# CHAPTER 6 / BÖLÜM 6

# IMMUNOMIC APPLICATIONS IN MEDICINE

# TIPTA İMMÜNOMİK UYGULAMALAR

**Demet KIVANÇ İZGİ*,**, Fatma SAVRAN OĞUZ***,****

*Research Assistant, Istanbul University Faculty of Medicine, Department of Medical Biology, Istanbul, Türkiye
**Istanbul University, Institute of Health Sciences, Department of Medical Biology, Istanbul, Türkiye
E-mail: demet.kivanc@istanbul.edu.tr

***Prof. Dr. Istanbul University Faculty of Medicine, Department of Medical Biology, Istanbul, Türkiye
****Prof. Dr. Istanbul University, Blood and Bone Marrow Donor Registry of Istanbul Medical Faculty, Istanbul, Türkiye
E-mail: oguzsf@istanbul.edu.tr

DOI: 10.26650/B/ET07.2023.005.06

**ABSTRACT**

Immune system is a complex system that includes many organs, special tissues, cells and a network of molecules. External factors such as viruses, bacteria, fungi or parasites may cause infection and disease in the organism in addition, exposure to foreign chemicals can cause toxic effects and pathogenic mutations. The main function of the immune system is to protect the organism from external and internal hazards and to provide the interface between the organism and its environment. The availability of high-throughput genomics, proteomics, and other "omics" methodologies, as well as accessibility to molecular databases, are forcing a significant shift in research and development strategies for biomedicine. The main sources of immunological data are public databases, various 'omics' data and published articles. In this context, genomics and proteomics have provided tremendous contribution and encouragement to biological sciences with the new biological data they contain. Currently, systems biology, the systematic study of complex interactions in biological systems, is closely related to the application and development of bioinformatics and biostatistics tools to genomic and proteomic data. Immunology databases provide data storage, extraction and analysis of immunological data. Standard bioinformatics tools such as sequence analysis and structural methods are routinely applied in immunological studies. However, due to the complexity of immune interactions, immunoinformatics methods and traditional research methods are largely limited to increase the efficiency of immunology research. In this context, immunomics can become a new and strong approach primarily applied in vaccine development, target identification and disease diagnosis. Use of immunonomics and T cell mapping in transplantation includes the discovery of self-immunogenic markers based on HLA immunogenicity, their association with graft antigens leading to rejection, and scoring for probability of rejection.

**Keywords:** Immunomic, epitope, transplantation, vaccination

**ÖZ**

Bağışıklık sistemi, pek çok organ, özel dokular, hücreler ve moleküller ağını içeren komplike bir sistemdir. Organizmada virüsler, bakteriler, mantarlar veya parazitler gibi dış etkenler enfeksiyon ve hastalığa neden olabilir, bunun yanı sıra yabancı kimyasallara maruz kalmak toksik etkilere ve patojenik mutasyonlara neden olabilir. Bağışıklık sisteminin ana işlevi organizmayı dış ve iç tehlikelerden korumak ve organizma ile çevresi arasındaki arayüzü sağlamaktır. Günümüzde immünolojik çalışmalar ve dolayısıyla immünololojik veriler büyük bir hızla artış göstermektedir. Moleküler veritabanlarına erişilebilirliğin yanı sıra yüksek verimli genomik, proteomik ve diğer "omik" metodolojilerin mevcudiyeti, biyotıp için araştırma ve geliştirme stratejilerinde önemli bir değişimi zorlamaktadır. İmmünolojik verilerin ana kaynakları, kamuya açık veri tabanları, çeşitli 'omik' veriler ve yayınlanmış makalelerdir. Bu bağlamda genomik ve proteomik, içerdikleri yeni biyolojik veriler ile biyolojik bilimlere muazzam bir katkı ve teşvik sağlamıştır. Biyolojik sistemlerdeki karmaşık etkileşimlerin sistematik bir çalışması olan sistem biyolojisi, şu anda biyoinformatik ve biyoistatistik araçlarının genomik ve proteomik verilere uygulanması ve geliştirilmesi ile yakından ilgilidir. İmmünoloji veri tabanları, immünolojik verilerin veri depolamasını, çıkarılmasını ve analizini sağlar. Dizi analizi ve yapısal yöntemler gibi standart biyoinformatik araçlar, immünolojik çalışmalar için rutin olarak uygulanır. Fakat immün etkileşimlerin komplike olması nedeniyle, immünoloji araştırmalarının verimliliğini arttırmak için immünoinformatik yöntemler ve geleneksel araştırma yöntemleri büyük ölçüde sınırlı kalmaktadır. Bu bağlamda immünomikler öncelikle aşı geliştirme, hedef belirleme ve hastalık teşhisinde uygulanan yeni ve güçlü bir yaklaşım haline gelebilmektedir. İmmünomik ve T hücre haritalamasının transplantasyonda kullanımı; HLA immünojenitesine dayalı olarak self immünojenik belirleyicilerin keşfi, bunların rejeksiyona yol açan greft antijenleri ile ilişkilendirilmesi ve rejeksiyon olasılığı için skorlamayı içermektedir.

**Anahtar Kelimeler:** İmmunomik, epitop, transplantasyon, aşı

## 1. Immunoinformatics

A host's immune system responds to pathogen invasion with a series of pathogen-specific responses involving many immune system elements such as antibodies, T cells, B cells, and antigen presenting cells. Antigen presenting cells (APC) have the ability to internalize pathogen-associated molecular patterns and initiate T-cell response by presenting these antigens to large histocompatibility complexes (MHC). Not all pathogenic peptide sequences are required for the initiation of an immune response. Only the specificpeptide sequences, called epitopes, from pathogen-specific antigens are sufficient to stimulate T and B cell responses. In this context, the antigenicity of a pathogen is defined by its epitopes and can be demonstrated by comparing genome sequences using immunoinformatic tools. Immunoinformatics is a field that connects computer science with experimental immunology and uses computational methods and resources to understand immunological information. Although it is mainly based on the interpretation of immunological results obtained in the laboratory using computational methods, informatics has also been associated with many immunological subjects, from disease prevention and diagnosis to drug discovery (Tomar & De, 2014).

Immunoinformatics also uses multiple immuno-biotechnological processes and predictive tools for immunomics to produce a variety of viable vaccines, kits, and biological products for the treatment of infectious diseases, allergies, and cancers. Identification of toxic compounds, facilitating tissue transplantations and large tissue compatibility complex (MHC) genotyping can be achieved more easily with the use of bioinformatics tools. In addition, bioinformatics provides better exploration of the functions and interactions of toll-like receptors (TLRs) (Hegde et al, 2018).

Immunoinformatic tools are also used in vaccine development. Vaccines are one of the best strategies for controlling infectious diseases. Classical ways of developing vaccines consist of mass-producing pathogens, inactivating them by physical or chemical means, purifying and formulating the antigen(Oli et al, 2020).

## 2. Immunomic

The term 'immunomic' was first coined by the researcher J. Klysik in 2001 and examines all immune-related molecules with their targets and functions, and examines the immune system's response and regulation process on pathogens (Kylsik, 2001). Currently, the term immunomics refers to the integration of molecular immunology, genomics, proteomics, transcriptomics and bioinformatics, effectively providing the needed link between these fields and providing an effective correlation between immunological research and clinical practice

(Doolan, 2011). Immunomics combines the understanding of molecular biology and cellular immune function with the application of laboratory tools to provide a detailed analysis of the immune system and its components of health and disease.

'Immunomics' ability to explore and manipulate the cellular microenvironment of the immune system makes it useful not only at the desk, but also at the bedside in the clinic (Tremoulet & Albani, 2005). The development of immunomics and its future clinical applications can be best understood by exploring the evolution of its predecessors, genomics and proteomics. Originally, genomics was defined as the study of the organization of all genes (McKusick & Ruddle, 1987). The meaning of genomics changed from the study of DNA structure to the study of gene function with the emergence of the draft sequence of the human genome in 2001 (McKusick, 1997; Baltimore, 2001).This has been further enhanced by the development of microarray technology, where the identification of gene expression patterns is associated with disease outcome and drug resistance(Lockhart & Winzeler, 2000; Wulfkuhle et al., 2004). The natural successor to genomics, proteomics explores the structure, localization and interactions of proteins encoded by the human genome (Smith & Bolouri, 2005). The first era of immunomics focused on the development of research tools that modeled the immune system (for example, algorithms for identifying T-cell epitopes and immunological synapse imaging) and monitored the immune function (Tetramers, artificial antigen presenting cells, and immunological databases, etc.). Its future lies in the ability to use these technologies to accelerate the development of new clinical treatments for the diseases such as cancer, infections or autoimmune disorders (Tremoulet & Albani, 2005).

One of the biggest advantages of immunomics over traditional methods is the ability to measure immune function in the development of treatments. Rather than waiting for clinical signs of toxicity, as is customary in trials for immunosuppressive agents at large in autoimmune diseases and cancer, immunomic tools allow the detection of subclinical immunological changes, such as reductions in the number or function of a particular cell line. Thus, it increases the detectability of potential toxicity. Unlike the immunosuppressive agents currently in use, antigen-specific therapy provides an opportunity to develop immunomodulatory therapies that only modulate disease-associated inflammation and preserves normal immune function (Tremoulet & Albani, 2005). The path to clinical application of immunomics requires a gradual ex vivo progression from peptide design to assessing the inflammatory response of peptides with biomarkers, to database mining to find correlations between immunological and clinical data that may determine clinical applicability (Tremoulet & Albani, 2005).

Guan and Kiss-Toth defined the immunomics of the innate immune system as a new perspective in immunology research by integrating the approaches of cellular immunology, bioinformatics, genomics, proteomics, immuno-informatics and other related scientific fields in order to derive integrated models of immune modulatory processes demonstrated the power of an integrated approach to characterize signaling pathways and identify novel components of the innate immune system. Scientific research of immunomics often involves screening for antigens and mapping epitopes that stimulate an immune response (Guan & Kiss-Toth, 2008).

### 2.1. Immunomic-based approaches

#### *2.1.1. Use of protein microarrays in serological antigen screening*

Using serum or plasma to screen protein libraries derived from genomic sequence data is a common immunomics-based approach (Henics et al., 2003). Analytical protein microarrays are a useful tool for profiling pathogen-specific antibody responses to identify immune-reactive proteins potentially involved in disease prevention as vaccine targets or serodiagnostic antigens (Doolan, 2011; Hall, Ptacek & Snyder, 2007). In this tool, pathogen-derived protein libraries selected for antigenic traits to be identified at all lifecycle stages are screened for antigen-antibody interactions using microarray chips. The arrays can be used to compare antibody responses or interspecies reactivity of susceptible and resistant individuals, populations of different ages, individuals from different endemic regions (Crompton et al., 2010; Doolan, 2011; Doolan, et al., 2008; Trieu, et al., 2011). In addition, microarray antigen recognition profiles can correlate with host resistance, susceptibility (Trieu, et al., 2011), clinical disease stage classification (Barbour, et al., 2008), or in vitro virus neutralizing activity (Davies, et al., 2007) allowing the identification of protection-associated antigens.

#### *2.1.2. T cell targeted antigen screening*

T cell targeted antigen screening measures the T cell activation by stimulating T cell lines or peripheral blood mononuclear cells from pathogen-exposed or infected animals or humans with libraries of whole proteome or in silico predicted partial proteome expression (Cardoso, et al., 2011; Jing, et al., 2008; Koelle, 2003; Lopez, et al., 2008). Other T-cell-targeted antigen screening approaches use the in vitro response to a specific peptide epitope to identify potential vaccine candidates (De Groot, 2006; Doolan, et al., 2003). Combined peptide libraries of predicted epitopes for a pathogen proteome or random peptide sequences can be applied for B-cell and T-cell epitope mapping, although no further datais available on antigen structure or sequence (Liu, Enstrom & Lam, 2003).

T cell epitope mapping has emerged as one of the most powerful new drug discovery tools for a range of biomedical applications. Epitope mapping is important in vaccine development and the mapping of T cell epitopes on protein antigens derived from pathogens refers to the identification of amino acid sequences recognized by CD4 or CD8 T cells. In the organism, T cells essentially monitor MHC-bound ligands expressed on the surface of all cell types. MHC ligands that trigger the T cell immune response are called T cell epitopes. Identification of these epitopes enables the monitoring, phenotyping and stimulation of T cells involved in immune responses in infectious disease, allergy, autoimmunity, transplantation and cancer (Peters, Nielsen & Sette, 2020). The specific T cell epitopes recognized in an individual are determined by genetic factors such as the individual's expressed MHC molecules in line with the individual's environmental exposure history. The complexity and importance of T cell epitope mapping has motivated the development of computational approaches that predict which T cell epitopes are likely to be recognized in a given individual or wider population. Such predictions guide experimental epitope mapping studies and provide computational analysis of the immunogenic potential of a particular protein sequence region (Peters, Nielsen & Sette, 2020). While initially T-cell-epitope discovery was focused on the development of vaccines for infectious diseases, more recently, the number of epitope mapping applications has been expanded to include reengineering of protein therapeutics, autoimmunity, endocrinology, allergy, transplantation and diagnostics (Anne, 2006).

Comparison of epitope sets identified for various pathogens between pathogen strains, species, or host immunoproteome can identify cross-reactive epitopes, help avoid molecular mimicry, and elucidate new insights into antigenicity and immune activation (Liu, Enstrom & Lam, 2003). In addition, consideration of human leukocyte antigen (HLA) supertypes may allow for a vaccine design that can be effective in all genetically heterogeneous host populations (Sette & Sidney, 1998).

## 3. Immunomics in the discovery of new targets for vaccines and therapeutics

Infectious diseases are one of the leading global causes of morbidity and mortality and there is an urgent need for effective approaches to develop vaccines, especially against complex pathogens (Schussek, Trieu & Doolan, 2014).

Most currently licensed vaccines use all live, attenuated or killed pathogens as immunogens and are derived from empirical methodologies pioneered by Edward Jenner and Louis Pasteur in the 18th and 19th centuries, respectively. However, numerous datasets and tech-

nological advances in the age of "omics" have led to the advancement of high-throughput approaches that enable antigen discovery for subunit vaccines. The availability of comprehensive genomic, proteomic and transcriptomic datasets has enabled the paradigm of vaccine development to shift from microbiological approaches to array-based approaches (Doolan, et al., 2003; Rappuoli, 2000).

In 1995, the first bacterial genome sequence was completed, laying the foundation for a new era of genomic knowledge-based vaccine development.The reverse vaccination and immunomic approaches pioneered at the beginning of the 21st century are based on genomic, proteomic, and transcriptomic datasets.Subsequent advances in mass spectrometry and high-throughput sequencing techniques have led to faster and more accurate identification and evaluation of vaccine candidates.Numerous high-throughput approaches and databases are currently available to predict, evaluate, and test vaccine candidates in silico, in vitro, and in vivo (Figure 1) (Schussek, Trieu & Doolan, 2014).

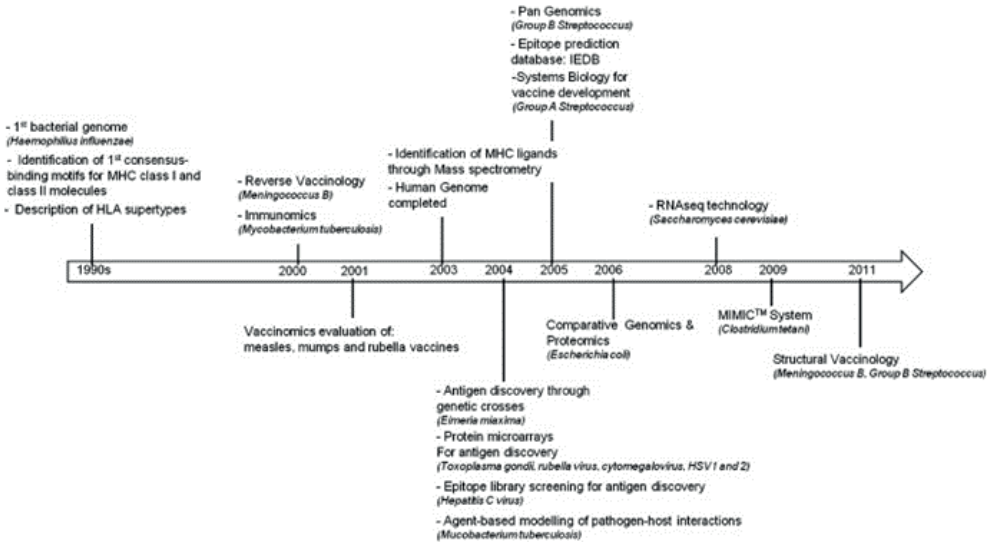


**Figure 1:** History of vaccine development methods (Schussek, Trieu & Doolan, 2014).

Bacterial genome sequencing studies have accelerated as a result of the lack of effective treatment for many infections, the emergence of multi-drug resistant bacteria, the need to improve the safety of conventional licensed vaccines, and the convergence of omics sciences such as genomics, proteomics, and immunomics (Fig. 2). Thus, the method called Reverse Vaccinology has emerged (Bagnoli, et al., 2011). In this method, firstly, the genome of the

pathogen is scanned and genes encoding proteins that can be vaccine targets are tried to be identified by using immunoinformatic methods. Appropriate targets are selected using mathematical and computational methods, their expression is ensured and are examined in terms of immunization by applying to animal models (Seib, Zhao & Rappuoli, 2012; Rappuoli, 2000). Thus, the method allows the vaccine candidate to be selected without the need for culture in the laboratory.

High-throughput sequencing and advances in technologies such as molecular biology, cytometry, and bioinformatics have facilitated the understanding of pathogen genomes and the identification of novel genes and their protein products, leading to a gene-to-vaccine approach in vaccine development. Proteomic and transcriptomic studies provide a better understanding of pathogen biology and can identify subsets of antigens such as surface, secretome, and stage-specific proteins for more rational vaccine development.The proteome paired with increased knowledge of protective host immune responses for potential vaccine candidate antigens can be screened using immunological-based studies (Schussek, Trieu & Doolan, 2014).

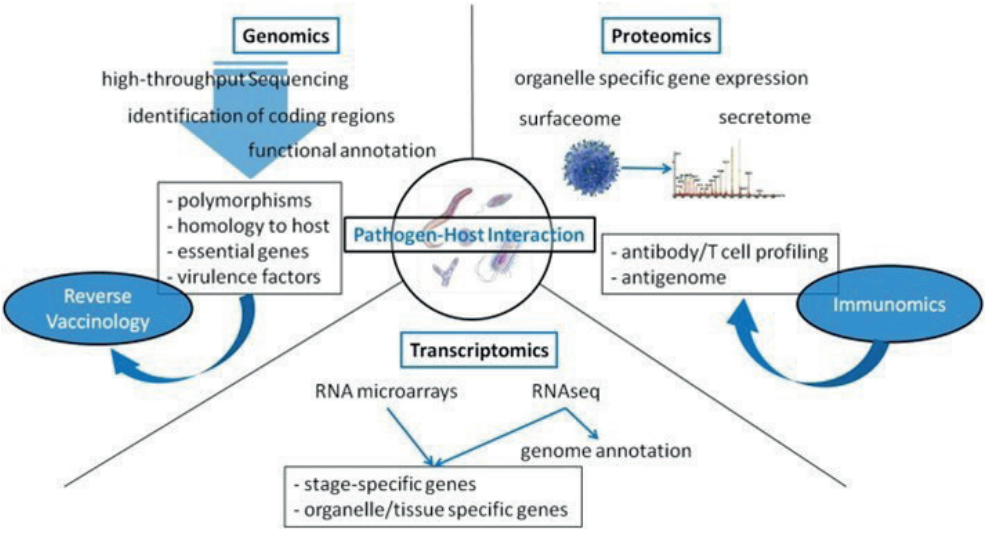


**Figure 2:** Effects of various 'omics' on next generation vaccine development (Schussek, Trieu & Doolan, 2014).

**Genomic:** The ever-growing and evolving genomic, proteomic, transcriptomic, and comparative genomic datasets provide the basis for studies that extract whole genomes and proteomes and identify genes encoding putative protective antigens as new targets for interventions against bacteria, fungi, and parasites (Doolan, 2011; Relman, 2011; Seib, Dougan & Rappuoli, 2009).

Genome analysis typically begins with identifying potential coding regions and attributing function to novel proteins based on sequence homology analysis and reverse genetic evaluation to identify the full repertoire of potential proteins and facilitate antigen discovery. Reverse genetic approaches can use DNA sequence information to identify essential proteins related to the survival or infectivity of pathogens and mutate putative genes by analyzing the resulting phenotypic effects. In addition, reverse genetics can be used to identify genomic regions that are critical for pathogen-host interactions that can affect severe disease. However, most reverse genetic techniques have been applied to pathogens with few genes for known antigens or site-directed mutations, so they may not be amenable to antigen discovery as they have a large number of possible target proteins of unknown function for pathogens with larger genomes that cannot be cultured in vitro or for which no animal models are available.

**Reverse vaccination:** In recent years,the computational infrastructure has been improved through the development of various databases, and advanced statistical tools and numerous bioinformatics algorithms have become available to organize, visualize, integrate and query large datasets (Vivona, et al., 2008). Pioneered by Rappuoli and colleagues, reverse vaccination involves in silico analysis of the genome to identify genes encoding proteins with vaccine-induced immunity-related properties and systematic evaluation of these proteins for immunogenicity (Rappuoli, 2000; Seib, Zhao & Rappuoli, 2012; Sette & Rappuoli, 2010). The advent of high-throughput sequencing has enabled the widespread availability of genomic information on multiple strains of the same species, enabling comparative genomic and pan-genomic studies (Medini, et al., 2005). Thanks to rapid advances in sequencing technology, the pan-genome can now offer the entire genetic repertoire of a given species, with multiple genome sequences or antigenically diverse isolates of different strains of a single species available Seib, Dougan & Rappuoli, 2009). The purpose of pan-genomic reverse vaccination is to identify vaccine candidates that are antigenically conserved in several species (Muzzi, Masignani & Rappuoli, 2007; Pajon, et al., 2009).

**Transcriptomics:** The transcriptome reveals the complete set of gene transcripts or RNA types that are transcribed in a particular cell type, tissue or organism for a particular physiological or pathological condition.It contains both coding RNA translated into proteins and non-coding RNA involved in post-transcriptional control, which further influences gene expression (Piétu, et al., 1996; Assis, et al. 2014). Transcriptomic research aims to interpret this important functional output of the genome by comparing cells or tissues under certain conditions or disease states to identify changes in gene expression (Kan, Shumyatcher & Himes, 2017; Wang, Gerstein & Snyder, 2009). Changes in gene transcript amount during

experimental conditions can be evaluated by differential expression analysis and identified through clustering analysis to provide information about co-regulated genes, biological mechanisms or pathways. Transcriptomics is therefore an important tool for the discovery of new diagnostic or therapeutic targets (Assis, et al. 2014).On the other hand, transcriptomic datasets can be used to construct cDNA libraries that reflect total or stage-specific transcription for multistage pathogens where expression of potential antigens at a given stage is considered essential for optimal vaccine efficacy (Kaiser, et al., 2004).

**Proteomics:** While the genome remains the same in a living organism, the proteome can undergo modifications and is variable. The proteome consists of genome expression, different forms of proteins belonging to a single gene, and a variety of post-translational modifications. After sequencing the human genome, researchers began to focus on proteins, which are the products of genes, after intense research to understand the function of all identified genes. Proteomics is concerned with the analysis, identification and functions of all proteins encoded by genes in organisms, tissues and cells under certain conditions, and its purpose is to identify the proteins involved in the pathogenesis of diseases, and to understand how their expression, structure and function cause disease. Proteomics has identified promising proteins for diagnostic or prognostic markers or therapeutic targets in many diseases, including cancer, post-transplant rejection, and infectious diseases (such as tuberculosis and malaria) (Kavallaris & Marshall, 2005). Because expression of a gene may not always correlate with protein translation, and post-translational modifications may play a role in antigenicity so proteomic discovery methods are challenging and may be more advantageous than transcriptomic methods (Larsson & Nadon, 2008).

**Immunomic:** In contrast to reverse vaccination approaches, the immunomic-based identification of vaccine candidates does not rely solely on in silico prediction algorithms, but uses biological samples from humans or animals immunized against the disease of interest to identify the immunoma of a pathogen. Therefore, immunomicis a systems biology-based approach that integrates informatics, genomics, proteomics, immunology, and clinical medicine to reveal the interface between the host immune system and the pathogen proteome (Klysik, 2001). Immunomic-based antigen identification strategies integrate and validate in silico and in vitro approaches by assessing whether preselected or newly identified proteins are the targets of clinically relevant immune responses such as production of specific cytokines, activation of cell types, and protection (Bagnoli, et al., 2011). This is achieved by describing the functional state of the host immune system at the time of infection with the pathogen and assessing the relationship between protective host immune responses and specific pathogen-derived anti-

gens. This approach requires cloning and protein expression or peptide synthesis of previously unknown proteins derived from the genomic sequence in order to screen serum or peripheral blood mononuclear cells (PBMC) of exposed, infected or immunized individuals and ideally distinguish between conserved and unprotected cohorts (Doolan, 2011).

**Immunomic and Transplantation**

Use of immunonomic and T cell mapping in transplantation includes the discovery of self-immunogenic markers considering HLA immunogenicity, their association with graft antigens leading to rejection, and scoring for probability of rejection. Measuring graft versus host (GvHD) immune responses following transplantation, improving allograft matches, and monitoring T-cell responses to opportunistic pathogens after transplantation are among the potential applications of T-cell-epitope mapping in the field of tolerance and transplantation. However, there are still large areas that remain unexplored regarding the role of epitope-specific regulatory T cells in the development and maintenance of tolerance, and perhaps T-cell epitope mapping tools can help us explore them (Anne, 2006).

In a study by Schieferdecker A. et al., the myeloma B cell immunoma was profiled to both investigate its predictive role in the context of autologous and allogeneic hematopoietic stem cell transplantation (HSCT) and to identify new immunotherapeutic targets. As a result of this study, it was reported that the search for transplant immunomas for potent myeloma surface binders could open important alternative avenues for myeloma immunotherapy (Schieferdecker, et al., 2016).

In addition, several authors have discussed the development of new guidelines in the field of evaluation of marginal donor kidneys for transplantation. Some authors argue that there are many new technologies available to study an organ at the molecular level, from proteomics to metabolomics and transcription studies, and that the use of these omic technologies should improve organ evaluation (Reese, et al., 2016; Scian, et al., 2012). Studies are ongoing to evaluate these technologies in donor urine or pre-transplant biopsies (Salvadori & Tsalouchos, 2019).

## 4. Conclusion

Scientists have recently combined imaginations and immunomic tools to explore the new world of the human immunoassay, and this approach has the potential to make significant advances in immunology and improve human health. Research is progressing towards evaluating the interface between self-antigens and the human immune system, pairing in silico

assays with in vitro assays such as binding studies, MHC-tetramers and ELISpot assays, and validating these discoveries in vivo using HLA transgenic mice. The orientation towards identifying protective antigens instead of immunogenic antigens is a more effective tool for vaccine development. In this context, immunomics-based approaches that can link whole genome data and related clinical outcomes and separate protective antigens will offer significant potential for rational vaccine development.

## References / Kaynakça


Assis, A., Oliveira, E., Donate, P., Giuliatti, S., Nguyen, C., Passos, G. (2014). What is the transcriptome and how it is evaluated? In: Passos G (ed) Transcriptomics in Health and Disease. *Cham, Springer International Publishing*, 3–48.

Bagnoli, F., Baudner, B., Mishra, R.P., et al. (2011). Designing the next generation of vaccines for global public health. *OMICS*, 15,545-66.

Baltimore, D. (2001). Our genome unveiled. *Nature*. 409, 814–818.

Barbour A.G., Jasinskas, A., Kayala, M.A., Davies, D.H., Steere, A.C., Baldi, P., et al. (2008). A genome-wide proteome array reveals a limited set of immunogens in natural infections of humans and white-footed mice with Borrelia burgdorferi. *Infect Immun*, 76 (8), 3374-89.

Cardoso, F.C., Roddick, J.S., Groves, P., Doolan, D.L. (2011). Evaluation of approaches to identify the targets of cellular immunity on a proteome-wide scale. *PLoS One*, 6 (11),e27666.

Crompton, P.D., Kayala, M.A., Traore, B., Kayentao, K., Ongoiba, A., Weiss, G.E., et al. (2010). A prospective analysis of the Ab response to Plasmodium falciparum before and after a malaria season by protein microarray. *Proc Natl Acad Sci U S A*, 107 (15), 6958-6963.

Davies, D.H., Molina, D.M., Wrammert, J., Miller, J., Hirst, S., Mu, Y., et al. (2007). Proteome-wide analysis of the serological response to vaccinia and smallpox. *Proteomics,* 7 (10), 1678-86.

De Groot, A.S. (2006). Immunomics: discovering new targets for vaccines and therapeutics. *Drug Discov Today*, 11 (5–6), 203-9.

Doolan, D.L. (2011). Plasmodium immunomics. *Int J Parasitol*., 41(1),3-20.

Doolan, D.L., Mu, Y., Unal, B., Sundaresh, S., Hirst, S., Valdez, C., et al. (2008). Profiling humoral immune responses to P. falciparum infection with protein microarrays. *Proteomics,* 8 (22), 4680-4694.

Doolan, D.L., Aguiar, J.C., Weiss, W.R., Sette, A., Felgner, P.L., Regis, D.P., et al. (2003). Utilization of genomic sequence information to develop malaria vaccines. *J Exp Biol*, 206 (Pt 21), 3789-3802.

Guan, H., Kiss-Toth, E. (2008). Advanced technologies for studies on protein interactomes. *Adv Biochem Eng Biotechnol.*110,1-24.

Hall, D.A., Ptacek, J., Snyder, M. (2007). Protein microarray technology. *Mech Ageing Dev*. 128 (1), 161-167.

Hegde, N. R., Gauthami, S., Sampath Kumar, H. M., & Bayry, J. (2018). The use of databases, data mining and immunoinformatics in vaccinology: where are we?. *Expert opinion on drug discovery*, 13(2), 117–130.

Henics, T., Winkler, B., Pfeifer, U., Gill, S.R., Buschle, M., von Gabain, A., et al. (2003). Small-fragment genomic libraries for the display of putative epitopes from clinically significant pathogens. *Biotechniques*. 35 (1),196-202.

Jing, L., Davies, D.H., Chong, T.M., Chun, S., McClurkan, C.L., Huang, J., et al. (2008). An extremely diverse CD4 response to vaccinia virus in humans is revealed by proteome-wide T cell profiling. *J Virol*, 82 (14), 7120-34.

Kaiser, K., Matuschewski, K., Camargo, N., Ross, J., Kappe, S.H. (2004). Differential transcriptome profiling identifies Plasmodium genes encoding pre-erythrocytic stage-specific proteins. *Mol Microbiol,* 51 (5), 1221-32.

Kan, M., Shumyatcher, M., Himes, B.E. (2017). Using omics approaches to understand pulmonary diseases. *Respir. Res.*18, 149.

Kavallaris, M., & Marshall, G. M. (2005). Proteomics and disease: opportunities and challenges. *The Medical journal of Australia*, 182(11), 575–79.

Koelle, D.M. (2003). Expression cloning for the discovery of viral antigens and epitopes recognized by T cells. *Methods*, 29 (3), 213-26.

Kylsik, J. (2001). Concept of immunomics: A new frontier in the battle for gene function? *Acta Biotheor.*, 49(3),191-202.

Larsson, O., Nadon, R. (2008). Gene expression — time to change point of view? *Biotechnol Genet Eng Rev*, 25, 77-92.

Liu, R., Enstrom, A.M., Lam, K.S. (2003). Combinatorial peptide library methods for immunobiology research. *Exp Hematol*, 31 (1), 11-30.

Lockhart, D.J., Winzeler, E.A. (2000). Genomics, gene expression and DNA arrays. *Nature*. 405, 827–836.

Lopez, J.E., Beare, P.A., Heinzen, R.A., Norimine, J., Lahmers, K.K., Palmer, G.H., et al. (2008). High-throughput identification of T-lymphocyte antigens from Anaplasma marginale expressed using in vitro transcription and translation. *J Immunol Methods*, 332 (1–2), 129-41).

McKusick, V.A., Ruddle, F.H. (1987). A new discipline, a new name, a new journal. *Genomics.* 1(1), 1–2.

McKusick, V.A. (1997). Perspective: genomics: structural and functional studies of genomes. *Genomics.* 45(2), 244–249.

Medini, D., Donati, C., Tettelin, H., Masignani, V., Rappuoli, R. (2005). The microbial pan-genome. *Curr Opin Genet Dev*, 15 (6), 589-94.

Muzzi, A., Masignani, V., Rappuoli, R. (2007). The pan-genome: towards a knowledge-based discovery of novel targets for vaccines and antibacterials. *Drug Discov Today*, 12 (11–12), 429-39.

Oli, A. N., Obialor, W. O., Ifeanyichukwu, M. O., Odimegwu, D. C., Okoyeh, J. N., Emechebe, G. O., Adejumo, S. A., & Ibeanu, G. C. (2020). Immunoinformatics and Vaccine Development: An Overview. *ImmunoTargets and therapy,* 9, 13–30.

Pajon, R., Yero, D., Niebla, O. Climent, Y., Sardinas, G., Garcia, D., et al. (2009). Identification of new meningococcal serogroup B surface antigens through a systematic analysis of neisserial genomes. *Vaccine*, 28 (2), 532-41.

Peters, B., Nielsen, M., Sette, A. (2020). T Cell Epitope Predictions. *Annu Rev Immunol*. 26;38,123-145.

Piétu, G., Alibert, O., Guichard, V., Lamy, B., Bois, F., Leroy, E., Mariage-Sampson, R., Houlgatte, R., Soularue, P., Auffray, C. (1996). Novel gene transcripts preferentially expressed in human muscles revealed by quantitative hybridization of a high density cDNA array. *Genome Res*. 6, 492–503.

Rappuoli, R. (2000). Reverse vaccinology. *Curr Opin Microbiol*, 3 (5), 445-50.

Reese, P.P., Hall, I.E., Weng, F.L., Schröppel, B., Doshi, M.D., Hasz, R.D., Thiessen-Philbrook, H., Ficek, J., Rao, V., Murray, P., Lin, H., Parikh, C.R. (2016). Associations between Deceased-Donor Urine Injury Biomarkers and Kidney Transplant Outcomes. *J Am Soc Nephrol*. 27,1534–43.

Relman, D.A. (2011). Microbial genomics and infectious diseases. *N Engl J Med*, 365 (4), 347-57.

Salvadori, M., Tsalouchos, A. (2019). Histological and clinical evaluation of marginal donor kidneys before transplantation: Which is best? *World J Transplant*. 26,9(4),62-80.

Schieferdecker, A., Oberle, A., Thiele, B., Hofmann, F., Göthel, M., Miethe, S., Hust, M., Braig, F., Voigt, M., von Pein, U.M., Koch-Nolte, F., Haag, F., Alawi, M., Indenbirken, D., Grundhoff, A., Bokemeyer, C., Ba-

cher, U., Kröger, N., Binder, M. A. (2016). transplant “immunome” screening platform defines a targetable epitope fingerprint of multiple myeloma. *Blood*. 23,127(25),3202-14.

Schussek, S., Trieu, A., Doolan, D.L. (2014). Genome- and proteome-wide screening strategies for antigen discovery and immunogen design. *Biotechnol Adv.* 32(2),403-14.

Scian, M.J., Maluf, D.G., Archer, K.J., Turner, S.D., Suh, J.L., David, K.G., King, A.L., Posner, M.P., Brayman, K.L., Mas, V.R. (2012). Identification of biomarkers to assess organ quality and predict posttransplantation outcomes. *Transplantation*. 94,851–85.

Seib, K.L., Dougan, G., Rappuoli, R. (2009). The key role of genomics in modern vaccine and drug design for emerging infectious diseases. *PLoS Genet*, 5 (10), e1000612.

Seib, K.L., Zhao, X., Rappuoli, R. (2012). Developing vaccines in the era of genomics: a decade of reverse vaccinology. *Clin Microbiol Infect*., 18,109-16.

Sette, A., Rappuoli, R. (2010). Reverse vaccinology: developing vaccines in the era of genomics Immunity, 33 (4), 530-41.

Sette, A., Sidney J. (1998). HLA supertypes and supermotifs: a functional perspective on HLA polymorphism. *Curr Opin Immunol*, 10 (4), 478-82.

Smith, K.D., Bolouri, H. (2005). Dissecting innate immune responses with the tools of systems biology. *Curr. Opin. Immunol.* 17, 49–54.

Tomar, N., De, R.K. (2014). Immunoinformatics: a brief review. *Methods Mol Biol.*, 1184, 23-55.

Tremoulet, A.H., Albani, S. (2005). Immunomics in clinical development: bridging the gap. *Expert Rev Clin Immunol.* 1(1), 3-6.

Trieu, A., Kayala, M.A., Burk, C., Molina, D.M., Freilich, D.A., Richie, T.L., et al. (2011). Sterile protective immunity to malaria is associated with a panel of novel P. falciparum antigens. *Mol Cell Proteomics*, 10 (9).

Vivona, S., Gardy, J.L., Ramachandran, S., Brinkman, F.S., Raghava, G.P., Flower, D.R., et al. (2008). Computer-aided biotechnology: from immuno-informatics to reverse vaccinology. *Trends Biotechnol*, 26 (4), 190-200.

Wang, Z., Gerstein, M., Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nat. Rev. Genet.* 10, 57–63.

Wulfkuhle, J., Espina, V., Liotta, L., Petricoin, E. (2004). Genomic and proteomic technologies for individualisation and improvement of cancer treatment. *Eur. J. Cancer*. 40(17), 2623–2632.