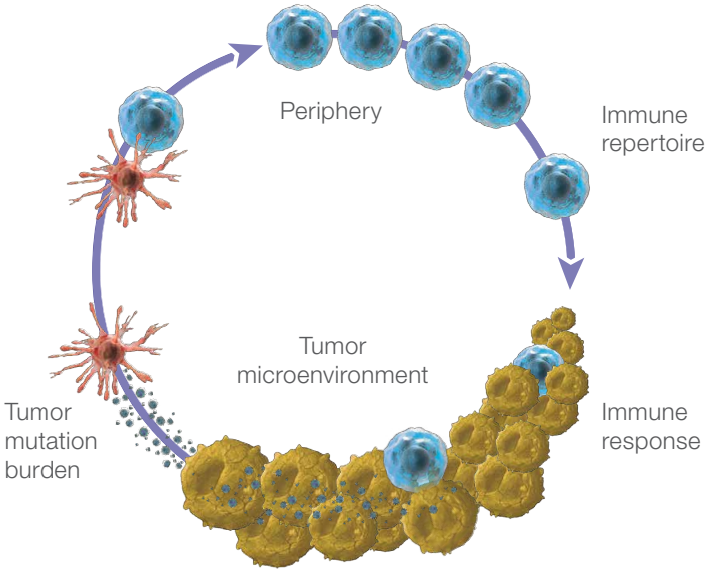
Multidimensional NGS solutions enable tumor-immune insights within IO research

Introduction

Immuno-oncology (IO) therapies are considered to be promising anticancer treatments and are generating hope and excitement among researchers. The Ion Torrent™ portfolio of next-generation sequencing (NGS) assays for IO enables an innovative, multidimensional approach to understanding and profiling tumor-immune interactions. Each genomic assay utilizes the sensitivity of NGS to decipher the hidden biology within precious samples. The assays can be implemented independently or together for a more holistic view to enhance the design of clinical research studies.



Oncomine Comprehensive Assay Plus

The enhanced Ion Torrent™ Oncomine™ Comprehensive Assay Plus is the only solution you need for comprehensive genomic profiling without compromises. This broad pan-cancer NGS assay helps enable biomarker profiling across more than 500 relevant DNA and RNA genes, and maximizes the genomic insights delivered from just 20 ng of precious FFPE tumor sample. With this assay, you can detect single-gene biomarkers across all variant types and multiple-gene biomarkers to characterize mutational signatures, including immuno-oncology biomarkers for tumor mutation burden and microsatellite instability. Additionally, genomic instability and homologous repair deficiency (HRD) status can now be characterized through assessment of homologous recombination repair (HRR) genes and loss of heterozygosity (LOH). Fusion detection capabilities are further enhanced with FusionSync™ technology, and this 3–5 day sample-to-report workflow can be automated to increase lab efficiencies.

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Oncomine Tumor Mutation Load Assay

Tumor mutation load (TML), also known as tumor mutation burden (TMB), is an emerging immuno-oncology biomarker that measures the number of somatic mutations present in a tumor genome. The Ion Torrent™ Oncomine™ TML Assay covers 1.7 Mb of genomic DNA across 409 cancer-driven genes relevant across major cancer types. It requires as little as 20 ng of tumor DNA, and the workflow to go from FFPE sample to streamlined analysis only takes 3 days. The assay highly correlates with exome mutation counts, eliminating the need for whole-exome sequencing, as shown in Figure 1. Sequencing with a targeted panel like the Oncomine TML Assay enables more samples to be evaluated while conserving sample quantity, enabling additional biomarker assessments.

Oncomine Immune Response Research Assay

The Ion Torrent™ Oncomine™ Immune Response Research Assay panel was carefully designed to monitor the tumor microenvironment (TME). This large pan-cancer assay profiles 395 genes associated with immune response and can be used for identification of biomarkers or to study the mechanism of action and other interactions emanating from combination therapy experiments. Figure 2 shows how this multi-pronged approach was tested through the combined evaluation of immune response with immune repertoire insights.

Oncomine TCR-Beta assays

Ion Torrent™ Oncomine™ TCR-Beta assays are a complementary pair of unique assays that enable immune repertoire profiling through sequencing of the complementarity-determining regions (CDRs) of the T cell receptor (TCR) beta chain.

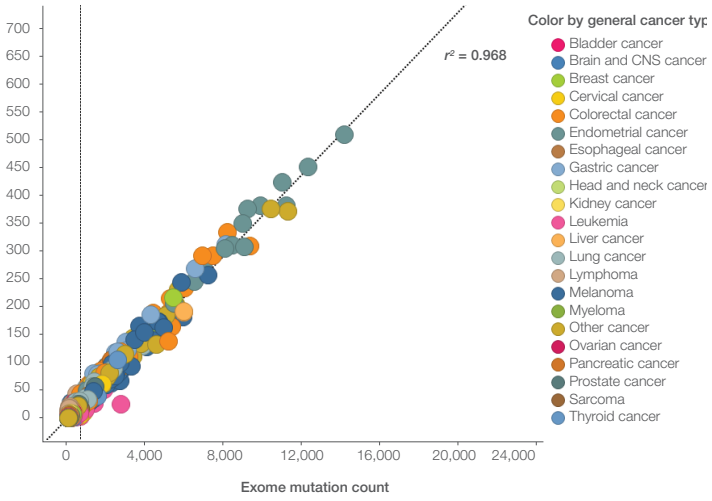

Figure 1. *In silico* comparison of the Oncomine TML assay and whole-exome sequencing (WES). WES data of 21,056 samples were downloaded from the COSMIC v80 database. Mutations were restricted to Oncomine TML targets. Mutation counts by WES strongly correlated (r^2 = 0.968) with those of the Oncomine TML assay.

Multidimensional insights to interrogate the mechanism of action of immune therapy candidates and the potential impact on immune response.

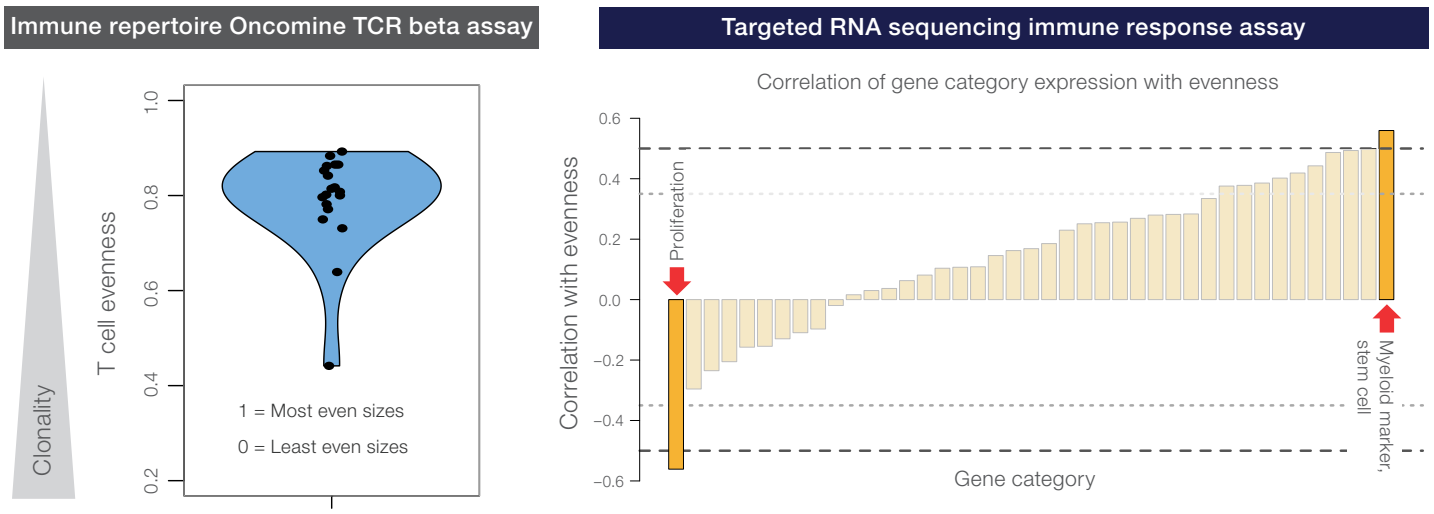

Figure 2. Multidimensional insights from an immune response NGS assay together with the Oncomine TCR Beta-LR Assay. The TME was profiled from 19 NSCLC fresh-frozen samples. **(Left)** While these samples span the range of T cell expansion from low to high, most demonstrate low clonality. **(Right)** The bar plot shows correlation of 36 gene signature categories of a targeted RNA sequencing immune response assay with T cell clonality. Lack of T cell clonal expansion correlates most strongly with a myeloid suppressor, like the gene expression signature shown on the rightmost bar.

Utilizing a newly developed long-read sequencing technology, the Ion Torrent™ Oncomine™ TCR Beta-LR Assay is designed to efficiently capture all three CDRs (CDR1, CDR2, and CDR3) with high accuracy. This approach may enable key applications such as predictive or prognostic biomarker discovery, T cell characterization, and identification of variable gene polymorphisms from samples such as RNA extracted from whole blood, fresh-frozen tissue, or FACS-sorted cells. Rare or abundant clones can be identified with as little as 10 ng RNA input, and this use of RNA template improves rare clone identification through the sequencing of productive and relevant variable (V), diversity (D), and joining (J) rearrangements. Additionally, this long-read immune repertoire assay enables profiling of TCR convergence, which may serve as a metric for quantifying tumor immunogenicity (Figure 3).

In comparison, the Ion Torrent™ Oncomine™ TCR Beta-SR Assay is a short-read immune profiling assay that specifically interrogates the CDR3 region of the TCR beta chain. Compatible with both formalin-fixed, paraffin-embedded (FFPE) DNA and RNA, this NGS assay characterizes the immune repertoire through analysis of T lymphocyte evenness (Figure 4), and also detects T cell minimal residual disease (MRD) in peripheral blood. The Oncomine TCR Beta-SR Assay offers a fast 2-day workflow that combines low sample input with superior informatics for accurate clonality and TCR beta chain CDR3 sequence assessment without interference from primer bias.

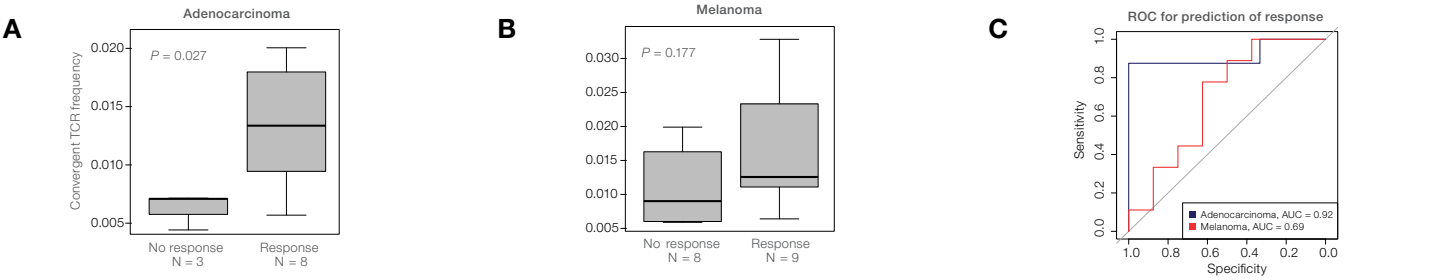

Figure 3. Peripheral blood TCR-beta convergence may help quantify tumor immunogencity, as demonstrated here in these research studies. Pretreatment convergence values for samples that demonstrated response or no response to PD-1 or CTLA-4 blockade for **(A)** adenocarcinoma or **(B)** melanoma. Response is defined as RECIST graded SD, PR, or CR following therapy. **(C)** Receiver operator characteristic (ROC) curves for prediction of response via pretreatment convergence values. Area under curve (AUC) is indicated in figure legend.

Figure 4. T cell content for three different FFPE tissues: (A) tonsil RNA—high T cell content; (B) thymus RNA—medium T cell content; (C) spleen RNA—low T cell content. These spectratyping plots demonstrate the ability of this assay to interrogate tissue samples with varying levels of T cell content.

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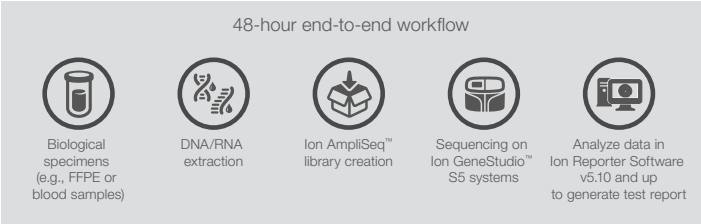
Streamlined workflow optimized for maximum results

Ion Reporter™ Software offers unique, customized workflows for each assay with specific features and analyses that result in relevant data and report output. Within the immuno-oncology suite of simple data analysis tools, results on the frequency and sequence features of clonotypes of repertoires can be analyzed. DNA and RNA workflows for the analysis of human TCR beta chain CDR3 regions amplified with the Oncomine TCR Beta-SR Assay can be compared. Data generated across different IO research assays such as the Oncomine TCR Beta-SR Assay and the Oncomine TCR Beta-LR assay can be compared as well. These analysis tools are designed to cover a growing number of research applications, including immuno-oncology research.



From sample extraction through library preparation, sequencing, and data analysis, the Ion Torrent NGS workflow is a true end-to-end solution. As the sole manufacturer of every workflow component, we offer an optimized solution that can help yield maximum results in your immuno-oncology research workflows.

Ordering information

Product	Cat. No.
Library preparation	
Dual Barcode Kit 1-96 (only for the Oncomine TCR Beta-SR Assay)	A39360
Oncomine Comprehensive Assay Plus	A49667 (automated)
	A48577 (manual)
Oncomine Tumor Mutation Load Assay	A37909 (manual)
	A37910 (automated)
Oncomine TCR Beta-LR Assay	A35386
Oncomine TCR Beta-SR Assay	A39072 (DNA)
	A39359 (RNA)
Oncomine Immune Response Research Assay	A32881 (manual)
	A32928 (automated)
Sequencing	
Ion GeneStudio S5 System Series	Please inquire
Analytics	
Ion Reporter Software	**ionreporter.thermofisher.com/ir**

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