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# THE QUEST

*A Biannual Multidisciplinary Refereed Journal*

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2023

Institute for Excellence in Higher Education

Kolar Road, Kaliyasot Dam, Bhopal - 462 016 Madhya Pradesh, India

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# Integration of AI, ML and Blockchain Technology: Transforming Codes into Life

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## Abstract

Data-driven approaches using AI, ML, and blockchain have revolutionized biotechnology and genomics. The present paper delves into the exciting possibilities that arise from the intersection of AI, ML, and blockchain technology with genomics, healthcare, and biotechnology. The use of these advanced technologies allows for the creation of radical applications that leverage machine learning, Big Data analytics, natural language processing, decision support, and reasoning under uncertainty. Such applications provide unprecedented avenues for improving human health and well-being.

By assimilating AI with biotechnology, researchers can develop cutting-edge applications that enable genomic sharing, next-generation sequencing, gene editing, clinical workflow optimization, risk prediction, diagnosis, and precision medicine. The potential applications of AI, ML, and blockchain in these areas are truly transformative, and have the power to revolutionize the future of healthcare. The survey showcases the significant impact of these technologies in improving patient outcomes, reducing costs, and increasing the efficiency of healthcare delivery. With the help of AI, ML, and blockchain, one can realize a future where healthcare is more personalized, effective, and accessible to everyone.

*Keywords: Artificial Intelligence, Biotechnology, Blockchain, Deep Learning, Digital Transformation, Machine Learning.*

## Introduction

Big data has become an essential aspect of modern society, with its significance spread across various industries and fields. In 2001, Gartner introduced the 3Vs of data: Volume, Velocity, and Variety. Since then, the field of data analytics has expanded on this concept by adding two more Vs - Value and Veracity. Here, volume refers to the massive amount of data, which is often complex and heterogeneous. Traditional database technology cannot handle this volume of data, leading to the need for advanced analytics to extract insights. Velocity refers to the speed at which new data is generated and moves around. Variety refers to the different types of structured, semi-structured, and unstructured data available, such as social media conversations and voice recordings. Veracity refers to the certainty, accuracy, relevance, and predictive value of the data, while value refers to the conversion of data into business insights.

The genomics and healthcare industry is one of the sectors that have been impacted significantly by big data and artificial intelligence (AI). Big data analytics and AI have become omnipresent across the entire healthcare spectrum, including payers, providers, policy-makers/ government,

patients, and product manufacturers. Healthcare fraud and abuse account for up to 10% of global healthcare expenditure, and AI-based tools can help mitigate this problem in payer programs [Joudaki, et al., 2015]. Medical coding errors and incorrect claims also account for substantial losses, and the reliable identification of these errors can save payers, providers, and governments significant amounts of money and time [Davenport and Kalakota, 2019].

AI is also used for evidence-based clinical decision support, detection of adverse events, and predicting patients at risk for readmission. Healthcare policymakers and government can use AI-based tools to control and predict infections and outbreaks.

With the advent of the global pandemic coronavirus disease 2019 (COVID- 19) in early 2020, AI models could be used to predict at-risk populations and provide additional risk information to clinicians caring for at-risk patients [Vaishya et al., 2020]. The big data analytics for patients and biotechnology/ healthcare products is a crucial aspect of healthcare and the future of healthcare will depend significantly on these technologies - AI, ML and Blockchain to provide efficient and effective care to patients.

## Artificial Intelligence Vs Machine Learning

Artificial Intelligence (AI) and Machine Learning (ML) are two terms that are often used interchangeably, but they represent different concepts. AI refers to the broad vision of generating computers and software that can perform tasks that require human intelligence. On the other hand, ML is a subfield of AI that involves training computers to perform tasks without explicit instructions using patterns and insights from data. Deep Learning (DL) is a subset of ML that uses artificial neural networks with many layers to learn and make decisions. It is particularly useful for tasks that involve analyzing large amounts of data [Garg, 2023a].

The AI field was initiated in 1956 when a group of computer scientists met at Dartmouth College in Hanover, New Hampshire. The group had ambitious goals to create machines that could simulate every aspect of human intelligence. Since then, AI has gone through many ups and downs, including an AI winter in the 1980s. However, the rise of statistical data-driven ML helped to revive the field of AI. Today, AI is experiencing a resurgence, and the latest natural language technology developed by OpenAI, called ChatGPT, is proof of what AI can do.

There are three major classes of ML: supervised learning, unsupervised learning, and reinforcement learning. Supervised learning aims to predict a classification or label of data points using a given set of labeled training examples. In contrast, unsupervised learning aims to learn inherent patterns within the data. Reinforcement learning is based on rewarding desired behavior and punishing undesired behavior of software agents.

DL models are more flexible than standard ML methods and can model more complex relationships between inputs and outputs [LeCun et.al, 2015; Zou et.al, 2019]. Different types of neural networks have been developed for specific tasks such as convolutional neural networks, which capture spatial dependencies, and recurrent neuronal networks, which handle sequential or time-series data.

Thus, AI aims to provide the theoretical fundamentals for ML to develop software that can learn autonomously from previous experience. To reach a level of usable intelligence, we need to learn

from prior data, extract knowledge, generalize, fight the curse of dimensionality, and disentangle the underlying explanatory factors of the data. The grand goal is to create software that can learn automatically without human intervention.

## AI in Genomics and Healthcare

Artificial Intelligence (AI) has revolutionized many domains, and healthcare is no exception. It involves the use of technology to create software that mimics human-like critical thinking [Ramesh et al., 2004]. AI uses techniques such as fuzzy expert systems and artificial neural networks [Hessler and Baringhaus, 2018; Mintz and Brodie, 2019] to provide personalized experiences where predictions are backed by mathematical data points. The field of AI in healthcare can be divided into two subunits, virtual and physical. The virtual aspect of AI involves electronic healthcare records [Esteva et al., 2019] and neural networks guiding patient treatments [McDonnell et al., 2021], while the physical aspect involves robots assisting in surgeries and AI-generated prosthetics for the disabled.

Over the past decade, AI has seen remarkable growth and acceptance in genomics and biotechnology. It provides rich opportunities for designing intelligent products, creating novel services, and generating new business models. The use of AI in medicine can introduce social and ethical challenges to security, privacy, and human rights.

AI technologies in medicine exist in many forms, from the purely virtual to cyber-physical. AI technologies have enabled many image-based detection and diagnostic systems in healthcare to perform as well or better than clinicians. AI-enabled clinical decision-support systems may reduce diagnostic errors, augment intelligence to support decision making, and assist clinicians with EHR data extraction and documentation tasks.

Emerging computational improvements in natural language processing, pattern identification, efficient search, prediction, and bias-free reasoning will lead to further capabilities in AI that address currently intractable problems [Biamonte et al., 2017]. However, the advances in the computational capability of AI have prompted concerns that AI technologies will eventually replace physicians.

Therefore, the term augmented intelligence [Ashby, 1957] may be a more apt description of the future interplay among data, computation, and healthcare providers, and perhaps a better definition for the abbreviation AI in healthcare.

## Insights into the Blueprint of Life

The human genome is the foundation for the expression of human traits, consisting of unique biological DNA that makes each individual distinct. The advent of genomics has revolutionized the field of molecular biology, enabling scientists to map the structure and function of genomes. Each human genome contains 20,000 to 25,000 genes, with every gene comprising a few hundred to 2 million DNA bases [International Human Genome Sequencing Consortium, 2001]. The mapping of the human genome in 2003 opened up numerous possibilities for using genomics in the medical field.

Gene expression occurs through transcription and translation, with RNA splicing in between, resulting in diversity in protein coding. However, errors in splicing or mutations can cause a range of diseases [Fletcher et al., 2013]. While protein-coding DNA accounts for only a small percentage of the genome, a significant portion is transcribed into non-protein-coding RNAs (ncRNAs) that regulate gene expression and transcription initiation and termination [Mattick and Makunin, 2006]. Genomic sequencing has revolutionized the way researchers read the genetic blueprint, but affordability and data management are two significant challenges that must be addressed.

## Challenges with Gene Sequencing

The ability to sequence DNA has provided researchers with unprecedented opportunities to understand human biology and develop new therapies for diseases. However, the cost of using genome sequencing in routine clinical care remains a significant challenge. At present, the cost of sequencing a single genome in a single laboratory is around $1000 [Schwarze et al., 2020]. This cost can be prohibitively expensive for many people, limiting access to potentially life-saving genetic information. To make genomic sequencing more affordable, researchers are developing new technologies that could reduce the cost of sequencing and improve the accuracy of results.

### 1. Data Management and Privacy

Another significant challenge in genomics is data management. The collection, sharing, ownership, and storage of genomic data are all complex and time-consuming processes that require special attention to detail, precision, and privacy. Genomic data contains highly personal information about an individual's past, present, and future generations. Therefore, researchers must take special care to ensure that this information is recorded and managed securely to prevent potential misuse or breaches of privacy.

### 2. Potential Misuses of Genomic Data

The potential misuse of genomic data is a significant concern in the field of genomics. This information could be used to develop harmful medicines or even commit crimes, highlighting the importance of managing and securing genomic data carefully. Researchers must be mindful of the potential consequences of any data breaches or misuses, and must take steps to minimize the risk of these occurrences.

## Transforming Genomics through ML

In the area of genome sequencing, machine learning can be used to identify patterns within high volume genetic data sets. These patterns are then used to create computer models that can help predict an individual's probability of developing certain diseases or inform the design of potential therapies. This is particularly useful in the field of precision medicine, where treatments are tailored to an individual's unique genetic makeup. By analyzing large data sets, machine learning algorithms can identify subtle differences in genetic patterns that may be associated with increased disease risk or specific treatment responses.

Advancements in genomics continue to offer insights into human health and disease. For instance, researchers have employed genomics to identify genetic variations that contribute to various diseases, such as cancer, diabetes, and Alzheimer's disease. By comparing the genomes of healthy

individuals to those with specific diseases, researchers can identify genetic differences and develop targeted treatments.

Even companies offering genomic sequencing services to individual consumers are using machine learning algorithms to gain a greater understanding of how an individual's genes may impact their health. By analyzing genetic data, companies can predict an individual's likelihood of developing certain conditions, such as weight gain, and provide personalized advice on diet and exercise to help individuals manage their weight.

Machine learning is also being used to predict pharmaceutical properties of drug targets and drug candidates. By analyzing large data sets on the molecular properties of potential drugs, machine learning algorithms can predict their effectiveness in treating specific diseases. This has the potential to greatly accelerate the drug discovery process, ultimately leading to more effective treatments.

Another important area of application is in the analysis of multimodal data from genomics and other omics fields, combined with clinical data [National Research Council, 2011]. By integrating large data sets from multiple sources, machine learning algorithms can generate new diagnostic and predictive models for diseases, including their underlying genetic causes. This has the potential to greatly improve disease diagnosis and treatment, leading to better patient outcomes.

Pharmacogenomics is another promising application of genomics, which helps doctors assign medication and corresponding dosage based on the patient's genetic markers. This technique has enabled specialists to provide more personalized care and improve patient outcomes. CRISPR is another revolutionary technology that has made it possible to treat chronic diseases like HIV [Xiao et al., 2019], β-thalassemia [Frangoul et al., 2021], cancers [Chen et al., 2019], leukemia [Tzelepis et al., 2016], and sickle cell anemia [Frangoul et al., 2021].

Despite ongoing debates on the ethics of genetic testing without a clear cure, the availability of genetic information through next-generation sequencing and direct-to-consumer testing makes personalized prevention and management of serious diseases a reality.

**1. Next Generation Sequencing**

Next Generation Sequencing (NGS) technology has revolutionized genome sequencing and emerged as the leading method. Compared to classic Sanger sequencing that took over a decade to complete the human genome, NGS allows researchers to sequence a whole human genome in just one day. Illumina sequencing is currently the most popular technology due to its cost, speed, and accuracy [Liu et al., 2012]. However, long-read sequencing technologies like those created by Oxford Nanopore [Green and Sambrook, 2018] and Pacific Biosciences [Rhoads and Au, 2015] generate longer reads that are thousands of base pairs long, but lower in quality than short-read sequencing.

NGS data has the potential to supplement other genomic sequencing methods and improve the effectiveness of precision medicine by better identifying disease risk and actionable genetic mutations in cancer patients. This technology can aid in the development of drugs targeting tumors and matching patients to therapy methods. Companies like Deep Genomics are using machine

learning algorithms to interpret genetic variation by identifying patterns in large genetic datasets and translating them into computer models.

DNA sequencing data is stored in the FASTQ format, which consists of four corresponding lines of text for each sequence. FASTA is another commonly used text-based file format for storing reference genomes. Algorithms map sequencing reads to reference genomes, and these results are stored in Sequence Alignment Map (SAM) or its binary equivalent (BAM) file formats [Hoogstrate et al., 2021]. While SAM files are readable by humans, BAM files are used to compress the data due to the large file sizes. Finally, variant call format (VCF) files describe the sequence variations, insertions, and deletions found in samples along with rich annotations [Zhang, 2016; Morash et al., 2018].

Although the efficacy of NGS data in precision medicine remains controversial due to experimental design, the technology's potential for development is immense. The improved methods for analyzing sequenced data can help in the development of precision medicine. NGS technology, combined with machine learning, can also help identify and interpret genetic variation and its effects on crucial cellular processes.

**2. Variant Discovery**

Variant discovery is a critical step in understanding the genetic basis of various diseases. Whole genome sequencing (WGS) is a technique that involves sequencing an individual's entire genome, including both protein-coding and non-protein-coding regions, while the entire exon sequencing (WES) focuses solely on the protein-coding regions [Petersen et al., 2017]. By using variant calling, researchers can identify various types of variants, providing valuable insights into disease diagnosis and prevention.

There are three main types of pipelines used for WGS and WES: cloud-computing, centralized, and standalone [Ahmed et al., 2021]. Cloud-computing pipelines are utilized in environments with on-demand compute resources provided by external vendors. On the other hand, centralized pipelines are used in local computers, while standalone pipelines are mainly used in high-performance computing environments. These pipelines have been designed to effectively collect and process data from WGS or WES, allowing researchers or medical professionals to recognize the links between genetic variants and diseases.

**3. Gene Editing**

Gene editing involves making targeted changes to DNA at the cellular or organism level. CRISPR is a gene editing technology that has made this process faster and less expensive. However, selecting the appropriate target sequence for CRISPR can be a challenging task. Luckily, the use of machine learning has the potential to significantly reduce the time, cost, and effort required to identify the right target sequence. Continued research and development in this area could revolutionize the field of gene editing.

At the intersection of AI and CRISPR, London-based software company Desktop Genetics has emerged. The company works with experimental or reference data uploaded to Google Cloud, which is then processed and formatted before being sent to their bioinformatics and machine

learning teams. By analyzing this data, they can design and conduct CRISPR experiments, develop new models, and generate FASTQ data that feeds back into the workflow.

Recently, the company published two significant findings from their research. Firstly, they found that an increased amount of training data improves the accuracy of the algorithm's ability to predict CRISPR activity. Secondly, they discovered that the model's accuracy decreases when applied to a different species, such as humans versus mice. Although these findings may not be surprising, they highlight the importance of ongoing research to continue improving processes and push the boundaries of how machine learning can impact CRISPR.

**4. Clinical Workflow**

In today's world, where technology has penetrated every aspect of our lives, it is no surprise that the genomics and healthcare industries are also reaping the benefits of technological advancements. With the help of artificial intelligence (AI) and machine learning, the healthcare sector is trying to revolutionize the way it functions.

One of the challenges that the healthcare sector faces is the availability of patient data to the various members of the healthcare team serving a patient. However, this challenge has sparked an interest in using machine learning to improve the efficiency of the clinical workflow process.

Intel, a major tech company, has created an Analytics Toolkit that integrates machine learning capabilities to evaluate factors like a patient's risk of developing multiple cancers. The algorithm utilized in the toolkit was created with four primary components, including a centralized genomic data database linked to clinical and patient data, electronic health record (EHR) access for all clinicians and genetic counselors, integration of all data from genetic tests into EHRs, and access to operational Clinical Decision Support tools (CDS). Examples of clinical decision support include family health histories, screenings, and past clinical data.

It has been reported that a sample workflow for a patient can be screened in just 3 to 5 minutes with the workflow model developed using machine learning. This has contributed to improved data accessibility. Despite the regulatory issues and complex sales cycles, many of the major players in artificial intelligence are recognizing the significant economic value of AI in healthcare.

**5. Direct-to-Consumer Genomics**

The market for predictive genetic testing and consumer genomics is set to expand dramatically, and is expected to touch $5 billion by 2025. This growth is fueled by the increasing awareness of how genomic testing can aid in identifying one's risk of developing certain illnesses. Proper guidance can make these tests a valuable tool in preventative healthcare, despite concerns regarding regulation and the need for health professionals to interpret results for patients.

Direct-to-consumer genomics is a rapidly expanding industry, especially as people become more conscious of their lifestyle and dietary habits. Personalized analyses of an individual's genetic makeup, taking into account factors like genotype, sex, age, and self-identified primary ancestry, can help determine how one's genetic material may impact their weight. However, there are still concerns about the regulation of these tests and the necessity of professional interpretation of results.

**6. Clinical Genomics**

Clinical genomics is a rapidly developing field that leverages sequencing techniques to identify genes associated with diseases. The approach can detect abnormalities in patients, predict their susceptibility to certain diseases, and facilitate the development of treatments for rare diseases. However, the usefulness of genomics data depends on how it is organized and assimilated.

One essential tool for organizing and assimilating genomic and phenotypic data is gene-disease databases. Despite the existence of approximately 18,000 gene-disease databases [Huang et al., 2018] only a few are approved by the American College of Medical Genetic and Genomics (ACMG). One significant challenge with these databases is their lack of standardization, which may lead to outdated or irrelevant information about diseases.

To address this challenge, researchers have developed IOS applications such as PAS-Gen and PROMIS-APP-SUITE. These applications provide a centralized database for genomic and disease information [Stenson et al., 2017], making it more accessible and practical for researchers and healthcare professionals. By providing standardized and up-to-date information, these apps can accelerate medical discoveries and aid in the development of treatments for various genetic diseases.

**7. Precision Medicines**

The integration of machine learning into genomics has brought about significant advancements in precision medicine. Machine learning algorithms have revolutionized the analysis of vast amounts of genomic data, enabling the identification of genetic mutations and patterns that are linked to various diseases and disorders. Such insights are used to develop patient-specific treatment plans, thereby improving outcomes and lowering healthcare expenses.

Precision medicine is an approach to patient care that takes into account an individual's unique genetics, behaviors, and environment. Its goal is to create tailored treatment interventions instead of a one-size-fits-all approach. For example, matching a patient in need of a blood transfusion to a donor with the same blood type can significantly reduce the risk of complications.

Despite the potential benefits of precision medicine, a significant obstacle to its widespread implementation is the high cost of collecting and analyzing patient data. Machine learning techniques are useful in reducing these costs by swiftly and effectively analyzing vast amounts of data. Furthermore, as the cost of genome sequencing continues to decline, genomics is becoming more accessible and affordable.

By leveraging machine learning techniques, genomics firms and researchers can hasten the pace of discovery and create more personalized treatment plans for patients. As the field of genomics progresses, we can anticipate exciting advancements in precision medicine and other aspects of healthcare. Overall, the integration of machine learning into genomics has the potential to significantly enhance patient outcomes and lower healthcare costs.

**8. Diagnostics**

The use of artificial intelligence (AI) in medical biotechnology has great potential to revolutionize the field. However, implementing AI algorithms in in vitro diagnostics (IVD) companies presents

significant challenges, particularly related to ethical and legal issues. Despite these obstacles, AI can be utilized in several ways to improve medical biotechnology.

*Drug target identification*: One way in which AI can be utilized in medical biotechnology is drug target identification. By analyzing genomic data and protein-protein interaction data, AI can identify potential therapeutic targets for the treatment of diseases. Machine learning algorithms can identify patterns and correlations that may not be apparent to humans.

*Drug screening*: Another application of AI in medical biotechnology is drug screening. AI can analyze data on the activity of potential drugs against different targets and identify those most likely to be effective. Machine learning algorithms can predict the likelihood of a particular drug being effective based on its characteristics and the characteristics of the target.

*Image screening*: AI can also be utilized in medical image screening. By analyzing CT scans and MRI images, AI can identify abnormalities and diagnose diseases. Deep learning algorithms can automatically segment and classify structures in medical images.

*Predictive Modeling*: AI can be used for predictive modeling. By analyzing data from electronic health records and wearable devices, machine learning algorithms can make predictions about an individual's health. This includes predicting the likelihood of an individual developing a particular disease or the likelihood of a particular treatment being effective.

**9. Cardiovascular Disease**

The field of cardiovascular medicine has a rich history of employing predictive modeling to evaluate patient risk. Recent advancements have enabled the prediction of heart failure and other cardiac events in asymptomatic individuals. When utilized in conjunction with personalized prevention strategies, these predictive models hold the potential to have a positive impact on disease incidence and its effects. The intricacy of diseases such as cardiovascular disease necessitates the integration of various factors, including gender, genetics, lifestyle, and environmental factors. As a result, it is critical to consider the heterogeneity of the data, and artificial intelligence (AI) approaches have exhibited promise in identifying intricate connections among a vast number of factors. A Vanderbilt study showcased the early successes of merging electronic health record (EHR) and genetic data, yielding favorable outcomes in cardiovascular disease prediction [Zhao, et al., 2019]. AI-powered recognition of phenotype features via EHR or images and correlating those features with genetic variants may enable more rapid genetic disease diagnosis [Gurovich et al., 2019].

## Future Prospects

The field of genomics is rapidly advancing, and machine learning is expected to have a significant impact in several areas. One of these areas is the development of patient-specific pharmaceutical drugs. Machine learning models are being used to determine stable doses of drugs including those commonly administered to patients following solid organ transplants to prevent acute rejection of the new organ. Pharmacogenomics is an emerging field that uses genetics to understand how individuals respond to drugs, and machine learning is expected to play a crucial role in this field.

Another area where machine learning is expected to have a significant impact is in newborn genetic screening. As this practice becomes more widespread, data collected at birth will be integrated into individuals' electronic health records. Non-invasive screening capabilities for diseases such as Down Syndrome may be available to women during pregnancy.

## Roadblocks

Managing, analyzing, and storing the large amounts of data generated by healthcare and genomics industries is a daunting task. Current data management systems face various challenges, such as data sharing, analysis cost, data ownership, privacy, and security. Researchers have developed different solutions to tackle these problems, including Data Cloud Architecture, Data Commons, and Data Ecosystem. However, these solutions still have scalability and flexibility issues.

Recently, blockchain technology has emerged as a promising solution to address these challenges. With its decentralized, distributed, and immutable nature, blockchain can provide secure and transparent data management solutions. Moreover, blockchain can reduce the analysis cost of genomics and healthcare applications by enabling faster and more efficient transactions compared to traditional processes.

Blockchain technology also offers pseudo-anonymity to ensure personal data security and privacy. Individuals can modify their data access permissions and use encryption methods, such as symmetric encryption, to secure their data further [Garg, 2023b]. Thus, blockchain technology has enormous potential to be a valuable cornerstone in building a Data Ecosystem for healthcare applications.

## Blockchain as a Way-out

Blockchain technology can revolutionize the healthcare industry by enabling secure, transparent, and efficient sharing of electronic health records (EHRs) and genetic test results. The use of blockchain technology has led to the development of several platforms such as Coral Health, Patientory, Medicalchain, and GemOS, all built on Ethereum and Hyperledger protocols.

Coral Health is a data sharing platform that creates a secure and accessible healthcare ecosystem through a precision medicine program. The system uses SMART and FHIR protocols to connect mobile devices of patients and other environments hosting their medical data [Coral Health, 2023]. EncrypGen and Gene-Chain are other platforms that use blockchain technology to de-identify genomic data and enable safe, traceable, and unhackable transactions of genomic data.

Health Nexus is an open-source blockchain protocol that offers a more efficient, trustworthy, and secure path for data to travel in the healthcare community. Medicalchain is an EHR sharing project that employs a dual blockchain structure with Hyperledger controlling access to health records and Ethereum underlies all the applications and services. MedRec and Opal are other encrypted platforms that use blockchain technology to manage authentication, confidentiality, accountability, and data sharing of sensitive healthcare information.

Nebula Genomics is a platform that leverages blockchain technology to empower individuals to own their personal genomic data, lower sequencing costs, and enhance data privacy. The platform uses an open protocol to enable data buyers to efficiently aggregate standardized data from many

individuals and genomic databanks [Nebula Genomics, 2023]. Zenome is another platform that focuses on genetic data sharing and has an Ethereum-based ZNA token [Kulemin, Popov & Gorbachev, 2017].

These platforms offer a scalable and flexible approach to patient-centric healthcare and personalized medicine and present an exciting opportunity for innovation in data transfer for the healthcare community.

## Discussion

In recent years, Artificial Intelligence (AI) has played a significant role in the biotechnology industry, particularly in fields such as drug discovery, drug safety, proteomics, pharmacology, and pharmacogenetics. These fields require the storage, filtering, analysis, and sharing of large amounts of data, and AI software solutions have provided support to increase speed and reduce manual errors [McAlister et al., 2017; Ginsburg and Phillips, 2018]. The adoption of new technologies and processes to improve efficiency, accuracy, and speed through digital transformation can further accelerate the development and use of AI in biotechnology.

In healthcare, the successful adoption of AI is dependent on three key principles: data and security, analytics and insights, and shared expertise. Shared expertise refers to the complementary relationship between AI systems and human professionals. Moreover, precision medicine, which aims to personalize care for every individual, is providing an equal or even greater influence than AI on the direction of healthcare. Precision medicine requires access to massive amounts of data, and the convergence of AI and precision medicine can accelerate the goals of personalized care and tightly couple AI to healthcare providers for the foreseeable future.

The future of biotechnology and healthcare is dependent on several key areas, including genomics, AI, big data, and blockchain. Precision medicine is one of the target areas in this field, with numerous advantages such as more accurate diagnoses, easy access to medical data, and a better understanding of diseases and their causes [Hasin et al., 2017]. However, implementing precision medicine can be challenging due to the lack of a system to compare multi-omics patient data and identify appropriate approaches to use with different types of medical data [Picard et al., 2021]. Ethical and logistical issues also need to be considered in clinical genomics, big data, and pharmacogenomics implementation.

To address these challenges, multiple approaches need to be integrated into precision medicine. By combining information from different fields, researchers can gain a more comprehensive understanding of a medical case and select an appropriate treatment method. For example, the integration of AI and genomics has led to significant developments in disease analysis and prediction, resulting in faster decision-making.

However, the integration of multi-omics datasets is crucial in capturing the complexity of each omics approach. More benchmark studies are needed to determine the best machine-learning strategy to implement. Multi-omics integrative models can help in understanding disease abnormalities that are not always possible with only genomic or other single-omics analysis.

The field of precision medicine has seen remarkable growth with the advent of advancements in AI, healthcare, clinical genomics, and pharmacogenomics. These developments have generated an

enormous amount of data, which can provide insights into personalized treatment options. However, incomplete or inaccurate healthcare-specific information in open access clinical data and claims data presents difficulties in determining how patient-specific treatment is appropriate or effective. The same limitation exists in genomic databases, where data cannot be easily transferred from one database to another. These limitations complicate the process of cross-referencing data between different databases, which can prove to be an obstacle to efficient patient treatment.

Pharmacogenomics focuses on an individual's reaction to specific treatments and medications rather than the disease itself. By correlating a patient's genomic makeup and their reaction to treatments, it allows for more precise and personalized prescription of treatment. However, this field is still developing and has not been utilized reliably.

The large influx of data generated in precision medicine presents an issue, as no reliable or standardized means of analysis has been developed. The use of AI and ML techniques alleviates this issue by allowing for efficient data management and the ability to recognize patterns in complex datasets. These techniques can predict pharmaceutical properties of drug targets and drug candidates, which is especially beneficial in clinical settings.

## Conclusion

The integration of artificial intelligence (AI), machine learning (ML), and blockchain technology in the fields of genomics, healthcare, and biotechnology has the potential to modernize these industries. AI can improve diagnosis accuracy, aid in clinical decision-making, and provide personalized patient experiences. However, ethical and social considerations must be taken into account.

ML is increasingly important in genomics research and may become an even more crucial tool in unlocking the secrets of the genome. In the healthcare sector, the integration of AI and ML can revolutionize patient care by improving access to data and using it more efficiently. Additionally, the development of precision medicine requires a combination of different approaches.

Blockchain technology can enhance security and transparency in the storage and sharing of patient data. However, energy and computation efficiency should be considered when implementing this technology. Despite the challenges that need to be addressed, the potential benefits of these technologies in improving patient care and disease prevention cannot be overlooked.

The impact of big data analytics and AI on the healthcare industry is enormous. AI-based tools can help mitigate healthcare fraud, medical coding errors, and improve patient care. Healthcare policymakers and government also use AI-based tools to control and predict infections and outbreaks. With the advent of COVID-19, AI models can predict at-risk populations and provide additional risk information to clinicians caring for at-risk patients. In our journey towards a progressively technology-driven future, it is crucial that we place utmost importance on the well-being of every individual inhabiting our planet.

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*Review Article*

# Immune Response to COVID-19: Understanding the Lethal Pandemic

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## Abstract

The SARS-CoV-2 virus causes COVID-19, a severe respiratory disease that can lead to death. The virus primarily invades the lungs' epithelium, specifically the pneumocytes. Mild infections are often asymptomatic, while severe infections result in various respiratory distress depending on the host's immune response, which varies by age. The present article explores the impact of COVID-19 on infected lungs and the innate and acquired immune responses involved. Alveolar and interstitial macrophages, monocytes, neutrophils, dendritic cells, natural killer cells, and T and B lymphocytes contribute to the specific immune response. Additionally, the article briefly discusses dendritic cells' role as professional antigen-presenting cells and the main interferon-producing signal in response to SARS-CoV-2, type I IFNs. Lastly, it provides a brief overview of vaccine development, a critical aspect of combating the infection.

*Keywords: Amphiregulin, Bronchoalveolar lavages, COVID-19, Desquamation, Edema, Lymphopenia, Megakaryocytes, Myeloid, Thrombocytes, Thrombocytopenia.*

## Introduction

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a single-stranded positive-sense RNA virus that belongs to the Coronaviridae family in the genus Beta coronavirus of the Orthornavirae Kingdom. This virus has caused a troublesome global pandemic that has resulted in the deaths of millions. The clinical manifestations of COVID-19 range from the asymptomatic or mild symptoms stage to severe stages of acute respiratory illness or respiratory failure. In the mild stage, symptoms such as cough, fever, and breathing problems are observed, while in severe cases, pulmonary edema, hyaline formation, and pneumocyte desquamation are observed.

The SARS-CoV-2 virus primarily invades human lung epithelial cells, making them one of the first targets. The virus is transmitted through droplets, which primarily infect upper airway epithelial cells that are rich in Angiotensin Converting Enzyme 2 (ACE2) viral receptors, leading to subsequent spreading to the lower airways in severe cases. Pulmonary pathology has been defined as diffused alveolar damage (DAD) and subsequent Acute Respiratory Distress Syndrome (ARDS) with severe and frequent thromboembolic complications with intravascular fibrin deposition. The systemic manifestations of the virus result from delayed immune response against the infection and a lack of knowledge about the virus and the immune response generated against it. The severity of COVID-19 correlates with comorbidities such as older age, cancer, pneumonia,

obesity, cardiovascular disease, diabetes, immunosuppression, and lung-related problems. The immune response against COVID-19 is an outcome of both innate and acquired immunity (Melenotte et al., 2020; Szekely et al., 2021).

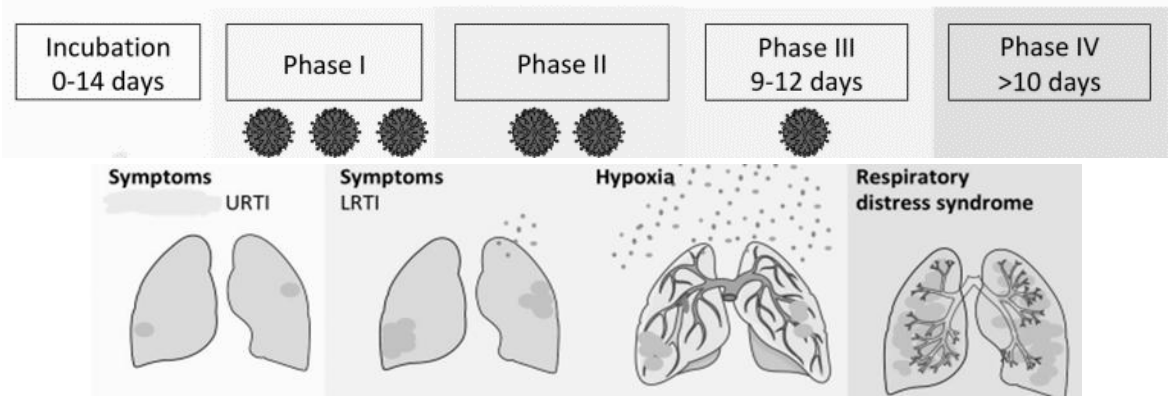


Fig-1: Natural history of COVID- 19 Infection

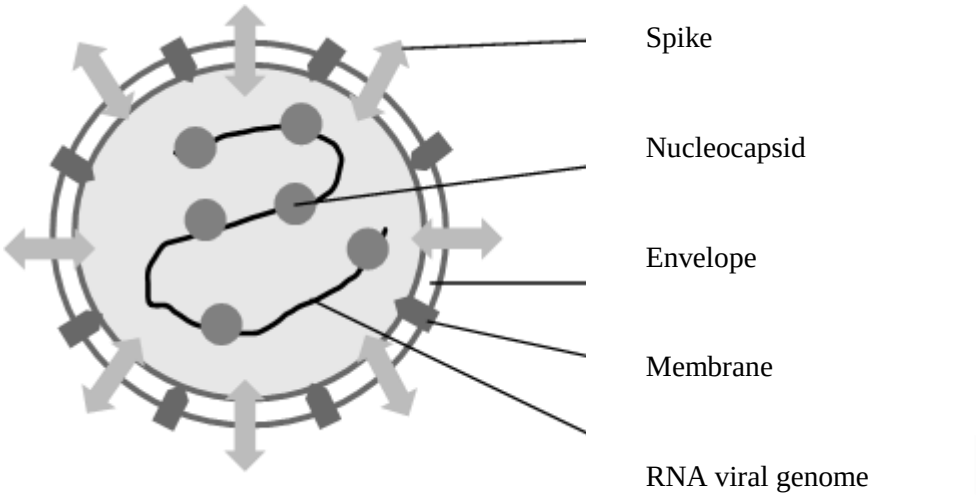


Fig: Structure of SARS-Cov-2

The corona virus structure is crown like having spikes on their surface in Latin this type of structure is called "Corona". Based on the structure of their genome they are divided into alpha, beta, gamma and delta subgroups out of which alpha and beta infect mammals. They are pleomorphic and enveloped (lipid protein) having positive sense single stranded RNA with RNA polymerase of size nearly 29.9kb having 6 extra open reading frames (ORFs) in genome. The genome code for four main structural proteins which are nucleocapsid protein, spike protein, envelope and membrane protein. Viral genome also codes for several non-structural proteins. Out of four structural proteins spike proteins identify host and enable virus to attach on specific receptors which poses various receptor binding domains which binds to AEC-2. Protease enzyme of host cell dissolve the viral membrane due to which RNAs are released into host cell taking over cell machinery and inhibiting immune response by producing virulence factors (Hosseini et al., 2020, King et al., 2020, Boopathi et al., 2021)

**Anatomy of lungs of the infected person**

The lungs are complex and important organ with specialised structure made for exchange of gases to fulfil the oxygen demand and release out carbon di oxide from human body. The study of abnormalities in lungs of COVID-19 patients is important as it is the main damaged organ due to infection. The lungs are divided into upper and lower respiratory tract containing structures like bronchioles and alveoli. The COVID- 19 infection initially takes place in upper respiratory tract of lungs and gradually spread in lower respiratory tract (Melenotte et al., 2020, Wang et al., 2020).

According to Szekely L et al. study, the lungs of infected persons were heavy and fluid filled, approximately 2.8 times heavier than normal lungs. Lungs of infected persons were firm to touch with rubbery consolidations. Lungs had negligible air content. The posterior part of the lower lobes of lungs were free from air and were fully consolidated (Szekely et al., 2021).

The mucous membranes of most of the patients were found unaffected. But some patients show purulent trachea bronchitis and some shows blood filled swollen mucous membrane. (Szekely et al., 2021).

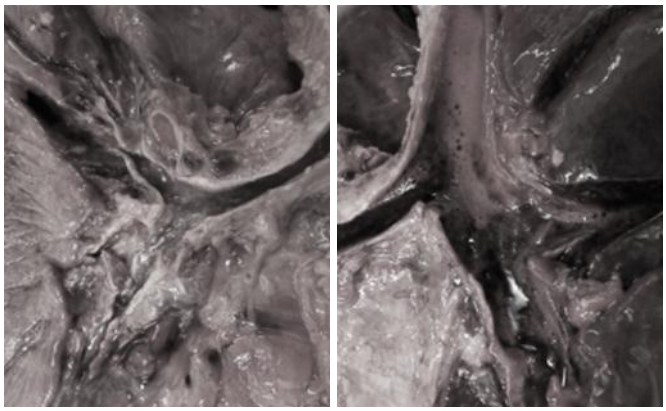

Fig: 3. Purulent tracheal bronchitis (left); Blood filled swollen mucous membrane (right)

The most severe pathological change that occurred in the lung parenchyma started with immense cytopathogenic effects on both Type I and Type II pneumocytes. On infection the pneumocytes were very much swollen and assembled as single cells. The damaged alveoli were often filled with edema fluid, plasma, coagulated fibrin, blood or coagulated blood and cells like macrophages, neutrophils and lymphocytes. The viral RNA mostly replicates in pneumocytes. The desquamated pneumocytes regularly showed advanced cellular abnormalities/atypia with nuclear heterogeneity and unusual and strange shaped cytoplasm. Due to these abnormalities in cellular structure pneumocytes were casually demonstrated as “COVID cells”. The regular denudation of the alveolar wall was often accompanied with the damage of the alveolar capillaries and release of plasma and blood into the alveolar space resulting in infection of alveolar epithelial cells. Diffuse alveolar damage in the patients of COVID- 19 was observed due to massive production of hyaline membrane structure which contained residues of the debris of alveolar epithelial cells, macrophages and serum proteins. (Szekely et al., 2021, Wang et al., 2020, Aguiar et al., 2020).

Large intra vascular thrombus formation in branches of pulmonary arteries was observed in some cases which were composed of aggregated thrombocytes. The conditions like trachea bronchitis/ pneumonia are observed induced by viral infection and aggravated by bacterial superinfections. Lung consolidation was accompanied with the accumulation of large number of macrophages and immature myeloid elements along with substantial proliferation in both epithelial and stomal components. The central part of lungs was mainly affected due to infection in comparison to peripheral parts which were less affected (Szekely et al., 2020, Aguiar Diego et al., 2020).

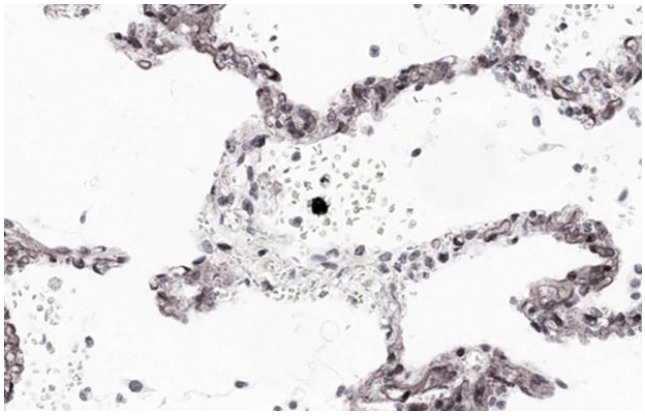

Fig-4: Damage of alveolar capillary wall due to COVID- 19 infection
that led to intra alveolar bleeding

## Immune response produced against COVID-19 Infection

### Innate Immune Response

Pulmonary innate lymphoid cells (ILCs) play important role in mitigating viral infection in lungs. Following types of ILCs take part in producing immune response against COVID-19 infection:

### 1. Macrophages

Pulmonary macrophages maintain lung homeostasis by clearing dead cells and invading pathogens and phagocytose the pathogen cells. There are two different types of macrophages present in lungs: (i) The Alveolar Macrophages (AMs) and (ii) Interstitial Macrophages (IMs). The AMs arise mostly from fatal hepatic monocytes. The IMs are located with dendritic cells and lymphocytes in the interstitium. IMs are of two types: CD206+ which are tolerogenic and secretes chemokine and CD206- which acts as antigen presenting cells. A unique subset of IMs known as "Nerve airway macrophages" (NAMs) mitigate viral infection through Interleukin-10. Broncho-alveolar lavages (BALs) from patients having sever infection contained more macrophages. Pulmonary macrophages specifically AMs can control resident memory T cells (TRMs) of lungs. TRMs are perfectly positioned against respiratory pathogen such as corona virus. Viral infection of epithelial cells leads to the activation of macrophages and dendritic cells which secret IL-12, IL-18 and type I-INF along with-it activating NK cells to exert effector function. Damaged epithelium sensed by AMs releases IL-25, IL-33 and TSLP alarmins acting on ILC-2 to stimulate their proliferation and effector function. ILC-2 derived amphiregulin (AREG) which is widely expressed transmembrane tyrosine kinase promote epithelial cell repair (Melenotte C et al., 2020).

**2. Monocytes**

Monocytes are haematopoietically derived innate immune cells whose function ranges from inflammatory to anti-inflammatory response. They are classified as classical (CD14high), non-classical (CD16high) and intermediate monocytes (CD14+CD16-). During COVID-19 CD14+ monocytes are overactivated which results in emergence of monocytic subsets including myeloid derived suppressor cells (MDSC) with in COVID-19 patients (Mukund et al., 2021).

**3. Low-Density Neutrophils**

It is the term used for heterogenous group of different types of neutrophil cells composed of a mixture of immature and low-density mature neutrophils, progenitor cells and granulocytic/ polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs). Low density neutrophils are highly proliferative and activated mostly in severe COVID-19 cases. Low density granulocytes are significantly proinflammatory and interferon-1 responsive. PMN-MDSCs play important role in the immune dysregulation in severe COVID-19 infection cases. Megakaryocytic expansion in severe COVID-19 cases also reported. Thrombocytopenia is associated with increased platelet demand and exhibit emperipolesis observed in COVID-19 cases is fulfilled by Megakaryocytes. (Mukund et al., 2021).

**4. Natural Killer Cells**

NK cells are non-phagocytic lymphocytes that respond rapidly to eliminate or control pathogens in host cell including tumour progression, microbes and viruses. During COVID-19 activation of NK cells with impaired cytolytic/osmotic lysis potential and reduced absolute cell counts, with increased severity in infection was reported. NK cells also show increased interferon signalling with increased expression of the inhibitory surface protein in moderate and sever patients (Mukund et al., 2021).

**Acquired Immune Response**

Acquired/Adaptive immunity is crucial for successful viral clearance and long-term immunity.

**1. T cells**

Virus clearance during a primary response induced against COVID-19 infection depends on coronavirus virus specific CD4+ and CD8+ T cell response. Compared with healthy individuals, the number of T-cells were reduced in severe COVID-19 patients. Higher proportion of CD8T/CD4T effector cells are observed in higher proportions in non-severe subjects as compared to healthy individuals but this number decreases with increase in severity. An increase in CD8T effector and CD4t naïve cells is reported in patients with non-severe subjects. In addition to this increase in CD4+ Tregs was also reported which are crucial for regulating immune homeostasis and autoimmunity, controlling the quality and magnitude if immune response. The cluster CD16+CDT8 subset showed significant increase in proportion of cells within both severe and non-severe COVID-19 patients. A notable decrease in cellular abundance of low frequency T subsets including Mucosal Associated Invariant T and Gamma Delta T cells was seen in COVID-19 individuals. MAITs are class of non-conventional T cells and respond to innate inflammatory signals including IL-12, IFN-γ and IL-18 with viral infection including COVID-19. Despite low abundance MAIT cells in severe and non-severe disease showed increased activation. T cell Lym-

phopenia include a sustained type I INF response and high level of stress-induced glucocorticoids together contributing to T cell apoptosis (Qin et al. 2020, Melenotte et al., 2020, Mukund et al., 2021).

**2. B cells**

B cells are observed to produce immune response against COVID-19 infection. The B cells play a crucial role against SARS CoV-2 by producing immunoglobulins that provide protection against protection from mild disease to severe stage. Immature B cells mature to naïve B cells and proliferate into memory cells and plasma B cells upon antigenic activation. With increase of severity B cell dysfunction with decrease in multiple cell types including naïve B cells is observed. Plasma B cells exceptionally showed expansion and heterogeneity with increasing disease severity. Exceptionally increase in Plasma B cells concentration is observed with increase in severity of infection (Qin et al.2020, Melenotte et al., 2020, Mukund et al., 2021).

**Antigen Presenting Cells (APCs)**

Classical Dendritic Cells (cDCs) acts as professional APCs that initiate and regulate the pathogen specific adaptive immune response by providing antigen for proliferation of lymphocytes and production of antibodies and associated factors. cDSc can be subdivided into cDC1 and cDC2 based on their expression of cell surface markers, gene expression profile, specific transcription factor required for their development and unique functions. During COVID-19 infection decrease in APCs is reported resulting decrease in DC subsets favouring lymphopenia. Highly polarized human lung epithelial cells infected by SARS-CoV-2 can modulate the intrinsic functions of monocyte-derived macrophages and dendritic cells (DC) respectively. They slow down the APC functions of DC and macrophage phagocytosis through a mechanism involving IL-6 and IL-8. In contrast, cDC1 resist to most viral infections, thereby maintaining their APC functions through their constitutive expression of vesicle trafficking protein RAB15 (Melenotte et al., 2020).

**Interferons**

Type I interferons is main INF signalling during COVID-19 which includes INF-α, β and ω cytokines, producing cellular antiviral response through the JAK-STAT signalling pathway and through the induction of interferon stimulated genes (ISGs). An impaired type I IFN response was observed in severe COVID-19 infection cases. Patient with low type I IFN plasma level reported high blood viral load. Human coronavirus encodes multiple structural and non-structural proteins that antagonize IFN and ISG response. Genetic factors may influence the IFN response and explain the individual variability in antiviral response. Pathogen associated molecular patterns (PAMPs) such as ssRNA or viral protein in case of COVID-19 trigger the activation of transcription factor leading to pro inflammatory cytokine and type I IFN induction. Viral replication is actually accompanied by a delayed type I IFN signalling that produce inflammatory responses and lung immunopathology. Type I IFN remained detectable until after the peak of viral titres, and delayed IFN-I signalling promoted recruitment of pathogenic inflammatory monocytes/macrophages, resulting in vascular leakage and impaired virus-specific T cell response (Melenotte et al., 2020).

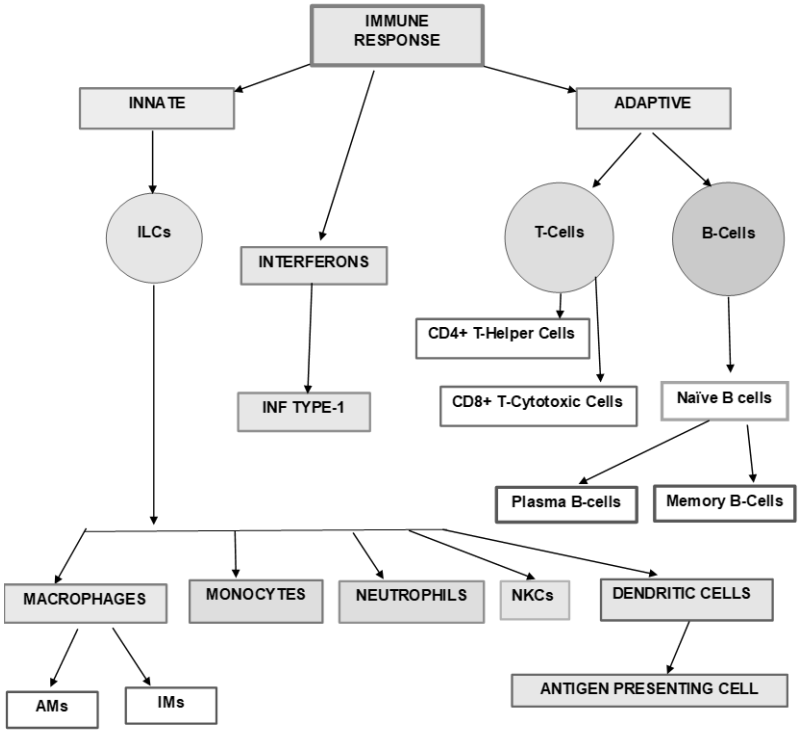


Fig-5: Immune Response during Covid-19 flow chart

### Vaccination

The idea behind vaccination is to expose the host body against the antigen that will not cause disease but will provide immune response against viral infection.

There are ongoing trails and development of vaccines mainly based on four broad types which are Protein Based Vaccines (PBVs), Virus Like Particles Vaccines (VLPs), Nucleic Acid Vaccines (NAVs) and Virus Vector Vaccines (VVVs). In PBVs direct injection of viral protein into host or injection of viral antigens developed from recombinant protein techniques take place. In VLPs weaker or inactive form of virus or self-assembled virus protein structure lacking viral genome is induced in host body. From NAVs proteins are developed or gene encoding viral antigenic components that are expressed by plasmid vector induced into host cell. In VVVs virus like adeno virus is genetically engineered to provide antigens for immune response against target virus. There are several vaccines developed based on various factors like eGFP-SARS-COV-2 which precent binding of virus at ACE2 receptors, recombinant adenovirus type-5 vaccine & BNT162b1 vaccine which produce antibodies against the virus and live attenuated vaccines which are considered as successful vaccines (Li et al., 2020, Melenotte et al., 2020).

## Conclusion

The COVID-19 infection served as most deadly pandemic to whole human kind. The disease had great impact on global health care system due to lack of knowledge about the life cycle of virus, detection and prevention measures. COVID-19 had a great impact on respiratory systems of infected individuals initially which later resulted into serious health problems or death. Study of

both virus infection induced cell damage and response of hosts of different age group should take place, as most of the deaths were of old age individuals.

Both innate and adaptive immune response immune responses induced virus clearance, inhibited virus replication and promoted tissue repair mechanism.

Both the type of immune responses and their interaction with interferons should be studied comprehensively. There is an urgent need for a high dimensional longitudinal follow up of the underlying immunological mechanisms across the different stages of the COVID-19 to make more rational and personalised therapeutic decisions. The current goal should be development of broad range of effective vaccines against coronavirus keeping in mind of different strains of the virus which are generated due to mutation.

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*Review Article*

# Impact of UV Radiation on DNA Repair Mechanism in Xeroderma Pigmentosum

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## Abstract

Xeroderma Pigmentosum, an autosomal recessive disorder first described by Kaposi in 1874, is caused by mutations in one or more of the 7-8 XP genes that are involved in the nucleotide excision repair system (NER). This disorder results in the accumulation of cyclobutane pyrimidine dimers or 6-4 photoproducts in the DNA of affected individuals due to high UV exposure. These individuals exhibit low tolerance to sunlight and may experience inflammation even with short periods of sun exposure. In affected organisms, the photolyase enzyme responsible for reversing thymine dimers becomes inactive, leading to excessive sunburns, lentigines, and dermal pigmentation on the skin.

The normal functioning of all 7-8 XP genes is crucial for proper DNA repair, and any alterations can result in various abnormalities. The first set of XP genes (XP-A, XP-C, and XP-E) recognize the affected DNA region, followed by XP-B and XP-D, which unwind the distorted area. XP-F and XP-G then act as endonucleases and remove the lesioned part of approximately 30 nucleotides.

The present paper provides detailed information on each XP gene in both its unmutated and mutated forms, as well as the various signs and symptoms associated with Xeroderma Pigmentosum.

*Key words: Xeroderma Pigmentosum, XP gene, Mutation, Thymine dimers, UV rays, Lesion, NER system, Cyclobutane pyrimidine dimer, 6-4 Photoproducts, skin cancer*.

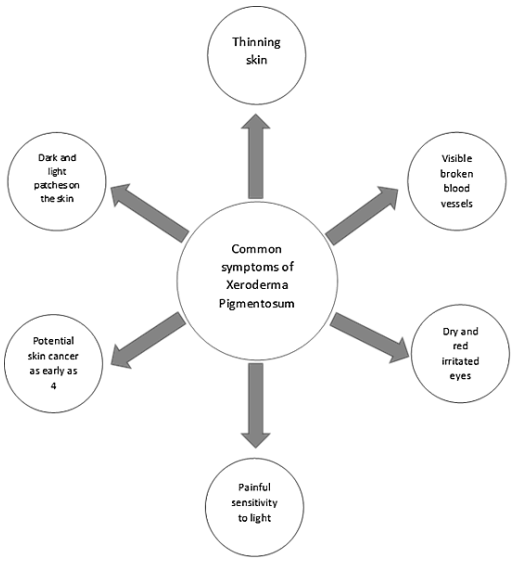
## Introduction

Xeroderma pigmentosum (XP) is a genetic disorder resulting from a defect in the nucleotide excision repair system, a crucial DNA repair mechanism (Black, 2016; Kraemer and Slor, 1985). Individuals with XP exhibit high sensitivity to environmental factors such as UV rays, which exacerbate their condition (Cleaver and Bootsma, 1975; Kraemer and Slor, 1985; and Norgauer et al., 2003).

XP is an autosomal recessive disorder, and its discovery dates back to 1874 when Moritz Kaposi first described it. It was not until 100 years later that James Cleaver reported that XP was caused by defective DNA repair in XP cells (Cleaver and Bootsma, 1975; Lehmann et al., 2011). Normal cells have seven complementation groups from XP-A to XP-G, which work in a coordinated manner, but XP cells have mutations in one or more of these genes, leading to the persistence of thymine dimers (Cleaver, 1968; Norgauer et al., 2003). Exposure to UV rays from sunlight induces cyclobutene pyrimidine dimers (CPDs) or 6-4 photoproducts (6-4 PPs) that alter DNA structure,

resulting in reduced minimal erythema dose during early life. Other symptoms of XP include excessive freckling, lentiginous pigmentation, poikiloderma, and degenerative changes in the skin and eyes, which can cause neoplasia. Liposomal encapsulated T4 endonuclease V or photolyase, which are supposed to repair CPDs through photoreactivation, are also ineffective in XP cells (Cleaver, 1968).

Data indicates that XP occurs more frequently in the Japanese population, affecting one in every 20,000 people, while in the US, it affects one in every 300,000 people, and in Western Europe, it affects about 2.3 per million people (Norgauer et al., 2003).




**XP genes in healthy individuals**

The eight risk genes including the variant type gene is responsible for causing different types of xeroderma pigmentosum in eukaryotic organisms. A cell with normal repair mechanism has all the XP genes playing their role accurately that repairs mutation in DNA. (Cleaver, 1968; Lehmann et al., 2011) Series of these risk genes, XP-A, XP-B, XP-C, XP-D, XP-E, XP-F, XP-G and a variant type as XP-V, removes the bulky chemical lesions from DNA. Accessory factors like DNA polymerase, PCNA, RPA and DNA ligase are required to reseal the DNA fragment through de-novo DNA synthesis. (DiGiovanna and Kraemer, 2012; Gratchev et al., 2003 and Sugasawa, 2008)

This genome repair system functioning in all eukaryotes, called nucleotide excision repair system functions when the UV induced CPDs or 6-4 PPs blocks the progress of RNA polymerase during pathway of molecular expression. (DiGiovanna and Kraemer, 2012) The mechanism is initiated when three of total XP genes recognizes the lesion. These genes are XP-A, XP-C and XP-E, with XP-C as central molecule. Once the area of lesion is determined, another pair of XP genes called XP-B that works along with ERCC-3 and XP-D that works with ERCC-2, functioning along with transcription factor II, starts unwinding the DNA surrounding the area of lesion via its helicase activity. (DiGiovanna and Kraemer, 2012; Sugasawa, 2008). The single stranded DNA is now worked upon by another set of XP gens called XP-F and XP-G having endonuclease activity. XP-F works with ERCC-1 on the 5’ side of distorted DNA and makes a cut about 8 nucleotides away, meanwhile XP-G makes a cut on 3’ side of target DNA resulting in the excision patch removal of around 30 nucleotides containing the lesioned part.

After the removal of DNA patch, XP-B and XP-D are no longer associated with the complex and the resultant gap is filled by polymerization activity of DNA polymerase delta accompanied by gap sealing activity of DNA ligase-ATPase complex. (DiGiovanna and Kraemer, 2012)

The entire system is so co-ordinated that mutation in even one of the XP gene can result in complete misfunctioning and hence persistence of CPDs or 6-4 PPs occurs in DNA causing Xeroderma Pigmentosum.

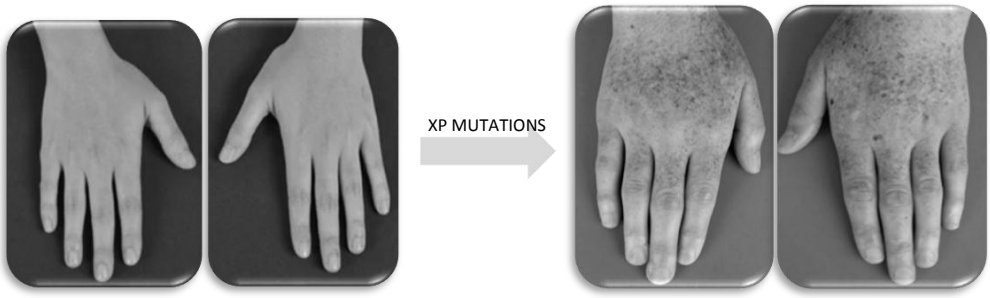



Fig-1: - A-Normal skin, B- Pigmented skin

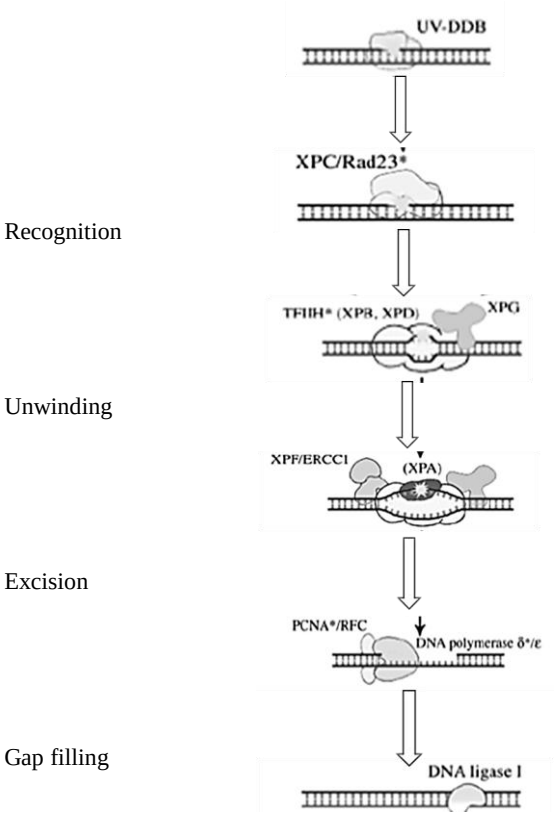



Fig-2: - Global genome repair (GGR) showing NER system

**Mutation in XP genes**

Out of all seven to eight XP genes, each one of them affect different steps of mechanism making it worse against the protection of UV.

**XP-A**

The gene product of XP-A, earlier thought to be responsible for identification of damaged DNA, is now considered to be recruited after TF II-H factor. (Sugasawa, 2008) Another protein called RPA works along with XP-A, this RPA-XP-A complex is known to bind and stabilize the unwinded part of DNA. (DiGiovanna and Kraemer, 2012; Sugasawa, 2008) Its location is precisely on the q arm of 9th chromosome at designated region of 22.3. (Xeroderma Pigmentosum 2019, Sugasawa, 2008) Mutation in XP-A gene product causes severe form of xeroderma pigmentosum as the ability to repair DNA vanishes, resulting in increased sensitivity towards UV light. People with XP-A mutation shows initiation of skin cancer at early age and severe neurological abnormalities. With mutation rate of only 25%, XP-A is majorly involved in formation of pre-incision complex also. (Sugasawa, 2008).

**XP-B/ERCC-3**

XP acts as co-worker of ERCC-3, it is one of the ten factors of TF II-H. XP-B protein has 3' - 5' helicase activity that is essential for unwinding the distorted part of DNA. Along with XP-D, XP-B moves on the DNA strand making it single stranded in the lesioned area. (Sugasawa, 2008) With the least frequency out of all XP types, affected individuals with mutated XP-B shows increased sensitivity to UV light. The appearance of symptoms of Cockayne syndrome might be the indicator of XP-B mutation. Located on q arm of 2nd chromosome's 21st region, its mutation can also result in mild neurological abnormalities.

**XP-C**

Specific for global genome repair, XP-C acts as central molecule that recognizes the distortion and recruits another XP gene products i.e., XP-A and XP-E. The heterotrimer structure of XP-C with two human orthologs of Saccharomyces cerevisiae as Rad23p and Centrin-2 is involved in stabilization of XP gene product. Out of these two, Centrin-2 has the ability to potentially identify the lesion. (Sugasawa, 2008) Located at p arm of 3rd chromosome's 25th position, XP-C prefers to bind at a junction of double and single stranded regions, hence XP-C recognition is best identified when DNA helical distortion is associated with local unwinding. (Xeroderma Pigmentosum 2019, Sugasawa, 2008) Mutation in XP-C is considered the most frequent type of XP in Caucasian population. Although its mutation shows increased sensitivity towards UV light, this variant has highest ability to repair DNA. Individuals with affected XP-C gene products show no neurological disability but may express a typical and severe lentigines at sun exposed area including ocular abnormalities.

**XP-D/ERCC-2**

Another component of TF II-H, XP-D is the helicase containing XP gene product that opens DNA in the direction of 5' to 3'. The helicase activity of XP-D is in the opposite direction to XP-B and both these XP gene products are considered to migrate together on double stranded DNA. (DiGiovanna and Kraemer, 2012; Sugasawa, 2008) With mutation rate of only 15%, affected individuals shows sensorineural deafness, ataxia and mental retardation. Located on q arm of 19th chromosome's 13.2-13.3 region, mutation at this position results in increased UV sensitivity. (Xeroderma Pigmentosum 2019) Additional functions of XP-D might involve formation of pre-incision complex and opening of promoter assembly. (Sugasawa, 2008).

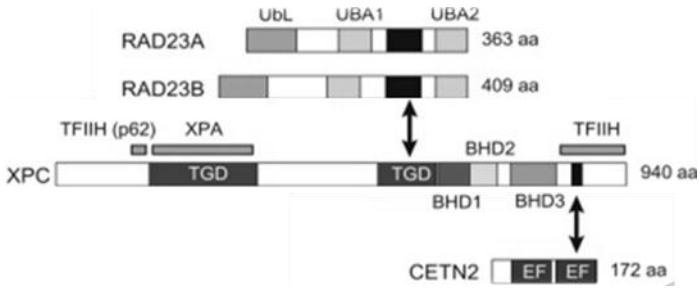



Fig-3: - XP-C gene on chromosome number 3

**XP-E/DDB-1; DDB-2**

Located on p arm of 11th chromosome's 11-12 position and q arm of 11th chromosome's 12-13 position, XP-E gene product plays a side role in identification of distorted part of DNA. With very low frequency of XP type, it is very aggressive and restricted to some dermal infections only. Skin cancer may develop at later stage. (DiGiovanna and Kraemer, 2012).

**XP-F/ERCC-4**

Once the pre-incision complex is assembled by XP-A, two gene products namely XP-F and XP-G are introduced to single stranded portion containing CPDs or 6-4PPs, making a cut at both the ends of lesion. XP-F specifically cleaves at the 5' end of single stranded DNA few nucleotides away from distortion. (Sugasawa, 2008) Located on p arm of 16th chromosome's 13.3 region, its mutation can cause cutaneious cancer in Japanese population.

**XP-G/ERCC-5**

Located on q arm of 13th chromosomes' 32-33 region, mutation in this occurs with very less frequency but is aggressive. (Xeroderma Pigmentosum 2019) Normally, XP-G making a cut at 3' end of single strand DNA belongs to flap endonuclease-I family. Depending on the type of lesions or dimers, the exact position of XP-F and XP-G cut can vary significantly, but the excised patch is usually ranged between 24-32 nucleotides. (DiGiovanna and Kraemer, 2012; Sugasawa, 2008).

| S. No. | XP gene | Related gene | Location | Normal function | Symptoms of mutation |
|---|---|---|---|---|---|
| 1. | A | - | 9q22.3 | Thymine dimer recognition | Early age skin cancer, neurological abnormalities |
| 2. | B | ERCC-3 | 2q21 | 3'-5' helicase | Characteristics of Cockayne syndrome |
| 3. | C | - | 3p25 | Initial damage recognition | Typical and dense lentigines, eye problems |
| 4. | D | ERCC-2 | 19q13.2-q13.3 | 5'-3' helicase | Sensorineural deafness, ataxia, mental retardation |
| 5. | E | DDB-1; DDB-2 | 11p11-p12; 11q12-q13 | Damage recognition | Cutaneous cancer |
| 6. | F | ERCC-4 | 16p13.3 | 5' endonuclease | Cutaneous cancer |
| 7. | G | ERCC-5 | 13q32-q33 | 3' endonuclease | Skin cancer, neurological abnormalities |

Table-1: Comparison of XP genes

Once the distorted part is removed off from the DNA, the gap filling activity is to be performed by DNA polymerase. Additional factors like PCNA and RFC enhances the polymerization activity. (Sugasawa, 2008).

Following this, the final gap between last two nucleotides is sealed by DNA ligase. Once the gap is filled and sealed, the CPDs or 6-4PPs is now being vanished and in place of that normal nucleotide inserted making it unaffected for later DNA replication.

**Conclusion**

Xeroderma Pigmentosum is a genetic disorder caused by mutations in the XP gene products, leading to significant impacts on affected individuals. Although not fatal, this condition manifests with various phenotypic defects, including neurological irregularities, CNS tumors, and malignant melanomas. One crucial indicator of Xeroderma Pigmentosum is the hyperplasia of melanocytes, resulting in severe lentigines on the skin.

Researchers have made substantial efforts to identify the precise locus of each XP gene and its functions in both healthy and affected states. Currently, the focus is on investigating the underlying causes of each mutation, and finding the most effective approach towards a cure. While significant progress has been made in developing medication strategies, further research is needed to understand the precise mechanism of each XP gene responsible for causing Xeroderma Pigmentosum. Therefore, it is imperative to prioritize ongoing research efforts to enhance our understanding of this debilitating condition and develop effective treatment options.

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*Review Article*

# Immunosuppressive Drug Therapy and its Effect on Mesenchymal Stem Cells

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## Abstract

The isolation of Mesenchymal Stem Cells (MSCs) was primarily carried out from bone marrow, but these cells have also been identified in other tissues such as adipose tissue, skeletal muscle, umbilical cord, and dental pulp. While MSCs can be found throughout the body, they are more frequently present in regions with inflammation, tissue damage, and lymphoid organs. In the context of vascularized composite allotransplantation (VCA), the use of MSCs has been investigated for their immunological potential in cell therapies. Due to their limited expression of class II Major Histocompatibility Complex (MHC) and stimulatory surface chemicals, MSCs are immune-privileged and possess immunosuppressive properties. As a result, they can regulate the immune system and exert diverse immunosuppressive effects during graft rejection. MSCs have been recognized as a valuable source of immunosuppressive cytokines, including IL-10 and TGF-ß, which are critical contributors to tissue regeneration. MSCs are capable of inhibiting the maturation of B cells, the release of antibodies, cytokine secretion, and cytotoxicity of T and NK cells, among other immune cell functions. However, it should be noted that polyclonal antibodies such as ATG or ALS, which are commonly used as induction agents, can be detrimental to stem cell therapies.

*Keywords: Mesenchymal stem cells, Immunosuppressive, Major Histocompatibility Complex, Inflammatory response, Antibodies, Immunomodulation, Anti- thymocyte globulin, Organ transplantation, Cell therapy.*

## Introduction

More than three decades ago, Mesenchymal Stem Cells (MSCs) were first isolated from bone marrow (Dazzi et.al, 2012), and since then, they have been identified in various tissues, including the spleen, heart, skeletal muscles, synovium, amniotic fluid, bone marrow, and nearly all postnatal connective organs. MSCs are composed of multiple organs and heterogeneous cell populations (Claudia et.al, 2014).

When MSCs are exposed to suitable inflammatory environments, soluble factors are triggered, while some factors are constantly secreted by MSCs, which are made up of numerous precursor cells, endothelium cells, and pericytes (Miguel et.al, 2012). These cells possess both immune-modulatory and immunosuppressive characteristics and have been shown to increase the longevity of skin and cardiac grafts in experimental investigations. MSCs have also been examined in clinical protocols to promote engraftment in hematological stem cell transplantation and to influence the recovery process (Dazzi et.al, 2012; Claudia et.al, 2014).

MSCs, also known as multipotent mesenchymal stromal cells, are non-hematopoietic, multipotent progenitor cells that can differentiate into mesenchymal lineages in vitro and in vivo. These cells are present in almost every organ and serve as the primary source for tissue repair and regeneration. It is believed that tissue damage and pro-inflammatory factors trigger MSCs.

Studies have shown that there are two-way interactions between MSCs and inflammatory cells. When triggered by an inflammatory response, MSCs give off immunosuppressive feedback that helps to restore tissue homeostasis and initiate the tissue repair process.

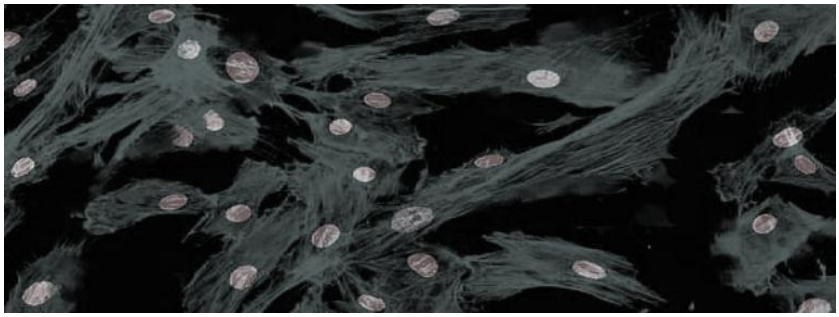

Fig 1: Blood-Derived Mesenchymal Stem Cell

## Mesenchymal Stem Cell and its Immunosuppressive Properties

In 2000, two groups demonstrated that human mesenchymal stem cells generated from bone marrow may persist for more than a year without being rejected in a fetal safe after developing immunological competence.

Even more amazing was the unanticipated immunosuppressive effects of MSC in mice, baboons, and humans in vitro and in vivo conditions, even in an MSC, independent way. After system injection, MSC is distributed to practically all organs, however, they are more frequently found in damaged areas, inflammatory regions, and lymphoid organs. Due to all these benefits, MSC treatment was taken into consideration for immunomodulatory purposes (Shi et.al, 2012; Wakako, et.al, 2015).

Many medications and antibodies have been developed for the induction treatment and maintenance of immuno-suppression in clinical SOT, and clinical VCA (vascularized composite allotransplantation) procedures which have been extensively utilized by them.

Clinical methods employ the addition of tolerance with lymphocyte-depleting antibodies like ATG (anti–thymocyte globulin) or alemtuzumab to reduce recipient all responses (Wakako et.al, 2015). Polyclonal antibodies like ATG or ALS (anti–lymphocyte serum) are well-known induction agents that appear harmful to stem cell treatments when administered concurrently with antibody therapy (Dazzi et.al, 2012). ALS is identical to ATG and has been investigated in preclinical models of SOT (solid organ transplantation).

MSC have greater plasticity, including the ability to differentiate into non-mesenchymal tissues. In periodontal tissue, clusters of differentiation (CD) 29, CD 44, CD 73, CD 90, CD 105, and chemokines receptor type 4 are the positive cells surface markers of Mesenchymal cells (Gronthos et.al, 2002; Claudia et.al, 2014).

To describe an inactive lesion, MSC molecules go through a process of proliferation and differentiation that involves cell fate markers. The MSC recruitment inhibition led to a reduction in the expression of inflammatory and anti-wound healing markers and an increase in the production of inflammatory and osteoclastogenic cytokines. MSC has been viewed as an essential source of immunosuppressive cytokines including IL-10 (Interleukin-10) and TGF-beta (transforming growth factor) which is a beneficial contributor to the process of tissue regeneration (Miguel et.al, 2012).

## Innate Immune Response and its Effect on MSC

The Innate response is crucial in the T cell response rejection of proinflammatory mediators. It is well established that acute T cell cytotoxicity rejection and Delayed type hypersensitivity (DTH) affect the adaptive immune response.

### 1. Dendritic Cells (DC)

Dendritic cells (DC) are antigen-presenting cells (APC) that are important in the early stages of the innate immune response. In the presence of Granulocyte-macrophage colony-stimulating factor (GM- CSF) and Tumor necrosis factor alpha (TNF- alpha), invitro DC may be produced from both cord blood/blood CD34+ cells and CD 14+ peripheral blood monocytes cells (Miguel et.al, 2012).

Tolerance is only induced by the mature DCs when costimulatory molecules do not bind and result in T cell anergy or death of the cell. MSC may stop DCs from properly maturing and differentiating from their progenitors. The expression of MHC and costimulatory molecules by DC in the presence of MSC may not change, but they may lose their frequency activity (Spaggiari et.al, 2009).

As a result, dendritic cells generate less interleukin-12 (IL-12) and are unable to activate T cells. MSC can also reverse the condition of DC from mature to immature when co-cultured.

### 2. Natural Killer Cells (NKs)

Because of their significant involvement in the cytotoxicity caused by MHC molecules, NKs are particularly relevant when the innate response is rejected. The danger signals that NKs perceive through their inhibitor receptor NKG2 are low or null levels of the MHC-1 molecule present on the surface of a cell, which is the typical tumor and virus-infected cells.

MSC can stop the activation of NKs, preventing their enormous IFN- Gamma and tumor necrosis factor-alpha (TNF- alpha) production and cytotoxic impact. NKs produce interferon-gamma and TNF-alpha when activated (Sotiropoulou et.al, 2006). With a little IFN-gamma release, non-activated NKs first excite to MSC, and these MSC inhibit the lytic capacity of NKs.

MSC can repress NKs and stop them from becoming cytotoxic and secreting cytokines. These receptors are downregulated when NK cells are co-cultured with their stimulators or target cells and MSC.

The expression of the MHC-I molecule is up-regulated in MSC after exogenous IFN-gamma exposure, preventing NKs from killing them. Allogenic and Autogenic Cells are capable of being killed by NKs previously stimulated by IL-2 or IL-15 and LFA-1, NKP- 30 and NKP-2D which mediate this interaction with cells (Crop et.al, 2011; Wakako et.al, 2015).

## Adaptive Immune Response and its Effect on MSC

Two important components of the adaptive immune response are the T and B cells. A naïve B cell uses an MHC-II molecule to recognize an Ag (Antigen) from a CD4+ T cell (Th2 – Lymphocyte T Helper 2) B cells are also prompted to start producing IgM at this time. When the Th2 hits this B cell, which was previously primed by an APC (antigen-presenting cell) with the same Ag, T cells release cytokines that cause B-cell growth and differentiation into plasma cells. The plasma cell converts, from the switching of IgM to IgG, IgA, and IgE antibodies against that particular Ag. B cells can also survive and function as memory B cells (Wakako, Jonas, and Schinder, 2015).

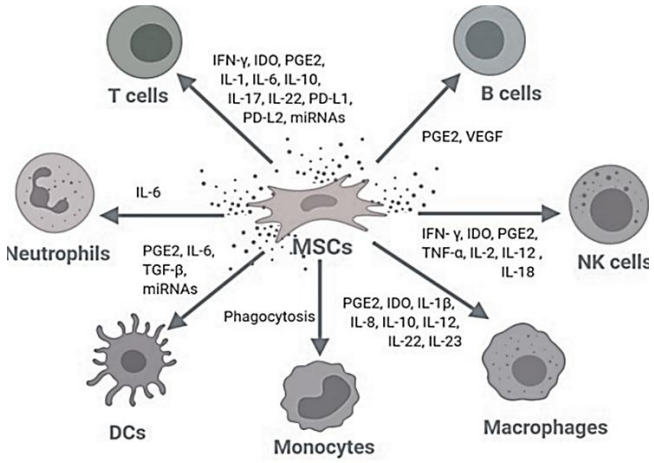


Fig 2: Mesenchymal Stem Cell Immunomodulation

### 1. B - Cells

MSCs may disrupt B cell proliferation, differentiation, maturation, and antibody production. When LPS, CpG, Oligodeoxynucleotides (types of drug), IL-2, and IL-4 are added to B cell cultures to stimulate them, MSC inhibits the multiplication of the B cells (Corcione et.al, 2006). B cell growth and immunoglobulin production are suppressed when condition media from a co-culture of Mice with either stimulating compounds or B cells is employed.

MSC-conditioned media alone has little impact on the growth of B cells. MSCs must therefore be activated to use their suppressive abilities. B lymphocyte's ability to deliver antigen is unaffected by MSC since these conditions have no impact on the expression of MHC- II, CD 40, or CD 80. When B cells downregulate their chemotaxis receptors CXCR4 and CXCR 5 in the presence of MSCs, migration caused by chemokines seems to be halted (Corcione et.al, 2006; Gronthos et.al, 2002).

### 2. T - Cells

Both autologous and allogeneic MSCs have been demonstrated to effectively block the T-cell response of allogenic cells, phytohemagglutinin (PHA), IL-2, and mitogens. In a dose-dependent way, MSCs selectively influence T-cell proliferation, IFN-gamma release, and cytotoxicity. If T cells have not been stimulated earlier, MSCs should not have caused a response. IFN-gamma,

which is released by T cells after being stimulated by allogeneic PBMCs (peripheral blood mononucleosis cells) or mitogens, is thought to be the catalyst for MSCs' immunosuppressive qualities (Gronthos et.al, 2002).

MSCs can produce anergy in T cells and cause them to enter the G1 cell cycle phase. The MSc-released substance known as IDO suppresses T lymphocytes in humans. IDO is a limiting enzyme in the breakdown of tryptophan (Trp). Trp metabolite production and degradation in the local environment are the factors that affect IDO-mediated immunosuppression.

The main effector molecule of MSC-mediated T cell immuno-suppression in mice is nitric oxide (NO). IFN- Gamma alone or in combination with TNF- alpha must activate MSC to have an immunosuppressive impact on T cells. These cytokines may originate from T cells that have been activated or from the inflammatory environment (Krampera et.al, 2003; Di Nicola et.al, 2002).

## In Vivo Advances: Preclinical Studies

Studies conducted in vivo have revealed a wide range of differences in MSCs' immunomodulatory abilities. The inhibition of the immune response in autoimmune and inflammatory illnesses and their ability to prevent or treat allograft rejection have been the two key areas of mainstream investigation of the advantages of transplanting MSCs.

### Autoimmune and Inflammatory Diseases

MSC treatment has been investigated in several clinical studies for autoimmune disorders, graft augmentation, and rejection.

#### 1. Experimental Autoimmune Encephalomyelitis (EAE)

The effectiveness of MSC therapy for autoimmune diseases was first noted when mice received intravenous injections of MSCs developed by EAE. Treatment was observed to reduce demyelination and leucocyte infiltration of the central nervous system (CNS) in the acute phase, hence preventing the clinical course of EAE (Zhang et.al, 2006).

#### 2. Experimental Diabetes

Experimental diabetes was improved in immunocompromised mice by systemic administration of human MSC. Similarly, in both allogenic and syngenic murine, MSC stopped and postponed the disease's development. MSC injections decreased the inflammatory infiltration in pancreatic islets and brought blood sugar and insulin levels back to their baseline.

### Cell and Solid Organ Transplants

#### 1. Heart Transplant

In mice, the immune-competent semi-allogenic heart transplantation model of donor MSC infusion slowed the graft rejection. MSC infusion, however, caused the rejection in a mouse model for an allogenic heart transplant (Krampera et.al, 2003). A synergistic effect was produced and tolerance was established for up to 100 days after transplantation when used in conjunction with rapamycin or mycophenolate mofetil.

**2. Kidney Transplant**

Allogenic kidney transplantation in mice was life-sustaining which was significantly improved by the donor MSCs. MSCs had to be administered many days in advance of organ transplant for both heart and kidney transplants, which implies that tolerance must be instilled before engraftment (Zhang et.al, 2006).

## Conclusion / Discussion

MSC has innate as well as adaptive immune responses and plays a therapeutic role in immunomodulation. DC, NKs, macrophages, and neutrophils are the major cells that act during the innate response of MSC while the, B and T cells are the important cells that play a major role during the adaptive immune response. Immune-mediated illnesses including autoimmune disorders and graft rejection can be treated with the help of mesenchymal stem cells' immunomodulatory characteristics.

More research is required on the origin of MSCs, and the predictive value of donor and host HLA (Human Leucocyte Antigen) matching. Preclinical findings indicate that combining immunosuppressive medication with MSC treatment would likely increase transplant survival and long-term function more than either therapy. MSC survival and an environment conducive to MSC activation would be enabled by immunosuppressive medications and transplantation.

On the other hand, MSC may lower the dosage of immunosuppressive medication, lowering the risk of adverse drug effects. MSCs were able to elicit T- cell anergy and tolerance in autoimmune, inflammatory, and transplantation experiments while not differentiating into cells of the injured tissue.

Due to their capacity to detect foreign molecules through their MHC molecules, MSCs in an infectious environment may not only lose their immunosuppressive properties but also increase inflammation.

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*Brief Review*

# Therapeutic Use of RNA Antisense Oligonucleotides

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**Abstract**

Antisense oligonucleotides (ASO) have the potential to serve as therapeutic agents for various diseases in living organisms by selectively targeting specific genes and altering their expression. By halting the production of defective proteins, ASOs can help to control the source of diseases. This field is gaining increasing importance in drug development and provides a positive alternative to downstream targeting processes. However, to translate ASO-based therapies into clinical success, it is crucial to address challenges such as off-target side effects and insufficient biological activity. This review paper aims to provide a comprehensive summary of ASOs, including their preparation, mode of action, and biological activities.

*Key Words: Human gene therapy, DNA vaccines, ASOs, ncRNA, miRNA, siRNA, RNAi pathway*.

## Introduction

In 1990, the first human gene therapy treatment was approved for adenosine deaminase deficiency. Gene therapy has shown success in curing genetic diseases, as demonstrated in patients with severe combined immunodeficiency in 2002. Research is currently underway to develop gene therapies for more complex disorders like Alzheimer's disease and polygenic cancers.

DNA-based therapeutics, on the other hand, have a wider range of applications beyond gene replacement. DNA vaccines are currently used primarily in veterinary clinics, but trials are underway to explore their potential for protecting against diseases such as malaria, tuberculosis, Ebola, HIV, and influenza.

Antisense oligonucleotides (ASOs) are synthetic RNA or DNA sequences designed to selectively bind to RNA that encodes the target gene. ASOs have been tested for a variety of disorders, and when they bind to their target, they can alter mRNA, prevent its attachment to ribosomes, or recruit RNase H to degrade it.

Non-coding RNA (ncRNA)-based gene regulation often depends on small, stable RNA molecules like miRNA. These molecules can be isolated from endosomes and micro-vesicles and used as biomarkers or therapeutics for many diseases. miRNA is a small, single-stranded ribonucleotide that regulates gene expression at the primary stages. They load onto an Argonauto (Ago) protein to form RNA-induced silencing complex (RISC), which represses mRNA translation by binding to complementary sites in the target transcript's 3' UTR. Human miRNAs have specific target sets and expression patterns, making them vital for controlling biological processes and developing drugs for clinical trials.

Short interfering RNA (siRNA), by contrast, are small, double-stranded complexes that trigger the RNAi pathway. While this pathway occurs naturally in some organisms, it does not occur naturally in humans and other mammals. Synthetic siRNAs can be utilized to bind and cleave RNA.

## Method for antisense oligonucleotides

One of the main techniques used to prepare oligonucleotides is called phosphoramidite synthesis (SPS). This method involves attaching the sequence to a solid support group or resin using a long chain linker. The synthesis cycle then begins with detritylation, which removes the DMT protection group.

In the second step, a nucleotide phosphoramidite monomer is activated with a catalyst, and the base functional group of the monomer corresponds to the next appropriate monomer. In the third step, the newly activated phosphoramidite monomer or base quickly reacts by joining the 5' end of the hydroxyl group of the previous base, which extends the growing sequence. The sequence is immobilized on a solid support or bound to the core structure used for liquid-phase synthesis, and this step is known as coupling.

After each coupling, each hydroxyl group at the end of the molecule is capped via acetylation to prevent deletion mutation, degradation, or the formation of unwanted side products. This process is called capping.

In the fourth step, oxidation occurs between the two nucleotides, forming a phosphotriester linkage. This cycle is repeated until the oligonucleotide reaches the desired length. This step is known as oxidation. Finally, the oligonucleotide is cleaved from its solid support using ammonolysis or ester hydrolysis, and protection groups are removed with caustic reagents.

### Mode of action

The action of antisense oligonucleotides (ASOs) can occur in two ways: either by causing RNA cleavage or by RNA blockage. RNA cleavage can happen through RNase H1 mediated cleavage or RNA interference (RNAi), where siRNA associated with RISC degrades mRNA. On the other hand, RNA blockage can happen through steric hindrance, where the ASO-mRNA complex blocks the interaction of mRNA with ribosomes, or splice modulation, which produces the correct form of protein by skipping mutated exons. These ASOs are designed with a unique genetic code that binds to specific sequences of nucleotides in the target mRNA, interfering with the production of abnormal proteins and ultimately stopping the disease progression. Numerous studies have confirmed that synthetic ASOs can work through RNA cleavages or RNA blockages.

### Applications

#### 1. SARS-CoV-2 antibody response

Various vaccines have been developed worldwide against SARS-CoV-2, using messenger ribonucleic acid (mRNA) that was prepared by inactivating the virus particle. The antibodies were created by collecting serum samples from MS clinics, aliquoting them into 1000 μL polypropylene tubes, storing them at -80°C, and then using Chemiluminescent microparticle immunoassay (CMIA) to quantify IgG antibodies against the receptor-binding domain (RBD) of the S1 subunit of the spike protein of SARS-CoV-2.

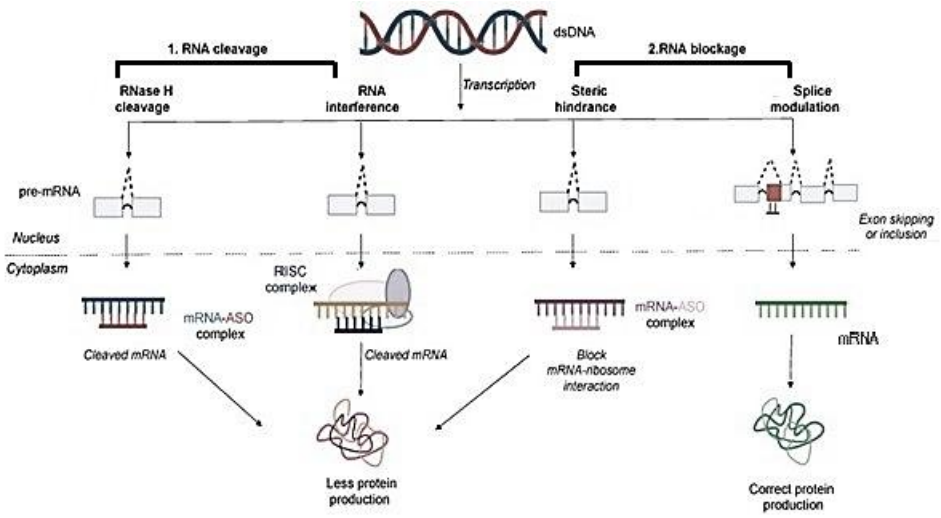


Fig - 1: RNA a1

## 2. Monogenic retinal degenerative disorders

Lipid-based nanoparticles (LNP) are nanostructures that contain various classes of lipids, such as cationic or ionizable lipids (CILs), PEG-conjugated lipids (PEG-lipids), and structural lipids like phospholipid or sterol. These can self-assemble under controlled microfluidic conditions when mixed in an aqueous solution containing nucleic acid. CILs, which are known for their amphiphilicity and positive charge, bind and encapsulate mRNA into organized LNPs. After delivering the mRNA to the back of the eye, the kinetics of gene expression show a rapid onset (within 4 hours) that persists for 96 hours. Gene delivery is cell-type specific, with most expression in the retinal pigmented epithelium (RPE) and limited expression in the Müller glia.

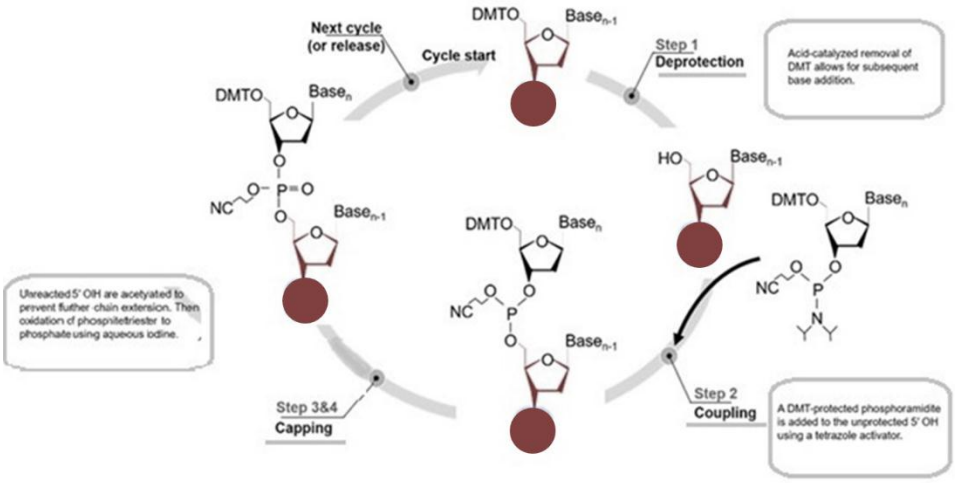


Fig - 2: RNA b1

### 3. mRNA vaccines for rabies

The first candidate for an mRNA rabies vaccine was CV7201, which consisted of lyophilized, temperature-stable mRNA made up of free and complexed mRNA that encoded rabies virus glycoprotein (RABV-G), along with a cationic protein protamine as stabilizer and adjuvant. The data showed a transient but significant increase in RABV-G-specific CD4+ T cells at day 42, which declined to baseline levels on day 91, consistent with the contraction and memory phase of the immune response, confirming the effectiveness of the mRNA vaccine.

### 4. Modified mRNA Vaccines against Zika Virus Infection

Modifying the preM-E RNA encoding mutation by destroying the conserved fusion loop epitope present in the E protein can produce a variant that can protect against ZIKV. LNP-encapsulated modified mRNA vaccines, which code for either wild-type or variant ZIKV structural genes, can be used for immunogenicity and protection. When two doses of these modified mRNA LNPs, which code for preM-E genes, are provided, they produce virus-like particles. As a result, high levels of antibody-neutralizing titers take place, which ultimately provide protection against ZIKV.

## Conclusion

This review paper presents an analysis of various antisense oligonucleotides (ASO) techniques and their pharmacological applications. ASO offers a wide range of processes, including RNAi, RNase H-mediated cleavage, splicing modulation, non-coding RNA inhibition, gene activation, and programmed gene editing, to regulate gene expression. ASO drugs have tremendous therapeutic potential and have gained recognition in recent years. However, the efficient delivery of oligonucleotides, especially to extrahepatic tissues, remains a significant challenge. This review provides an overview of oligonucleotide-based drug platforms, focusing mainly on chemical modification, bioconjugation, and nanocarrier-based approaches to address the delivery challenge. These approaches offer more selective action, conceptual simplicity, low toxicity, fewer side effects, and permanent cure activities. Hence, this review advocates the use of antisense oligonucleotides as a viable treatment option for various diseases.

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# Life Elements in the Fictions of Ernest Hemingway

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## Abstract

Ernest Hemingway is widely regarded as one of the greatest American writers of the 20th century. He won the Pulitzer Prize in 1952 and the Nobel Prize in literature in 1954. Despite his literary success, Hemingway remains a controversial figure, with some critics arguing that his narrow focus on violence and machismo, and his limited range of characters, make his fiction shallow and insensitive. Others see a complex and fully realized world beneath the deceptively simple surface of his writing.

Hemingway's characterizations evolved over time, reflecting both his personal struggles and his changing literary style. His male characters initially suffered from low self-esteem, inner conflict, and an inability to connect with others. However, his later heroes were more self-assured, formed meaningful relationships, and were driven by an idealistic code of conduct. Hemingway's early female characters were criticized for lacking depth and agency, but he later developed more complex and sophisticated female characters who expressed independent thought and will. Although Hemingway's female characters were initially divided into "nurturing" and "destructive" types, his later works challenged these simplistic categorizations, and included characters who defied gender norms and expressed free thought.

*Key Words: Ernest Hemingway, fiction, novels, chronological study, characterization, male characters, female characters, life elements.*

## Introduction

Ernest Hemingway, an acclaimed American writer of the 20th century, is well-known for his understated prose style. He was awarded the Pulitzer Prize in May 1952 (Meyers, 1985) and later the Nobel Prize in English literature. Despite his literary achievements, Hemingway's novels and short stories have been subject to extensive critical commentary due to his narrow range of characters and thematic focus on violence and machismo (Witalec, 2004). Some critics view his fiction as shallow and insensitive, while others argue that there is a complex and fully realized fictional world beneath the surface (Baker, 2005)

### Evolution of Characters

Hemingway's works exhibit a unique approach to character development. He portrays his heroes through minor details and sketches, allowing the reader to draw their conclusions without ready-made judgments (Madina, 2010).This paper aims to explore the characters in Hemingway's fiction and examine the development of characterization chronologically in the context of his life to draw conclusions. Hemingway's male heroes range from the frivolous Scripps and Yogi to the serious Jake and Frederic, with Robert Jordan and Santiago as the universal heroes.

**Early Novels**

The characters of Hemingway's first novel have puzzled dialogue delivery, loose in some places, and confused and bewildered presence. Yogi Johnson is a lovable character with an unresolved trauma that is sorted out at the end, while Scripps O'Neill deliberately upsets his life by running around with women and has low self-esteem. Scripps exhibits manipulative and dependent behavior, while Yogi displays a delightful child-like behavior that sometimes enters into a parent-protecting stage with the Wood Indians. The characters exhibit contrasting attitudes in the plot, with Scripps displaying inferior feelings and a lack of self-esteem, while Yogi poses superiority over others.

Hemingway's first novel is a parody itself, with no place for serious fiction, but his idea to float satire and target Sherwood Anderson is achieved. The heroines in The Torrents of Spring, Diana, and Mandy, are less expressive than the heroes, with Diana being a thin, gray, and aging character who clings to the hero for emotional support. She has average intellect and is jealous of Mandy, who lures Scripps away from her. Mandy is a bubbly, effervescent heroine with manipulative skills to lure men. Psychologically, Diana and Mandy cannot be placed in exact ego states, with Diana mostly in a parent state and Mandy in a child ego state, but both manipulate Scripps from their ends. The hero is feeble, drifted by their attempts and charms, as he is not a steady character.

In The Sun Also Rises, Jake, the protagonist, is a pure Hemingway hero with a genuine code. He is a newspaper man who mingles with other characters that seem lost and driven by their trauma or weaknesses. Jake has a phallic wound but takes it in stride. He is watchful of his words and actions, checking himself with a seemingly confident demeanor. Jake is troubled only when he sees Brett, whom he loves and feels vulnerable in her presence. Otherwise, he gets along well with Bill Gordon, Mike Campbell, and Robert Cohn. Different codes operating in the fiction bring versatility to the plot, but the characters are jaded due to their lost hopes.

Jake is emotionally stable except for his soft spot for Lady Brett. He temporarily loses his composure when he helps her be with Romero. Otherwise, he keeps his emotions under control in the company of others. Bill is the only character who does not fall for Brett's seductive ways. He genuinely cares for his friends, except for Robert Cohn. Cohn's broken marriage, going out with Brett, following her to Pamplona, and yet not leaving others' company, all indicate his imbalanced emotional state. Mike is a rough and risky character who puts his emotions at stake. He feels hurt when Brett leaves him for Romero but puts on a pretentious devil-may-care attitude for all. His emotional outbursts often come out during drinking sessions.

Robert Cohn seems indifferent to emotional upheavals. He persistsently trails people without feeling insulted and boasts about his accomplishments with superiority. This suggests that he is tough or pretends to be to cover up inner feelings. He only shows dejection once in the plot and hits Jake for sending Brett to Romero, but later regrets and cries over his folly. He addresses Jake as a ‘pimp’ which is a downright derogatory term (Hemingway, 2004). Romero is a perfectly balanced individual whose emotional stability lends him grace, skill, and a superior presence in the plot. He exhibits vulnerability when he falls in love with Brett and tries to be possessive towards her, but she resists.

**Reflection of Life Elements**

In the plot, Jake acts in a parent ego state with Brett when he checks her moves, and in an adult state with his other friends. His behaviour is sensible with a constraint. Bill is a sensible adult, while Mike is a manipulative child-like character who, along with Robert Cohn, manipulates people and circumstances. Cohn operates from the parent state, reflecting superiority from his being but inwardly insecure about being a Jew and feeling like an odd man out. Romero, like Bill, is in a balanced adult state that helps him make the right decisions regarding his profession. His stability unnerves Brett, who finds Mike more understanding.

On the temperamental stage, Jake is intelligent with negative shades, Bill is a sharp positive individual, Robert Cohn is average with negative tones, Mike is a little dull, reckless character, and Romero is a sensible positive one. The post-war generation of lustful, drunkard people who are disappointed with values and hope seem to bundle together, shifting from country to country and bottle to bottle, in search of love and peace. Hemingway has attempted to highlight relationship complexities through the female character, Lady Brett Ashley.

Brett is a charming and dominant individual who possesses the ability to seduce men. Her emotions fluctuate often due to her sensitive heart, which fancies one man after the other. She confides her guilt and changing affection to Jake, whom she is amiable with. Her psychological complexity is showcased as she portrays a child who wants everything in life from luxury to men. Brett maneuvers charmingly and can sway people with her approach. She has a sharp understanding of the traits of people, and her approach can be both confident and naïve, depending on the situation. Brett's relationships develop and break easily due to her erratic mood swings and behavior. Although she appears to be well-behaved and compliant outwardly, her inner mindset is disturbed. She does not evolve from her weaknesses, but at the end, she shows some positive aspects of her personality by leaving Pedro. Hemingway's personal experiences at Pamplona and a brief affair with Lady Tyson appear to be reflected in the story (Nagel, 1996). He married Hadley Richardson and took her to Pamplona with a couple of friends. Hemingway's genuineness in his diverse writing of fiction gave him instant success in 'The Sun Also Rises.' The author established himself as a fiction writer with this novel as he was the most successful in capturing the time and place in it. (Aldridge, 1990)

Next in line was 'A Farewell to Arms' (FTA), in which Frederic Henry, the male character, is treated by Hemingway as himself, depicting his experiences in Italy as an ambulance driver. Frederic is a serious hero who simply tries to clear up doubts regarding the war and his future in it. Although he is to be decorated with medals for his bravery on the war front, he is not eager about the entire thing. He loves Catherine Barkley and tries to establish a separate peace within himself by escaping from the Italian Army. The code hero, Frederic, is a questioning hero whose thoughts indicate constant dilemma as if to decide about the future. Even at the end, he is helpless about the future with Catherine passing away in childbirth. Hemingway does not describe Frederic and Rinaldi's physical features. The hero, Frederic, faces emotional upheavals and trauma of indecision throughout the plot. His condition is more vulnerable when in love with Catherine. To stay and fight as an Italian soldier or to quit the army, to marry or not to marry Catherine, is a source of concern. His confusions, weak decisions, dwindling professional and moral ethics, put a question to his emotional bearing.

Upon psychological analysis, one finds Frederic Henry in an obedient child ego state firmly under the supervision of parent and nurse, Catherine Barkley. At one instance, readers may find Catherine very submissive and complying, mistaking her to be a weak female guided by the male, Frederic. On close study, it becomes apparent that Catherine is manipulating Frederic and governing his life. He is cut off from friends and leads a hideous life of just survival under her supervision. The intelligence of Frederic is subdued due to blinded confusion and negativity infused in him due to war circumstances and his illicit relationship with Catherine. He is unable to plan, execute, and remain in command of the profession and relationship. When the decorations are to be bestowed on him due to valor, he quits the army. He flees with Catherine and lives a life of seclusion from society. The attitude of this character goes from bad to worse when he is left without Catherine at the end.

The author skillfully crafted Frederic and Catherine's characters in a unique way that distinguishes them from the mixed masculine and feminine traits portrayed in his posthumous novel, Garden Of Eden. However, Frederic's character seems to be controlled as a puppet by Catherine, causing him to become withdrawn, hopeless, and secluded from society after her death. Catherine, on the other hand, is a beautiful heroine with tawny skin and grey eyes who loves wholeheartedly with complete surrender, exhibiting genuine feelings and a sacrificing temperament. Although she is a complex character still stuck in the past, some critics argue that she undergoes change towards the end of the plot. (www.Studymode.Com)

Hemingway's motivation for writing this novel remains unclear; he may have been reflecting on his marital conflicts or attempting to depict his parents' stifled relationship. He had divorced Hadley and married Pauline before writing this novel but could not sustain this relationship after its release. Faulkner's comment that "For every new novel, Hemingway would require a new woman" seems to hold some truth. (Timeless Hemingway, 2009) Hemingway, tired of war and its after-effects, turned his attention to the fishermen and war veterans of Key West, realizing that people can sustain their livelihoods through means other than war. Thus, he wrote To Have and Have Not, focusing on the privileged and underprivileged sections of society.

The protagonist, Harry Morgan, initially a fair fisherman, turns to illegal activity after being cheated by an unfair customer. He becomes a hard-core planner, tough smuggler, and tries his best to get money from Chinese dealers and Cuban revolutionaries cum bank robbers to provide for his family. Although he has a tough exterior, he is soft on the inside and is never seen in an emotional dilemma except when thinking of his wife and family. Psychologically, he seems to be a mature adult who prioritizes his family's well-being. His uncanny ability to scheme and attack his opponents unnerves readers. Despite being a lone man, his sharp intellect, strong physique, and family bonding are his strengths that make him unique. His attitude is positive and down-to-earth, with shades of cynicism and depression at times. However, Hemingway worked on a different concept after THHN, recognizing that Morgan could not achieve his goals alone.

In "For Whom the Bell Tolls," Hemingway created the universal hero Robert Jordan who has resolved his inner conflicts and views his mission with confidence. Jordan loves Maria without mingling his personal and professional lives, and he trains his guerrilla band like a professional. The author also created the character of Anselmo, an old man who embodies genuine hope and discipline despite the corrupt system of values.

Physically, Jordan is stocky with sun-streaked hair and a sunburnt face, and emotionally stable. His psychology is that of an adult, and he conducts his mission to blow up a bridge with a clear and sensible strategy. He is unafraid of his enemies and impending death and has a sharp intellect, especially after falling in love with Maria.

Hemingway also shaped female characters in Pilar and Maria, who have distinctive features and are supportive of each other. Pilar is a masculine woman who is supportive of tribe members while Maria is more feminine and compliant with orders. Hemingway reached a zenith in character sketching with his distinctive characters and iceberg theory of writing.

In "Across the River and Into the Trees," Hemingway crafted the character of Colonel Cantwell, a lovable old man who is charming and bold. He spends quality time with his young lover Renata in Venice, reminiscing about his war experiences and looking for something enjoyable in his remaining life. (Meyers, 1985). The plot gently brings out the condition of Venice and its residents after WWI. Cantwell is unlike other old men in Hemingway's works as he is physically and emotionally weak but maintains a stoic facade. Renata is a decisive and enduring young girl who never wavers in her emotional support for Cantwell.

In Hemingway's masterpiece, The Old Man and the Sea, Santiago embodies the quintessential code hero. Hemingway artfully depicts Santiago's character development as a symbol of his own post-war journey towards healing. Despite being unable to catch fish for 84 days, enduring ridicule from young fishermen, and confronting fierce natural forces, Santiago remains steadfast in his pursuit. He bravely battles sharks to save the giant marlin he catches, ultimately succumbing to defeat. Yet, his humility and unwavering spirit remain unbroken as he returns to shore, never boasting of his heroic feat. Physically frail, with deep creased scars, Santiago's admirable endurance and undefeated, sea-colored eyes are a testament to his emotional stability and connection to nature. Unaffected by jeering and adversity, Santiago's peaceful heart and temperate demeanor ensure that he is never defeated.

Santiago's parent ego state, child ego state with Manolin, and occasional healthy adult interaction level demonstrate his well-rounded temperament. His awe of nature and strong communion with it bring out his childlike wonder, yet his peace and balance within help him overcome external challenges with ease. Hemingway alludes to Christ-like qualities in Santiago's humility, simplicity, and contentment (Brenner, 1991). Manolin serves as Santiago's soulmate, providing comfort and assistance. Santiago's limited supplies are met with a positive outlook towards life.

Hemingway's own personal conflicts, such as troubled childhood, marital discord, lack of esteem, war, unrequited love, disappointment, criticism, injuries, and diseases, are either resolved or accepted with calm resignation. Santiago is only spiritually connected to God when he speaks of the Seven Commandments and Mary, otherwise he finds solace in nature and its objects. Santiago's struggle with glory is so wonderful that readers rejoice in it. The novel's dictum, "Man can be destroyed but never defeated," (Hemingway, 2004) is beautifully embodied by Santiago's character and philosophy, which he imparts to the young Manolin.

Hemingway's protagonists share certain common traits, but the author has skillfully diversified these characteristics in a unique and loving way (Madina). These traits encompass their psychological, physical, temperamental, and emotional makeup. In the present paper, the author explores Hemingway's vision of his characters displaying different ego states in the 19th century,

using Transactional Analysis (Muriel and Dorothy, 1971) as a tool. Through his fiction, Hemingway depicts everyday people in Europe during that time period. For instance, The Torrents Of Spring offers a glimpse into the extravagant lifestyle of the roaring twenties, initially starting as a playful parody of Sherwood Anderson's depictions of mechanized folks. Hemingway's work also includes portrayals of Indians, club life, waitresses, bartenders, telegraph operators, and factory workers. The dark humor of Hemingway's writing, characterized by its eerie chapter endings, is cleverly conveyed in a distinctive voice that captivates and amuses readers.

**Transformation in Characterization**

If we trace the characters chronologically through Hemingway's life, two phenomena become apparent. First, as expected, the author's style of characterization improves over time. Second, Hemingway's wounded and ailing spirit, torn by war, slowly recovers and gains strength to overcome the forces of nature.

In earlier fiction, male characters were portrayed as complex and often had low self-esteem. Their dialogues were muddled, their thoughts lacked development, and their trauma hindered their personal growth. Characters like Scripps, Jake, and Frederic suffered from inner turmoil, lacked substance, and struggled to find harmony in their lives. They often feared and resented society and sought refuge in nature and work, running away from civilization (Madina). Some characters made peace with their lives but pursued materialistic gains and sensuous pleasure, projecting inflated egos with little regard for women.

Over time, Hemingway's heroes became simpler, more resolved, and had greater self-esteem. Their dialogues were clearer and more precise, and they developed relationships with people from various backgrounds more willingly. Hemingway shifted his focus from a hedonistic code of conduct to an idealistic one, promoting the idea that evil can be defeated by targeting society's problems (Bryant, 1981). Characters like Harry Morgan, Robert Jordan, Cantwell, and Santiago were more mature and had resolved thoughts. They drew energy from nature and possessed the power to change the world. These characters exhibited more value systems, corrected moral conduct, and had strength of character. They were respectful towards women and had less animosity towards their opponents. The transformation from chaos, unrest, disorder, and degenerate feelings to general well-being, joy, peace, and contentment was evident in the characters and their stories. The concept of spirituality was hinted at but not fully explored.

Hemingway initially faced significant criticism for his portrayal of female characters, who were often criticized as being one-dimensional and amoeba-like. However, he did not rest at this stage and worked hard to give strength to his female characters, whether dominant or submissive. Despite the criticism, he continued to refine his craft and his female characters became more complex and sophisticated over time, with greater attention paid to their personalities, appearances, and dress.

While some of Hemingway's female characters could be classified as either nurturing or destructive types, such a classification is an over-simplification and does not do justice to the full complexity of these characters. Characters like Diana and Mandy could be seen as mindless, while others like Brett and Catherine Bourne were made bad by their circumstances. Renata and Maria, on the other hand, were loving and trusting females.

Catherine in A Farewell to Arms and Maria in For Whom The Bell Tolls are peripheral characters who are mirror images of each other, representing Hemingway's idealized version of the perfect lover: submissive, shallow, selfless, and self-effacing. While both characters have deep psychological wounds, they are willing to risk public shame and humiliation to serve their men.

Despite these portrayals, Hemingway's later works, such as Garden of Eden, show a shift towards more complex, independent female characters with free thought of expression. Hemingway sympathizes with women in failed relationships, and the woman he values most is one who complicates his life the least. However, such a woman is rare, and a marriage with her often ends unhappily.

**The Ultimate Mark of Appreciation**

Hemingway's Golden Period is believed to have spanned from "For Whom the Bell Tolls" to "The Old Man and the Sea," during which he was honored with the Nobel Prize for his last novel. After a lifetime of searching, the author ultimately found peace, contentment, and happiness. He learned to live with his trauma, resolve his thoughts, and come to terms with adverse people and circumstances by developing a deep faith in himself and humanity's creator. However, fate dealt him a cruel blow when he was involved in a plane crash on his way to Africa, causing him to suffer from pain and injury and bringing him back to a troubled physical and mental state (Baker, 1972). Despite his deteriorating health, Hemingway continued to write, producing novels such as "Islands in the Stream" and "Garden of Eden."

In "Islands in the Stream," Hemingway created the character of Thomas Hudson, a professional painter who is sensitive, mature, and close to nature. In the first part of the novel, he spends time with his three sons during their vacation. In the second part, he becomes disillusioned after the death of his sons, and in the last part, he chases smugglers. Unfortunately, the novel fails to create the magic of Hemingway, as it is loosely spun and has broken parts. Perhaps the author's spirit was wilting or had entered unknown territory without recognition.

Similarly, in "Garden of Eden," Hemingway introduced David Bourne and Catherine, a honeymoon couple whose relationship becomes complex due to gender transformation and the introduction of another woman. Although the concept was novel, it did not generate the desired response from readers.

In contrast, Hemingway's posthumous heroes, such as Thomas Hudson and David Bourne, have more variations in their personal and professional lives. Thomas Hudson endures trauma from a broken marriage but conceals it by remaining disciplined and committed to his profession. He reflects different characteristics in the three sections of the novel, maintaining a healthy adult ego state for his sons and friends. He buries his thoughts of the past in routine tasks and never manipulates or plans adversely in the first half. In the second half, he becomes a complex and degenerate character, maintaining a neutral philosophy of life and exercising caution in building intimate relationships.

David Bourne possesses a sensitive nature and has the ability to create vivid experiences and emotions. However, he struggles to cope with his wife's sudden gender transformation, initially accepting it without complaint but later breaking off under the strain. When facing his wife Catherine, he often exhibits a child ego state, displaying non-confrontational behavior and a help-

less attitude, indicating his inferior presence before her superior complex personality. The temperament of David and Catherine contrast greatly, with Catherine displaying a destructive parent form that upsets the healthy environment of the family. Hemingway has crafted Catherine's character in all shades, experimenting with her dressing and accessories to attract David and the readers. Although emotionally insecure and sensitive, Catherine wears a mask of rudeness and unconcern that later crumbles when she is unable to cope with her trauma. Her frequent mood swings upset her relationship with David, causing temperamental problems. She has an extremely sharp intellect, which is dulled later when she is unable to cope with her mental and emotional struggles, leading her to attack people with criticism and eventually leave David and Marita to live together.

Hemingway's posthumous works, although regarded as inferior, leave much to be desired. The pattern of his evolution and gradual senility of spirit is evident in his later works after The Old Man and The Sea. Hemingway was unable to avoid the two dangers he diagnosed, one being his tendency to deceive and live up to his popular image, and the other being the degeneration of his writing skills in later stages, leading to his corruption and slow yet steady destruction of spirit.

## Conclusion

Although Hemingway was an ailing spirit and could not carry on with his deterioration in mental and physical health, he lived life on his own terms, following the ideals of honor, courage, and endurance in a chaotic, stressful, and painful world. Ultimately, his strength could not go on any further, and the forces of nature defeated him. Nevertheless, Hemingway will be remembered forever as the "Father of Characterization" in English literature. Despite his suicide (Reynolds, 2000) and the bulk of unpublished material he left behind, Hemingway's career graph grew and amounted to numerous works across various genres, characterized by his realistic portrayal of live, simple, and distinct characters, reflecting the author's own personality as a Nobel Laureate.

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