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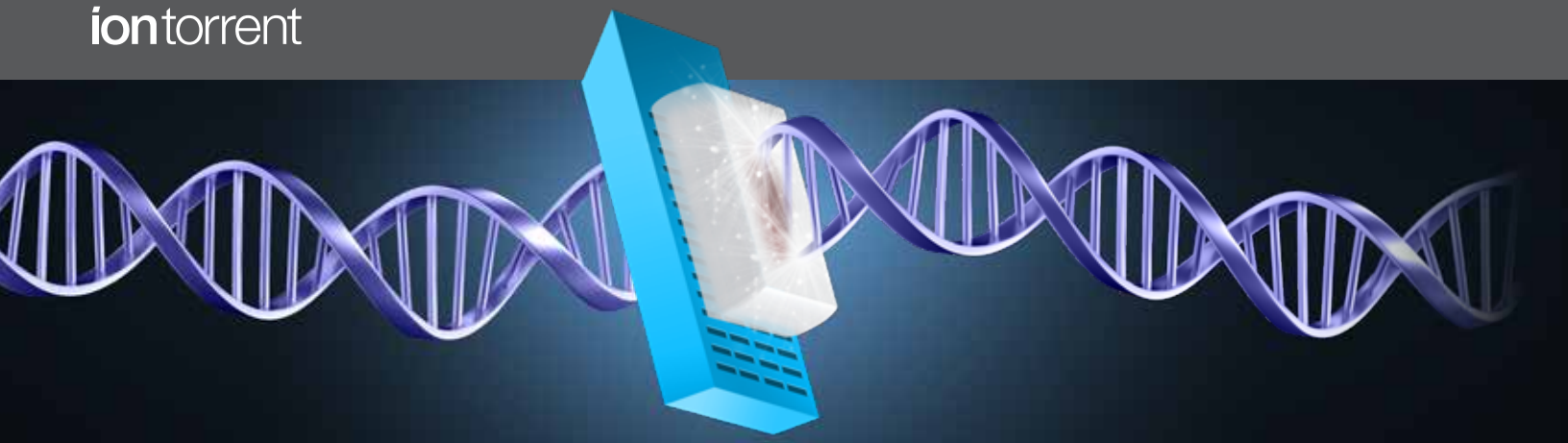

Oncomine Tumor Mutation Load Assay

A next-generation sequencing assay for an emerging immuno-oncology biomarker

Introduction: the need for a targeted panel in immuno-oncology research

Immunotherapy is now widely accepted as a key component of oncology therapeutic strategies. Utilizing the body's own immune system to recognize, control, or potentially eliminate cancers is revolutionizing therapeutic concepts and changing the standard of care for cancer treatment. Moreover, immunotherapy has the potential to affect a clinical cure, or convert cancer from a fatal disease into a non–life-threatening or chronic disease.

However, despite the current success of immunotherapy not all patients respond, and even those who do often experience toxicities. Thus, there is a growing need to identify predictive and prognostic biomarkers of immune toxicity and immune response.

Clinical biomarkers have been shown to be as important as the therapies they guide in our quest to help match patients with appropriate treatments. Since no single biomarker is fully predictive, it follows that interrogating the tumor microenvironment using a multidimensional approach may provide the most comprehensive insights. Recent advancements in next-generation sequencing assays further enable a deeper understanding of a tumor's molecular profile.

Tumor mutational burden (TMB), a measure of the number of mutations within a tumor genome, is a promising emerging biomarker that has historically been assessed by whole-exome sequencing (WES). However, recent studies demonstrate that TMB can be effectively estimated using targeted sequencing panels and show high concordance with WES.

The Oncomine solution

The **Ion Torrent™ Oncomine™ Tumor Mutation Load Assay** measures this important biomarker, enabling research that explores the correlation between TMB and future therapeutic response in several cancers.

Key highlights:

- Targeted NGS assay (1.65 Mb) for simultaneous assessment of tumor mutational burden and variant profiling in one run
- Low input requirement (as little as 20 ng of DNA) to enable testing on cytological and fine-needle biopsy samples
- Sample-to-report solution with tumor mutation burden and relevant variant insights with Ion Torrent™ Oncomine™ Reporter software

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Robust assessment of TMB and variant profiling for clinical research

In order to determine comparability with WES, the Oncomine Tumor Mutation Load Assay was sequenced and analyzed with a tumor-only workflow, and compared with WES using tumor/normal samples (Figure 1). Results show a high correlation of $r^2 = 0.925$, confirming that targeted sequencing is a sufficient replacement for WES across different cancer types.

The assay also enables reproducible results that are vital to its clinical-use potential. In the first study, TMB was assessed on 10 pairs of replicates using the Ion GeneStudio™ S5 System and the Oncomine Tumor Mutation Load Assay. The TMB values were highly correlated ($r^2 = 0.9827$) and were reproducible across multiple cancer types with high multiplexing capability (Figure 2A). In a second study, 8 replicates of 5 different FFPE cell lines were analyzed by multiple laboratories. Results demonstrated high reproducibility across the different sample types (Figure 2B).

The Oncomine Tumor Mutation Load Assay can also effectively stratify samples with high TMB from samples with low TMB, without the need for a matched normal sample, as demonstrated with colorectal cancer research samples (Figure 3).

"We used the Oncomine Tumor Mutation Load Assay on a retrospective colon cancer cohort, and were able to separate high– and low–mutation load samples, with results correlating well with the MSI status of the tumors. The assay yielded rapid and robust results with its streamlined informatics. Together with other Oncomine assays, we truly have a comprehensive solution for tumor samples."

Prof. José Carlos Machado, PhD
Group Coordinator, IPATIMUP
Porto, Portugal

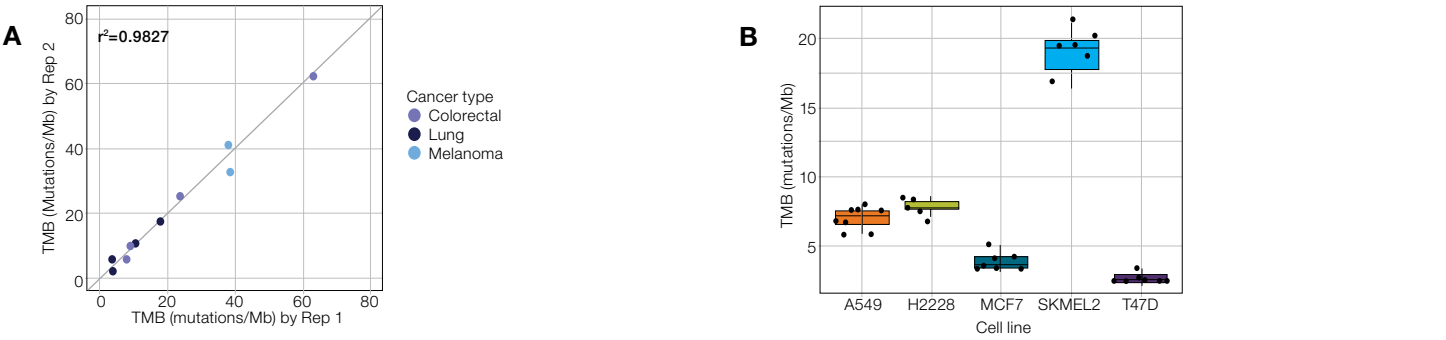

Figure 2A. Reproducibility of Oncomine Tumor Mutation Load Assay on (A) different cancer types and (B) different cell lines.

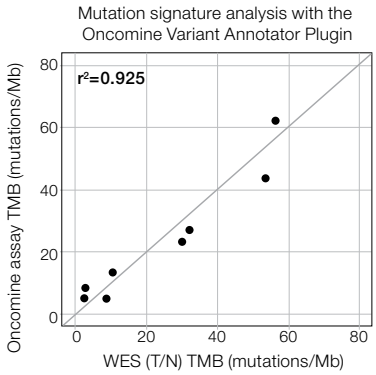

Figure 1. *In silico* analysis of tumor-only somatic mutation counts detected by the Oncomine Tumor Mutation Load Assay, vs. whole-exome mutation count using tumor/normal (T/N) samples.

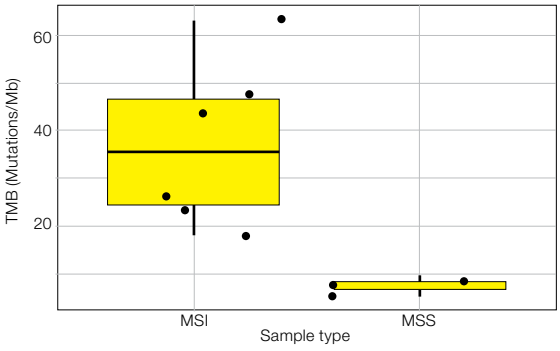

Figure 3. Excellent stratification of colorectal cancer research samples based on assessment of TMB.

Sample-to-report workflow for TMB assessment and variant profiling

A streamlined targeted sequencing workflow

For fast TMB insights, the Oncomine Tumor Mutation Load Assay workflow consists of three key steps (Figure 4). Library and template preparation can be done manually or automated with the Ion Chef™ System. Samples are multiplexed using the Ion 540 Chip or Ion 550 Chip, and sequenced on the Ion GeneStudio S5 Systems. Integrated informatics takes you from variant caller to a finished report that provides contextual insight on sample-specific variants and their use with respect to labels, guidelines, and current global clinical trials. This process transforms data into knowledge, helping you gain insight into the number of acquired somatic mutations in a tumor, and how these mutations may play a role in responsiveness to future treatments.

The Ion Reporter™ Software advantage

NGS can make sequencing faster, easier, and more affordable. However, the amount of sequencing data and bioinformatics can be overwhelming. Ion Reporter™ Software can help you overcome the bioinformatics bottlenecks of NGS data analysis to streamline your process and enable you to focus on finding the biological meaning of your data.

Ion Reporter analysis for the Oncomine Tumor Mutation Load Assay has been designed to generate results with a tumor-only workflow. Using germline filtering with 1,000 genomes, 5,000 exomes, and ExAC databases, nonsynonymous variants are accurately called and assessed for TMB (mutations/Mb). A simple readout is provided in addition to detailed analysis of mutation signatures (Figure 5), providing meaningful insights for understanding response to immunotherapies.

Personalizing precision medicine

Ion Torrent™ Oncomine™ Reporter software produces clear and intuitive reports customized to support informed decision-making by summarizing relevant biomarkers with information from targeted therapies, guidelines, and global clinical trials.

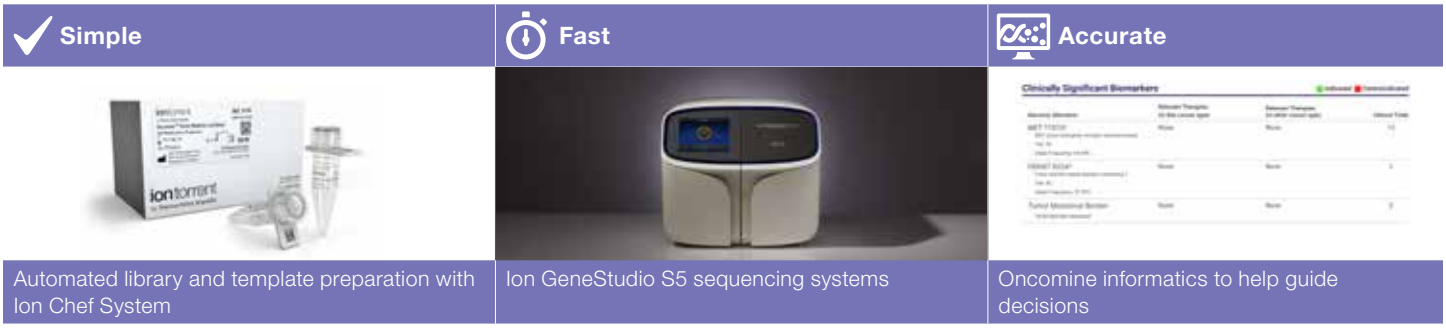

Figure 4. Streamlined sample-to-data NGS solution to assess TMB, with Ion Reporter software and Oncomine Reporter.

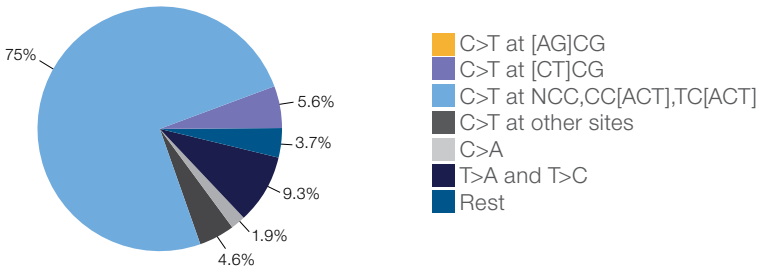

Figure 5. Our carefully designed bioinformatics workflow solution provides insights such as Somatic Mutations Across Substitution Types for researching disease etiology.

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Oncomine assay gene content

The Oncomine Tumor Mutation Load Assay is a large targeted NGS assay designed to provide clinical researchers with an accurate assessment of TMB (mutations/Mb) from limited formalin-fixed, paraffin-embedded (FFPE) samples. The assay covers 1.65 Mb across 409 oncogenes relevant across major cancer types (Figure 6).

A

Categorized by somatic alteration type		Categorized by relevance	
1.65 M	Total bases	4	Genes on 25 labels
1.2 M	Exonic bases	9	Genes in 12 guidelines
43	Driver genes annotated for deleterious mutations	123	Genes used in >680 global clinical trials
61	Driver genes with hotspot mutations		

Oncomine Reporter, September 2018.

B

ABL2	*CD79A*	*EPHB1*	*GRM8*	*LIFR*	*MYH9*	*PMS1*	*SOX2*	*WAS*	*GNAS*	*ATRX*	*TSC2*
ACVR2A	*CD79B*	*EPHB4*	*GUCY1A2*	*LPHN3*	*NCOA1*	*POT1*	*SSX1*	*WHSC1*	*HFN1A*	*BAP1*	*WT1*
ADAMTS20	*CDC73*	*EPHB6*	*HCAR1*	*LPP*	*NCOA2*	*POU5F1*	*STK36*	*WRN*	*HRAS*	*CDK12*	
AFF1	*CDH1*	*ERCC1*	*HIF1A*	*LRP1B*	*NCOA4*	*PPARG*	*SUFU*	*XPA*	*IDH1*	*CDKN2A*	
AFF3	*CDH11*	*ERCC3*	*HLF*	*LTF*	*NFKB1*	*PPP2R1A*	*SYK*	*XPC*	*IDH2*	*CDKN2B*	
AKAP9	*CDH2*	*ERCC4*	*HOOK3*	*LTK*	*NFKB2*	*PRDM1*	*SYNE1*	*XPO1*	*JAK2*	*CEBPA*	
APC	*CDH20*	*ERCC5*	*HSP90AA1*	*MAF*	*NIN*	*PRKAR1A*	*TAF1*	*XRCC2*	*KOR*	*CHEK1*	
ARID2	*CDH5*	*ERG*	*HSP90AB1*	*MAFB*	*NKX2-1*	*PRKDC*	*TAF1L*	*ZNF384*	*KIT*	*CHEK2*	
ARNT	*CDK8*	*ETS1*	*ICK*	*MAGEA1*	*NLRP1*	*PSIP1*	*TAL1*	*ZNF521*	*KRAS*	*CREBBP*	
ATF1	*CDKN2C*	*ETV1*	*IGF1R*	*MAGI1*	*NOTCH4*	*PTGS2*	*TBX22*	*ABL1*	*MAP2K1*	*DNMT3A*	
AURKA	*CIC*	*ETV4*	*IGF2*	*MALT1*	*NSD1*	*PTPRD*	*TCF12*	*AKT1*	*MAP2K2*	*FANCA*	
AURKB	*CKS1B*	*EXT1*	*IGF2R*	*MAML2*	*NUMA1*	*PTPRT*	*TCF3*	*AKT2*	*MAP2K4*	*FANCD2*	
AURKC	*CMPK1*	*EXT2*	*IKBKB*	*MAP3K7*	*NUP214*	*RALGDS*	*TCF7L1*	*AKT3*	*MAPK1*	*FBXW7*	
BAI3	*COL1A1*	*FAM123B*	*IKBKE*	*MAPK8*	*NUP98*	*RARA*	*TCF7L2*	*ALK*	*MET*	*MLH1*	
BCL10	*CRBN*	*FANCC*	*IKZF1*	*MARK1*	*PAK3*	*RECQL4*	*TCL1A*	*AR*	*MPL*	*MSH2*	
BCL11A	*CREB1*	*FANCF*	*IL2*	*MARK4*	*PARP1*	*REL*	*TET1*	*AXL*	*MTOR*	*MSH6*	
BCL11B	*CRKL*	*FANCG*	*IL21R*	*MBD1*	*PAX3*	*RHOH*	*TFE3*	*BRAF*	*MYC*	*NBN*	
BCL2	*CRTC1*	*FANCJ*	*IL6ST*	*MCL1*	*PAX5*	*RNASEL*	*TGFBR2*	*CBL*	*MYCN*	*NF1*	
BCL2L1	*CSMD3*	*FAS*	*IL7R*	*MDM2*	*PAX7*	*RNF2*	*TGM7*	*CCND1*	*NFE2L2*	*NF2*	
BCL2L2	*CTNNA1*	*FH*	*ING4*	*MDM4*	*PAX8*	*RNF213*	*THBS1*	*CDK4*	*NRAS*	*NOTCH1*	
BCL3	*CTNNB1*	*FLCN*	*IRF4*	*MEN1*	*PBRM1*	*RPS6KA2*	*TIMP3*	*CDK6*	*NTRK1*	*NOTCH2*	
BCL6	*CYLD*	*FLI1*	*IRS2*	*MITF*	*PBX1*	*RRM1*	*TLR4*	*CSF1R*	*NTRK3*	*NPM1*	
BCL9	*CYP2C19*	*FLT1*	*ITGA10*	*MLL*	*PDE4DIP*	*RUNX1T1*	*TLX1*	*DDR2*	*PDGFRA*	*PALB2*	
BCR	*CYP2D6*	*FLT4*	*ITGA9*	*MLL2*	*PDGFB*	*SAMD9*	*TNFAIP3*	*EGFR*	*PDGFRB*	*PIK3R1*	
BIRC2	*DAXX*	*FN1*	*ITGB2*	*MLL3*	*PER1*	*SBDS*	*TNFRSF14*	*ERBB2*	*PIK3CA*	*PMS2*	
BIRC3	*DCC*	*FOXL2*	*ITGB3*	*MLLT10*	*PGAP3*	*SDHA*	*TNK2*	*ERBB3*	*PIK3CB*	*PTCH1*	
BIRC5	*DDB2*	*FOXO1*	*JAK1*	*MMP2*	*PHOX2B*	*SDHB*	*TOP1*	*ERBB4*	*PTPN11*	*PTEN*	
BLM	*DDIT3*	*FOXO3*	*JAK3*	*MN1*	*PIK3C2B*	*SDHC*	*TPR*	*ERCC2*	*RAF1*	*RADSO*	
BLNK	*DEK*	*FOXP1*	*JUN*	*MRE11A*	*PIK3CD*	*SOHD*	*TRIM24*	*ESR1*	*RET*	*RB1*	
BMPR1A	*DICER1*	*FOXP4*	*KAT6A*	*MTR*	*PIK3CG*	*SEPT9*	*TRIM33*	*EZH2*	*ROS1*	*RUNX1*	
BRD3	*DPYD*	*FZR1*	*KAT6B*	*MTRR*	*PIK3R2*	*SGK1*	*TRIP11*	*FGFR1*	*SF3B1*	*SETD2*	
BTK	*DST*	*G6PD*	*KDM5C*	*MUC1*	*PIM1*	*SH2D1A*	*TRRAP*	*FGFR2*	*SMO*	*SMARCA4*	
BUB1B	*EML4*	*GATA1*	*KDM6A*	*MUTYH*	*PKHD1*	*SMAD2*	*TSHR*	*FGFR3*	*SRC*	*SMARCB1*	
CARD11	*EP300*	*GATA2*	*KEAP1*	*MYB*	*PLAG1*	*SMAD4*	*UBR5*	*FGFR4*	*ARID1A*	*STK11*	
CASC5	*EP400*	*GATA3*	*KLF6*	*MYCL1*	*PLCG1*	*SMUG1*	*UGT1A1*	*FLT3*	*ASXL1*	*TET2*	
CCND2	*EPHA3*	*GDNF*	*LAMP1*	*MYD88*	*PLEKHG5*	*SOCS1*	*USP9X*	*GNA11*	*ATM*	*TP53*	
CCNE1	*EPHA7*	*GPR124*	*LCK*	*MYH11*	*PML*	*SOX11*	*VHL*	*GNAQ*	*ATR*	*TSC1*	

Annotated for gain-of-function mutations Annotated for loss-of-function mutations

Figure 6. Gene content of the Oncomine Tumor Mutation Load Assay. (A) Categories by somatic alteration type and relevance. **(B)** Full list of genes assayed.

Ordering information

Product	Cat. No.
Oncomine Tumor Mutation Load Assay, Manual Library Preparation	A37909
Oncomine Tumor Mutation Load Assay, Chef-Ready Library Preparation	A37910

For more information about the Oncomine Tumor Mutation Load Assay, go to **thermofisher.com/tmb**

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