

The DGP-QUIP PD-L1 and TMB EQA program

Manfred Dietel

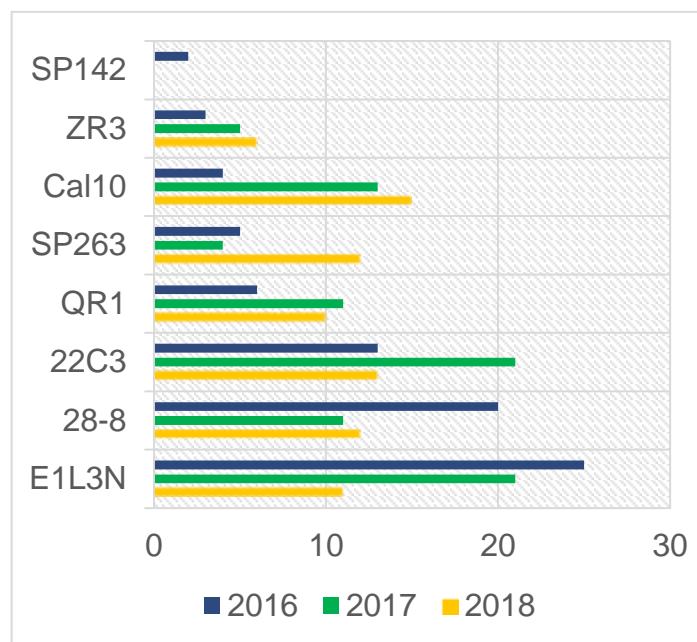
QUIP PD-L1 scheme

PD-L1 activities 2016-2019

- Since 2016: 5 PD-L1 proficiency tests
 - 3 NSCLC
 - 1 Malignant Melanoma
 - 1 Urothelial Carcinoma
- Live trainings
- Online trainings (webinar + webcast)
- PD-L1 Website

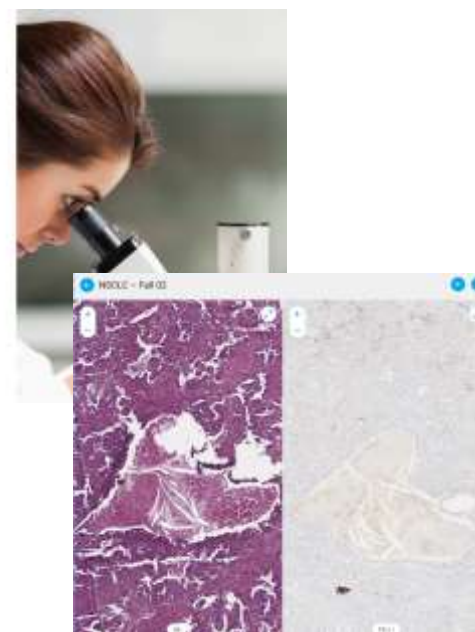
PD-L1 NSCLC: Used antibodies

AK	2016	2017	2018
E1L3N	25	21	11
28-8	20	11	12
22C3	13	21	13
QR1	6	11	10
SP263	5	4	12
Cal10	4	13	15
ZR3	3	5	6
SP142	2	0	0



Live training with digital scoring

- Since 2016 3 live trainings in Berlin
- 2016/2017 focus on NSCLC
- 2018 including Urothelial Carcinoma and HNSC
- Since 2018 with digital scoring and digital TED-voting (tablets)
- 3-5 speakers with different specialisations



Webinar/Webcasts

- 2 different types
 - As a training before a proficiency test:
 - explanation of scores and cutoffs
 - Possible pitfalls
 - Training of example cases –live microscopy and virtual TED vote
 - As a feedback tool after a proficiency test:
 - Scan of all participants slides
 - Analysis of actual pitfalls
 - Antibody analysis
 - live microscopy of anonymous participant slides



TMB assessment: Two independent efforts to harmonize assays and align standards



- Participating organisations:
 - **Pharma:** BMS, MSD, Roche
 - **Diagnostic companies:** Illumina, NEO New Oncology, Qiagen, Thermo Fisher Scientific, Foundation Medicine



- Participating organisations:
 - **Pharma:** AstraZeneca, BMS, EMD Serono, Genentech, MSD, Pfizer
 - **Diagnostic companies:** ACT Genomics, Foundation Medicine, Guardant Health, Illumina, NeoGenomics, OmniSeq, PGDx, Qiagen, Thermo Fisher Scientific
 - **Others:** FDA, MSKCC, NCI

These initiatives collectively aim to better understand the parameters used for TMB assessment, establish standards, and compare existing assays to understand how they relate to one another

Tumor Mutational Burden (TMB) Standardization Initiative: Establishing a Consistent Methodology for TMB Measurement in Clinical Samples

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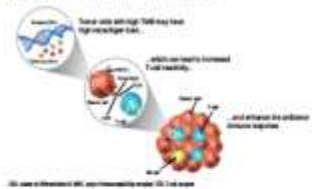
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Background

TMB as a biomarker for response to immune checkpoint inhibitors

- TMB is the total burden of somatic mutations in a defined region of a tumor genome and varies according to tumor type and staging patterns^{1,2}
- For some tumors, evidence is emerging for the association of TMB with cancer responsiveness³
- Numerous risk factors influence TMB, such as anti-programmed death-1 (PD-1) and anti-programmed death-1 ligand 1 (PD-L1) inhibitor treatment, T immunohistochemistry (IHC), and previous tumor history (prior resection for primary or secondary cancer, locally advanced disease, recurrence)⁴
- Patients with high TMB and high response rates may be more likely to achieve durable benefit from treatment with immune checkpoint inhibitors
- Recent clinical studies have identified an association in some tumor types between elevated TMB and improved outcome in patients treated with these agents^{5,6}
- Thus, TMB is an emerging predictive biomarker for response to immune checkpoint inhibitors

Figure 1. TMB association with the anti-PD1 response



The need for standardization and harmonization of TMB assessment

- Recent TMB data are promising and will continue to accumulate
- The number of published studies investigating immune checkpoint inhibitors that include TMB assessment has increased over recent years
- Ongoing epidemiologic research addresses whether clinical trials with TMB assessment registered on the ClinicalTrials.gov database are increasing (27 trials as of July 18, 2018) (registered 2013-2017 are 12 registered 2018-2019 are 15)⁷
- Currently, methods of TMB estimation and reporting vary widely across clinical studies
- TMB can be measured using a range of next-generation sequencing (NGS) methods
- Published studies including TMB assessment have used different approaches and methodologies⁸
- Multiple parameters include sample type, genomic coverage, genomic coordinates, bioinformatics pipeline, read length, and sequencing method⁹⁻¹¹
- This highlights a need for standardization and harmonization of TMB assessment across research and clinical
- Harmonized TMB assessment is critical for consistent identification of patients who are likely to benefit from immune checkpoint inhibitors¹²

Friends and QiP[®] TMB Standardization and Harmonization Initiatives

The international collaboration between Friends of Cancer Research (Friends) and QiP[®] has been working to establish a consistent methodology for TMB assessment in clinical samples across various cancer types and stages.

Key objectives:

- Collaborate to investigate efforts to improve standardization for assessing consistency in TMB assessment in clinical samples between stage and across diseases
- Use multidisciplinary approaches to develop recommendations to standardize TMB estimation and reporting across various TMB studies

Initiatives overview:

- Friends and QiP[®] have partnered with a number of academic institutions and diagnostic pharmaceutical companies to bring together key experts such as pathologists, physicians, and patient advocacy groups (Figure 2)
- Evidence-based risk factor utility multidisciplinary approaches (Figure 3) will assess consistency of TMB estimation, assay sensitivity, and TMB cutoff values for predictive clinical use
- Recommendations will inform the design community of best practices for TMB assessment in clinical samples to guide patient treatment decisions and achieve maximum clinical benefit for patients

Results of the Friends and QiP[®] TMB initiatives

Key findings that can impact TMB assessment

- Review of the published literature indicates that several factors influence TMB assessment (Figure 3)
- Some factors have greater impact than others on TMB estimation and reporting
- Findings analyzed from Friends and QiP[®] highlight how variation in sequencing and bioinformatics parameters can impact TMB estimation (Table 1)

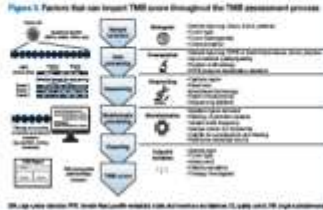


Table 1. Key factors that can impact TMB across throughout the TMB assessment process



Factor	Recommendation	Impact on TMB
Sequencing platform	Target high depth (100x) on targeted NGS or 50x on whole-genome NGS	High depth sequencing is essential for accurate TMB estimation
Genomic coverage	Target 100% coverage for all regions of interest	High genomic coverage is essential for accurate TMB estimation
Sequencing depth	Target 100x depth for all regions of interest	High sequencing depth is essential for accurate TMB estimation
Bioinformatics pipeline	Use standard, validated pipelines	Consistent bioinformatics pipelines are essential for accurate TMB estimation
Reporting method	Report TMB as a ratio of somatic mutations per megabase (mut/mb)	Standardized reporting is essential for accurate TMB estimation

Future Directions

- Evidence-based clinical analyses are underway to:
 - Assess predictive utility of TMB
 - Evaluate TMB assessment by NGS with TMB measured by either targeted panels or NGS
 - Assess consistency of TMB measurement
 - Validate harmonized and consistent methodology
- Studies by other organizations are ongoing and compare the findings of Friends and QiP findings to assess the predictive utility for TMB assessment by Cheng et al, ESMO 2018 poster 141P

Conclusions

- Standardization and harmonization of TMB assessment are essential for relative use of TMB as a predictive biomarker to effectively guide patient treatment decisions
- To address the critical need for standardization and harmonization of TMB estimation and reporting, Friends and QiP have coordinated efforts to propose recommendations for consistent methodology for TMB measurement in clinical samples
- Preclinical analyses highlight the importance of targeted gene panels and consistent bioinformatics pipelines for reliable TMB estimation
- These recommendations will help ensure consistency of TMB assessment in clinical samples and inform consistent TMB studies, which is critical for reliable and reproducible use of TMB as a biomarker to identify patients likely to benefit from immune checkpoint inhibitor therapy

Recommendations for the Standardization and Harmonization of TMB Assessment

- Friends and QiP have developed recommendations for the reliable assessment of TMB and use to inform variability, to inform the consistency in TMB estimation and reporting (Table 1)
- Publishing the recommendations will improve consistency of TMB assessment in clinical research across design and reporting

Parameter	Sample priority	Recommendation
Sequencing platform	High	Target high depth (100x) on targeted NGS or 50x on whole-genome NGS
Genomic coverage	High	Target 100% coverage for all regions of interest
Sequencing depth	High	Target 100x depth for all regions of interest
Bioinformatics pipeline	Medium	Use standard, validated pipelines
Reporting method	High	Report TMB as a ratio of somatic mutations per megabase (mut/mb)

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A. Stenzinger et al. ESMO 2018 Abstract 141P

Overview on Test Approach – Technical Comparability Study

Included TMB panels (sorted by company):

- * **ThermoFisher Assay**: Oncomine Tumor Mutation Load Assay
- * **Qiagen „TMB-Panel“** QIAseq Targeted DNA IO Panel (12); **“MSI booster panel“** QIAseq Targeted DNA Booster Panel (96)
- * **NEO New Oncology**: NEOplus v2 RUO
- * **Illumina**: Illumina TruSight™ Oncology 500 (TSO500)
- * **Foundation Medicine**: FoundationOne (F1) Panel



Panels assessed by test centers

Test centers	Panel 1 st run	Panel 2 nd run	Panel 3 rd run
Charité Hospital Berlin	ThermoFisher	Qiagen	
University Hospital Dresden	Qiagen		
University Hospital Erlangen	Illumina	Qiagen	Academic (Agilent)
University Hospital Halle		Neo NewOncology	
Hambur, Inst. for Hematopathology	Neo NewOncology		
University Hospital Heidelberg	Illumina	ThermoFisher	Academic (Agilent)
University Hospital Cologne	Illumina	Neo NewOncology	Academic (Agilent)
Technical University of Munich	Illumina	ThermoFisher	
LM University of Munich		ThermoFisher	
University of Regensburg	Neo NewOncology	Qiagen	
University Hospital Zurich		F1	

Tumor mutational burden standardization initiatives: Recommendations for consistent tumor mutational burden assessment in clinical samples to guide immunotherapy treatment decisions

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Preliminary data analyses showed a considerable interassay complexity with assay and tumor type specific characteristics. This means that a harmonization and conversion of different assays as well as an adaptation to different tumor types – i.e. there is no tumor-agnosticity - is essential to get reliable and reproducible results.

Center, Johns Hopkins University, Columbia University, Thermo Fisher Scientific Inc; SeraCare Life Sciences Inc; Regeneron Pharmaceuticals Inc; QIAGEN NV; Pfizer Inc; Personal Genome Diagnostics (PGDx) Inc; OmniSeq LLC; NeoGenomics Laboratories Inc; Merck & Company Inc; Illumina Inc; Guardant Health Inc; Genentech Inc; Foundation Medicine Inc; EMD Serono Inc; Caris Life Sciences Inc; AstraZeneca LP; ACT Genomics Company Ltd; Bristol-Myers Squibb Company Inc

be followed to minimize variability in TMB estimation and reporting, which will ensure reliable and reproducible identification of patients who are likely to benefit from immune checkpoint inhibitors.

KEYWORDS

biomarkers, immune checkpoint inhibitors, neoantigens, next-generation sequencing, tumor mutational burden/load

es & Cancer

[IQN Path Selects SeraCare Life Sciences for First-Ever Tumor Mutational Burden External Quality Assessment Program](#)

Posted on [March 27, 2019](#)

International expert group focused on improving quality of clinical biomarker testing launching pilot TMB proficiency program in Spring 2019

SeraCare Life Sciences, a manufacturer and leading partner to global in vitro diagnostics manufacturers and clinical laboratories, announced today their successful selection as a technology partner in support of the first ever tumor mutational burden (TMB) external quality assessment (EQA) program being developed by the International Quality Network for Pathology (IQN Path). Under the agreement with IQN Path, SeraCare Life Sciences will develop, manufacture, and supply a range of **highly-characterized cell line genomic DNA** and **formalin-fixed, paraffin embedded (FFPE) standards** with confirmed low, mid, and high **levels of mutational burden** within their exome regions. Beginning in June 2019, this material will be sent to about 30 labs as part of the **first-ever EQA program** for clinical labs who are monitoring TMB as part of their cancer tumor profiling assays by next-generation sequencing (NGS).

Studies to understand the clinical utility of TMB as a biomarker by NGS have been hampered by the lack of available standards and cost-effective assays for laboratories to implement. Last fall, SeraCare entered this space by joining **the Friends of Cancer Research consortium** on TMB harmonization as a **key partner** and provided standards for their **harmonization efforts**. Interest in TMB standards and harmonization is currently a very important topic of clinical research, as numerous pharma companies have clinical immuno-oncology (I-O) programs and seek to accurately and consistently assess TMB scores using NGS panels deployed in labs around the world in order to direct I-O therapeutics.