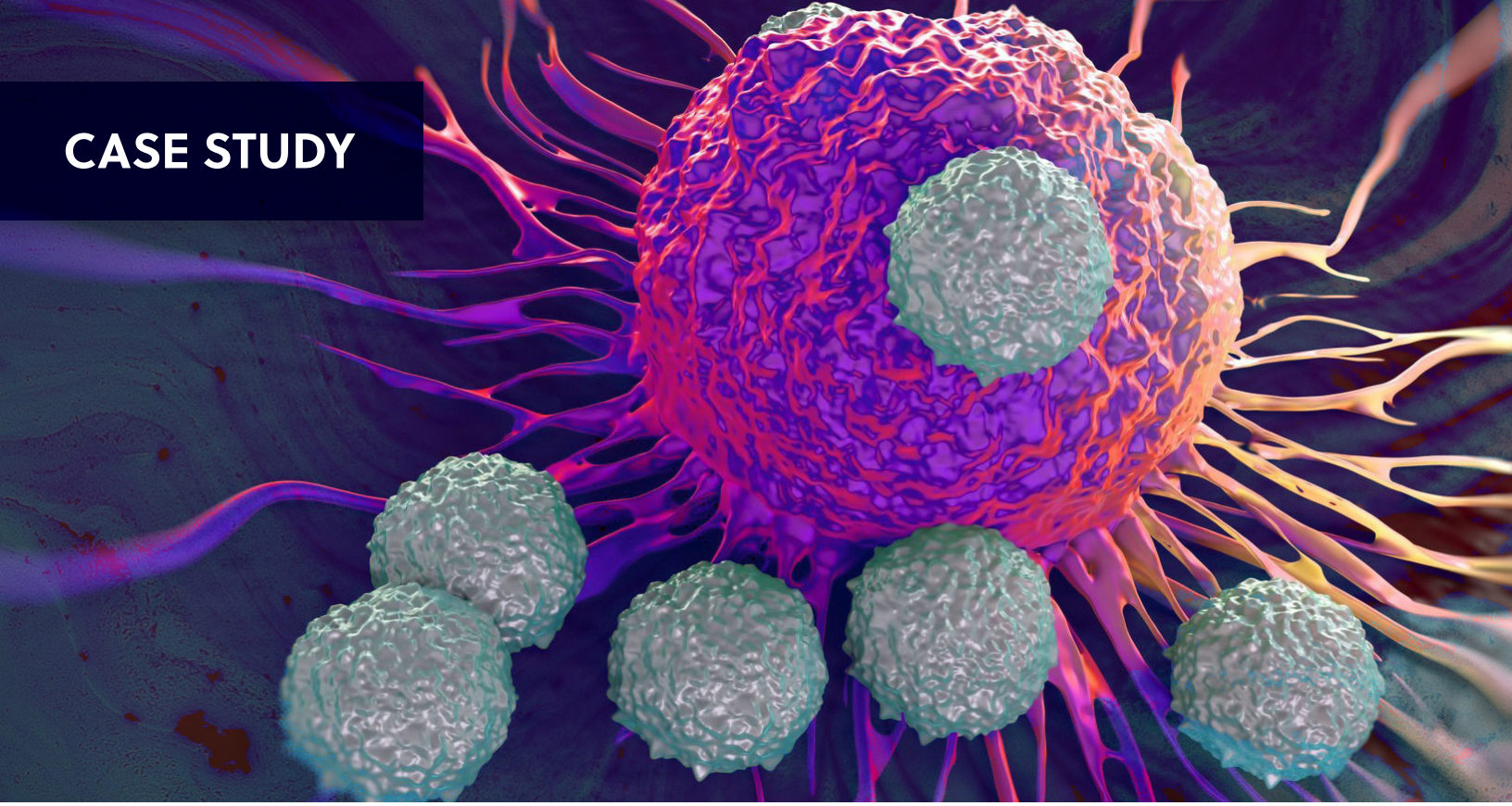


CASE STUDY

Immune Repertoire Sequencing for Pre- and Post-Treatment Monitoring in Cancer

THE IMMUNE REPERTOIRE IS A COMPREHENSIVE INDICATOR OF HEALTH

The adaptive immune system is largely characterized by the prevalence of T cells and B cells, which together constitute the immune repertoire. When T cells or B cells encounter an antigen or disease marker that matches their receptor, a cascade of events is triggered within the immune system to eliminate the disease. Two significant steps include activation and subsequent proliferation of the specific T cells and B cells whose receptors recognize that disease marker. As these particular T cells and B cells clonally expand, the composition and diversity of the immune repertoire shifts over time.

Immune repertoire diversity is a powerful barometer for disease status and response. Under normal circumstances, a highly diverse repertoire is indicative of good health, because it suggests that the immune system is poised to respond to a large number of diseases. An expansion of specific receptors in

response to immune activation can indicate the body is fighting off an existing disease, resulting in a decrease of immune repertoire diversity.

Immune repertoire sequencing is a powerful tool for analyzing the immune repertoire, because it quantitatively and qualitatively measures the diversity of the immune system. This diversity can largely be attributed to the structure of B and T cell receptors, which are composed of multiple polypeptide chains containing a unique "variable region." Sequencing the variable region can thus potentially lead to the identification of clinical indicators of various disease states[1–3].

ANALYZING THE IMMUNE REPERTOIRE FOR DIAGNOSTIC/ PROGNOSTIC PURPOSES IN CANCER

There are several examples in the literature in which immune repertoire diversity was correlated with disease presence or progression in cancer. Recently, researchers at the Chinese Academy of Medical Sciences showed that TCR-gamma CDR3 diversity is significantly reduced in patients with lung cancer carcinoma[4]. Similarly, individuals with renal cell cancer exhibit reduced repertoire diversity compared to normal controls **(see the poster)**. In another example, comparison of immune repertoires from pediatric and adult patients with acute myeloid leukemia showed that clonal expansion of both B and T cells occurs in diseased patients[5]. In the case of nasopharyngeal carcinoma, however, higher immune cell receptor diversity (the absence of specific receptor proliferation) correlates to worse prognosis[6].

Together, findings such as these indicate that immune repertoire characterization has the potential to provide a powerful diagnostic tool in the study of cancer. Immune cell repertoires can be evaluated within the context of particular cancers to better understand how the immune system changes in the case of progression or remission. Ultimately, these characterizations may even help inform clinicians as to the best treatment options.

Cancer treatment is often riddled with complexities. The uncontrolled and destructive cell growth of the disease state is often the result of random mutations within the body. Moreover, mutations continue to occur as the disease progresses, generating heterogeneous tumors in which some tumor cells may be unaffected by the course of treatment.

An amazing feature of the immune system is the ability to recognize new mutations and to generate specific T cells and B cells to target those newly mutated tumor cells. Then, by analyzing the immune response via immune repertoire sequencing, clinical researchers can evaluate changes in tumor growth and determine which treatment options are best suited to different disease pathologies. Indeed, immune repertoire sequencing has been used to monitor and predict treatment response in a variety of different disease and treatment contexts discussed in the proceeding section.

PREDICTING AND MONITORING TREATMENT EFFICACY IN CLINICAL TRIALS VIA IMMUNE REPERTOIRE SEQUENCING

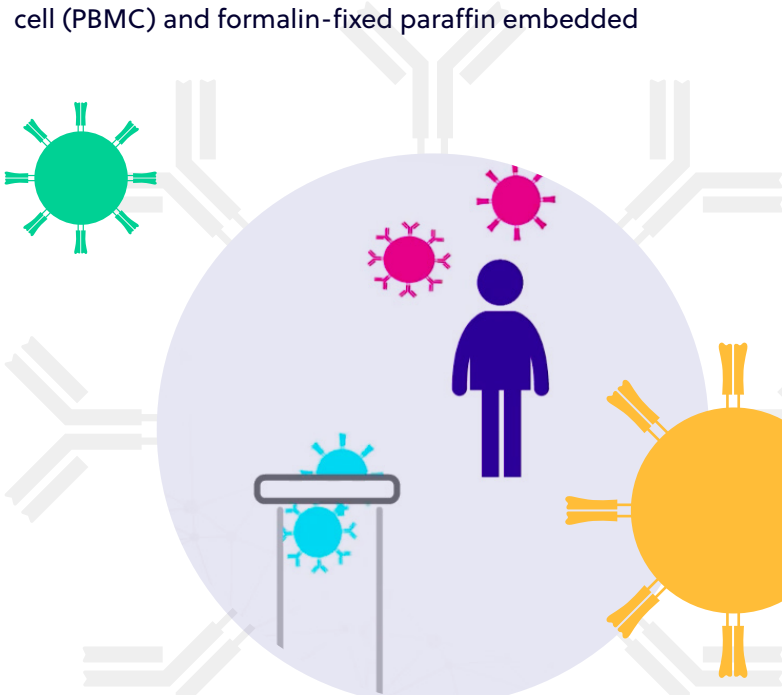
There are several examples in the literature in which immune repertoire sequencing was conducted after cancer treatment and expression patterns were found to be correlated with treatment efficacy. In a study on large cell neuroendocrine lung carcinoma, Christopoulos et al. evaluated patients' TCRs pre- and post-treatment, and patients were tracked over time to measure their long-term outcomes[7]. Patients whose TCRs normalized after treatment had better prognoses. Similarly, a study evaluating patients who were undergoing treatment for colorectal cancer revealed that variations in TCR repertoires normalize for patients in remission, whereas patients experiencing tumor progression continue to have unbalanced TCR expression[8].

Another recent study used TCR repertoire sequencing in concert with serum cytokine levels and lymphocyte subpopulations to measure the immune responses of patients with various different types of cancer (ovarian, pancreatic, gastric, colorectal, cervical, or endometrial) to a combination of therapeutic techniques[9]. Increased TCR diversity was deemed beneficial and correlated with what the investigators describe as "favorable" shifts in cytokines.
A study by Victor et al. on melanoma also revealed that radiation treatment expands the TCR diversity found in intratumoral T cells[10]. When used in concert with other treatments that activate other immune mechanisms in melanoma patients, this increased diversity in the TCR repertoire promotes an immune response to melanoma-based tumors.

In a seemingly contradictory finding, a study on metastatic melanoma treatments revealed that low diversity of TCRs correlated to longer survival in patients treated with PD-1 blockade, but poor response to CTLA4 inhibition[11]. Together, these results seem to suggest that normalization of TCR diversity after treatment is suggestive of positive prognoses, but high versus low TCR diversity in general is not a clear cut predictor of treatment efficacy. Therefore, immune repertoire sequencing both pre- and post-treatment may give the clearest picture of each individual patient's response.

Because treatment efficacy is reflected in immune repertoire signatures, it is theoretically possible to use immune repertoire status prior to treatment to predict response. In the case of the Christopoulos et al. study, patterns in the TCR at diagnosis were predictive of prognosis, treatment response, and long-term survival[7]. Another study on non-small cell lung cancer similarly revealed that patients with a higher TCR diversity showed better treatment response and progression-free survival compared to patients with low TCR diversity[12].

A case study on renal cell cancer employed the novel technique referred to as dimer avoided multiplex PCR **(dam-PCR)** to analyze all seven BCR and TCR loci from patients in a single quantitative multiplex reaction **(see the poster)**. Samples were taken from both peripheral blood mononuclear cell (PBMC) and formalin-fixed paraffin embedded



(FFPE) patient samples, and repertoire analysis was performed on patients before and after treatment with hydroxychloroquine and Aledsleukin (IL-2). The data revealed that positive treatment outcomes were correlated with high TCR alpha- and beta- diversity prior to treatment, as well as B-cell/T-cell expression ratios. Cases such as these, demonstrating correlated positive outcomes, suggest that immune repertoire sequencing can effectively be used to predict (and inform) treatment outcomes.

FUTURE OPPORTUNITIES

Characterization of the immune repertoire has proven informative in analyzing treatment responses and clinical outcomes in patients with a variety of conditions[9]. By examining the immune repertoire of cancer patients, it is possible not only to predict treatment responses and outcomes[7,8,12], but also to monitor the progress of immune cell-related therapies in clinical trials[13,14]. Dam-PCR is a particularly efficacious tool for analyzing immune repertoires in the context of pre- and post-treatment cancer monitoring, as it enables simultaneous analysis of all seven chains of the immune repertoire quantitatively. Ultimately, immune repertoire sequencing approaches, including dam-PCR, can potentially inform a path forward in clinical trials for cancer treatment by indicating how participants are likely to respond pre-treatment and providing a clearer picture of their response post-treatment.

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