APPLICATION NOTE
IMMUNO-ONCOLOGY

Immunotherapy: The next generation of cancer treatment

Learn how researchers are leveraging next-generation sequencing to advance immuno-oncology research and develop personalized immunotherapy

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Introduction

Over the past decade, immunotherapy has revolutionized the landscape of cancer treatment for advanced stage cancers. Illumina next-generation sequencing (NGS) has shown remarkable utility in cancer and immunology research, and contributed to the development of individualized immunotherapies. High-throughput NGS has dramatically improved our knowledge of the cancer genome and the complex cellular mechanisms involved in tumor progression and metastasis.[1] Current methods of tumor analysis effectively reveal new epitopes, or neoantigens, that are potential targets for the immune system.[2] Sequencing can also be used to investigate the immune repertoire as a real-time, highly sensitive monitor of clonal expansion and contraction of cell populations in response to tumor progression or treatment.[3,4]

The immune system has the innate ability to recognize tumor-specific antigens and protect the host from cancer progression via activation of a T-cell response against these antigens. Therefore, cancer can only be successfully established when the tumor cell is able to evade the host immune response. Because immune evasion is a hallmark of cancer, intensive research has been aimed at understanding the complex interactions between tumors and the immune system. This research can potentially lead to improved strategies for cancer treatment.[5]

Guided by the wealth of new information that genomic methods provide, manipulation of the immune response has resulted in promising therapies by boosting the ability of the immune system to target cancer or by limiting the ability of tumors to evade the natural immune response. Further advances in NGS technology have increased knowledge of the intricate pathways that regulate the immune response (Figure 1) and improved methods used to identify tumors that are appropriate candidates for specific immunological therapies.

This application note highlights recent advances in immuno-oncology, including evolving trends, needs of researchers, and genomic technologies available to aid in this rapidly advancing field. The promise and challenge of current immunotherapeutics, as well as emerging approaches and targets are presented.

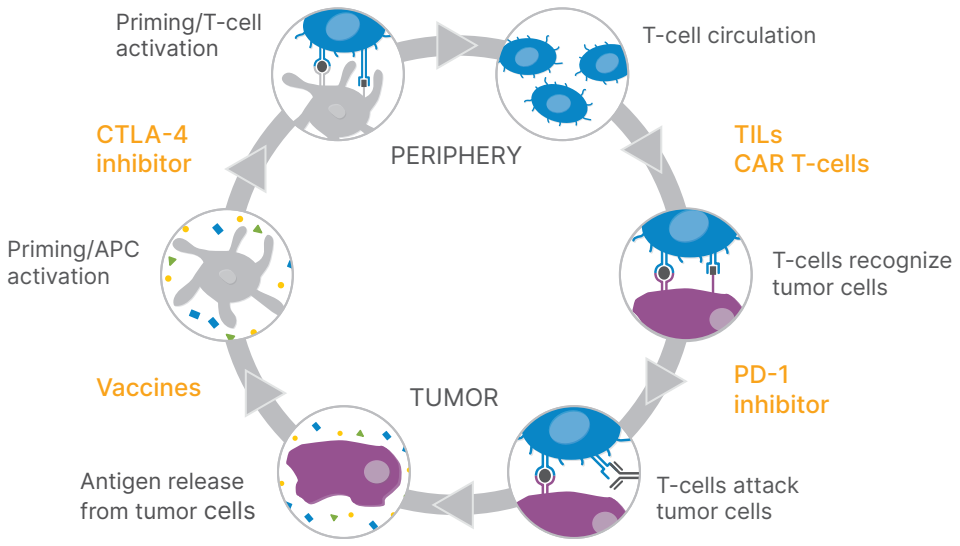

Figure 1: Targeting T-cell–mediated immunity in cancer treatment—Several steps are necessary for establishing a successful immune response that can be augmented with immunotherapies. Tumor-specific antigens are released from dead tumor cells. Neoantigens recognized by antigen-presenting cells (APC) are presented to T-cells, which are activated when they bypass immune checkpoints. Activated T-cells circulate in the bloodstream until tumor infiltration. When tumor recognition occurs, additional checkpoints must be surpassed before systemic T-cell response is established. Orange text indicates immune modulatory treatments described in this application note. Figure adapted from Chen and Mellman.[6]

Immunotherapeutic approaches in cancer treatment

Current immunotherapies are aimed at mobilizing the host immune system, particularly T-cell–mediated immunity, to target tumors.[5] Approaches used in the clinic include immunomodulators, such as checkpoint inhibitors, adoptive cellular therapies that introduce tumor-targeting immune cells into the body, and cancer vaccines that may have either prophylactic or therapeutic activity against specific cancers.

Immune checkpoint inhibitors

Immune checkpoints are negative regulators of T-cell activation and frequently exploited by tumor cells to evade immune surveillance.[7] Manipulation of these checkpoints, particularly cytotoxic T lymphocyte antigen-4 (CTLA-4), programmed cell death receptor-1 (PD-1), and programmed cell death ligand-1 (PD-L1), have shown great promise in the clinical oncology setting (Figure 2).[8] In 2011, the FDA approved a monoclonal antibody against CTLA-4 (ipilimumab) for clinical use in metastatic melanoma.[9] Two anti-PD-1 antibodies (pembrolizumab and nivolumab), approved in 2014, have now become the standard of care for multiple advanced cancers,[10] with more inhibitory drugs currently in development.[11]

With the success of checkpoint inhibitors, it has become a top priority to identify and characterize factors that predict response to these therapies.[12,13] The efficacy of these new drugs depends, in part, on the level of immunogenicity of each tumor. The expression of individual checkpoint mediators does not always correlate directly to positive clinical responses. However, the mutational load of the tumor may determine response to specific checkpoint inhibitors. The predictive power of tumor mutational burden (TMB) can be boosted when combined with information about the tumor mutational landscape.[14] For instance, TMB and T-cell gene expression profile together effectively identify candidates for pembrolizumab therapy.[15] Another study reported that human leukocyte antigen class-I (HLA-I) type and diversity paired with TMB data accurately predicted response to checkpoint inhibition.[16] Molecular profiling using NGS approaches have been implemented to characterize biomarker profiles that indicate a good match for specific therapeutic regimens.[17,18]

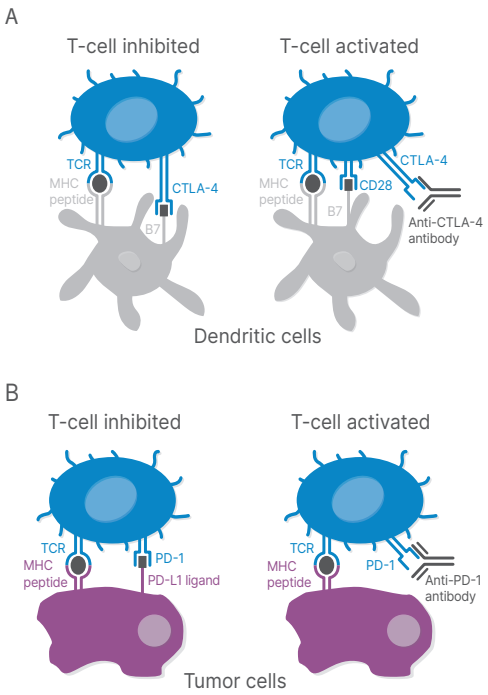

Figure 2: Checkpoint inhibition for cancer treatment— Natural mechanisms dependent upon binding of ligands to PD-1 and CTLA-4 receptors exist to suppress the T-cell response. Monoclonal antibodies to these receptors have shown positive clinical outcomes, but only in certain patients. (A) Priming of T-cell activation by antigen-presenting cells requires binding of costimulatory ligand B7. Binding of B7 by CTLA-4 receptor inhibits costimulation, while blocking of CTLA-4 with an antibody allows costimulation. (B) Recognition of tumor-specific ligand is not sufficient for T-cell activation when inhibitory ligand PD-L1 binds to the PD-1 receptor on the T-cell. Anti-PD-1 antibodies block this interaction and allow T-cell activation.

RNA sequencing (RNA-Seq), for example, has been used to identify key aspects of the tumor microenvironment, such as inductive and inhibitory cytokines, local recruitment of other cells types that can inhibit the T-cell response, and microbial composition in the gut, that can influence the effectiveness of checkpoint therapies.[16-22]

Adoptive cell transfer

Adoptive cell transfer (ACT) therapy involves selection of tumor-specific lymphocytes and infusion of these cells to induce an antitumor response in patients (Figure 3). ACT clinical trials have been successful for targeting melanoma and certain leukemias and are currently being applied to other types of cancers.[23]

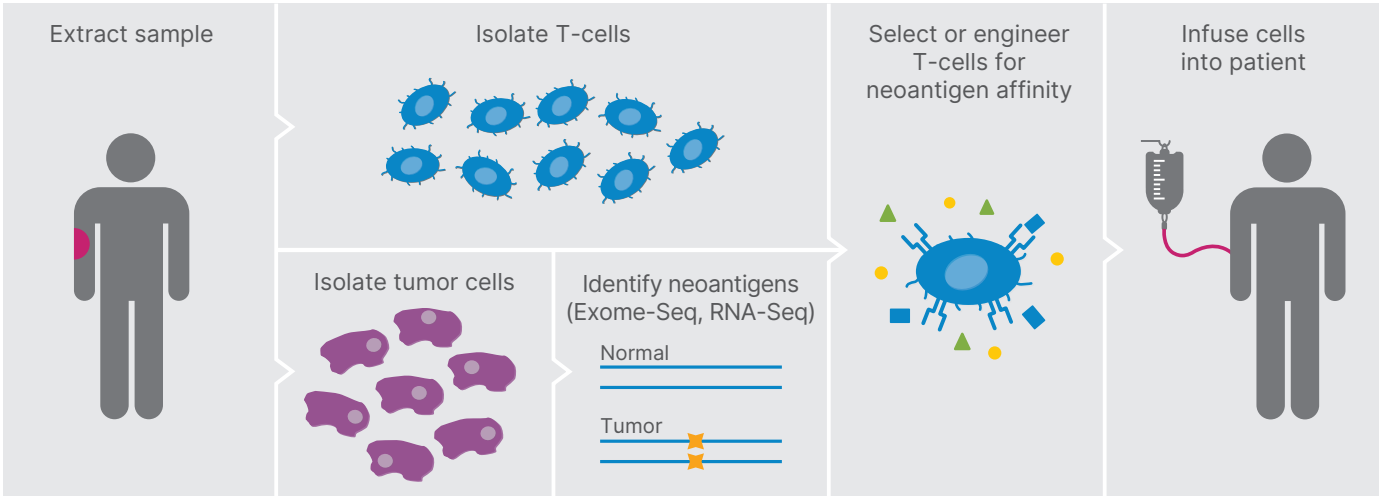

Figure 3: Adoptive T-cell transfer—Improved methods for neoantigen selection have been used to screen lymphocytes that exhibit tumor-specific recognition. Lymphocytes can be isolated from the patient (tumor-infiltrating lymphocytes, TILs) or engineered (CAR T-cells). Purified cells are expanded *ex vivo* prior to infusion into the patient for an augmented antitumor immune response.

ACT can be categorized into chimeric antigen receptor (CAR) T-cells and tumor-infiltrating lymphocytes (TIL) based on the cell type that is used for adoptive transfer.

Chimeric antigen receptor (CAR) T-cells

T-lymphocytes can be modified *ex vivo* to express a CAR directed at tumor-specific antigens. The modified CAR T-cells are then injected back into the patient for *in vivo* targeting of tumors that served as the original source of the antigen. This method has led to several reports of infused CAR T-cells expanding 1000-fold, indicating an antigen-specific response. Furthermore, these methods led to positive clinical outcomes, with persistent CAR expression, and evidence of persistence of immunologic memory cells.[24,25]

Tumor-infiltrating lymphocytes (TILs)

Characterization of tumor exomes has facilitated the discovery that TILs can recognize and target products of cancer mutations.[26] When grown and activated *in vitro*, TILs can be screened before reinjection into patients, leading to active tumor targeting. The adoptive cell transfer of neoantigen-targeting TILs into patients is an important emerging therapeutic approach for many cancer types. Treatment of melanomas has yielded the best results, though further screening has identified the existence of TILs that can recognize neoantigens in other types of solid tumors.[27-28]

Vaccine Immunotherapy

Mutations occurring in protein-coding genes of cancer cells are a source of potential neoantigens that can be targeted by the immune system. Recent advances in NGS, such as exome and RNA-Seq, have enabled the predictive selection of neoantigens that are likely to elicit a tumor-specific response (Figure 4). Neoantigen selection is facilitated by improved bioinformatics tools to analyze specific mutation profiles. Computer algorithm-guided epitope prediction models enable intelligent selection of mutations likely to result in high-affinity epitopes that bind to major histocompatibility complex (MHC) molecules.[29]

Further advances in recombinant DNA technology, such as transduction of neoantigen-expressing RNAs into antigen-presenting cells, have led to success in triggering tumor-specific immune responses.[30,31] Recent successes in clinical trials, facilitated by the rapid turnaround time for tumor analysis and vaccine development, indicate a possible increase in the use of these types of therapies in the near future.

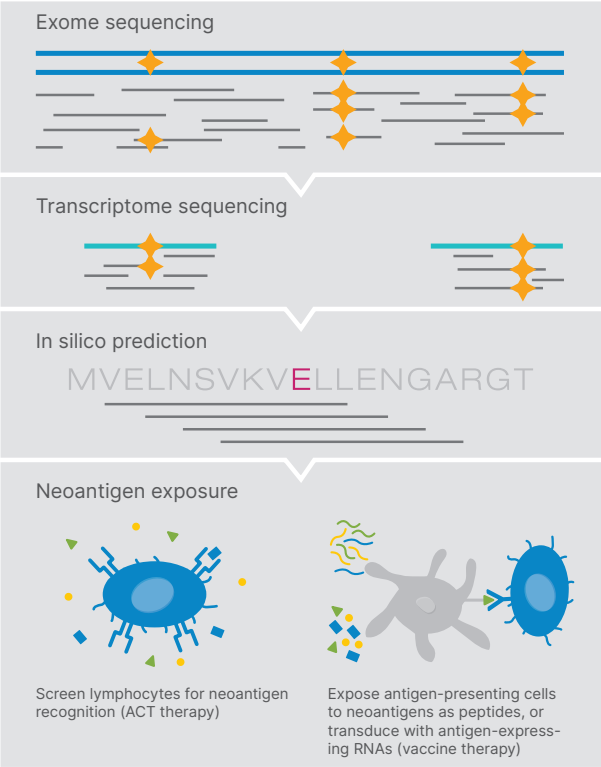

Figure 4: Exome and transcriptome sequencing in the development of personalized immunotherapies—Neoantigens are identified by sequencing the coding regions and expressed genes of tumor cells. Improved predictive algorithms further refine the selection of epitopes with affinity for human leukocyte antigen (HLA) receptors. Small sets of selected neoantigens are then used for vaccine development or adoptive cell transfer.

NGS-based immuno-oncology research solutions

Our understanding of the complex pathways used by cancer cells to evade immune detection continues to expand rapidly. However, it is becoming increasingly clear that strategies to identify tumor susceptibility need to be developed to maximize the therapeutic potential of available immunotherapies.[13] The field of immuno-oncology research will benefit from a comprehensive assessment of the dynamic factors that determine tumor growth and therapy response.

NGS-based methods can monitor key alterations in immune checkpoint expression, TMB, tumor microenvironment, and gut microbial composition, with high analytical sensitivity (Table 1). Leading researchers in the field are leveraging NGS technologies to discover biomarkers and develop analytical tools that will guide personalized immunotherapeutics. The key to this approach is to identify the appropriate neoantigen-reactive T-cells that can mediate a durable antitumor immune response.

Tumor microenvironment and immune composition

Tumors vary considerably in their microenvironment, and the existence of other cell types can influence the ability of the T-cell to infiltrate and attack tumor cells. NGS analysis has been used to characterize the immune repertoire, various cell populations in the microenvironment, and expression of genes that can suppress or improve the T-cell response. RNA analysis has been used to delineate functional subtypes of cancer-associated fibroblasts in the tumor microenvironment, with distinct clinical responses to targeted therapies for lung cancer.[32] RNA-Seq has also been applied to understanding how host genetics can influence the tumor immune microenvironment and determine response to immune checkpoint blockade. [33]

Single-cell RNA sequencing (scRNA-Seq) is being increasingly used to investigate the transcriptomic profile of cancers and tumor microenvironments at single-cell resolution.[34] The number of TILs and the degree of specific clonal expansion provide critical information about how immunosurveillance in the tumor microenvironment is modulated. Using scRNA-Seq, researchers can elucidate specific T-cell receptor (TCR) sequences that drive neoantigen recognition, clonal expansion, and TCR diversity. TCR sequencing, in combination with single-cell transcriptomics, has been applied to understand the key factors in cancer immunosurveillance and resistance to immunotherapy.[28]

Spatial transcriptomics (ST) is a technique that characterizes the localization of gene expression within tissue architecture. This approach provides researchers with critical morphological context when studying intratumor heterogeneity and tumor-microenvironment interactions.[35] Sequencing-based ST has been applied to assess tumor immune infiltration and predict response to immunotherapy in multiple solid tumors.[36,37]

Table 1: NGS-based immuno-oncology applications

Clinical research relevance		Applications				
		Neoantigen, mutational burden	Expression profiling	Microbiome (16S) sequencing	TCR/BCR[a] profiling	Epigenetic profiling
Therapeutic applications	Checkpoint inhibitors	X	X	-	X	X
	Vaccines	X	X	-	X	X
	Adoptive cell therapy	X	X	-	X	X
Prognostics	Microbiome	-	-	X	-	-
	Immune repertoire	-	X	-	X	X
Monitoring		X	X	-	X	-

a. TCR, T-cell receptor; BCR, B-cell receptor

Tumor mutational burden and neoantigen prediction

The number of somatic mutations within the coding region of a tumor genome, known as TMB, is an emerging biomarker that may influence response to immunotherapy. Recent studies indicate that a high TMB increases the likelihood that immunogenic neoantigens expressed by tumor cells may induce a response to immunotherapy.[38] Mutational profiling, by exome and transcriptome sequencing, can help identify tumor-specific neoantigens that may be capable of inducing an immune response, a critical first step in developing effective neoantigen-based vaccines.[27] Whole-genome sequencing (WGS) coupled with RNA-Seq can also be applied to uncover neoantigens as potential targets for immunotherapy, particularly in tumors with low TMB and minimal pretreatment TILs.[39]

Comprehensive genomic profiling of tumors, using the Illumina TruSight™ Oncology 500 assay, has been applied to determine TMB and microsatellite instability (MSI) in various solid tumors and predict response to PD-1 blockade.[40,41] Liquid biopsy is an emerging approach that involves analyzing circulating tumor DNA (ctDNA) in plasma samples for assessing TMB and tumor heterogeneity, and studying treatment response. The TruSight Oncology 500 ctDNA assay uses NGS to detect low somatic variants and complex immuno-oncology genomic signatures with high analytical sensitivity in plasma samples.[42]

Gut microbial composition in cancer

Emerging evidence suggests that the host microbiome influences the strength of the response to immunotherapy.[43] Diet and drugs can disrupt microbiome diversity and key species in the microbiome can cause local or systemic influences on host immunity. For example, microbes that influence cytokine release and inflammation can exert either a positive or negative effect on tumor growth, or the ability of the immune system to suppress tumor growth. In one study, resistance to CTLA-4 blockade therapy was associated with the absence of a specific gut bacterium.[19] However, the outcome improved with several combinatorial approaches, such as bacterial reconstitution, using bacterial antigens for immunization, or adoptive transfer of bacterial antigen-specific T-cells. Microbial 16S rRNA sequencing identified another microbe that mediated the effects of anti-PD-L1 treatment that could be modulated by microbiota manipulation.[20]

NGS enables simultaneous comparison of thousands of microbial community species in samples from healthy individuals and those with cancer.[44] With the development of bioinformatic tools to manage large volumes of novel data, this shift from single organism analysis enables accurate assessment of species diversity and measurement of dynamic fluctuations in microbial communities.

For details on NGS-based immuno-oncology methods, see Methods guide for cancer research

Multiomic approaches in immuno-oncology research

Multiomic studies integrate high-dimensional data sets from genomic, epigenomic, transcriptomic, metagenomic, and proteomic approaches, often using computational and network biology to interpret the vast amounts of data generated by these techniques. Applying multiomics to clinical data amplifies the discovery power of existing 'omics' methodologies to uncover new biomarkers and immunotherapy targets (Figure 5).

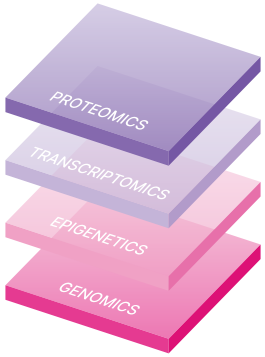

Figure 5: Multiomics in oncology research—Multiple layers of information provide novel insights into tumor biology that cannot be resolved by single omics studies alone.

Recent studies used multiomic approaches, integrating data from WGS, transcriptomics, epigenetics, scRNA-Seq, spatial transcriptomics, and TIL profiling, to correctly predict response to treatment in patients with advanced cutaneous melanoma[45] and metastatic breast cancer.[46] Multiomic profiling of tumor microenvironments was used to identify specific immune regulatory pathways in metastatic pancreatic cancers, offering new insights into treatment resistance.[47] Machine learning analyses of multiomics data collected from the Cancer Genome Atlas are currently underway to determine tumor susceptibility to immunotherapy across 20 solid tumor types.[48]

Multiomic approaches provide a comprehensive view of the molecular profile of the tumor and microenvironment, with the potential to redefine the oncology treatment paradigm by stratifying patients and optimizing therapy, ultimately improving treatment outcomes.

Data analysis solutions

Illumina continuously makes available user-friendly informatics tools that address new challenges in medicine. Comprehensive data analysis solutions include the Illumina DRAGEN™ (Dynamic Read Analysis for GENomics) Bio-IT Platform, supported by Illumina Connected Analytics and an expanding BaseSpace™ Informatics Suite. These tools allow researchers to analyze large volumes of data generated by multiomic approaches with high-accuracy and speed.

Summary

As personalized medicine becomes an increasingly desirable approach, Illumina strives to align trends in immunotherapy with the evolution of genomic technologies that compliment and enable the promise of this field. With a broad range of NGS applications available, Illumina provides researchers with flexible, accurate, and reliable options for analyzing the genome, exome, epigenome, metagenome, transcriptome, and proteome. Through partnerships with leading oncology experts and collaboration with national and international cancer organizations, Illumina continues to expand the portfolio of cancer-focused research solutions.

Learn more

NGS applications for immuno-oncology research, illumina.com/areas-of-interest/cancer/research/cancer-immunotherapy-research

Cancer transcriptome analysis with RNA-Seq, illumina.com/areas-of-interest/cancer/research/sequencing-methods/cancer-rna-seq

Multiomics in cancer immunotherapy, illumina.com/events/webinar/2021/multi-omics-in-cancer-immunotherapy

TruSight Oncology 500 portfolio, illumina.com/products/by-type/clinical-research-products/trusight-oncology-500

Illumina informatics products, illumina.com/products/by-type/informatics-products

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