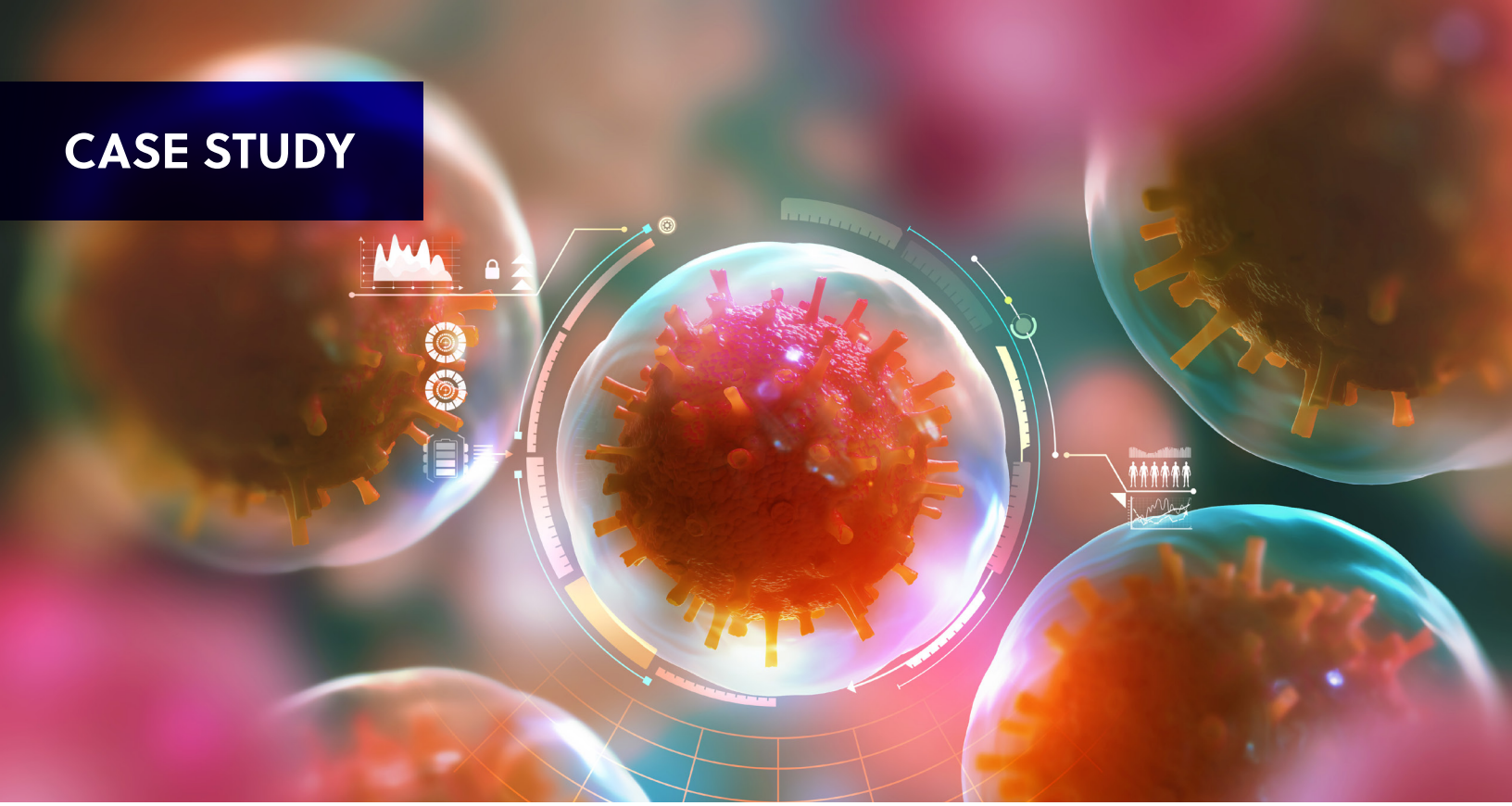
CASE STUDY



Predicting and measuring patient response to cell therapy with single cell TCR sequencing

Cancer is both a widespread and leading cause of death with a wide range of treatments and prognoses that vary from individual to individual. Cancer cell-based immunotherapies, including neoantigen vaccines and adoptive cell therapies, have attracted great attention recently for their therapeutic effects.

However, patient response to these therapies remains limited. For example, over 50% of metastatic melanoma patients do not benefit from anti-PD1 and/or anti-CTLA4 treatment, despite a positive clinical outcome in patients who do respond[1]. This inconsistency in patient response represents a massive waste in terms of resources and cost. As immunotherapy costs reach upwards of $100,000 per patient per year[2], it is critical to predict how patients will respond to such a therapy in order to effectively personalize treatment for optimal clinical outcome while maintaining lower costs.

To better understand what dictates patient response to immunotherapies, we must first look to the immune system of the individual. Everyone has their own unique immune repertoire with its own capacity to recognize

neoantigens, like those associated with tumors and other cancer cells. The backbone of this repertoire is the diversity of the T cell receptors (TCRs) that are responsible for the recognition of these neoantigens. Indeed, improved TCR diversity has been linked to better prognostic outcomes for patients undergoing immunotherapies[3-5].

Immune repertoire sequencing is a cutting-edge technology first developed and commercialized by iRepertoire in 2009. This technology, which has now been extended to include single cell sequencing, can be used to elucidate what the TCR repertoire of each individual has to offer. Analyzing the individual immune repertoire can provide insight into how and why these cells contribute to certain prognostic outcomes. This information can then, in turn, be used to determine prospective therapies to better suit individual needs, resulting in better clinical outcomes. Recent studies also suggest that immune sequencing can be used to demonstrate treatment efficacy in pre-clinical studies. In this paper, we will look at two recent applications of immune sequencing and how it has been used to shed light on the role of the immune repertoire in immunotherapy response.

TRACKING NEOANTIGEN RESPONSE WITH TCR SEQUENCING

In one investigation, researchers from Neon Therapeutics tracked the progression-free survival of 21 melanoma patients before, during, and after drug and vaccine treatment[6]. TCR repertoire profiling was done on each sample and compared to samples from 11 healthy individuals.

RNA library preparation and sequencing were done on-site or handed off to iRepertoire for processing as part of their iPair service. iPair is a single cell, consumer-based service designed for capturing physically paired variable regions in T and B cells. Alongside the iPair system, researchers made use of the iR-Profile Reagent System to prepare bulk TCR libraries from isolated RNA.

Analyzed TCR repertoires were assigned into categories based on their relative diversity, and each patient was followed for 9 months post-treatment to look at subsequent tumor progression and patient survivability. Researchers found that patients with increased baseline clonal TCR profiles were more likely to survive the treatment in the long-term. Additionally, TCR repertoire sequencing showed an increase in large and hyperexpanded TCR clonal frequencies following administration of the neoantigen vaccine, NEO-PV-01. This result implicates the role of TCR diversity in the longevity of patients after cancer treatments and supports neoantigen vaccines as a possible treatment.

PROFILING ANTIGEN-SPECIFIC T CELLS IN RESPONSE TO A NOVEL THERAPY

Another study conducted by Cue Biopharma looked at the ability of CUE-101, a novel therapeutic fusion protein, to prime patient TCRs for response to HPV16-driven tumors[7]. TCR repertoire diversity was examined by single-cell TCRα-chain and TCRβ-chain paired sequencing of tetramer+ CD8+ T cells using iRepertoire's iPair service, the iRmap VDJ pipeline, and the iPair Analyzer.

Researchers found that CUE-101 demonstrated selective binding, activation, and expansion of HPV16 E711-20-specific CD8+ T cells from peripheral blood mononuclear cells, relative to nontarget cells. In vivo studies confirmed selective expansion of tumor specific cytotoxic CD8+ T cells, induction of immunologic memory, and a favorable safety profile. This resulted in a positive anti-tumor effect in mice especially when used in combination therapies, implicating the role of selective TCR expansion in improving clinical outcomes and enabling progression of CUE-101 to phase I clinical trials.

BRINGING TCR SEQUENCING INTO THE FOREFRONT OF IMMUNOTHERAPY

Taken together, both studies highlighted here implicate the role of TCR diversity in better prognostic outcomes for patients undergoing immunotherapy. Future research could expand on this role further, but there is strong evidence suggesting a high baseline TCR diversity is necessary for a strong immunotherapeutic response. Immune repertoire sequencing for each patient thus becomes a very important tool for predicting their clinical outcome to immunotherapy.

Similarly, immune repertoire sequencing has become a useful tool in looking at the efficacy of immunotherapeutic drugs and treatments seeking to boost the immune repertoire. In both of the highlighted studies, researchers saw positive results in subjects when subjected T cell expanding treatments. These results were found using immune repertoire sequencing and demonstrate its potential for monitoring the patient immune repertoire for the entire length of immunotherapy studies. Not only may immune sequencing serve as a prognostic indicator to gain insight into patient responsiveness to treatment, but it may also be used to monitor the efficacy of those treatments, making it a powerful tool in the development of cancer immunotherapies.

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