**Immunotherapy, the Next Generation of Cancer Treatment**

NGS-guided assessment of interactions between tumors and the immune system leads to new discoveries in immuno-oncology.

**Introduction**

Traditionally the basis of cancer treatment has consisted of surgery, radiation therapy, and chemotherapy. More recently, several promising treatment options have emerged in the field of immuno-oncology that point to the possibility of developing new methods of anticancer therapy. High-throughput, next-generation sequencing (NGS) has shown remarkable utility in cancer and immunology research, and contributed to the development of individualized immunotherapy. For example, NGS has dramatically improved our knowledge of the cancer genome and the intracellular mechanisms involved in tumor progression. Current methods of tumor analysis can effectively reveal new epitopes (neoantigens) that are possible targets for the immune system. Sequencing can also be used to determine the immune repertoire as a real-time, highly sensitive monitor of clonal expansion and contraction of cell populations in response to tumor growth or treatment.

The immune system has the innate ability to recognize mutations in tumor cells and protect the host from cancer progression via activation of a T cell response against tumor-specific antigens. While the immune system routinely eliminates potential tumors originating from the host, cancer can only be successfully established when the tumor cell manages to evade the immune response. Therefore aggressive research has been aimed at understanding the complex interactions between tumors and the immune system, which may lead to improved forms of cancer treatment.

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 spotlight highlights recent advances in the immuno-oncology field, including evolving trends, needs of researchers, and genomic technologies that are available to aid in this rapidly advancing field. Three promising fields of immunotherapy are reviewed here, including checkpoint inhibitors, vaccine immunotherapy, and adoptive cell transfer.

![Figure 1: T Cell Mediated Immunity](image)

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Checkpoint Inhibitors

The immune system has an elaborate network of mechanisms to recognize foreign pathogens or mutated epitopes that tumor cells present. Efforts made over the past decades have informed the current understanding about immune cell-intrinsic checkpoints that can prevent T cell activation. These are commonly used by tumors to evade the immune response. Manipulation of these checkpoints has shown great promise in recent therapeutic approaches. One key checkpoint occurs during T cell priming by antigen presenting cells, which requires costimulatory binding of the B7 ligand. Binding of B7 by the CTLA-4 receptor results in suppression of the T cell response (Figure 2A). In 2011, the FDA approved a monoclonal antibody against this receptor (ipilimumab) for clinical use in metastatic melanoma. Another key checkpoint is the binding of PD1 to PDL1, for which 2 anti-PD1 antibodies (pembrolizumab and nivolumab) were approved in 2014 (Figure 2B), and more inhibitory drugs are under development. Both of these therapies have shown good clinical responses a portion of the time. However, relatively few tumors have endogenous T cell responses that can respond to immune checkpoint blockade.

With the success of checkpoint inhibitors, it has become a top priority to identify and characterize factors that predict response to these therapies. The efficacy of these new drugs depends in part on the level of immunogenicity of each tumor. But more information about interactive pathways in the tumor microenvironment will be necessary to improve the ability to predict response. Sometimes, the expression of individual checkpoint mediators correlates directly to positive clinical responses, but in other cases the complexity of the dynamic immune response is less understood. A positive correlation between the mutational load of the tumor and response to individual checkpoint inhibitors has been observed, but is not by itself predictive. Therefore approaches in exome sequencing and RNA sequencing have been implemented to develop ways to characterize biomarker profiles that indicate a good match for specific therapeutic regimens. RNA analysis, for example, has been used to identify other aspects of the tumor microenvironment that can influence the effectiveness of checkpoint therapies, 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.
Vaccine Immunotherapy

Mutations occurring in protein coding genes of cancer cells are a source of potential new antigens (neoantigens) that the immune system may target. To boost tissue-specific T cell immunity, vaccines have been explored since the 1980s. Limited success of early attempts in this field was possibly due, in part, to the presentation of antigens as whole cell lysates, in which highly effective immunogens were diluted with immunologically irrelevant antigens. Recent advances in NGS have enabled the predictive selection of neoantigens that are likely to elicit a tumor-specific response.

A quality versus quantity approach is now showing promise. Characterization of the DNA and/or RNA of cancer cells is efficiently done by exome sequencing or transcriptome sequencing, focusing on mutations that are likely to be presented to immune cells. Neoantigen selection is facilitated by improved bioinformatics tools to analyze specific mutation profiles (Figure 3).\(^{16-18}\) Computer algorithm-guided epitope prediction models enable intelligent selection of mutations likely to result in high-affinity epitopes that bind to MHC molecules.\(^{19,20}\)

Further advances in recombinant DNA technology, such as the transduction of neoantigen expressing RNAs into antigen presenting cells, have led to success in triggering tumor-specific immune responses.\(^ {17,21}\) Recent successes in clinical trials, facilitated by rapid turnaround time for tumor analysis and vaccine development, indicate a possible increase in the use of these types of therapies.

Adoptive Cell Transfer

Adoptive cell transfer (ACT) therapy involves selection of tumor-specific lymphocytes. Similarly to vaccine development, neoantigen selection may be done prior to the screening or engineering of lymphocytes (Figure 4). Trials in ACT have been successful for targeting melanoma and certain leukemias, and are currently being applied to other types of cancers.\(^ {22}\) Two subcategories of ACT are characterized by the cell types that are utilized.

CAR T-Cells

T lymphocytes can be modified ex vivo to express a chimeric antigen receptor (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.\(^ {23,24}\)

Tumor Infiltrating Lymphocytes (TILs)

Characterization of tumor exomes has facilitated the discovery that tumor infiltrating lymphocytes (TILs) can recognize and target products of cancer mutations. When grown and activated in vitro, TILs can be screened prior to reinjection into patients, leading to active tumor targeting. 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 tumors.\(^ {25,26}\)

Informed Development of Personalized Immunotherapies.

Various clinical trials have shown that approaches combining targeted therapy or chemotherapy with immunotherapy can successfully augment the natural immune response with greater results than either approach alone.\(^ {27,28}\) Several phases in the cycle of T cell immunity (Figure 1) can be manipulated simultaneously. It is clear that more information will be necessary to develop strategies to discern which tumors are appropriate candidates for personalized therapeutic regimes. To attain this goal, the field will benefit from methods designed to assess a comprehensive view of numerous factors, including but not limited to, expression of immune checkpoint molecules, mutational load of tumors, predicted neoantigens, tumor microenvironment, and microbial composition in the gut. Leading researchers in the field are continually using NGS technologies to discover new biomarkers, and new analytical tools that will guide a more personalized medicine approach.
Another microbe that mediated the effects of anti-PD-L1 treatment.  

An independent study used microbial sequencing (16S RNA-Seq) to identify for immunization, or adoptive transfer of antigen-specific T cells. An approaches, such as gavage with the bacteria, using bacterial antigens for Research Use Only. Not for use in diagnostic procedures.

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For more information on NGS applications in immunotherapy, visit www.illumina.com/immuno-oncology

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Because the presence of neoantigens does not necessarily correlate to induction of T cell immunity, interrogation of aspects other than the tumor mutational profile will likely help to identify new prognostic indicators.  

The successes of current immunotherapies also reveal there is still much to learn about the complexities of the immune system. While durable clinical responses have been seen in a proportion of advanced cancer patients, much more understanding is needed to manipulate the immune system with a personalized medicine approach. A holistic view of the host environment may be appropriate for many cases in the future. Beyond the scope of neoantigen profiles, the search for biomarkers both in and around the tumor cells should lead to new prognostic indicators, and new therapeutic approaches. RNA sequencing of the localized tumor environment can lead to detection of both supportive and inhibitory cell populations with regard to mediating T cell responses.  

A body of evidence has implicated epigenetic modulation of genes involved in tumorigenesis. Further investigation will possibly indicate similar mechanisms causing increases and/or decreases in expression of genes that mediate the immune response. For example, epigenetic silencing has been found to reduce expression of HLA genes in certain cases, which in turn impairs T cell mediated immunity. Although RNA analysis can detect the aberrant expression of immune modulatory genes, the advances of NGS-based epigenetic analyses enable rapid investigation of samples to define better the cause of abnormalities. Also the analysis of regulatory noncoding RNAs (small RNA-Seq) may help to identify mechanisms of tumor evasion. This knowledge may in turn point to other modes of combinatorial therapy, such as informed use of epigenetic modulatory drugs within the therapeutic regimen.  

Other methods that have been used routinely to monitor tumor progression are also appropriate in immunotherapeutic approaches. NGS applications provide a broad range of sensitive, high-throughput tools for obtaining information in a comprehensive fashion (Table 1).

**Applications**

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**Conclusion**

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. The scale of data delivered by Illumina sequencing systems supports a range of cancer research goals. With a broad range of NGS applications available, Illumina provides researchers with flexible, accurate, and reliable options for analyzing the genome, exome, transcriptome, and epigenome. Illumina continuously makes available user-friendly informatics tools that address new challenges in medicine, with an expanding BaseSpace® Informatics Suite. 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**

For more information on NGS applications in immunotherapy, visit www.illumina.com/immuno-oncology
References


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Application Spotlight: Cancer