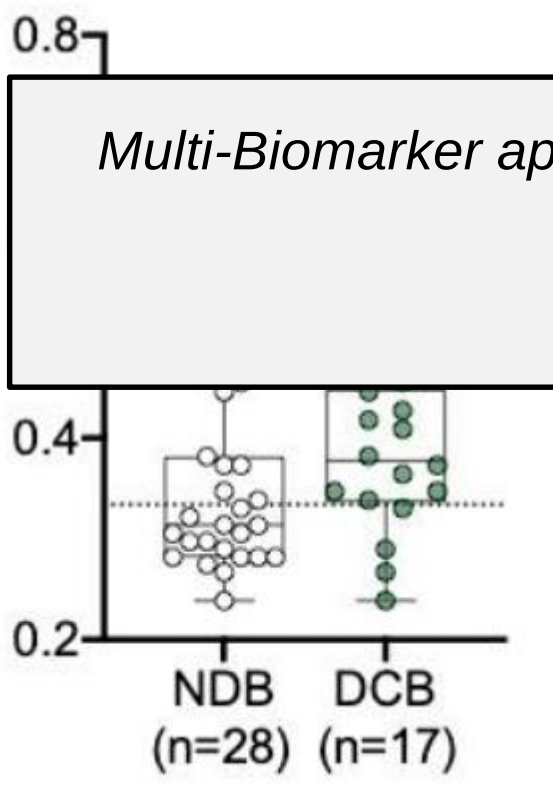
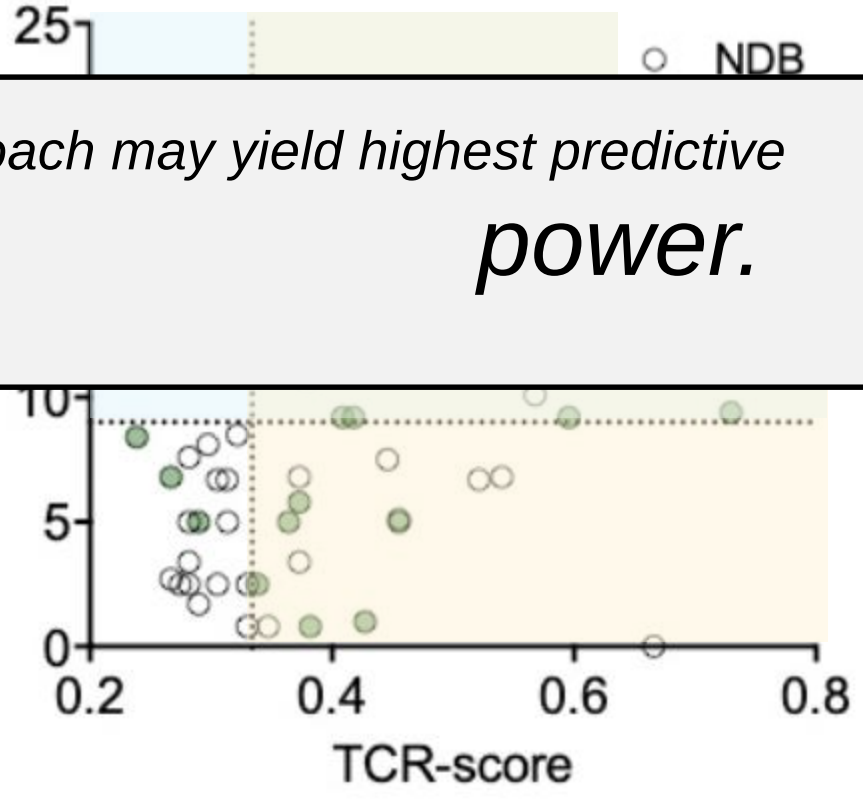


TCR Score vs. TMB



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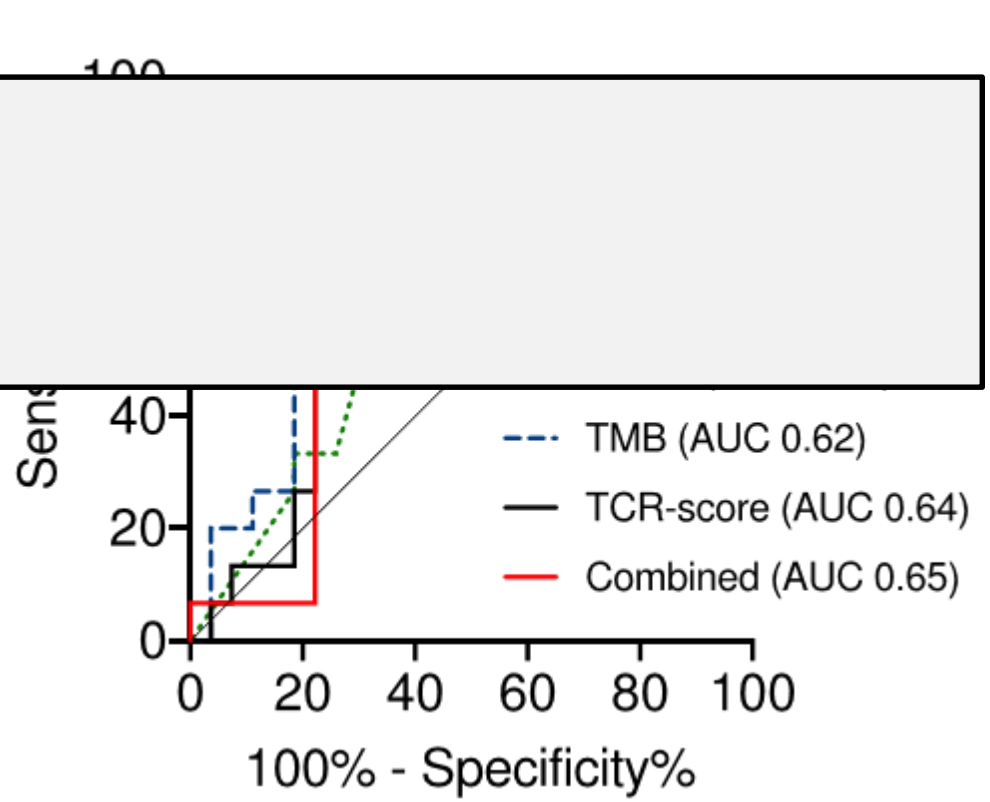


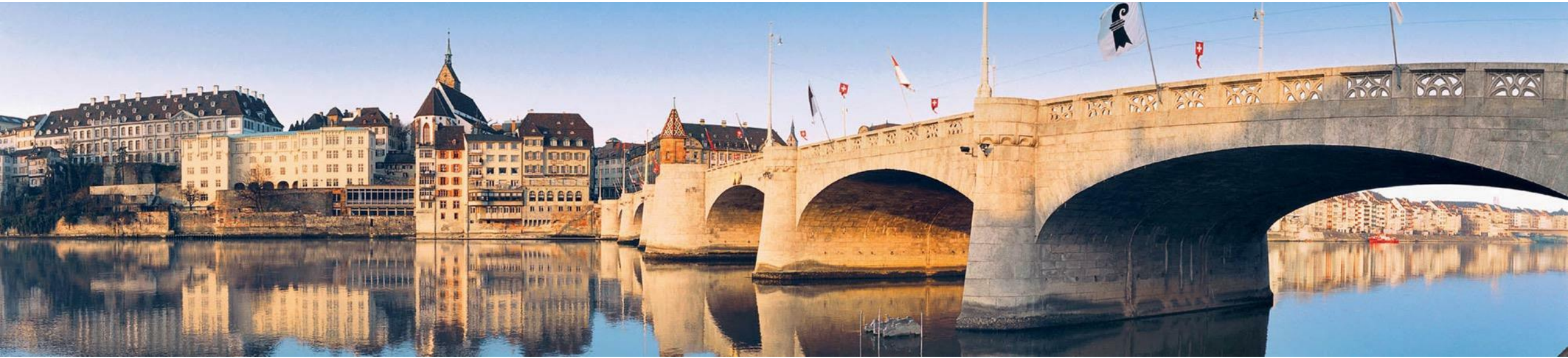
Multi-Biomarker approach may yield highest predictive power.

*DCB: PFS > 6 months

Universitätsspital Basel

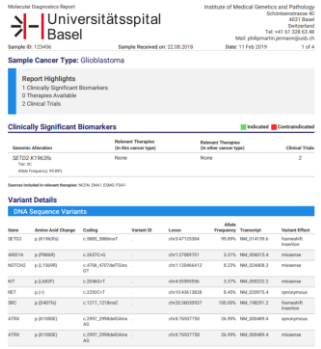
What's in store for the future?

First-hand experience with the new Genexus Integrated Sequencer



The Oncomine™ Assay Workflow

A step-by-step overview



FFPE Block → DNA / RNA Extraction → Library Preparation → Sequencing → Data Analysis

*Currently **5-7 days** from sample to report*

**For Research Use Only Not For Use in Diagnostic Procedures*

The Future of Diagnostic NGS in Basel

The new Genexus Integrated Sequencer

- ✓**Fully automated** library-prep, sequencing, and analysis
- ✓From sample to report in **1 day**
- ✓**Cost-efficient scaling** from 1 to 32 samples per run
- ✓only **10ng** input material

Oncomine™ Precision Assay for tissue and liquid biopsies

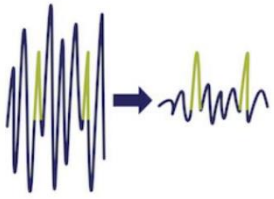
50 Genes

- Mutations, CNVs, and fusions
- Driver and resistance variants

Fusion Detection

- Targeted isoform design
- Novel fusion detection

Molecular Tagging



- Enhanced sensitivity
- Key for liquid biopsy

Tissue and Plasma

- One assay, multiple sample types

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The Future of Diagnostic NGS in Basel

The new Genexus Integrated Sequencer

The Genexus System

Highly reduced turnaround time

The Present
5-7 days TaT

FFPE Block → DNA / RNA Extraction → Library Preparation → Sequencing → Data Analysis

The Future
1-2 days TaT

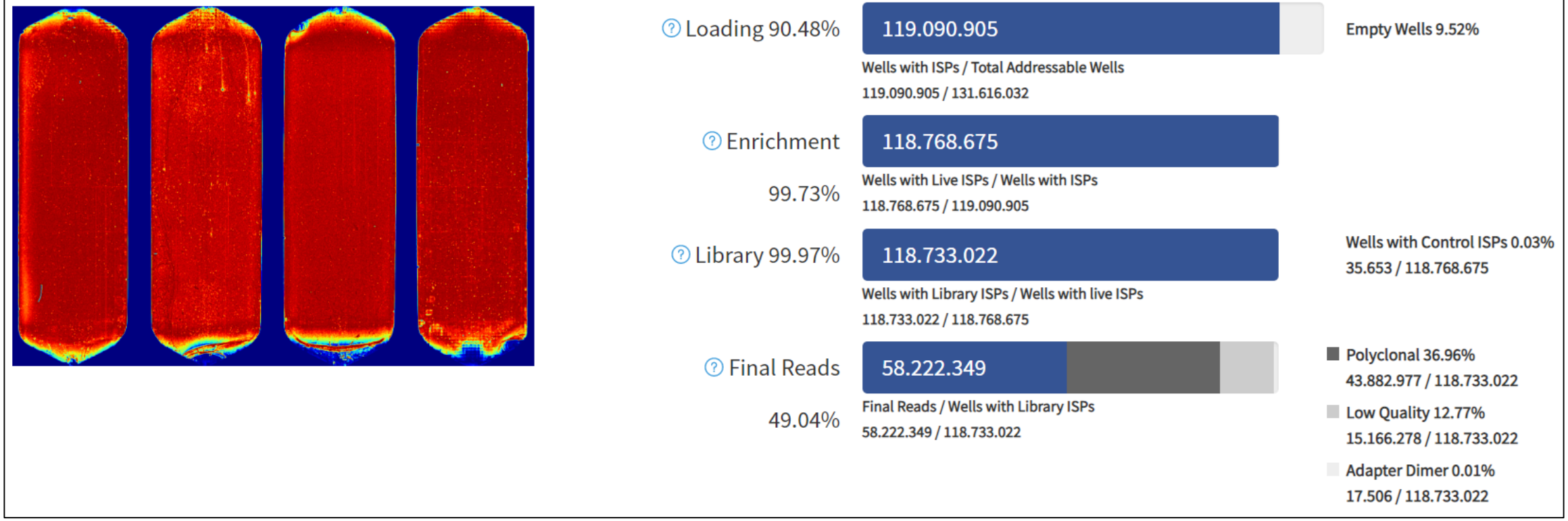
FFPE Block → DNA / RNA Extraction → Library Prep. / Seuqencing / Data Analysis

**For Research Use Only Not For Use in Diagnostic Procedures*

The Genexus System

First experience in Basel

- ***Up to 60 million reads per chip, divided in 4 lanes***
- ***Each lane can be run separately***
- ***Chip and reagents stable on-instrument for 1 month***



The Genexus System

The Oncomine Precision Assay on Tissue samples

Fully automated IR-like data analysis pipeline included in Torrent Suite Software

N511_N254 | QC Status : ✔ | Assay: OPA-GX5-SolidTumor-DNAandFusions-1.3.6 - 3M | Run Plan : OPA_Run2

Sample Details

Sample Name:	N511_N254	**Collection Date:**	05 NOV 2019
Gender:	Unknown	**Sample Type:**	DNA & RNA
Disease Category:	Cancer	**Cancer Type:**	Non-Small Cell Lung Cancer
Cancer Stage:	Unknown	**% Cellularity:**	35

Metrics

Average Base Coverage Depth:	1644
Uniformity Of Base Coverage:	94.99%
% Base Reads On Target:	88.64%

Variant Summary

A default filter has been applied. Go to SNVs/Indels, Fusions, CNVs pages to remove or modify variant filter.

Filter Chain Applied: *Variant Matrix tab Summary*

SNVs/Indels

3 Detected

Gene	AA Change	Allele Freq	Oncomine Variant Class
BRAF	p.V600E	0.321	Hotspot
ERBB2	p.V842I	0.383	Hotspot
TP53	p.G244C	0.024	Hotspot

Fusions

1 Detected

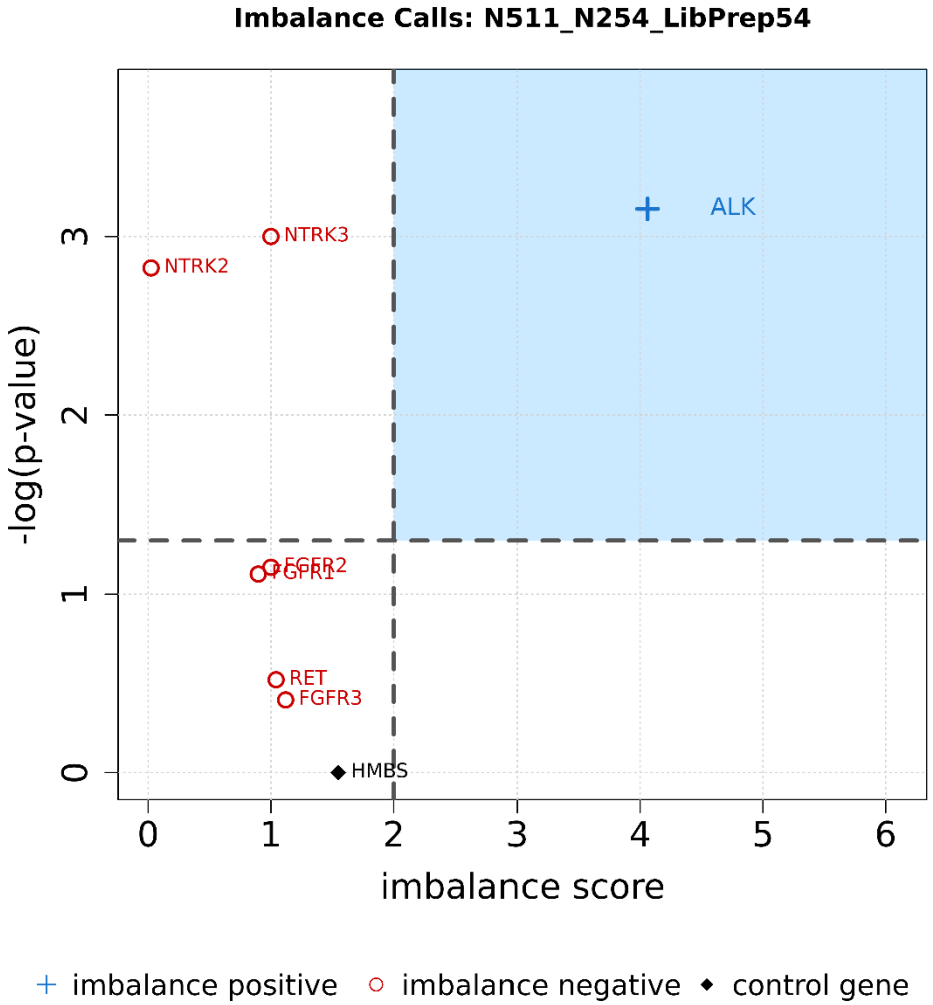
Oncomine Driver Gene	Evidence Level
ALK	Expression Imbalance

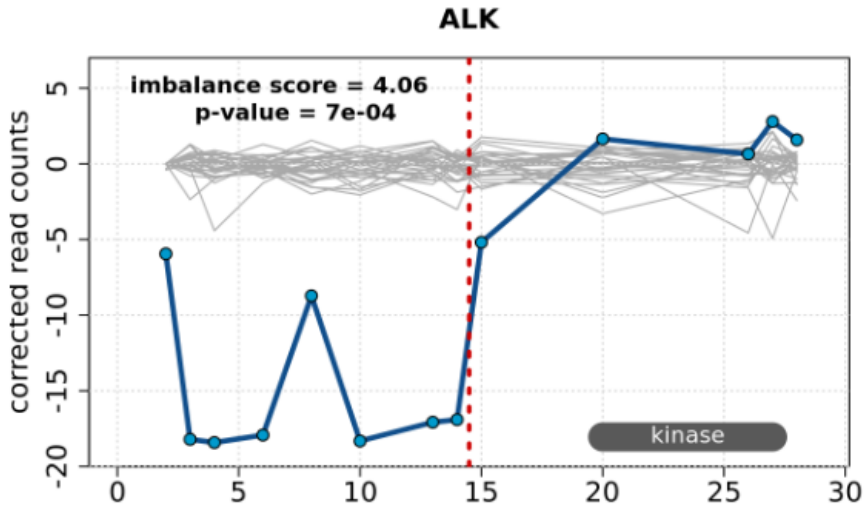
CNVs

0 Detected

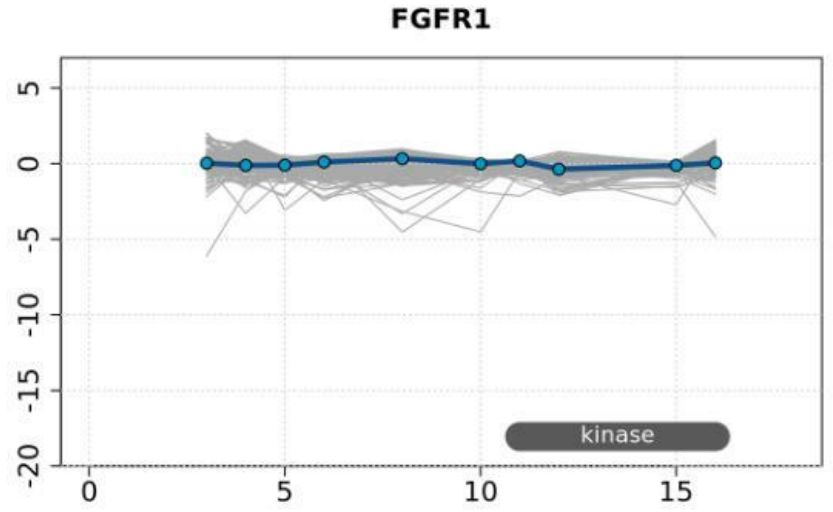
The Genexus System

Improved 5’ 3’ Imbalance Assay





Imbalance detected



No imbalance

The Genexus System

Liquid Biopsy using the Oncomine™ Precision Assay

Same assay can be used for Liquid Biopsy due to AmpliSeq HD technology

Sample Details

Sample Name:	I155	**Collection Date:**	12 NOV 2019
Gender:	Unknown	**Sample Type:**	cfDNA
Disease Category:	Cancer	**Cancer Type:**	Non-Small Cell Lung Cancer
Cancer Stage:	Unknown	**% Cellularity:**	null

Metrics

Average Base Coverage Depth:	46060
Uniformity Of Base Coverage:	93.59%
% Base Reads On Target:	93.64%
Median Molecular Coverage:	1022

Variant Summary

A default filter has been applied. Go to SNVs/Indels, Fusions, CNVs pages to remove or modify variant filter.

Filter Chain Applied: *Variant Matrix tab Summary*

SNVs/Indels

1 Detected

Gene	AA Change	Mol Freq %	Oncomine Variant Class
KRAS	p.G12C	0.8168	Hotspot

Fusions

0 Detected

CNVs

0 Detected

The Genexus System

What will it change in practice?

- *Shorter Turnaround time →Overnight NGS results together with ICH →Quicker treatment decisions*
- *Fully automated library prep to result workflow →Reduced workload on lab staff, easy to set-up in new lab*
- *Chip reusable →Cost efficient sample volume scaling*