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HYPOTHESIS

Harnessing Variability Signatures and Biological Noise May Enhance Immunotherapies' Efficacy and Act as Novel Biomarkers for Diagnosing and Monitoring Immune-Associated Disorders

Tal Sigawi, Adir Israeli, Yaron Ilan

Faculty of Medicine, Hebrew University and Department of Medicine, Hadassah Medical Center, Jerusalem, Israel

Correspondence: Yaron Ilan, Faculty of Medicine, Hebrew University and Department of Medicine, Hadassah Medical Center, Jerusalem, Israel, Email ilan@hadassah.org.il

Abstract: Lack of response to immunotherapies poses a significant challenge in treating immune-mediated disorders and cancers. While the mechanisms associated with poor responsiveness are not well defined and change between and among subjects, the current methods for overcoming the loss of response are insufficient. The Constrained Disorder Principle (CDP) explains biological systems based on their inherent variability, bounded by dynamic boundaries that change in response to internal and external perturbations. Inter and intra-subject variability characterize the immune system, making it difficult to provide a single therapeutic regimen to all patients and even the same patients over time. The dynamicity of the immune variability is also a significant challenge for personalizing immunotherapies. The CDP-based second-generation artificial intelligence system is an outcome-based dynamic platform that incorporates personalized variability signatures into the therapeutic regimen and may provide methods for improving the response and overcoming the loss of response to treatments. The signatures of immune variability may also offer a method for identifying new biomarkers for early diagnosis, monitoring immune-related disorders, and evaluating the response to treatments.

Keywords: immune system, variability, artificial intelligence, immunotherapy

Introduction

There has been a growing interest in immunotherapy in the last decades. Immunotherapy, including immune cell therapy and targeted therapy, is gradually developed through the ongoing discovery of molecular compounds or immune cells.[1] Selecting the best approach or combination of target compounds and immune-cell therapy is challenging for clinical scientists and clinicians. Colorectal cancer (CRC) ranks among the most prevalent malignancies affecting the gastrointestinal tract.[2] The infiltration of CD8+ T cells significantly influences the prognosis and progression of tumor patients.[2]

Passive immunotherapy involves using anti-CD20 and anti-TNF agents, bispecific antibodies with well-defined specificity and subclasses, and antibody-drug conjugates (ADCs). Another approach involves T cells transduced with chimeric antigen receptors (CAR) to expand tumor-infiltrating lymphocytes. In active immunotherapy, patients are given B- or T-cell-based immunity against selected antigens or induced active tolerance against allergens, autoantigens, and alloantigens. Combining both approaches is utilized to release the brakes on T cells that have already responded to antigens through T cell checkpoint control blockers.[3] However, the current response rate to immunotherapies could be more satisfactory. Many patients suffer from primary non-responsiveness, and among those who initially respond, there is a large segment of secondary non-responders.[4–6] The lack of response is associated with the dynamicity of both the host and the disease.

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Introducing variability patterns was proposed to enhance the clinical response to immunomodulatory agents.[7] The current paper aims to introduce the concept of constrained disorder principle (CDP) in light of recent data on the immune system's natural variability. Developing second-generation treatments based on artificial intelligence (AI) is essential to enhance the response to immunotherapies.

The Principle of Constrained Disorder Explains How Complex Systems Operate Within Specific Boundaries

The CDP defines systems based on their inherent variability." It differentiates living organisms by their variability, which is bounded by dynamic boundaries enabling systems to respond to perturbations.[8] The CDP is based on the variability that characterizes biological systems at the gene, cell, and whole organ levels.[9–33] In homeostasis, systems aim at returning to their setpoint post perturbations, and in allostasis, systems change their setpoint in response to triggers. The CDP views noise as inherent to the proper function of biological systems. Perturbations are answered by widening or tightening the noise boundaries in a system. Per the CDP, diseased states are described by a reduction in noise or an increase beyond the borders.[8,33–35]

Variability is Inherent in the Immune Response: Mechanisms of Intra- and Inter-Individual Variability in the Immune System

The immune system exhibits remarkable inter-individual variability as well as intra-individual variability.[36] Evolution maintains diversity within the immune system, which provides a powerful defense against pathogens. Nevertheless, immune-associated diseases may develop based on immune variation.[37,38] The dynamic nature of the immune system is influenced by various internal and external factors, including genetic variations, age, sex, environmental influences, microbiome, and disease states.[36] Many of these variables are inextricably intertwined, making it challenging to assign definitive contributions to them.[37,39,40]

Genetic polymorphism in immune-related genes can lead to diverse immune phenotypes.[37] The heritable contribution to the variability in immune cell frequencies is estimated to be between 20–50%.[37,41–44] As a result of the polymorphic genes that control the immune system and the sensitive environmental sensors that shape it, immunity can be pushed into various functional configurations.[44] One of these configurations is an intrinsic bias toward a particular type of immune response. Most healthy humans can produce type I interferons, type II and type III cells, Th17 cells, type I helper T cells, and inflammasome activation. However, the extent to which individuals are predisposed to a particular functional configuration varies significantly from person to person.[45,46] The interindividual differences continuously expand with age and are somewhat stable and resilient to perturbation.[36,37,47,48]

Genetic variations in human leukocyte antigen (HLA) genes have been linked to varying susceptibility to autoimmune diseases, infectious diseases, and response to immunotherapies.[37] Specific HLA alleles, such as HLA-DRB1 in rheumatoid arthritis (RA) and HLA-DQB1 in type 1 diabetes, are lined with disease susceptibility.[37,49] Interferon α (IFNα) levels are increased in systemic lupus erythematosus (SLE) and their healthy first-degree relatives, implying genetic influences on cytokine expression.[36]

Sexual dimorphism is observed in various aspects of the immune system, leading to inter-individual variability.[42] Overall, women appear to have higher immune cell counts and immunoglobulin levels, although some studies inconsistently demonstrated higher counts of specific immune cells and immunoglobulin subgroups in males.[42,50–52] Sex-specific differences in disease prevalence, clinical outcomes, and response to immunotherapies have been observed, highlighting the importance of considering sex-based differences in immune profiles.[1,2] Clinically, many autoimmune and inflammatory diseases show female predominance, while ankylosing spondylitis is more prevalent in male patients.[36] The response to vaccines is often more intense in women, attributed to the immune-enhancing effects of estrogen and immune-depressing effects of testosterone.[36,37,42,53] Several studies did not show a significant sex-related inter-individual immune variability.[50,54]

The immune system is relatively stable within a healthy individual, as implied by the steady immune cell and protein profiles of blood samples drawn weeks to years apart from healthy subjects.[36,50,54–56] Variations in immune function over

time are an individual feature.[41,57] Individual variations not caused by reported infections are a feature of the immune system, causing more significant shifts in phenotypes from one point to another.[41,50,58] A few cellular subpopulations showed intra-individual variability in consecutive tests of healthy individuals.[55] Healthy older individuals may show enhanced intra-individual immune variability over time.[47,54] This highly variable phenotype may be linked to a diminished flu vaccine response.[54] Also, high intra-individual variability in immune components was associated with a morbid metabolic profile and markers of cardiovascular morbidity and possibly mortality.[41,47]

A year-long study was conducted on healthy adults aged 50 to 65 years. The study monitored 750 plasma proteins and 115 immune cell populations every three months. It helped in understanding the relationships and fluctuations in the blood-immune system. Immune systems are usually stable, but the level of longitudinal variability varies from individual to individual. In the absence of apparent infections, individuals with the most variation exhibited variances in metabolic health markers, suggesting a link between immunologic and metabolic regulation.[41] Cell composition changes radically during early life due to environmental exposures, suggesting a distinctive cell composition developed during this critical period.[59–61]

Neutrophils are more abundant in the circulation of older individuals but exhibit impaired migration and phagocytosis.[54,62] Cytotoxic NK cell count rises with age, with diminished cytotoxic capabilities.[54,62] Self vs non-self antigen recognition disruptions due to altered function and amount of the antigen-presenting dendritic cells are also noted.[62] Involving the thymus leads to a decrease in naïve T cells, a reduction in peripheral T cell diversity, and an increase in memory and regulatory T cells.[42,43,54,55,62] Lymph nodes, splenic degeneration, and bone marrow dysfunction may also interrupt the homeostasis of lymphocyte populations and the humoral response of older individuals.[42,50,54,62] These changes, recognized as immunosenescence, increase susceptibility to infections, cancer, and inflammatory conditions and decrease vaccine responsiveness in older adults.[36,37,43,53,62,63] Aging is linked to low-grade inflammatory phenotype (CLIP), inflammaging.[62] It is characterized by elevated levels of inflammatory cytokines such as IL-6, IL-18, tumor necrosis factor (TNF), and CRP.[36,50,52,62] This chronic inflammation disrupts immune homeostasis, impairs immune cell function, and underlies the development of age-related diseases.[52] Age is also associated with inter-individual immune variability, as younger individuals show more inter-individual similarity of immunological traits than the diverse phenotypes of older adults.[37,41,54,63] Monozygotic twin pairs show higher inter-individual variability with older age, attributed to non-heritable factors.[43] This age-related variability is attributed to an increased cumulative environmental exposure and to direct age effects.[37,41]

Non-heritable factors are the dominant source of immune variability.[37,41–44] These factors occur throughout an individual's life course, thus contributing to intra-individual variability on various time scales. Additionally, they constitute a distinct input reflecting an individual's lifestyle, consequently influencing inter-individual variability.

Several studies demonstrated the seasonal variability of immune cell frequencies and plasma protein concentrations,[42,51,52] while others showed no seasonal effects.[41] Clinically, the incidence of type-1 diabetes mellitus is higher during winter;[34] allergies and viral infections show seasonal variations;[37,48] RA symptoms exhibit seasonal variations, as well as a circadian pattern with morning symptoms, correlated to a spike in serum IL-6 levels.[36]

Variations in microbiota composition can contribute to immune system variability and influence susceptibility to immune-related disorders and vaccine response.[36] The imbalance in the microbial community, bacterial dysbiosis, is typically linked to reduced microbial diversity and is related to various immune disorders such as inflammatory bowel disease(IBD) and asthma.[36] Diet can alter immune system modulation. Deficiencies or nutrient imbalances can compromise immune responses and increase susceptibility to infections.[37] Nonetheless, the impact of normal dietary variations on the immune response is uncertain.[37] Dietary alterations may also exert their effects indirectly through modifications in the microbiome or body weight.[37,52] Nutritional supplementations may influence immune response, as was demonstrated in measles-vaccinated iron-deficient children treated with iron.[37] Salt-rich diets are associated with increased autoimmunity.[37] Obesity has been linked to increased inflammatory markers such as IL-6 and altered NK cell count and function, attributed to the lipid-rich environment.[37,50] Long-term exposure to pollutants may lead to chronic inflammation, immune dysregulation, and increased susceptibility to immune-related disorders.[36,37]

Cigarette smoking increases leukocyte counts, reduces total immunoglobulin levels, and increases autoantibody titers.[36,37] Exposure to various infectious agents can shape the immune system. CMV-seropositivity contributed to inter-individual variability significantly.[64,65] Monozygotic twins with discordant CMV seropositivity showed higher inter-individual variability in many immune parameters than concordant seropositive or seronegative twin pairs.[44] Certain medications, vaccines, and immune therapies can affect the immune system, leading to variability within and between individuals.[36]

The data suggest that internal and external factors affect the immune system's inter and intra-individual variabilities. This variability emphasizes the need for personalized medicine to address immune-mediated pathologies effectively. Furthermore, fluctuating immune function may indicate the aging process and may be associated with mortality, suggesting the potential clinical relevance of its monitoring.[66] The design of variability-based digital twins is being developed to improve the accuracy of diagnosis and monitoring.[67]

Variability is Essential to the Proper Function of the Immune System

The immune system's primary purpose is to protect the host from the uncountable diversity of constantly changing and evolving pathogens. Therefore, dynamicity, plasticity, and variability are required characteristics of the immune system.[64] However, variability is not confined solely to the whole system or organism level. Instead, the immune cell itself can be perceived as a complex system that is subjected to constantly changing various inputs (eg, cytokines, chemokines, medications, toxins, and pollutants) aiming to generate a specific output or behavior (cytokine production, receptor expression, migration, and differentiation). Intercellular variability is a characteristic of all major immune cells.[65,68,69] Characterization of single-cell "omics", quantifying intra-individual intercellular variability, even among a well-defined, allegedly homogenous cellular subset, unravel previously concealed variability.[70] The high-resolution single-cell analysis identified intra-individual daily variability of immune cellular composition among healthy individuals, challenging the previous dogma of intra-individual stability over time.[68,71]

The immunological inputs fluctuate, manifesting noise and stochasticity.[68] Noise is a variation in a specific input signal that does not induce a different output by itself.[68] However, accumulating noisy signals may alter the cellular output, demonstrating noisy properties.[68] Stochasticity is another expression of randomness in cellular behavior that regards the choice of a distinct cellular outcome from a restricted repertoire of possibilities in response to a fixed input.[68] Noise and stochasticity are evident at the level of intracellular molecules, various cellular processes, and intercellular interplay and account for intercellular variability.[70]

Intracellular molecular stochasticity mechanisms are fundamental in B and T cells.[65,72] Through V(D)J recombination, these cells produce diverse B cell receptor (BCR) and T cell receptor (TCR) chains, allowing recognition of a wide range of antigen-major histocompatibility complex (MHC) combinations.[65,72] Epigenetic modifications generate variability in the single-cell level phenotypes and show stochastic properties.[65] Inter-cellular heterogeneity in interleukins and interferons mRNA expression in response to pathogens or vaccines was observed, stemming from stochasticity and probabilistic events in gene expression processes.[64,65] Protein level variation was also noted for intracellular signaling molecules and surface receptors, contributing to inter-cellular diversity.[65,70] Multiple intracellular stochastic events may determine the differentiation trajectory of a single immune cell.[64,65] Cellular variability creates heterogeneity within a single immune cell type population that enables a gradual activation of the immune response.[64,65]

For an effective threat-appropriate and dose-dependent response, the immune response necessitates the maintenance of a varied array of responsive cell states.[68] Variations in protective antibody production following vaccination have been linked to high plasmablast activity within a week of vaccination.[73] CD38+ B cell subsets are a strong predictor across many cohorts and studies, accompanied by specific gene-expression signatures.[74] Different activations of the interferon pathway have consistently been linked to increased antibody production in subsequent immune responses, as demonstrated across various vaccines.[75] Positive innate responses at baseline, mediated primarily by plasmacytoid dendritic cells, are also possible.[74] It is intriguing to note that a similar interferon signature predicts clinical flare-ups of SLE, indicating a common immunological variation depending on the context of activation.[37,76]

Elevated intercellular phenotypic variability can serve as a biomarker of aging and a quantitative trait, facilitating individual comparisons and intra-individual surveillance.[70] Uncontrolled cellular variability can detrimentally impact tissue function, notably in cancer development and autoreactivity.[64,65] With age, there is an increase in transcriptional variability, which has been demonstrated to disrupt synchronized immune responses.[77] Single-cell examination has been used in studying immune disorders, such as RA,[78,79] SLE,[79,80] multiple sclerosis,[81] IBD,[82] asthma,[83] and cancers.[84,85] Single-cell proteomics showed that alterations in macrophage prevalence have predictive value for RA treatment effects.[69] These methods revealed new immune cellular subtypes, advanced understanding of disease pathogenesis and clinical diversity, and potential individual therapeutic targets. Single-cell-based research established the boundaries between normal and pathological cellular heterogeneity of the ongoingly recognized immune cells, focusing on inter-individual variability.[51] Intra-individual variability analysis utilizing these novel markers is a potential tool for monitoring disease progression and treatment response.[86,87]

Per the CDP, the variability kept within dynamic boundaries is essential for the proper function of systems.[35] Variability characterizes biological systems and can be seen at the level of the DNA,[9] tissues,[12,13] and whole organs, including heart rate,[10,11] blood pressure,[14] respiratory,[15] gait,[16] and brain functions.[17–19] In several of these systems, it was proposed that they be associated with improved function by providing biological systems with an ability to adapt to perturbations in their continuously changing environments.[9,36,88]

Similarly, variation can confer unique advantages on the immune system.[37,89] As a protective mechanism, evolution has been selected for immune diversity rather than a homogeneous state of infection resistance.[90,91] Possessing an immune system wired differently from the previous host can provide an evolutionary advantage when pathogens can quickly specialize in taking advantage of a fixed niche.[34,37,92] Therefore, immune variation during immune responses is an essential mechanism mandated for effectiveness.[34,93] Both antibodies' structure, with a variable part of their structure, the multi types of cells cellular immunity, and cytokines panel, which are involved in all types of responses, are examples of the inherent variability that enables a relatively rapid response to perturbations.[29,94,95]

Thus, the variability is altered under changing host and environmental conditions, allowing the immune response to better deal with foreign antigens. Per the CDP, immune-associated diseases may evolve from a lack of variability or form a higher degree of variability.[8] It is possible to develop treatments targeting these mechanisms to modulate the immune response by understanding how individuals' immune systems differ, improving the immune response to vaccines, pathogens, and tumors, or alleviating immune-mediated disorders.[96,97] By optimizing modifiable environmental conditions, it may be possible to enhance the long-term immunological health of all populations by gaining a better understanding of how and when the immune system achieves a stable state.[36,98,99]

The Challenge to Achieve a Sustained Effect of Immune-Mediated Therapies: Primary and Secondary Non-Responsiveness: Time Dimension in Disease Pathogenesis and Host Response

Lack of response to immunotherapies poses a significant challenge in treating immune-mediated disorders and cancer.[100,101] Patients may not respond to initial therapy, primary non-response (PNR), or may lose response over time after initial improvement, with secondary loss of response (SLR).[102]

Tolerance against immunotherapies can result from forming anti-drug antibodies (ADA). Using drugs with high immunogenicity can generate ADA in up to 70% of the patients, depending on the drug.[103] ADAs can be generated by T-cell-dependent or independent B-cell activation pathways. ADAs are produced in the T-cell-dependent pathway when a T helper cell (Th) differentiates into a Th1 or Th2 phenotype, ultimately creating plasma cells that secrete ADAs. It is clinically essential as infliximab-specific T-lymphocytes have been detected in the serum of subjects who received the drug, and there has been a correlation to ADA formation in those patients.[104] Recent research examined risk factors for ADA formation against Infliximab. Smoking and RA encouraged ADA creation, whereas higher infliximab doses and higher serum infliximab concentrations reduced the risk of immunogenicity.[105]

PNR occurs in 10–40% of IBD patients, and it is usually declared after 12–14 weeks of therapy.[102] Switching treatments between anti-TNF formulations can be effective in over 50% of PNR patients, suggesting that it is not entirely a class-effect phenomenon.[106] SLR may result in treatment intensification or discontinuation in up to 50% of patients after 12 months. It may exacerbate symptoms of active IBD during maintenance therapy in a patient with controlled disease following induction treatment.[107] A confirmation of SLR usually requires the recurrence of symptoms mediated by the inflammatory disease exacerbation of.[7,108] The recent PANTS study tested 1610 active Crohn's disease subjects receiving adalimumab or Infliximab to predict clinical factors leading to PNR and SLR. PNR at 14 weeks of treatment occurred in 23%. Non-remission after 54 weeks occurred in 63% of patients. In multivariable analysis, low drug levels at week 14 were an independent factor related to PNR and SLR. Low drug levels were also a predictive value for the immunogenicity of both drugs. Obesity, smoking, low albumin concentrations, higher baseline disease activity markers, and immunogenicity development were associated with low drug concentrations, which mediated non-remission.[109]

Among patients with rheumatoid arthritis, anti-TNF drugs are the first-choice treatment for patients who fail to respond to methotrexate therapy.[110] Nonetheless, it is estimated that only 60% of subjects will attain a long-term response to these agents, while 30% of patients do not have an adequate primary response.[7] In the DREAM registry, 6% of patients achieved remission according to the European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) criteria. In comparison, 27% achieved a Disease Activity Score (DAS28) of less than 2.6, a less strict measure.[111] The heterogeneity of patients with RA makes it challenging to predict who benefits from anti-TNF treatment. A recent study tried to predict response before treatment with anti-TNF, using machine learning models to assess DNA methylation and gene expression profiling in peripheral mononuclear cells. An accuracy of 79–88% in predicting response, based on the EULAR criteria for disease response, was reported.[112] Unlike PLR, there is a lack of consensus on the definition of SLR in RA. SLR can be considered when there is an increase in EULAR response or DAS28>0.6 during the last six months.[7,113] The average survival on anti-TNF in subjects with RA is estimated to be 47 months.[114]

Using immune-mediated therapy for cancer treatment also encounters challenges in achieving long-term effects. The reasons for treatment suspension vary from side effects to NPR and SLR. Despite performing a preliminary selection of patients who are candidates for Immune checkpoint inhibitor (ICI) treatment according to histo-pathology characteristics of the tumor tissue, only 13% of subjects are responsive.[115–117] Prediction of ICI response is a complicated and evolving endeavor. It includes biomarkers such as intensity of PD-1 expression, tumor mutational burden, and analyzing signaling pathways in tumors as Interferon-γ (IFN-γ). More recent attempts for prediction include microbiome analysis using machine learning.[118] One of the latest attempts is to examine the effects of epigenetics on NPR. Several studies have demonstrated that mutations in genes associated with the SWI-SNF chromatin remodeling complex can increase the sensitivity of human tumors to ICIs.

Additionally, treatment with an epigenetic modulator enhances the ICI-mediated anti-tumor effect.[115,118] Resistance to ICIs is not fully understood due to the complexity of the immune response and its reliance on the host. While the response to ICIs varies depending on the specific disease, approximately 70% of patients are considered non-responders or experience disease progression after initially responding to these treatments.[119]

One suspected significant factor in resistance to ICI among cancer patients is the evolution or selection of tumors acquiring mutations in crucial pathways involved in the checkpoint blockade response.[115] A tumor microenvironment (TME), which comprises factors extrinsic to cancer cells, can also prevent the ICI effect. It was shown that the TME can obtain Immune-suppressive cells and inhibitory cytokines, thus undermining the ICI effect.[120,121] What was mentioned is only a fraction of how an SLR can occur in cancer patients, adding more challenges to finding an effective bypass to regain response.

Current Methods for Overcoming Immunotherapy Non-Responsiveness

Overcoming immunotherapy's non-responsiveness is a significant challenge, considering the heterogeneity of the disease and multiple mechanisms of PNR and SLR. In cancer, non-response can occur through TME, creating a non-favorable condition around the tumor and preventing the drug effect. There are various strategies to overcome the

TME effect. In triple-negative breast cancer, suppressing assays targeting annexin A1 (ANXA1) in mice in vivo experiment led to Treg function impairment and helped reduce the tumor size.[122] Hypoxia also plays an essential role in TME deregulation and resistance to therapy. Recent research on HER2+ breast cancer in a pre-clinical model demonstrated that interfering with AXL tyrosine kinase receptor reduces It was also found that a pharmacological combination of inhibition of Axl, with anti–PD–1, assisted in reducing the size of the tumor and the spread of cancer.[123] It is now essential to use additional drugs and biochemical agents to help CAR-T cells. It implies that compounds that affect different signaling pathways are given along with CAR-T cells. For example, Olaparib, a PARP inhibitor, significantly improved the activity of EGFR-specific CAR-T cells in a breast cancer model.[124] Combining drugs is also an essential way to improve response to ICIs. Cytotoxic drugs are a pillar in treating advanced cancer and metastases. Essential cytotoxic drugs such as carboplatin, cisplatin, fluorouracil, and oxaliplatin can upregulate PD-L1 in malignant cells by generating danger signals.[125]

They enhance anti-tumor immunity via cytotoxic T cell activation, antigen-presenting cells maturing, immunosuppressive regulatory T cell depletion, and myeloid-derived suppressor cell expansion.[126] Recent attempts to overcome ICI resistance include a gut microbiome therapeutic strategy. Several studies have demonstrated that microbial metabolites regulate anti-tumor immunity.[127,128] Short-chain fatty acids (SCFAs) are produced when gut microbes ferment dietary fiber. These acids interact directly with CD8+ T cells, boosting their ability to fight tumors. Clinical studies have revealed that solid cancer patients treated with nivolumab had higher levels of fecal SCFAs if they responded positively to the treatment, suggesting a longer time without the cancer progressing.[129] The benefits of SCFAs-producing microbiota can become another option for improving the response rate to ICI treatments.

Immune-mediated therapies, especially anti-TNF, are essential for many immunological diseases such as RA. However, as mentioned, 30–40% of patients cease the treatment due to PNR or SLR. Nonetheless, there are still no defined rules which indicate what to do in case of a treatment failure. European League Against Rheumatism (EULAR) recommends that a different biological disease-modifying anti-rheumatic drug (bDMARD) and anti-TNF be used instead in case of failure. Results of randomized controlled trials and observational data indicate that switching to a bDMARD with a different mechanism of action increases the likelihood of clinically significant improvements and improves drug retention rates.[130] The GO-AFTER study discovered that golimumab reduced the symptoms of active RA and improved physical function in patients previously treated with TNFα inhibitors.[131] An NJM study found that using abatacept in subjects with insufficient response to anti-TNF was clinically effective and had an acceptable safety profile.[132] A recent study compared the effectiveness of second-line therapies for rheumatoid arthritis (RA), precisely anti-TNF drugs, and other biologics (abatacept, BDMA, rituximab, tocilizumab). The study found that BDMA showed greater sustainability as a second-line treatment than TNFi, especially in seropositive RA patients. However, seronegative patients did not experience the same drug survival advantage with BDMA. The study also highlighted that the failure and adverse effects of TNFi could be attributed to the development of anti-TNF antibodies, leading to a higher rate of treatment withdrawal.

Nonetheless, either BDMA or TNF can be chosen in seronegative and seropositive RA subjects exposed to anti-TNF or if the duration of TNFi was shorter than 2 years.[133] This study is consistent with a previous study that determined the effectiveness of using a second-line anti-TNF drug compared with a non-TNF group. A non-TNF biologic agent achieved non-statistically better results than a second-line anti-TNF agent at 24 weeks.[134]

In IBD, dose intensification (DI) is often necessary due to loss of response. It is typically used in the context of SLR in case of low therapeutic drug levels and low ADA levels.[7] In a meta-analysis, the drug intervention requirement rate was 28% in treatment-naïve patients and 39% in non-naïve patients. The short-term response to the empirically prescribed drug intervention was 63% and 58% in naïve and non-naïve subjects, respectively.

Additionally, no differences were found in comparing UC vs CD, the use of anti-TNF drugs, or even between intensification regimens.[135] Another option is switching between anti-TNF drugs with success rates of about 50%. Shifting from adalimumab to Infliximab was beneficial in subjects with LOR and untraceable adalimumab levels, and half of them mandated DI after six months and 75% after 12 months of infliximab therapy. At 12 months, 81% still were

on infliximab.[136] Besides a change of anti-TNF, there is a question of whether adding an immunosuppressive drug will increase response. A study compared switching to a new anti-TNF in IBD subjects after experiencing LOR versus switching to a new anti-TNF treatment with the addition of Azathioprine. The study found that combining Azathioprine with the new anti-TNF treatment was more effective.[137]

A Second-Generation AI Platform Based on the CDP Can Overcome Drug Tolerance

Immune variation during health and disease must be considered when developing methods to overcome the loss of responsiveness to immunotherapies.[36,37,138] In precision medicine or personalized therapy, patients are treated differently based on their disease mechanisms and specific treatment needs. It is possible to achieve better outcomes by determining the requirements for each patient.[139] Based on the CDP, the second-generation AI systems are designed to incorporate personalized variability signatures into treatment regimens.[140–142] These systems quantify inherent variabilities using biological sensors (eg, heart rate, blood pressure, and respiratory variabilities) or test variabilities (eg, cytokines secretion and subsets of inflammatory cells variabilities) and implement them into therapeutic regimens. This method may overcome the loss of response to chronic treatments.[7,66,143–160]

Implementing variability signatures in treating patients with congestive heart failure who have developed resistance to diuretics has successfully overcome the resistance. Treated patients showed improved clinical and laboratory parameters, significantly reducing hospitalizations and emergency room admissions.[161] Similar beneficial effects were described for patients suffering from chronic pain who developed tolerance to painkillers and those with multiple sclerosis receiving immunotherapy.[35] This platform improves the response to Lenvatinib in head and neck cancer patients.[162] Long-term studies are required to ensure the sustainability of these effects.

The data shows potential for addressing medication response challenges through personalized variability signatures in treatment plans. Personalization of the treatment plans needs to be dynamic as both the host response and the disease pathogenesis keep changing. It mandates using algorithms, which can alter the variability in a way that deals with changing internal and external environments.[34,140–142,163]

Changes in Immune Variations as Early Signs for Disease and Monitoring: The Development of Variability-Based Biomarkers for Immune-Associated Diseases

Currently used laboratory tests and clinical scores are insufficient for early diagnosis and monitoring of the response to therapies in subjects with immune-associated diseases. The intra- and intersubject variability of the immune systems creates a significant challenge in identifying early diagnostic and valid monitoring biomarkers.

The lack of proper early diagnostic tools impacts the prognosis of many immunological diseases as it is associated with the late start of therapies.[164–167] Similarly, the lack of valid biomarkers provides a challenge for personalizing immunotherapies when faced with a dynamic immune system that alters the pathogenesis of the disease over time within and among patients treated with immunotherapies.[168] The current biomarkers are insufficient to select the ideal immunotherapy from the available immunotherapies. Moreover, as the host and the disease change, a dynamic system must provide time-dependent personalizing immunotherapies.[169,170]

The CDP-based early signs system may provide a variability-based method for the early diagnosis of immune-mediated disorders. Variances in the immune system, if too low or high, can predict disease. Alerts in variability levels can also monitor disease progression and therapy response. Both autoimmune diseases and cancers have a time factor in their pathogenesis and response to immunotherapies, which evolve from host and disease alterations. Therefore, immune-variability signatures are anticipated to offer a more accurate way to follow up on these patients and determine their treatment response.[67]

Long-term studies using CDP-based AI systems are required to show their use in monitoring the improvement of outcomes.

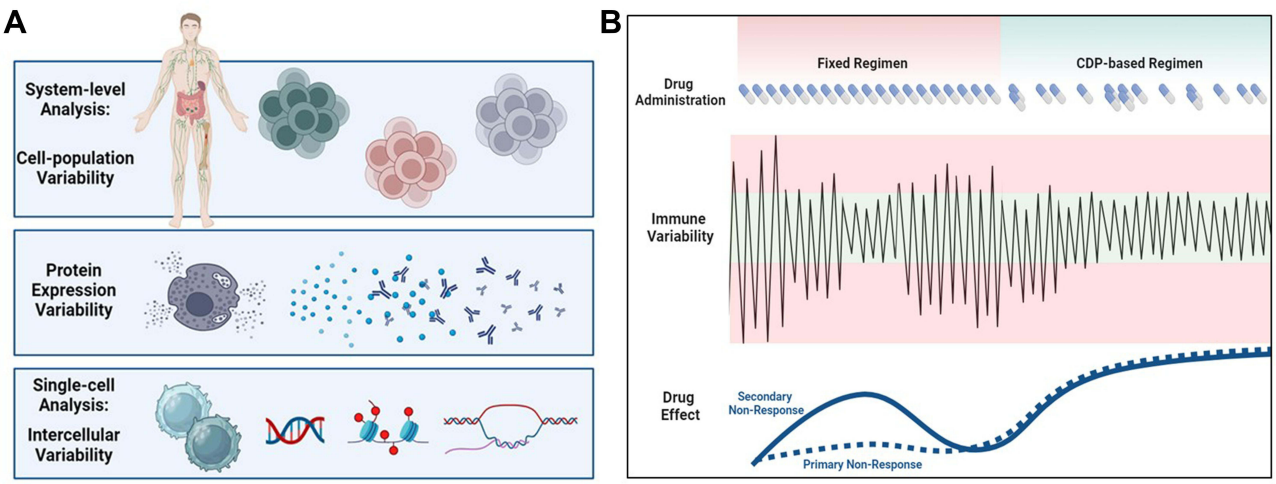

Figure 1 (**A**) Schematic presentation of the hypothesis of how changes in variability signatures can serve as a method for early signs of disease. (**B**) Schematic presentation of the hypothesized CDP-based treatment plan based on changing the degree of variability. The changes in the variability signatures may enable the selection of immunotherapies and change them over time while serving as biomarkers for disease progression, response to therapies, and prognosis.

Using CDP-Based Platforms for Improving the Response to Immune-Based Treatments

The second-generation AI system based on CDP is designed to address the lack of response to immunotherapies and the loss of response. Figure 1 shows a schematic presentation of the changes in variability signatures that can serve as a method for early signs of disease (A) and an schematic presentation of a CDP-based treatment plan based on changing the degree of variability (B). The changes in the variability signatures may enable the selection of immunotherapies and change them over time while serving as biomarkers for disease progression, response to therapies, and prognosis. Box 1 shows several potential biomarkers that can serve as biomarkers.

The platform is being developed in three stages. Initially, a pseudorandom number generator picks the administration time and dose from a pre-defined dosing scheme. This open-loop system operates independently of the outcome and is not personalized. For using anti-TNF Adalimumab in colitis or arthritis, the algorithm can select between 40 and 80mg, and a range between ten and sixteen days between administrations, rather than a fixed 80mg every fourteen days, is proposed to improve the response. In the second stage, a closed-loop system is implemented. Clinical and laboratory endpoints provide feedback to select dosing and administration times dynamically. In the third step, the quantification of biological signatures of variability is implemented into the algorithm to improve the response further.[140–142]

Future studies are designed to evaluate the overarching framework and assess the necessity of potential mergers to improve the clinical outcomes of using this method.

Box 1 Potential variability signatures as novel biomarkers

These can be measured periodically (e.g., daily or monthly).

a. Alterations in cytokine secretion: pro and anti-inflammatory patterns.
b. Alterations in B cells and immunoglobulin secretion.
c. Alterations in subsets of innate and adaptive T cells.
d. Functional immune assay for B cells and T cells.
e. Immune-related protein expression and secretion.
f. Apoptosis and necrosis biomarkers.
g. Inflammatory serum biomarkers include acute phase reactants (C reactive protein, ferritin, sedimentation rate).

Summary

The immune system's intra and inter-subject variability is a significant challenge for immunotherapies and current diagnostic and monitoring modalities. The CDP-based second-generation AI system may offer a new platform to address these challenges. Ongoing studies determine the ability to implement these modalities into clinical practice to improve the diagnosis, monitoring, and responsiveness to therapies.

Abbreviations

CDP, constrained disorder principle; ADCs, antibody-drug conjugates; HLA, human leukocyte antigen; TNF, tumor necrosis factor; RA, rheumatoid arthritis; PNR, primary non-response; SLR, secondary loss of response; LOR, loss of response; ADA, anti-drugs antibodies; IBD, inflammatory bowel disease; ICI, immune checkpoint inhibitor; EULAR, European League Against Rheumatism; bDMARD, biologic disease-modifying anti-rheumatic drugs; DI, dose intensification.

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